A Mosquito Parasite Is Locally Adapted to Its Host but Not Temperature

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ABSTRACT: Climate change will alter interactions between parasites and their hosts. Warming may affect patterns of local adaptation, shifting the environment to favor the parasite or host and thus changing the prevalence of disease. We assessed local adaptation to hosts and temperature in the facultative ciliate parasite *Lambornella clarki*, which infects the western tree hole mosquito *Aedes sierrensis*. We conducted laboratory infection experiments with mosquito larvae and parasites collected from across a climate gradient, pairing sympatric or allopatric populations across three temperatures that were either matched or mismatched to the source environment. *Lambornella clarki* parasites were locally adapted to their hosts, with 2.6 times higher infection rates on sympatric populations compared with allopatric populations, but they were not locally adapted to temperature. Infection peaked at the intermediate temperature of 12.5°C, notably lower than the optimum temperature for free-living *L. clarki* growth, suggesting that the host's immune response can play a significant role in mediating the outcome of infection. Our results highlight the importance of host selective pressure on parasites, despite the impact of temperature on infection success.

Keywords: host-parasite coevolution, climate change, local adaptation, parasitism.

Introduction

Climate change is likely to have a significant impact on species interactions such as those between hosts and parasites. In particular, warming temperatures have been associated with changes in the prevalence and intensity of disease outbreaks (Altizer et al 2013; Lafferty and Mordecai 2016) and rates of parasitism (Pardikes et al. 2022). Variation in the thermal sensitivities of parasite population performance may play an important role in determining where warming will have the largest effect (Cohen et al. 2020). Specifically, parasite populations can be locally adapted, performing best in climates similar to their home environments. For example, some populations of chytrid fungus show a signal of local adaptation to temperature (Stevenson and Pike 2013). Species exhibiting local thermal adaptation—that is, they perform best at the temperature that corresponds with their natal temperature—may be better able to adapt to warming and persist under climate change (Angilletta 2009). Conversely, species with minimal between-population variation in thermal tolerance may have a reduced capacity to adapt. Understanding not only the sensitivities of parasites and pathogens to temperature but also the variation in thermal sensitivities among populations is critical for predicting how climate change will impact disease dynamics (Sternberg and Thomas 2014).

Parasites and pathogens may also be locally adapted to their hosts. Hosts and parasites are often engaged in a reciprocal evolutionary interaction (i.e., an evolutionary arms race) in which hosts can evolve immune or behavioral defenses and parasites can evolve to overcome them (Dybdahl and Lively 1998; Brodie et al. 2002; Gandon 2002; Thompson 2005; Koskella and Parr 2015). The outcome of host-parasite coevolution, whether parasites or hosts are winning the race, depends on generation time, genetic variation, and dispersal (Gandon 2002; Gandon and Michalakis 2002; Kawecki and Ebert 2004). Studies have shown that in some cases, hosts are more resistant to sympatric parasites compared with allopatric parasites (Eizaguirre et al. 2012). However, more commonly, parasites demonstrate greater infection success on sympatric versus allopatric hosts (Greischar and Koskella 2007). For instance, in an experimental evolution study, Bedhomme et al. (2012) observed rapid adaptation of a plant RNA virus to four host species. Typically, parasites are expected to be locally adapted to their hosts because of their shorter generation

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times, but low migration rates and low genetic diversity in the parasite can theoretically reverse this prediction (Greischar and Koskella 2007; Koskella and Brockhurst 2014).

Parasites experience strong evolutionary pressures from both biotic and abiotic sources. A combination of physical environmental characteristics and host characteristics have been found to influence rates of parasitism by water mites on odonates and helminth parasite diversity in rodents (Preisser 2019; LoScerbo et al. 2020; Hasik and Siepielski 2022). Adapting to the physical environment may be equally as important as adapting to hosts, especially for parasites of ectotherms or those with life cycles involving free-living forms. Despite this, previous studies are limited in that they examine local adaptation to hosts or local adaptation to temperature but not both. To our knowledge, only one prior study has experimentally examined local adaptation of parasites to both hosts and temperature (Laine 2008), and it only briefly mentions temperature adaptation in a single population and was limited in its spatial extent with <1°C difference in temperature among populations. More typically, studies of host-parasite local adaptation consider temperature as an ecological stressor rather than an evolutionary driver of local adaptation. In these cases the evidence is mixed as to whether temperature significantly affects the pattern of host-parasite local adaptation (e.g., Blanford et al. 2003; Laine 2008) or whether there is a consistent pattern of local adaptation across temperature (e.g., Landis et al. 2012).

The western tree hole mosquito *Aedes sierrensis* and its facultative ciliate parasite *Lambornella clarki* present an ideal system for investigating patterns of adaptation of a parasite to its host and to its thermal environment. *Aedes sierrensis* is a broadly distributed mosquito, inhabiting water-filled tree holes across the western United States. *Aedes sierrensis* is commonly infected by *L. clarki*, a facultative ciliate parasite that invades the host's cuticle, replicates inside, and is released upon the death of its host. Mosquito larvae can eat free-living ciliates, driving potentially strong selective pressure on the ciliate that likely promotes parasitism as a predation avoidance strategy (Washburn et al. 1988). Both species occur across a broad latitudinal temperature gradient, where not only temperature varies but also many other environmental factors, and they can be easily reared in the lab (Hawley 1985; Washburn and Anderson 1986; Broberg and Bradshaw 1997). Using this model system, we aim to test (i) whether parasitism is temperature dependent and (ii) whether parasites are locally adapted to hosts, temperature, or both. We employ a common-garden experimental design that measures the interaction between parasite population, host population, and temperature, comparing sympatric and allopatric host-parasite pairs, in the laboratory.

