Journal of Medical Entomology, 58(3), 2021, 1188–1196
doi: 10.1093/jme/tjab009
Advance Access Publication Date: 11 February 2021
Research

Field Evidence of Mosquito Population Regulation by a Gregarine Parasite

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Subject Editor: Patricia Scaraffia

Received 24 November 2020; Editorial decision 23 December 2020

Abstract

Although parasites are by definition costly to their host, demonstrating that a parasite is regulating its host abundance in the field can be difficult. Here we present an example of a gregarine parasite, *Ascogregarina taiwanensis* Lien and Levine (Apicomplexa: Lecudinidae), regulating its mosquito host, *Aedes albopictus* Skuse (Diptera: Culicidae), in Bermuda. We sampled larvae from container habitats over 2 yr, assessed parasite prevalence, and estimated host abundance from egg counts obtained in neighboring ovitraps. We regressed change in average egg count from 1 yr to the next on parasite prevalence and found a significant negative effect of parasite prevalence. We found no evidence of host density affecting parasite prevalence. Our results demonstrate that even for a parasite with moderate virulence, host regulation can occur in the field.

Key words: parasite, host, population regulation, mosquito, *Aedes*

By definition, a parasite must have a negative influence on its host's fitness. A large body of theoretical literature has developed around the ability for parasites to play a primary role in population regulation of hosts (e.g., Anderson and May 1979, May and Anderson 1979, Tompkins et al. 2011), but actual regulation by a parasite alone has been more difficult to show. Especially in the field, where it is possible that parasitized individuals are already suffering from other regulatory forces, such as competition or predation, parasitism may be acting in a compensatory fashion, rather than as the primary driver of host regulation (Beldomenico et al. 2009). For population regulation of a parasite to be possible, the parasite must influence the host in a density-dependent manner (Anderson and May 1979, May and Anderson 1979).

Specifically, the response of the host population growth rate must be an inverse function of parasite abundance or prevalence, which reaches equilibrium at some level of parasite prevalence between 0 and 1. This latter criterion distinguishes between adversity imposed on a host population and regulation, because mere adversity may not be sufficient to bring the host population to an equilibrium level. Additionally, parasite abundance in field conditions must be shown to approximate the abundance necessary for the parasite to bring the host population to equilibrium. These criteria apply to either microparasites or macroparasites, although theoretical bases for them are derived differently (Anderson and May 1978, 1979; May and Anderson 1979). In either case, an inverse relation between host rate of change and parasite abundance (quantified either as the intensity of parasitism, i.e., the number of parasites per host or the prevalence of parasites, i.e., the fraction of hosts bearing parasites) is a paramount requirement. Examples are illustrated in Fig. 1.

Despite a strong theoretical basis pointing toward the potential population regulation of hosts by parasites, relatively few studies have been able to demonstrate the effects of parasites on wild hosts. Those that do typically manipulate parasite loads in host populations, such as the classic study by Hudson et al. (1998) in which the removal of a nematode from Red Grouse populations in the United Kingdom demonstrated that the parasite was limiting host population growth (Hudson et al. 1998). Other studies have manipulated parasites to find that host survival may be reduced by the parasite (e.g., Brown et al. 1995, McKilligan 1996, Gonzaga et al. 2015) or that host clutch size/number of offspring declines (Bize et al. 2004, Marzal et al. 2005, Gooderham and Schulte-Hostedde 2011), which could lead to population regulation. Yet, not all such studies have been able to detect fitness cost in the field (e.g., Forbes et al. 2014, Raveh et al. 2015), and others have shown that parasite removal could have negative consequences (Roby et al. 1992, Van Oers et al. 2002). In a meta-analysis by Watson (2013), of the 38 papers analyzed which manipulated host parasite loads in field populations, there was an overall moderate and negative effect of parasites on their hosts, but 11 studies found no effect or a positive effect of parasites, and there was some evidence that publication bias might exist

