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ARTICLE
Disease Ecology

Identifying individual and spatial drivers of heterogeneous transmission and virulence of malaria in Caribbean anoles

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Abstract

Heterogeneous distributions are a fundamental principle of ecology, manifesting as natural variability within ecological levels of organization from individuals to ecosystems. In disease ecology, variability in biotic and abiotic factors can result in heterogeneous patterns of transmission and virulence—broadly defined here as the negative consequences of infection. Still, our classic theoretical understanding of disease dynamics comes from models that assume homogeneous transmission and virulence. Here, we test this assumption by assessing the contribution of various sources of individual and spatial heterogeneity to patterns of transmission and sublethal measurements of virulence in two lizard–malaria systems: a three-parasite assemblage (*Plasmodium floridense*, *Plasmodium leukocytica*, and *Plasmodium azurophilum*) infecting the lizard *Anolis gundlachi* in the rainforest of Puerto Rico and a single-parasite system (*P. floridense*–*Anolis sagrei*) in Florida. Using a Bayesian model selection framework, we evaluated whether individual host differences (i.e., body size and sex) or spatial variability (i.e., habitat type and local-scale host spatial structure) drive heterogeneity in the probability of infection or its associated health costs (i.e., body condition, blood chemistry). We found that the probability of infection increases with increasing lizard body size in both systems. However, in Florida, we found the relationship to be subdued in deforested habitats compared to the adjacent urban hydric forests. Furthermore, infection was spatially clustered within sampling sites, with "hot" and "cold" spots across the landscape. Nevertheless, we found no clear evidence of costs of infection on lizard health in any of the measures assessed and hence no grounds for inference regarding heterogeneous virulence. Ultimately, the consistency of our results across systems suggests prominent roles of individual and spatial heterogeneities as driving factors of transmission of vector-borne diseases.

KEYWORDS
Anolis gundlachi, *Anolis sagrei*, blood chemistry, Florida, hematology, *Plasmodium azurophilum*, *Plasmodium floridense*, *Plasmodium leukocytica*, Puerto Rico, reptiles, urbanization, vector-borne disease

INTRODUCTION

The general theory of ecology is guided by the fundamental principle of heterogeneous distributions. This heterogeneity manifests as natural variability within ecological levels of organizations ranging from individuals to ecosystems (Scheiner & Willig, 2011). Heterogeneity applies to many constitutive ecological theories, including disease ecology, where variability in biotic and abiotic factors can result in heterogeneous patterns of transmission and virulence (Combes, 2001).

The traditional theoretical understanding of disease transmission is rooted in models that have historically assumed homogeneous mixing in which all individuals have an equal probability of infection (Reiner et al., 2013). Still, empirical evidence from many systems suggests that transmission is often heterogeneous (Dietz & Hadeler, 1988; Hagenaars et al., 2004; Lajmanovich & Yorke, 1976). The role of heterogeneity in transmission may be amplified in vector-borne disease (i.e., *Plasmodium*) systems in which the addition of a vector and its ensuing contact patterns with hosts provide new mediums for variability to arise. Vector-borne transmission often varies by the individual due to heterogeneous biting across individual host traits such as body size, sex, and blood type (Murphy et al., 2001; Port et al., 1980; Shirai et al., 2004; Takken, 1999; Verhulst et al., 2011; Woolhouse et al., 1997), as well as spatially where host and vector densities dictate contact rates and create "hotspots" of prevalence (Bousema et al., 2012; Keymer & Anderson, 1979). Therefore, identifying how various sources of heterogeneity mediate transmission is key to improving our understanding of parasite transmission in natural settings.

Transmission is theoretically linked to virulence—the reduction in host fitness due to infection (Alizon et al., 2009; Read, 1994)—through the virulence-transmission trade-off (Anderson & May, 1982). This theoretical formulation predicts that virulence is an unavoidable cost of transmission because the parasite replicates at the expense of host resources (Alizon et al., 2009). Some even argue that virulence in vector-borne diseases could be higher than in contact diseases because transmission is done by the vector and thus is less dependent on host conditions (Ewald, 1994). Theoretical models of virulence are derived from traditional transmission models, inheriting some of the same assumptions, including homogeneous mixing. Still, the costs of parasitic infections to their hosts—which materialize as pathological conditions that, in severe cases, can cause reductions in host fitness—can vary across hosts with different traits such as age or sex and in different environmental conditions such as temperature, nutrient availability, or pollutants (Acevedo et al., 2019; Bedhomme et al., 2004; Coors & De Meester, 2011; Endy et al., 2002; Gordon et al., 2005; Morrison et al., 2010; Murdock et al., 2012).