Material and Methods

Study System

In the Mediterranean climate of the Pacific Coast of North America, winter rains fill tree holes with standing water. Soon after, *Aedes sierrensis* mosquito larvae hatch from dormant eggs, and *Lambornella clarki* ciliates emerge from their dormant state to begin replicating as free-living cells (Washburn and Anderson 1986). Cued by the presence of mosquitoes, *L. clarki* transform into parasitic forms and are able to infect only the larvae stages of the mosquito. Infections usually kill the host in its larval stage, but on occasion, established infections can continue into the pupal stage and adulthood (Egerter et al. 1986). *Aedes sierrensis* is *L. clarki's* main and potentially only natural host, but the ciliate can also live in a free-living state, consuming bacteria and detritus in the water (Washburn and Anderson 1986). Another important ecological interaction between these species is that mosquitoes are predators of *L. clarki*, ingesting them as a food source, and it is thought that this was a selective force that led *L. clarki* to evolve to be facultative parasites (Washburn et al. 1988). Previous studies have documented significant intraspecific variation in *L. clarki* thermal performance and in temperature-dependent infection rates (Ismail et al. 2024) but very little variation in thermal tolerance between populations of *Ae. sierrensis* (Couper et al. 2024).

Collection of Mosquito Larvae and Parasites

From November 2021 to April 2022, we collected larval *Ae. sierrensis* mosquitoes from field sites across California and Oregon to obtain individuals infected with *L. clarki*. For this experiment, we used populations of *Ae. sierrensis* and *L. clarki* collected from nine sites (fig. 1), which spans the majority of the range of *L. clarki* (Washburn and Anderson 1986; Broberg and Bradshaw 1997). The sites spanned from San Diego, California, to Portland, Oregon (33°N–45°N), and in elevation from coastal to the Sierra Nevada mountains (up to 1,250 m elevation). The annual mean temperature ranged from 11°C to 18°C, and the average temperature of the wettest quarter of the year—the activity period for *Ae. sierrensis* and *L. clarki* in tree holes—ranged from 5°C to 13°C (coordinates and temperatures are given in table S1, available online). Once collected, mosquitoes were brought back to the lab, maintained in the dark at 7°C, and examined under a microscope for the presence of *L. clarki* infections. The parasite was then isolated by transferring roughly three to five infected mosquitoes into vials containing an autoclaved barley seed and 1 mL of autoclaved media made of 1 L of distilled water, 1 protozoan pellet, and 0.38 g of Herptivite (Fukami 2004). The *L. clarki* vials were propagated for

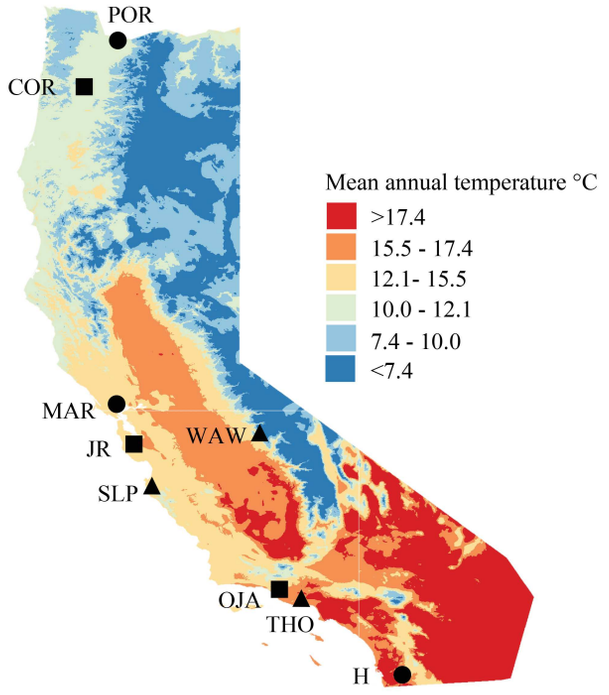

Figure 1: The nine populations used in the experiment span a wide climatic gradient and most of the species' geographic range. The map is colored by mean annual temperature (°C). Point shapes represent the three groups used in the experiment, within which all possible host-parasite pairs were tested.

3–5 months in the dark at 21°C, and new media was added weekly until the next generation of mosquitoes was available to start the experiment.

To obtain large numbers of first-instar mosquito larvae used in the experiment, we reared field-collected mosquitoes to the next generation. Larval mosquitoes were brought back from the field and maintained in 100-mL plastic containers and fed a 4% solution of high-protein cat chow, 36% bovine liver powder, and 14% brewer's yeast (Maïga 2017) weekly. While each tree hole typically contains thousands of larvae from many maternal families, to ensure that we captured variation within a population, we grouped mosquitoes and parasites from tree holes from the same general habitat area as a single population, which included between two and five tree holes, depending on the population. On average, populations spanned a radius of 5 km, but were as large as 20 km, a relatively small distance compared with the distance between populations used in allopatric treatments, which were separated by on average 275 km. While individual mosquitoes may disperse less than 5–20 km on average, we expect there to be gene flow at this scale. Any population structure within our "populations" would serve to reduce the extent of local adaptation of parasites on hosts, resulting in conservative estimates of parasite local adaptation on hosts. For each site, we raised mosquitoes from all tree holes within this radius except for those where infected larvae were found. Doing so was the only way to ensure that there was no cross contamination of field-derived parasites in the experiment before our addition of specific parasite populations. However, we recognize that using this sample of hosts had the potential to bias our result in that it could lead to lower infection rates and obscure patterns of host local adaptation. Once emergence began, adults were kept in collapsible aluminum cages (BioQuip, Rancho Dominguez, CA) and fed a 10% sugar-water solution twice per week and defibrinated sheep's blood once per week. Females laid eggs on damp filter paper placed inside black oviposition cups. Egg papers were labeled in plastic bags and maintained in the dark at 5°C for 1–3 months. To begin the experiment, eggs were hatched by submerging the egg papers in a solution of 500 mL of Arrowhead distilled water, 300 mL of autoclaved tree hole water, and 1 teaspoon of brewer's yeast (Schwan and Anderson 1980).