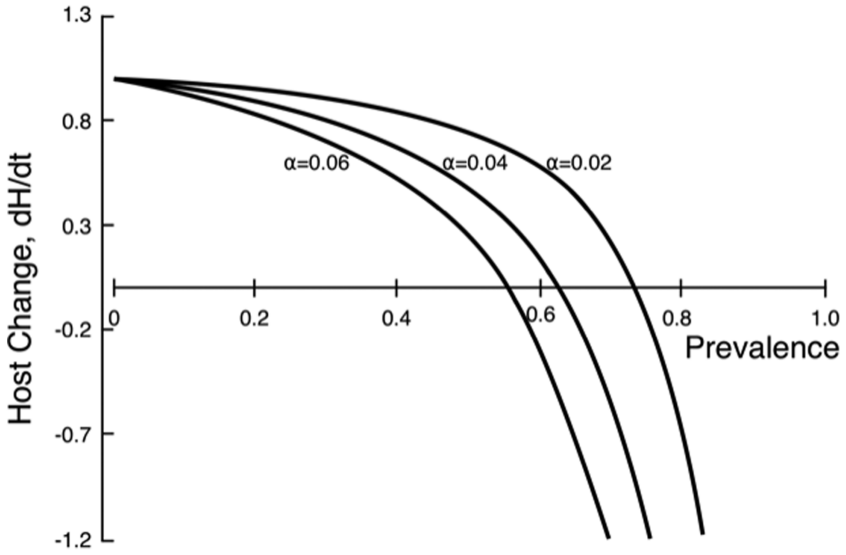

Fig. 1. Examples of functions obtained for the macroparasite model of Anderson and May (1978) for situations in which the parasite can regulate its host population. Scenarios with different degrees of virulence (α) are shown for mean host density of 10, and a negative binomial $k = 0.62$. Parasite abundance, the variable used in the microparasite model, is converted here into prevalence based on the host mean density and the negative binomial k value (see Supp Text [online only] for derivation). Note that functions decline monotonically, and that they all intercept the horizontal axis between 0 and 1.

in the literature (Watson 2013). Thus, while there is evidence of parasite regulation of host populations in the field, the matter is far from settled; evidence for a regulatory effect at the population level is rare, and additional evidence is needed to assess the degree to which parasite regulation actually occurs in the field. The daunting practical difficulties of testing for regulation by any mechanism using field census data alone has been emphasized by Murdoch (1994).

The biological control literature provides numerous examples of the introduction of natural enemies to control pest populations, which presumably constitute examples of pest population regulation (e.g., Murdoch et al. 1995). In a preponderance of cases, successful control has been achieved either through the use of parasitoids or predators rather than parasites (Huffaker 1971, DeBach and Rosen 1991).

Manipulation of parasite load in the field is not equivalent to observation on the natural effects of a parasite on a host. Load manipulation, while a valuable tool for assessing the effects of a parasite, is nonetheless a manipulation of field conditions and thus is not necessarily representative of the outcome of field conditions alone. Thus, field observations on the effects of parasites on wild hosts without manipulation still hold considerable value. Often, studies can demonstrate a cost of the parasite, as in Hakkarainen et al. (2007), where body mass of bank voles on isolated islands was negatively associated with the presence of a coccidian parasite, or where mortality rates were negatively associated with shore crabs infection with acanthocephalan parasites (Latham and Poulin 2002). However, demonstrating host regulation by the parasite itself is another matter, in no small part because it can be difficult to disentangle species interactions in ecological communities. For instance, in Latham and Poulin (2002), mortality effects of shore crabs infected with acanthocephalan parasites declined in times of year when birds were absent, suggesting an interaction with predation. Thus, simple systems may provide valuable insight into important concepts in disease ecology by limiting complicating interactions. Here we present just such an example, using an *Ascogregarina* parasite in its invasive mosquito host, with both species now established on Bermuda.

The Bermuda Islands (United Kingdom) form an archipelago in the North Atlantic located <1,100 km south-southeast off the coast of North Carolina. The archipelago has 181 islands and, at the northernmost fringes of the tropics, has a subtropical climate consisting of hot, humid summers, and mild winters. The total landmass of Bermuda is less than 54 square km, almost all of which is encompassed by the four main islands connected by roadways: the Main Island, Somerset Island, St George's Island, and St David's Island. The fauna of Bermuda is presently dominated by invasive and introduced species; of an estimated 1,600 resident plants and animals, approximately 430 are native (Sterrer et al. 2004).

One such naturalized insect invader is *Aedes albopictus*, which was found in Bermuda in 2000 and rapidly displaced *Aedes aegypti*. *Aedes albopictus* is an enormously successful invasive disease vector whose range has expanded from Asia to every continent (Bonizzoni et al. 2013). In a matter of 5 yr, *Ae. albopictus* completely replaced *Ae. aegypti* throughout Bermuda, as tracked from egg surveillance data (Kaplan et al. 2010). The Bermuda Ministry of Health maintains a robust vector monitoring program, tracking more than 580 ovitraps on a weekly basis, whose oviposition slats can be used for identification of species or to track population size through time (Kaplan et al 2010).