Infection Experiment

We conducted a common-garden experiment in the laboratory to examine patterns of parasite local adaptation to hosts and to temperature. We split the nine populations into three groups (fig. 1), each containing a warm site (annual mean temperature: 16.6°C–18.0°C; temperature of the wettest quarter of the year: 12.9°C–14.0°C), a temperate site (annual: 14.4°C–15.6°C; wettest quarter: 10.4°C–11.8°C), and a cold site (annual: 11.4°C–12.4°C; wettest quarter 6.0°C–6.6°C). To test for local adaptation of parasites to their hosts, each population of *L. clarki* was introduced into one sympatric population and two allopatric populations of mosquito larvae. The nine pairs of populations were split into groups of three (each with a warm, medium, and cool site) and not tested in a full factorial design, as testing only twice as many allopatric pairs as sympatric pairs has been shown to be the most powerful design in local adaptation experiments (Blanquart et al. 2013). To test for local adaptation to temperature, we used three temperatures (7°C, 12.5°C, and 17.5°C). We chose 17.5°C as our warm temperature, as this was the mean annual temperature at warm sites. We chose 7°C as our cold temperature, as larvae reared at temperatures colder than this fail to pupate (Couper et al. 2024) and this was similar to the temperature of the wettest quarter at cold sites. We chose 12.5°C as our moderate temperature, as this was roughly the midpoint. Although we were able to test only three temperatures to maintain experimental tractability, rather than a unique temperature representing each population, the pattern of local adaptation would also be

expected at this coarse level, in which populations perform best on the temperature most similar to their home environment. We had five replicates for each of the 81 *L. clarki–Ae. sierrensis* pair and temperature treatments, except for the 18 treatments with *Ae. sierrensis* from the OJA and POR populations, which had four replicates because of lower numbers of larvae that hatched. We had 387 experimental units (*L. clarki–Ae. sierrensis* pair by temperature treatment by replicate) in which each experimental unit contained five larvae, for a total of 1,935 larvae.

We filled six-well nontreated Falcon plates with five first-instar mosquito larvae in each well. To each well, we introduced 4 mL of *L. clarki* culture at a standard inoculation concentration of 320 cells/mL. The plates were maintained in a 14L:10D cycle, and each well was fed with 50 μL of 4% larval food suspension after each check for infection, which was chosen so that mosquitoes were not food limited (Maïga 2017). We monitored infection a total of four times before the first day the larvae were expected to pupate. Because larval development is faster at higher temperatures, the 17.5°C treatment was checked on days 3, 6, 9, and 12; the 12.5°C treatment was checked on days 5, 10, 15, and 20; and the 7°C treatment was checked on days 8, 16, 24, and 32. Thus, the experiment lasted 12, 20, and 32 days for the 17.5°C, 12.5°C, and 7°C treatments, respectively. These time points were chosen according to temperature-sensitive larval development times observed in a previous experiment that documented the time to pupation across a gradient of temperatures (Couper et al. 2024).

During the examinations, we recorded the presence of cuticular cysts, melanization spots, internal infection, and larval survival. Cuticular cysts form as the parasite attacks the mosquito, and melanization spots are the mosquito immune response to these cysts. As a final assay of infection, all dead larvae and larvae surviving through the fourth check were stained with black amide dye for 30 min as an additional check for *L. clarki* cysts on the cuticle of the host (Soldo and Merlin 1972; Washburn et al. 1988). All mosquitoes were inspected under a dissecting microscope at ×40 magnification.

Free-Living Thermal Performance Experiment

As *L. clarki* is a facultative ciliate parasite that can complete its life cycle as a free-living organism, we also conducted an experiment to examine local adaptation of the free-living form to temperature. We measured the exponential growth rate of each population at seven temperatures: 5°C, 7°C, 12°C, 18°C, 21°C, 23°C, and 28°C. The temperatures above 18°C are warmer than those *L. clarki* typically experience in nature (table S1), as this range was chosen to capture the full thermal performance curve based on pilot experiments that found that *L. clarki* was unable to grow below 4°C and above 30°C. For each temperature and population of *L. clarki*, we had five replicates (for a total sample size of 315), initiated by adding 2 mL of a low-density culture (3 cell/100 μL) and one barley seed to a 4-mL vial. We measured density daily for 6 days by plating 100 μL in small droplets on a petri dish and counting the number of cells.

Statistical Analysis

For the infection experiment, we report results of the number of larvae with cuticular cysts, melanization spots, and internal infection, as well as survival rates. To examine whether parasites are locally adapted to hosts, we included a binary independent variable for sympatric versus allopatric pairs. To examine whether parasites are locally adapted to temperature, we included a binary independent variable for a parasite in the experimental condition that was matched to its source environment versus one that was mismatched. A mismatched experimental condition was one in which a population was exposed to a temperature typically outside of its normal range based on mean annual and wettest-quarter temperatures (e.g., a cold population is matched to 7°C and mismatched to 12.5°C and 17.5°C). To examine whether there was an effect of parasite population, host population, or temperature, these terms were also included as categorical variables in the model.

We ran generalized linear models using the glm function in the stats package in R version 4.1.1. We ran Poisson regressions for the response variables: number of larvae with cuticular cysts, number of larvae with melanization spots, and number of larvae with internal infection. Because these responses happen sequentially, we chose to analyze the time point when each response peaked (i.e., time 1 for cysts, time 2 for melanization, and time 4 for internal infection). For survival, we calculated the percentage of all larvae that had died by time point 4, and because this percentage was so low, we did not conduct further analysis on this variable. For each response variable, our main model included parasite population, host population, temperature, sympatry/allopatry, and matching/mismatching temperature. To determine whether there was an effect of Falcon plate, we included this in the model, but it was not significant and was dropped. Additionally, to examine whether there was a temperature-mediated effect on local adaptation to hosts, we also included an interaction between temperature and sympatry/allopatry, but this did not significantly improve the model and was dropped. We evaluated model residuals by visually inspecting plots of the residuals versus fitted values and Q-Q plots. We determined significance using likelihood ratio tests

and report deviance and P values. In assessing the significance of the P values in our generalized linear models, we applied a Bonferroni correction for an adjusted α level of .01 to account for the increased risk of type I errors.

For the growth rate experiment, we subset the data to include only days 1–5 to analyze the exponential phase of population growth. We then calculated the exponential growth rate for each replicate using nls in R. We fitted thermal performance curves to these growth rates using both the Brière 1999 model and the Rezende 2019 model, with the latter producing a much better fit to our data (Brière et al. 1999; Rezende and Bozinovic 2019). The Rezende 2019 model visually produced a good fit to all of the populations and was bootstrapped using residual resampling to get 95% confidence intervals using the rTPC package (Padfield et al. 2021). To test for local adaptation to temperature, we calculated Pearson's correlation coefficient between annual mean temperature of the site (table S1) and peak growth rate temperature and a weighted linear regression, with weights equal to the inverse variance of bootstrapped estimates of peak growth rate temperature.

Results

Documenting the Progression of Infection

We observed the infection process starting with the introduction of *Lambornella clarki* as free-living cells, which quickly transformed and attacked the cuticle of larvae to form parasitic cysts. In response, some larvae initiated a melanization defense. If this defense was unsuccessful, *L. clarki* cells entered the body cavity of the larvae and began replicating inside. As expected, the proportion of wells containing free-living *L. clarki* declined over time (fig. 2*A*). By the first time point, infective cysts were present, and we observed the highest number of larvae with cuticular cysts (fig. 2*B*). At the second time point, we observed the highest number of larvae with melanization spots (fig. 2*C*). The number of infected larvae steadily rose over time (fig. 2*D*). These temporal trends were the same across temperature treatments, except for cuticular cysts, which were highest at the second time point for the 7°C and 17.5°C treatments, and for melanization, which was highest at the third time point for the 7°C treatment (fig. S1; figs. S1–S7 are available online). Because so few larvae died by the end of the experiment (11%), we did not use larval survival as a response variable in any further analysis (fig. 2*E*).

Local Adaptation

We found that parasites were locally adapted to their hosts (fig. 3; for individual parasite populations, see fig. S2). Sympatric populations had 1.2 times more larvae with cysts ($\chi^2_1 = 15$, $P < .001$) and 2.6 times more larvae that were infected ($\chi^2_1 = 62$, $P < .001$) compared with allopatric populations. Also, sympatric populations had fewer larvae that were melanized ($\chi^2_1 = 3.8$, $P = .05$). Hence, not only did *L. clarki* infect sympatric hosts at a higher rate than allopatric hosts but also sympatric *L. clarki* were less melanized, suggesting either a weaker immune response from the host or a better ability of parasites to evade detection. We also examined the relationship between the number of larvae infected and the geographic distance and the climate distance between host and parasite pairs, because we expect that as pairs become more distant or climatically dissimilar, infection should decline (fig. S3). As expected, the number of infected larvae decreased with both climatic distance ($R^2 = 0.12$, $P = .04$) and geographic distance between host and parasite, although the latter was not statistically significant ($R^2 = 0.09$, $P = .08$). Additionally, there was no evidence that temperature mediates the signal of local adaptation to hosts: on average the number of larvae infected in sympatric populations was higher than the number infected in allopatric populations at all temperatures (fig. 4). Including an interaction term between temperature condition in the experiment and the binary sympatry variable did not significantly improve the model ($\chi^2_2 = 2.3$, $P = .31$).

In contrast to the signal of host adaptation, parasites were not locally adapted to temperature at the three temperatures tested (fig. 5; for individual parasite populations, see fig. S4). There were no significant differences in the number of larvae with cysts ($\chi^2_1 = 0.12$, $P = .73$), the number of larvae that were infected ($\chi^2_1 = 0.74$, $P = .39$), or the number of larvae melanized ($\chi^2_1 = 0.23$, $P = .63$) between parasites that were placed at a temperature that matched their source environment versus those placed at mismatched temperatures (fig. 5).

Parasite populations varied significantly in the number of larvae they infected (figs. 6*A*, S5). This is often referred to as a "deme effect" in the local adaptation literature, signifying that certain populations are overall more fit than others, regardless of habitat. Parasites varied in cysts ($\chi^2_6 = 344$, $P < .001$), melanization ($\chi^2_6 = 53$, $P < .001$), and infection ($\chi^2_6 = 269$, $P < .001$). We found that one population (SLP) performed especially poorly, producing the lowest number of infections of all nine populations. Host populations also varied significantly in cysts ($\chi^2_6 = 22$, $P < .001$; fig. S5), melanization ($\chi^2_6 = 18$, $P < .01$; fig. 6*B*), and infection ($\chi^2_6 = 36$, $P < .001$; fig. S5). Host population can be considered one aspect of habitat quality, with certain host populations being more or less resistant to infection. Although there was variation in the amount of cyst formation, melanization, and infection for both host and parasite populations, this did not