The displacement in Bermuda largely mirrored that of the Southeastern United States, where initial observations on larval competition had suggested that *Aedes albopictus* was a superior competitor (Juliano 1998, Braks et al. 2004), but the speed of displacement made competition alone seem unlikely to be the sole cause of displacement (Kaplan et al. 2010). More recent studies have suggested that male *Ae. albopictus* mating interference and harassment of female *Ae. aegypti* could be responsible for the displacement (Tripet et al. 2011, Bargielowski et al. 2013, Soghigian et al. 2014). Briefly, researchers considered other options, such as apparent competition, in which the parasite *Ascogregarina taiwanensis*, introduced with *Ae. albopictus* to the United States (Munstermann and Wesson 1990) could have been responsible for *Ae. aegypti*'s decline. Although researchers have since dismissed *A. taiwanensis* as a causative agent in the collapse of *Ae. aegypti* due to low mortality effects of the

parasite on this non-native host and the relative rarity of finding *Ae. aegypti* infected with *A. taiwanensis* in the field (Blackmore et al. 1995, Juliano 1998), *A. taiwanensis* remains a subject of study today because of the importance of *Ae. albopictus* as a disease vector and gregarines' ubiquitous distributions with their hosts and competing species (Beier and Craig 1985, Westby et al. 2019a, Westby et al. 2019b).

Ascogregarina are protist parasites of Culicidae first described by Ross (1895) in the gut of *Ae. aegypti*. *Ascogregarina* primarily infect container-dwelling *Aedes* mosquitoes, such as *Aedes albopictus*, which is naturally infected by *A. taiwanensis* (Chen 1999). Infection by *A. taiwanensis* begins when filter-feeding larvae of any instar ingest the oocyst, which releases infectious sporozoites into the larval midgut, which burrow into host epithelial cells, and the parasites gather host mitochondria to their cell membranes as they develop intracellularly within their host (Chen et al. 1997). The parasite responds to host molting hormones and exits host cells, migrating to the malphigian tubules (Huang et al. 2006). Once there, parasites fuse in syzygy to form a gametocyst, from which oocysts 'bud off' (Chen et al. 1997). These oocysts remain in the host until eclosion, at which point some portion of them remain with the adult and others are released back into the habitat (dependent on the sex of the mosquito; Soghigian and Livdahl 2017).

Ascogregarina species can be detected either through larval dissections or through polymerase chain reaction (for morphological identification, see Munstermann and Wesson 1990, Reyes-Villanueva et al. 2001; for polymerase chain reaction techniques, see Morales et al. 2005, Erthal et al. 2012). Virulence of *A. taiwanensis* within its natural host can be described as low-to-moderate, due to low mortality costs under laboratory conditions. Consequently, some authors have suggested that these parasites are largely benign and that they would be insufficient for use in biological control in their natural hosts (Beier and Craig 1985, Reyes-Villanueva et al. 2003, Tseng 2007). However, environmental factors such as food availability and the amount of parasite exposure can lead to elevated mortality rates in aquatic stages of the mosquito, longer development times, and reduced body size in adults (Comiskey et al. 1999a, Tseng 2004, Soghigian and Livdahl 2017). Thus, evidence suggests that these parasites have a low-to-moderate virulence, resulting in deleterious effects on important elements of life history such as body size and time to emergence, and thus could regulate host abundance.

In Bermuda, *Aedes albopictus* population size appears to have stabilized since 2002 (Kaplan et al. 2010). To date, *Ae. albopictus* remains the only exclusively container-dwelling mosquito in Bermuda (personal observations), although *Culex pipiens* complex mosquitoes are also present and sometimes utilize the same habitats as *Ae. albopictus*. These two species compete asymmetrically; studies have shown that *Ae. albopictus* responds to intraspecific density in experimental microcosms but not to *Cx. pipiens* density, while *Cx. pipiens* responds to *Ae. albopictus*' density (Carrieri et al. 2003, Costanzo et al. 2005).

Thus, because we had previously detected *A. taiwanensis* in Bermuda, and Bermuda larval habitats are relatively simple in terms of larval species complexity, together with the regular oviposition data available to us through the Bermuda Ministry of Health, we found this an excellent study location to attempt to observe population regulation by a parasite in its host. Furthermore, due to oviposition data available and our ability to sample sites alongside ovitraps, we could observe the effect of parasite prevalence on local host abundance through the number of eggs in ovitraps. Based on previous experimental evidence demonstrating moderate virulence of this parasite, we hypothesized that we would find a negative relationship between disease prevalence and change in egg counts in ovitraps.

Our approach takes advantage of the patchy distribution of the *Ae. albopictus* host, which may be best described as a metapopulation (sensu Hanski and Gilpin 1997): discrete patches of mosquitoes occupying clustered habitats, each of which may have a high extinction probability, with unoccupied patches that can be colonized readily by mosquitoes from neighboring patches. This metapopulation structure presents possibilities for variation in parasite prevalence, which we can exploit to test for the parasites' potential role in local host population change.

Methods

Field Sampling

We sampled sites across Bermuda in October 2012 (week 40 in Fig. 2) by traveling from east to west, seeking small container habitats with assistance from Bermuda's Vector Control unit of the Ministry of Health. During 2012, we sampled one container in each of 18 sites that had *Ae. albopictus* larvae (Fig. 3); most of these were residential areas and all were artificial containers (Table 1). We sampled by taking up to twenty third or fourth instar larvae per habitat using pipettes or turkey basters and placing them in vials with ethanol. We returned to our 2012 sites where we found *Ae. albopictus* in 2013 and, where possible, sampled in the same approximate area, limiting ourselves to within a few blocks from the original sampling area. As a result, we gathered samples from fewer sites in 2013 than in 2012 (11 sites total; Table 1).

Quantifying Parasite Prevalence

We had previously confirmed that the parasites in Bermuda were *A. taiwanensis* (see Erthal et al. 2012 for details). In the present study, we quantified parasite prevalence using molecular methods customized here but based on the primers of Morales et al. (2005). For each mosquito in each sample, we separated larvae and rinsed them in distilled water. We identified larvae to species using Darsie and Ward (2005), and found only *Ae. albopictus* and *Culex pipiens* complex mosquitoes. We included only samples that had *Ae. albopictus* larvae.

We extracted DNA using the EZNA Forensic DNA Extraction Kit from Omega Biotek. From these DNA extracts we amplified parasite or host material from each extraction in a reaction mix containing 12.5 µl of Promega GoTaq Green MM2 buffer (Promega), 1.25 µl of 10 µM forward and reverse primers (AU and AT or 5.8S and 28S; see below), either 7 µl (parasite) or 9.5 µl (host) of nuclease free water for parasite reactions, and either 3 µl of sample (for parasite detection) or 1 µl of sample (for host DNA amplification). The reaction mixture was placed in a thermal cycler with PCR conditions of an initial denaturing phase of 95°C for 2 min followed by 35 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 1 min, with a final extension step of 5 min. These reaction conditions, using the primers AU (5′-ACC GCC CGT CCG TTC AAT CG-3′) and AT (5′-GAG AAG CCG TCG TCA ATA CAG C-3′), amplified 450 base pairs of the ITS region in *A. taiwanensis* (Supp Fig. 1 [online only]). For all samples, we also confirmed that our extractions had been successful by amplifying the ITS2 region of the host using previously published primers which bound to the 5.8S region and the 28S region of host rRNA genes and amplified the ITS2 region (Collins and Paskewitz 1996). In *Ae. albopictus*, this band corresponded to a nucleotide length of approximately 550

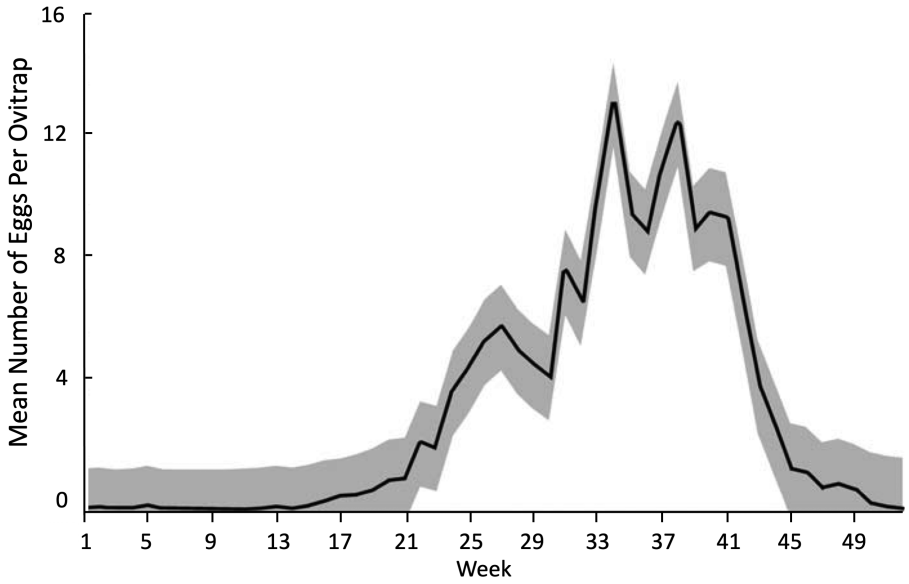

Fig. 2. Mean weekly egg production of *Aedes* mosquitoes pooled across years for 2002–2007 with standard error. Reprinted with permission from Kaplan et al. 2010. Our sampling took place in week 40, while our ovitrap data was from week 38, 39, and 40 for 2012, 2013, and 2014.