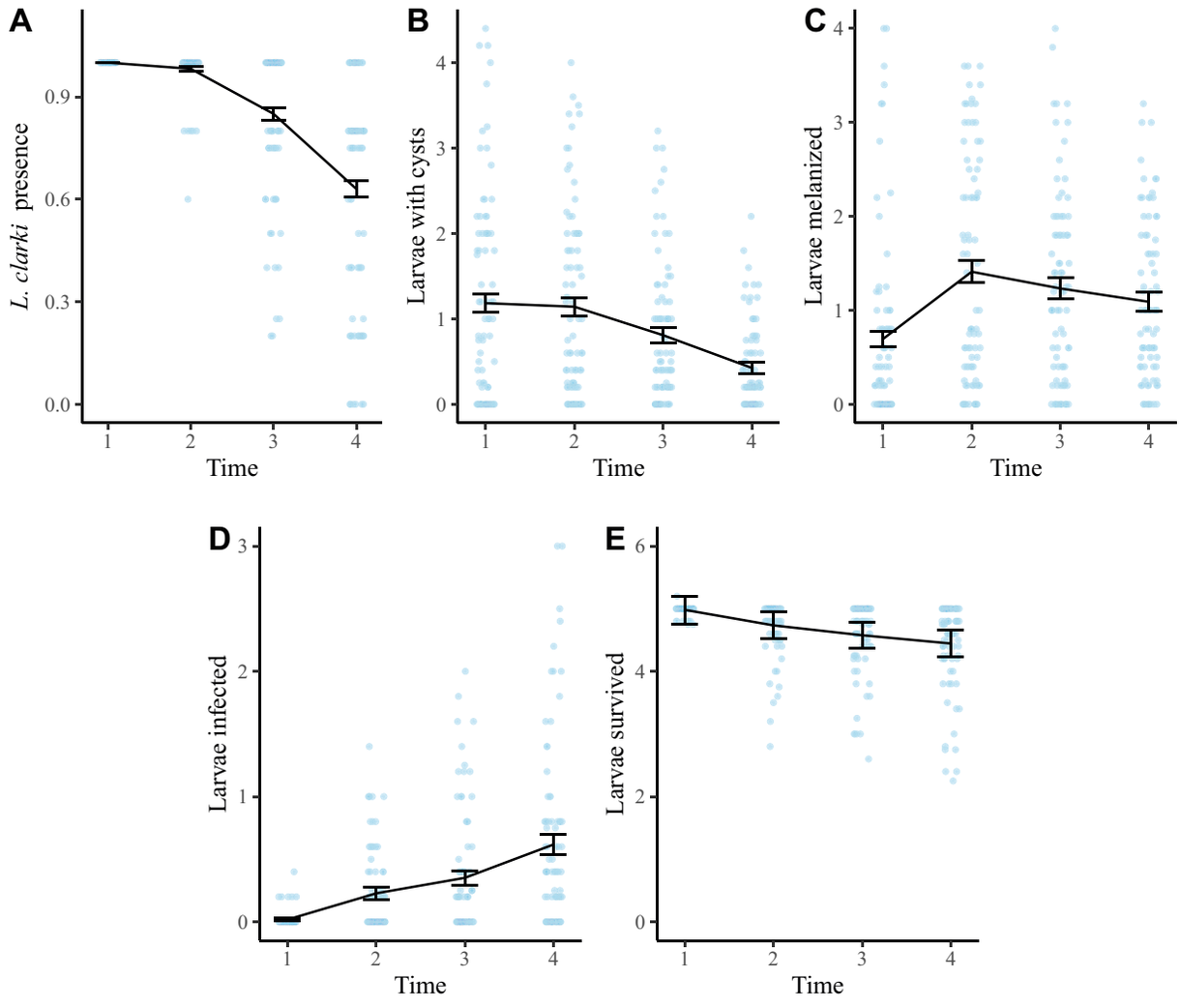

Figure 2: Progression of infection over time. Proportion of experimental units with free-living *Lambornella clarki* present (*A*) and the number of larvae with cysts (out of five; *B*) peaked at the first time point. The number of larvae melanized (*C*) peaked at the second time point. The number of larvae infected (*D*) increased over time, and the number of larvae that survived (*E*) decreased over time. Lines represent averages across all population pairs, temperatures, and replicates; error bars represent ±1 SE. Points represent the mean of each treatment combination.

correlate with the latitudinal or temperature gradient from which they were collected.

Temperature significantly affected infection, with a peak in the number of larvae infected occurring at the intermediate temperature of 12.5°C ($\chi^2_2 = 74$, $P < .001$; fig. 6*C*), as expected from principles of thermal biology. Temperature also significantly affected cyst formation and melanization, with the highest number of larvae with cysts occurring at 12.5°C and the highest number of larvae melanized occurring at the warmest temperature of 17.5°C ($\chi^2_2 = 15$, $P < .001$; fig. S5).

Although there was no indication that parasitic *L. clarki* are locally adapted to temperature, we did find evidence of free-living *L. clarki* local adaptation to temperature. Growth rates of free-living *L. clarki* from warmer sites peaked at higher temperatures than those from colder temperatures (figs. 7, S6). Peak growth rate temperature was strongly positively correlated with annual mean temperature of the collection site ($r = 0.80$, $P < .01$). Moreover, the temperatures of peak free-living ciliate growth (16.5°C–23.5°C) were substantially higher than the temperature that maximized infection in the experiment

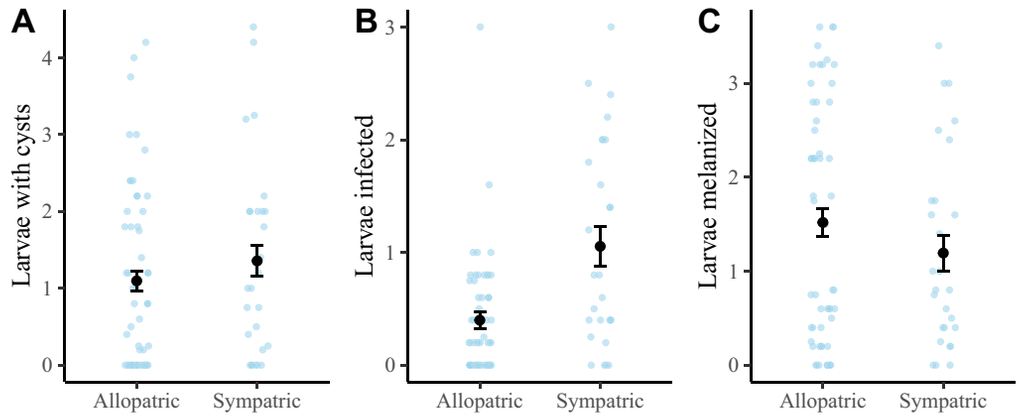

Figure 3: Parasites are locally adapted to hosts. The number of larvae (out of five) with parasitic cysts (*A*) and infection (*B*) was higher in sympatric than allopatric populations. The number of larvae melanized (*C*), a sign of immune response, was higher in allopatric than sympatric populations. Large points represent averages across all replicates and temperature treatments, error bars represent ±1 SE, and small points represent the mean of each treatment combination. The effect of sympatry on cysts was significant even though there is overlap between error bars, as there were other variables accounted for in the model.

(12.5°C), suggesting a strong role of the host response mediating infection success across temperatures.