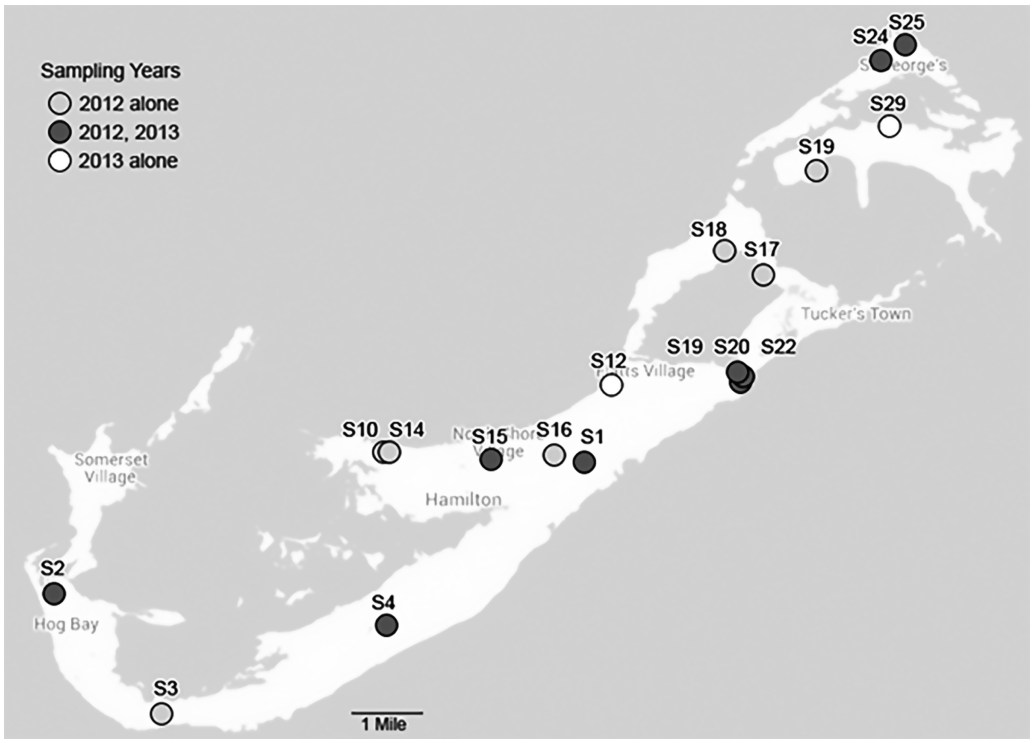

Fig. 3. Geographic location of all trapping sites. Light gray sites were only sampled in 2012, dark gray sites in both years, and white only in 2013. Map made in Google Maps.

base pairs, while it was approximately 400 in *Culex pipiens*. We considered a sample positive if we saw a parasite band, and we only considered samples negative if we saw a band for host DNA (indicative of a successful extraction) but no band for parasite DNA (Supp Fig. 1 [online only]). We considered an extraction failed if we detected no host DNA, which occurred in 8 of 428 extractions.

We chose these methods because it was more efficient to process a large number of samples than individual larval dissection, and we have previously shown that PCR techniques are more sensitive for detecting parasite presence, albeit for oocysts (Erthal et al. 2012).

We then estimated the prevalence for each sample site by dividing the number of positive *Ae. albopictus* samples by the total number of successful *Ae. albopictus* extractions.

Scoring Relative Abundance

The Vector Control unit of Bermuda's Ministry of Health maintains more than 580 ovitraps island wide, which allowed us to estimate change at a site between years. The ovitraps consist of an amber colored glass jar, with water and a Masonite paddle for oviposition within. Vector Control collects the paddles weekly from

Table 1. Collection information for each sampling site

Site ID	Year	Site type	Container type	*N*	Number *Ae. albopictus*	Number *Culex*
S1	2012	Residential	Vase	13	13	0
S2	2012	Residential	Vase	8	8	0
S3	2012	Agricultural	Bucket	16	3	13
S4	2012	Residential	Base of pot	20	17	3
S8	2012	Residential	Bucket	20	15	5
S10	2012	Residential	Flower pot	20	15	5
S15	2012	Residential	Vase	9	9	0
S16	2012	Residential	Base of pot	17	12	5
S17	2012	Residential	Base of pot	15	7	6
S18	2012	Cemetery	Flower pot	17	17	0
S19	2012	Residential	Base of pot	18	12	4
S20	2012	Residential	Flower pot	18	4	13
S22	2012	Residential	Dog water bowl	10	10	0
S24	2012	Cemetery	Flower pot	15	13	2
S25	2012	Empty Lot	Empty paint tray	20	20	0
S27	2012	Dump	Tire	13	6	4
S1	2013	Residential	Flower pot	3	3	0
S2	2013	Residential	Planting tray	20	19	1
S4	2013	Residential	Umbrella post holder	13	9	4
S12	2013	Residential	Vase	20	11	9
S15	2013	Residential	Base of pot	13	12	1
S19	2013	Residential	Tray	17	16	1
S20	2013	Residential	Umbrella post holder	13	13	0
S22	2013	Residential	Vase	20	20	0
S24	2013	Cemetery	Flower pot	20	18	2
S25	2013	Empty Lot	Bucket	20	18	2
S29	2013	Boat Yard	Bucket	20	12	8