Discussion

Our study examined the extent to which parasites are adapted to their biotic and abiotic environments, revealing that parasites are locally adapted to their hosts but not to temperature (figs. 3, 5), despite ciliates being locally adapted to temperature in their free-living form (fig. 7). Local adaptation to hosts is consistent with the theoretical expectation that most parasites are ahead in the evolutionary arms race when they have shorter generation times than hosts, although many other untested factors, such as genetic variation, can impact the outcome of coevolution. At the same time, our finding that *Lambornella clarki* is adapted to its mosquito host is surprising for several reasons. First, a review of experiments found that parasite local adaptation to hosts is relatively rare, seen in only roughly a third of cases (Greischar and Koskella 2007). In contrast to many previous studies analyzed in the review, our experimental design includes many populations ($N = 9$), spans the entire range of the focal organism, and tests the interaction under multiple environmental conditions, providing a more comprehensive picture of parasite local adaptation. Second, *L. clarki* is a facultative parasite.

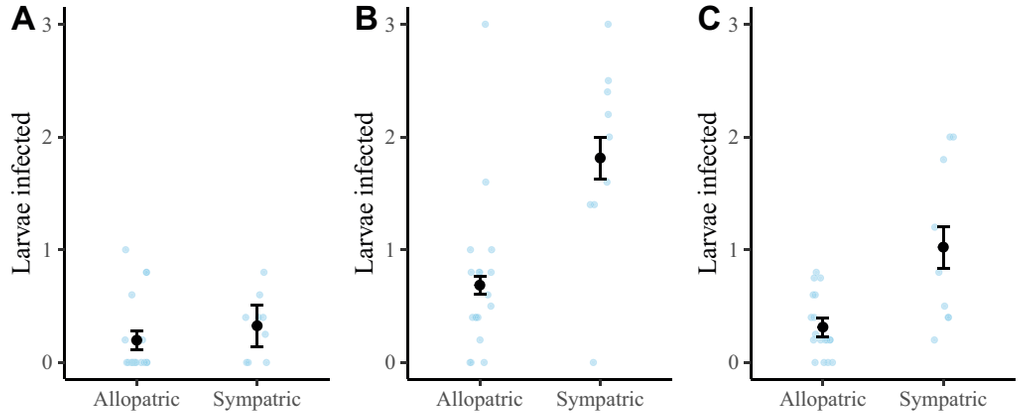

Figure 4: The interaction between temperature and local adaptation to hosts was not significant. Comparison of the number of larvae infected out of five for allopatric and sympatric populations at 7°C (*A*), 12.5°C (*B*), and 17.5°C (*C*). Large points represent averages across all replicates and temperature treatments, error bars represent ±1 SE, and small points represent the mean of each treatment combination.

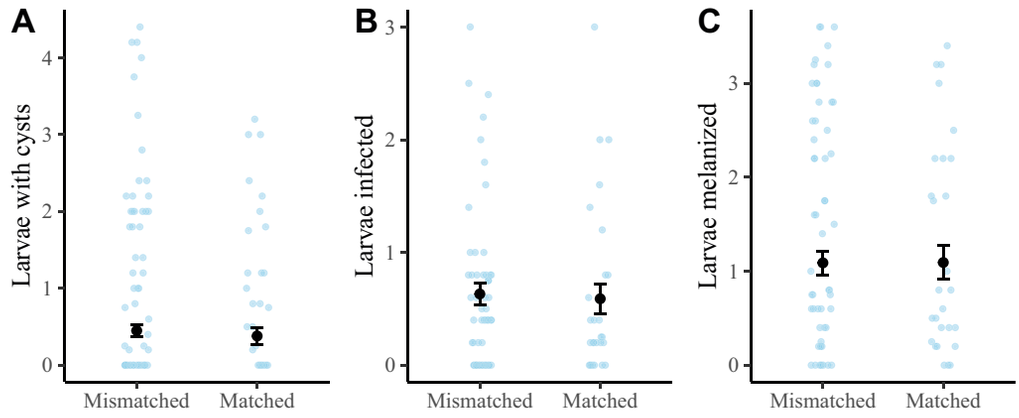

Figure 5: Parasitic *Lambornella clarki* are not locally adapted to temperature. The number of larvae (out of five) with cysts (*A*), infection (*B*), and melanization (*C*) did not significantly differ between populations that were matched versus mismatched to their source thermal environment. Large points represent averages across all replicates and sympatric/allopatric population pairs, error bars represent ±1 SE, and small points represent the mean of each treatment combination.

Therefore, despite existing and reproducing as a free-living organism in the absence of mosquitoes, it experiences such strong selective pressure from mosquito predation that it has evolved a parasitic stage that has further adapted locally to mosquito populations. Third, given that local adaptation is influenced by the relative migration rates of host and parasite, it is noteworthy that *L. clarki* parasites are likely more dispersal limited than their host, which has a flying adult life stage. Only on rare occasions does the parasite infect the reproductive tract of an adult female mosquito, which can then travel to other tree holes and deposit the parasite (Egerter and Anderson 1985). Other dispersal strategies of free-living *L. clarki* are unknown. Despite greater host dispersal, the parasite is ahead in the evolutionary arms race.

The pattern of parasites being locally adapted to hosts was consistent across temperatures. That is, parasites caused higher infection in sympatric, relative to allopatric, hosts across all temperature treatments (fig. 4). This finding that the effect of keeping coevolution intact versus breaking it did not depend on temperature suggests that in this system, adaptation to hosts can be considered independently of adaptation to the abiotic environment. This result aligns with previous findings in a fish-trematode parasite system, in which the parasite is evolutionarily ahead of the host even under heat stress (Landis et al.