each trap and counts eggs on each paddle. We were provided these oviposition data by the Bermuda Ministry of Health for the week of our sampling and 2 wk prior for each of the 2 yr of sampling and the subsequent third year. For each site per year, we used the three ovitraps nearest to the site and the three total weeks of oviposition data and calculated an average egg count per ovitrap per week, which we considered our relative abundance value. We chose to use an average of the 3 wk of oviposition data as the larvae present in the one habitat we sampled would likely be representative of recent local adult activity, rather than just a single week in that time span, and we felt that 3 wk better accounted for stochasticity than a given week. We calculated the rate of abundance change in ovitrap count from 1 yr to the next by subtracting the average eggs per ovitrap in the year of sampling from the relative abundance in the following year.

Statistical Methods

All statistical analyses were performed in R. We used Type II linear regressions, as implemented in the sma function from the package smatr, to evaluate combinations of explanatory and response variables (Warton et al. 2012). The sma function provides confidence intervals for estimated Type II slopes and intercepts, and can test several null hypotheses depending on input variables, such as that the variables are correlated, or whether factor levels of some group factor have different slopes in a multiple regression.

We used the prevalence of *A. taiwanensis* in *Ae. albopictus* as our explanatory variable and the difference in average ovitrap count between years as our response variable to test the hypothesis of parasite regulation of host abundances, that is, that from year to year parasite prevalence at the height of abundance of the host (Fig. 2) would be negatively correlated with host abundance in the subsequent year. As the sma function can also be used to evaluate whether levels of a categorical variable have different slopes in a multiple regression, we also tested whether the two sampling years had different slopes for the regression of prevalence and change in average ovitrap count. We also tested the relationship between relative abundance of *Ae. albopictus* in the year of larval sampling and prevalence of *A. taiwanensis* in *Ae. albopictus* to test the hypothesis that *A. taiwanensis* prevalence was dependent on host density. Finally, we built additional models to test whether the presence of *Culex* had any effect on relative abundance of *Ae. albopictus* between years by regressing the change in average ovitrap count against the proportion of *Culex* in a sample. In each case, we tested model residuals for normality using shapiro.test in R, and homoscedasticity using Bruesch-Pagan's test from the package lmtest. When either assumption was violated, we bootstrapped 95% BCa (DiCiccio and Efron 1996) confidence intervals around slope estimates (β) and assessed whether these slopes differed from zero. We also evaluated whether an ordinary least squares regression which provides a more direct test of the hypothesis of parasites acting as regulators of host density, would yield similar results to our Type II regression, with prevalence of *A. taiwanensis* in *Ae. albopictus* as our explanatory variable and the difference in average ovitrap count between years as our response variable.

We also tested whether there was a difference in average egg count per trap over our 3 yr of ovitrap data using a Kruskal–Wallis test, chosen because non-normal distributions warranted against a one-way ANOVA.

Results

We found a significant relationship between change in average ovitrap count and parasite prevalence in host ($\beta = -52.69$, $R^2 = 0.48$, $P < 0.001$), but there was no significant effect of sampling year on

the relationship between change in average ovitrap count and prevalence in host (LR = 0.03, df = 1, $P > 0.84$). Since the slopes did not vary with sampling year, we interpreted our results based on the results of our bivariate regression alone (Table 2, Fig. 4). The function obtained by regression passes through the horizontal axis at a prevalence of 0.65 (95% CI: 0.57, 073). This estimate overlaps with the overall prevalence estimate for the island-wide population of 0.61 (95% CI: 0.52, 0.71). The island-wide population did not change significantly between the 2 yr (mean change = 0.65 eggs/trap, 95% CI: −3.10, 6.73). These results were qualitatively the same as in our OLS regression, where there was a significant change in average ovitrap count with parasite prevalence in the host ($F_{1,25} = 8.935$, $P < 0.007$; see Supp Table 1 [online only]). We found no evidence of the number of eggs in an ovitrap and parasite prevalence at sites (Supp Fig. 2 [online only]), indicating that it was the change from year to year that was associated with parasite prevalence, and not total host abundance.

We also found no relationship between the change in average ovitrap count and proportion of *Culex* in a sample (β = −48, $R^2 = 0.01$, $P > 0.66$). Due to violations of assumptions (Table 3), we bootstrapped the regression of prevalence and average ovitrap count in the year of sampling. We found that the confidence intervals for the slope of this regression overlapped zero and thus we concluded that there was not a significant relationship between average ovitrap count in the year of sampling and the prevalence we observed in the field (β = 0.032, lower limit = −0.04, upper limit = 0.04; Supp Fig. 3 [online only]). Finally, we found no relationship between average egg count per trap and year ($\chi^2_2 = 1.526$, $P > 0.45$; Supp Fig. 3 [online only]).