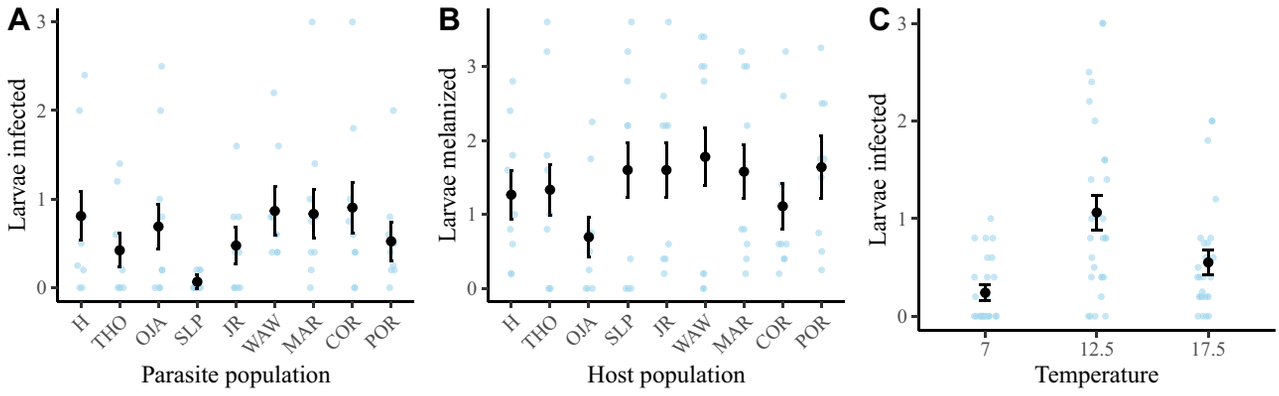

Figure 6: Infection peaks at intermediate temperature and varies among parasite and host populations. Variation in the number of larvae infected between parasite populations, averaged across temperatures, host populations, and replicates, illustrating a deme effect (*A*); melanization between host populations, averaged across parasite populations, temperatures, and replicates, illustrating a biotic environment effect (*B*); and infection rates across temperature, averaged across host and parasite populations and replicates, illustrating an abiotic environment effect (*C*). Populations are ordered by increasing latitude. Error bars represent ±1 SE, and small points represent the mean of each treatment combination.

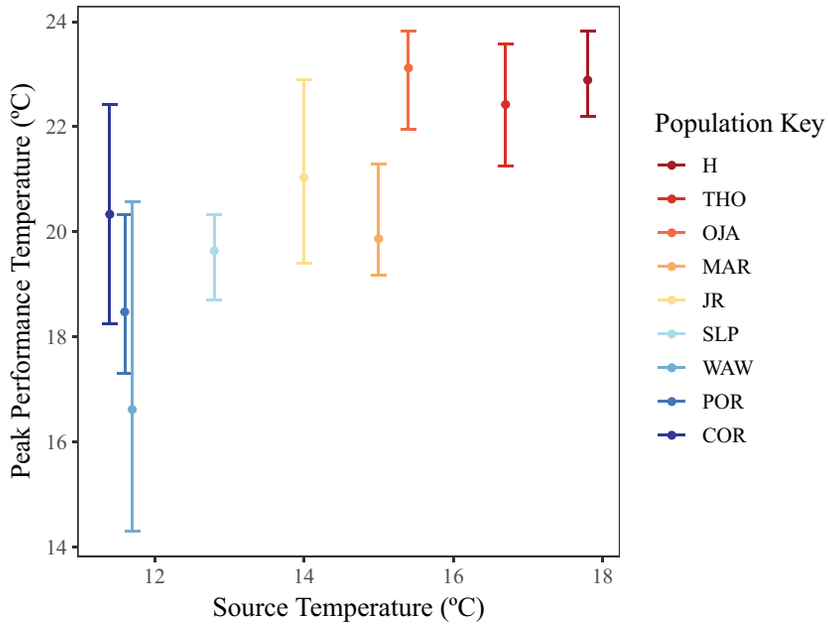

Figure 7: Free-living growth of *Lambornella clarki* populations show a signal of local adaptation to temperature, in contrast to parasitic forms. The peak growth rate temperature (y-axis) is positively correlated ($r = 0.80$, $P < .01$) with the mean annual temperature of the parasite's source environment (x-axis). Populations are colored in order of decreasing mean annual temperature. Error bars represent bootstrapped 95% confidence intervals.

2012). However, in other systems, such as a pea aphid fungal pathogen (Blanford et al. 2003) and a plant fungal pathogen (Laine 2008), temperature mediated patterns of local adaptation, even changing the direction of adaptation. *Lambornella clarki* parasites appear to be adapting to hosts both by increasing their attack rates, evidenced by more sympatric larvae with cysts, and by evading the immune response or inducing a weaker response of the host, evidenced by fewer sympatric larvae with melanization, resulting in overall higher infection success.

Despite inhabiting locations that vary by more than 8°C in their annual mean temperature, and despite evidence of local thermal adaptation in the free-living form (fig. 7), we did not find any evidence that *L. clarki* populations are locally adapted to temperature in their parasitic form (fig. 5). This suggests that *L. clarki* parasites may face stronger selective pressures from hosts than from the thermal environment. That free-living *L. clarki* growth rates were locally adapted to temperature (fig. 7) suggests that temperature is still an important selective pressure in the ciliate life cycle. Studies of the free-living stages of other parasites have found evidence of local thermal adaptation in some species (Mazé-Guilmo et al. 2016) but not others (Aleuy et al. 2020). However, one limitation of our study was the small number of temperature treatments in the infection experiment. By testing only three temperatures, we may have missed fine-scale differences in local adaptation of infection to temperature—for example, if the optimum temperatures for infection varied by only a few degrees between populations (but note that the optimal temperatures for free-living growth varied by ~7°C among populations). Our temperature treatments also did not test for local thermal adaptation to the maximum and minimum temperatures experienced at these sites, which can temporarily approach or exceed the thermal limits of host and parasite (Couper et al. 2024).

When we consider the performance of parasite populations on average across treatments, we find that some populations have consistently high attack and infection rates while others have consistently low attack and infection rates (fig. 6; i.e., a deme quality effect; Blanquart et al. 2013). Populations that perform poorly in any environment may have poor genetic quality owing to factors such as inbreeding and drift in small populations, or they may be poorly adapted to the laboratory experimental environment. Although we may expect populations near the range edge, such as our northernmost (Portland, OR: POR) or southernmost (Hollenbeck, CA: H) population to perform poorly relative to those in the center (Bontrager et al. 2021), we did not find evidence of this or any consistent pattern in deme quality across space. We also considered that a low-fitness parasite population might be better adapted to free-living growth

than to infection, but we did not detect a trade-off between infection and maximum free-living growth rate (fig. S7). In addition to variation among parasite populations, we also found variation among host populations in their susceptibility to attack and infection and in their melanization response (fig. 6). Similar to parasites, we did not find a consistent pattern in host quality—measured as the average rates of cyst formation, melanization, and infection across parasite and temperature treatments—across geographic or source temperature gradients. Host-parasite and predator-prey systems can also display geographic mosaics of hot spots and cold spots of coevolution rather than a continuous gradient (Thompson 2005). For example, in the classic case study of newts and garter snakes, areas where newts are the most toxic coincide with areas where garter snakes are most resistant to the toxin (Brodie et al. 2002). However, in this study, we found that locations with the most resistant hosts are not the locations with the most infective parasites. Going forward, to fully understand the coevolutionary dynamics between host and parasite, obtaining multiple years of data or performing a time-shift experiment in which hosts are exposed to parasites from a different time point, will be necessary (Thompson 1999; Penczykowski et al. 2016).

Overall, we found that infection is temperature dependent, peaking at the intermediate temperature of 12.5°C. For most ectotherms, performance declines away from the thermal optimum toward their thermal limits, and parasites are no exception (Lafferty and Mordecai 2016). Although our experiment was limited to three temperature treatments, we captured a nonlinear relationship between temperature and infection for *L. clarki*. We observed a peak infection temperature that was lower than the peak temperature for free-living *L. clarki* growth rates (i.e., 12.5°C vs. 16.5°C–23.5°C). Because infection involves the thermal performance of both parasite and host (Gehman et al. 2018), the difference in optimal temperatures between free-living growth and parasite infection may be explained by the relative influence of temperature on parasite attack, ciliate free-living growth, and host defense. Specifically, the melanization response of mosquitoes increased with temperature, which adds support to other studies demonstrating that mosquito immunity involving melanization is highest at 18°C (Murdock et al. 2012). A consequence of a hump-shaped relationship between parasite infection and temperature is that we should not expect climate change to uniformly increase or decrease disease prevalence (Rohr et al. 2011). Instead, the effects of warming on infection will differ across a species range, with climate change potentially driving geographic shifts in prevalence such as that observed in white pine blister rust (Dudney et al. 2021), where parasitism increases in currently cooler regions and decreases in warmer areas.

In summary, our study documents a coevolutionary interaction between a ciliate parasite and its mosquito host in the context of a temperature gradient, where the parasite appears to be ahead in the evolutionary arms race. Despite a large body of literature on host-parasite coevolutionary arms races and theory suggesting that these interactions drive evolutionary and population dynamics in both players, our experiment provides some of the first clear evidence of range-wide parasite local adaptation to its host. Although the parasite is ahead in the arms race, the host also plays a role in the outcome of the interaction, evidenced by a much lower optimal temperature for parasite infection than for free-living ciliate growth. The host immune response, which peaked at higher temperatures, appears to moderate the high free-living growth rates at warm temperatures and drive down the thermal optimum for parasitism. Interestingly, we did not find evidence that *L. clarki* is locally adapted to temperature in its parasitic form, despite finding this evidence for its free-living form. Adaptation of *L. clarki* parasites to hosts but not temperature suggests that the selective pressure on parasites from hosts may be stronger than that of the thermal environment. This result provides a contrast to a recent meta-analysis, which found that local adaptation to biotic interactions is rare (Hargreaves et al. 2020). We also captured some evidence of a nonlinear response of infection to temperature, demonstrating that although there was not local thermal adaptation at the temperatures we measured, temperature remains an important factor mediating host-parasite interactions.

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Statement of Authorship

K.L. and E.A.M. designed the study; J.F., K.L., and L.C. collected the samples; K.L. conducted the experiment and analysis; K.L. wrote the first draft of the manuscript; and all authors contributed substantially to revisions.

Data and Code Availability

The data and code supporting the results have been archived in the Dryad Digital Repository (https://doi.org/10.25338/B80W73; Lyberger et al. 2024).

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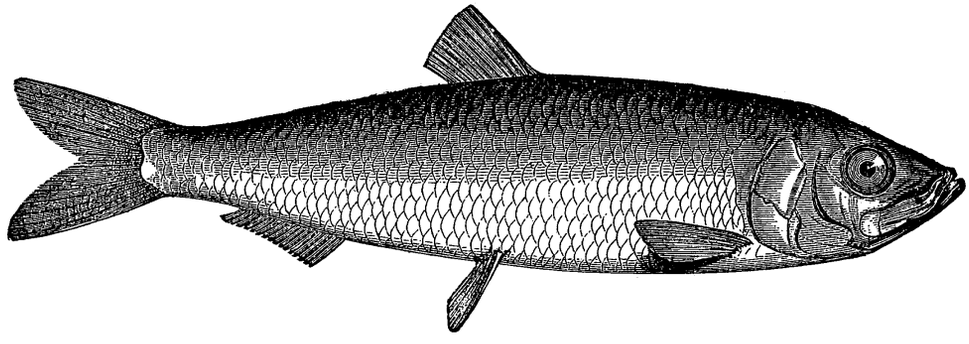

"In respect to our smaller fishes, the Herring (*Clupea elongata*), etc., we observe a considerable decrease in the numbers which now annually visit our shores, as compared with their former numbers. The Poggy (*Alosa Menhaden*) and the Herring (*Clupea elongata*) [figured] have comparatively almost deserted the waters about Provincetown, where I have formerly seen them in immense schools very near the shore." From "The Habits and Migrations of Some of the Marine Fishes of Massachusetts" by James H. Blake (*The American Naturalist*, 1870, 4:513–521).