Discussion

The key features of a regulating interaction between *Ae. albopictus* and *A. taiwanensis* appear to be in place in Bermuda. These include

Table 2. Regression coefficients for different combinations of response and explanatory variables

Model		Slope	Intercept	*P*-value
Response	Explanatory			
Change in average ovitrap count	Host prevalence	−52.69	34.2	6.10×10^{-5}*
Change in average ovitrap count	Proportion Culex in sample	−48.92	12.32	0.66
Host prevalence	Egg count in sampling year	0.03	0.39	**

*Indicates values below 0.05.

**Indicates slope test via bootstrapping; slope overlapped zero (lower limit = −0.04, upper limit = 0.04).

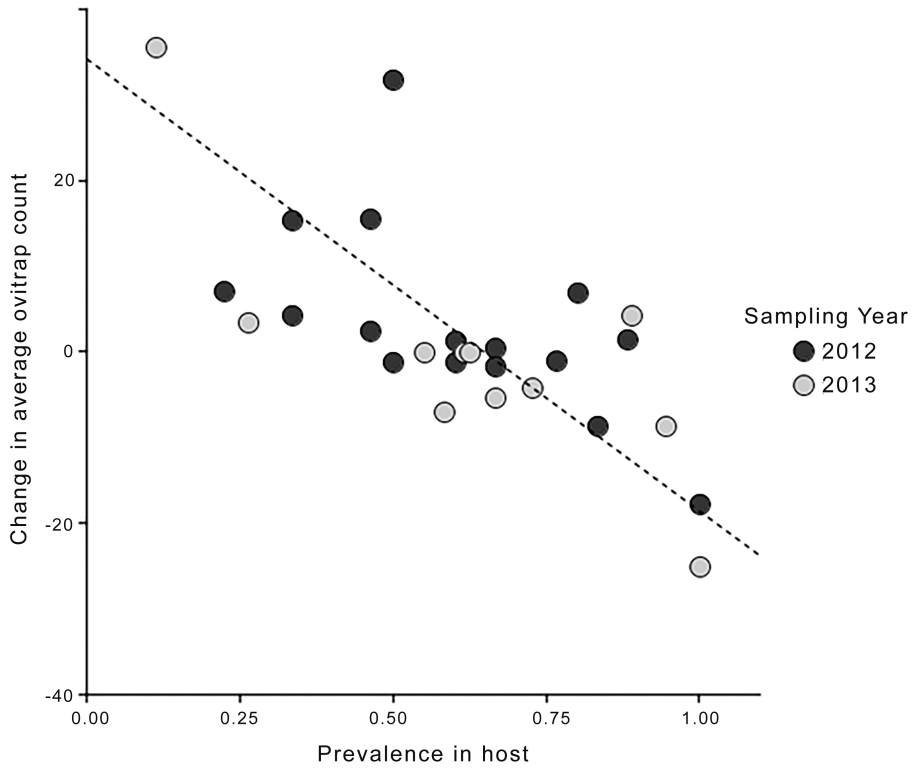

Fig. 4. The regression of change in average ovitrap count from year 1 to year 2 with prevalence of the host. Change in average ovitrap count was based on the change from year of sampling to the subsequent year—see Methods for additional details. The black dotted line is predicted relationship from the Type II linear regression. Sampling year was not significant and was dropped from the final model, but is shown here for comparison.

Table 3. Assumption test results for all statistical models

Model		Shapiro-Wilks test result		Bruesch-Pagan test result		
Response	Explanatory	*W*	*P*-value	BP	df	*P*-value
Change in average ovitrap count	Host prevalence	0.97	0.620	0.820	1	0.360
Change in average ovitrap count	Proportion Culex in sample	0.93	0.053	0.005	1	0.940
Host prevalence	Host abundance in sampling year	0.89	0.004*	4.778	1	0.028*
Average ovitrap count	Year	0.85	0.001*	0.042	1	0.837

*Indicates significant test result and a violation of assumptions.

a negative slope (Fig. 4), combined with a passage of the function through the prevalence axis at a value between 0 and 1. Also, we see that there was no significant change overall in the density of the host, and that the mean island-wide prevalence did not differ significantly from the prevalence we would expect when the host is at equilibrium. That we found no difference in slope between sampling years is further evidence that the phenomenon we observed is indeed evidence of host regulation.

These findings are remarkable given the difficulties inherent in field work, and the multitude of factors that complicate inference on natural populations, including patchy distributions, adult dispersal/migration across the island, species interactions, and weather patterns. Mosquitoes are continually transported by humans via containers or with human hosts over Bermuda (Kaplan et al. 2010), complicating accuracy of estimates of host or parasite abundance. Bermuda's Vector Control operations act in a density-dependent manner, as they will seek and remove larval habitats in areas where egg counts pass fifty in a given week, and residents are more likely to complain if there are more mosquitoes present (personal communication, Bermuda Vector Control). However, vector control acts only on host and not parasite abundance, and our analysis included sites across host abundance levels, not just at high abundance. Had we found a relationship between abundance of host and parasite prevalence, we would be more concerned that vector control is indirectly influencing the relationship we observed. However, because we found no such relationship, we do not suspect that vector control is substantially affecting the relationship we observed, but they could be contributing to some of the unexplained variance in our models.

Seasonality and weather are likely important contributors to host/parasite population structure. Although our study did not take weather into account, by estimating abundance in the same weeks of successive years, we helped control for seasonal variation. In one of the few other examples of observations of parasites regulating host populations, Pioz et al. (2008) found that, after accounting for density-dependent factors in the host, the prevalence of parasite antibodies was negatively correlated with annual variation in reproductive success, as well as weather conditions such as temperature and precipitation (Pioz et al. 2008). Latham and Poulin (2002) found seasonal variation in mortality rates of infected crabs. In *A. taiwanensis* infecting *Ae. albopictus* in Louisiana, prevalence and infection intensity were highest in September and October (Comiskey et al. 1999b), and while Bermuda *Ae. albopictus* exhibit strong seasonality in abundance (Fig. 2), the degree to which seasonality plays a role in *A. taiwanensis* in Bermuda is unknown.

In the present study, we estimated prevalence as *Ae. albopictus* positive for *Ascogregrarina* DNA as a way of assessing parasite abundance. While this does mean that we are not explicitly demonstrating active infections, just the presence of parasite DNA, previous studies have shown that *A. taiwanensis* can develop in any instar of *Ae. albopictus* (Roychoudhury and Kobayashi 2006) and thus active infections are likely present or developing when we detect parasite DNA in *Ae. albopictus*. While we did detect some *Culex* infected with *A. taiwanensis* (Table 2), we do not consider these to be active infections because *Ascogregarina* are cleared from *Culex pipiens* complex mosquitoes with known complications to the host (in *Culex quinquifasciatus* with *A. taiwanensis*, Garcia et al. 1994).

Our findings also suggest that parasites need not have high virulence to exert regulation over their hosts. Due in large part to relatively low increases in mortality rates, as indicated by Stapp and Casten (1971), Gentile et al. (1971), *Ascogregarina* have been dismissed as potential biocontrol agents for their natural hosts (Beier and Craig 1985, Tseng 2007). Indeed, Beier and Craig (1985) went so far as to claim that 'there is no evidence that gregarines can be used to control mosquitoes, and no evidence that gregarines in their natural habitat have a significant negative impact on populations of their normal host.' (p. 182). That claim was made despite the presentation of evidence to the contrary within the same paper, in which *Ae. triseriatus* infected by *A. barretti* showed reduced recapture rates after releases in the field, consistent with reduced adult survival. However, population growth responds to effects in addition to mortality (e.g., generation time, fecundity) and our results suggest that, while they may not induce mortality sufficient to make them suitable as strong biological control agents alone, they do induce population regulation. While *Ae. albopictus* appears to invade new localities with its gregarine parasite readily (e.g., North America, Munstermann and Wesson 1990; Bermuda, Kaplan et al. 2010; South America, Passos and Tadei 2008), if populations are found that harbor no gregarines, the introduction of *A. taiwanensis* to an *Ae. albopictus* population seems likely to result in some depression in host abundance.

Given the complexity of natural systems and the potential community-level interactions that are commonplace in the field, our results stand as a notable example of parasite regulation. While further studies in Bermuda should be undertaken to continue to assess the effects of the parasite, including assessing density of hosts in container habitat and the abundance of parasites at a finer scale, this study establishes the role of even moderately virulent parasites as regulators of host abundance, and further demonstrates the utility of island systems in the study of disease ecology. Future studies confirming these effects in other similar locales, such as other island populations of *Ae. albopictus* and related species, could provide further evidence in how gregarines structure host communities.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

Acknowledgments

We thank D. Kendell, A. Thomas, and R. Furbert of the Ministry of Health for their enormous help with this project; L. Valsdottir for assisting in field

collection 1 yr; M. Notarangelo for DNA extractions and ID of specimens; and L. Seeley, G. Fuentes, and R. Gaimari for assisting with DNA extractions. This project was partially funded by the Biology Department at Clark University and National Institutes of Health (NIH) grant 1R15AI092577-01A1.

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