Recent syntheses emphasize the need to better understand the contribution of individual and spatial variability to the heterogeneous transmission and virulence of vector-borne diseases as a key gap in our knowledge (Mandal et al., 2011; Perkins et al., 2013; Smith et al., 2012, 2014; Vazquez-Prokopec et al., 2016). Given that we know that these heterogeneities exist in many systems, our aim is to inform on which variables, if any, might be important to include as sources of heterogeneity in epidemiological models. To address this, here we test for the roles of individual and spatial variability as drivers of transmission and sublethal proxies of virulence in two Caribbean lizard–malaria systems: *Plasmodium floridense* infecting a common urban lizard in Florida, the brown anole (*Anolis sagrei*), and an assemblage of *Plasmodium* parasites including *P. azurophilum*, *P. leucocytica*, and *P. floridense*, infecting yellow-chinned anoles (*Anolis gundlachi*) in tropical moist forests in Puerto Rico. We hypothesize that individual host characteristics (body size and sex) and spatial attributes (habitat type and local-scale host spatial structure) are potential sources of heterogeneity. The *Plasmodium–Anolis* system is ideal to test for the roles of these individual and spatial factors in disease outcomes because lizard hosts are sexually dimorphic and vary both in size and in spatial use of their habitat (Cook, 2019; Lister, 1976; Paterson, 2002; Schoener & Schoener, 1980, 1982; Tokarz, 1985). Assessing these dynamics in a multiple-parasite assemblage as well as across two comparable but distinct systems provides insight about the generalizability of the results. Specifically, we ask: Are classic models that assume homogeneous transmission and virulence useful in these systems of *Plasmodium* infection in lizards? And if not, which individual and spatial factors best explain heterogeneity in (1) parasite transmission quantified as the probability of infection, and virulence quantified using measures of sublethal health costs such as changes in (2) body condition, (3) hematocrit, and (4) the chemical composition of the blood of the host? Although we do not conduct a direct measurement of fitness, the expectation of this research is that the multiple measurements of health we take—some never tested before in anole–malaria systems—will reveal sources of heterogeneous pathology that can inform the development of more robust epidemiological models of virulence.

METHODS

Study systems

A. sagrei is native to Cuba, Jamaica, the Bahamas, and several other northern Caribbean islands (Campbell & Echternacht, 2003). It is a trunk-ground specialist that primarily occupies disturbed and edge habitats in proximity to urban areas (Edwards & Lailvaux, 2012; Lever, 2003; Losos, 2009). This anole is an invader around the world (Norval et al., 2002), but has found the greatest invasive success in Florida, USA. The only malarial parasite known to infect *A. sagrei* in Florida is *P. floridense*. This species commonly infects red blood cells (RBCs) of *A. sagrei* as well as a variety of other species of anoles (from distantly related taxonomic groups) in the northern Caribbean and Southeastern United States (Telford, 1989; Thompson & Huff, 1944). The parasite's presence in distantly related species across a wide geographic range spanning numerous islands suggests an ancient evolutionary association between parasite and host that was established at the origin of each species of *Anolis* (Staats & Schall, 1996). Although *Plasmodium* infection has been shown to have detrimental health effects on reptiles including severe anemia (Telford, 1989) or premature death (Schall, 1996), research is yet to find significant costs to host fitness from *P. floridense* infections in *Anolis* species (Bonneaud et al., 2017; Schall, 2002). Still, *P. floridense* has been observed to induce some pathology yet to be directly correlated to fitness, increasing the relative levels of immature RBCs in *A. sabanus* on the Caribbean island of Saba (Schall & Staats, 2002) and of white blood cells in *A. sagrei* and *A. carolinensis* in Central Florida (Bessa et al., 2020; Doan et al., 2019).

A. gundlachi is a trunk-ground specialist (Rand, 1964) that occurs exclusively in the interior wet forests of Puerto Rico (Rivero, 1998), where it is the most common anole in the forest understory (Reagan, 1992). It is infected by three species of *Plasmodium*: *P. azurophilum*, *P. leucocytica*, and *P. floridense*. The most recent *Plasmodium* phylogeny groups *P. azurophilum* and *P. leucocytica* as sister taxa (Galen et al., 2018), even though *P. azurophilum* infects RBCs and *P. leucocytica* white blood cells. *P. floridense* also infects the RBCs of *A. gundlachi* (as it does *A. sagrei* in Florida), but *P. floridense* is the least common and the most distantly related of the three. In contrast to lizard malaria in California and Africa, there is no evidence yet of a relationship between anole fitness and infection by these Caribbean *Plasmodium* species (Bonneaud et al., 2017; Schall, 2002), although similar pathologies to those mentioned above have been shown for all three parasites in other Caribbean anole species (Ayala & Hertz, 1981; Bessa et al., 2020; Cruz et al., 2022; Doan et al., 2019; Schall, 2002; Schall & Staats, 2002).

Generally, the vector species of saurian *Plasmodium* infection are unknown; the only known vector is the *Lutzomyia* sand fly for *P. mexicanum* in western fence lizards (Ayala, 1970). Yet, most *Plasmodium* species are transmitted by mosquito vectors. The vector species of *Plasmodium* in both the Floridian and Puerto Rican systems are yet to be identified, but the working hypotheses are that the vectors are species of *Culex* mosquito. For *P. floridense* in Florida, the species are presumed to be either *C. erraticus* (Klein et al., 1987), *C. atratus*, or *C. pilosus* (Burkett-Cadena et al., 2008; Cohen et al., 2009; Cupp et al., 2004; Edman, 1979; Reeves et al., 2019). In Puerto Rico, *Culex* is the most common mosquito genus at the site and there is evidence of the presence of multiple *Plasmodium* species in this mosquito genus (M. Acevedo, unpublished). Moreover, previous studies of this three-parasite *A. gundlachi* system hypothesized that there may be more than one vector species transmitting the parasites; however, the exact species are yet to be identified (Otero et al., 2018; Schall et al., 2000).

Data collection

A. sagrei–P. floridense (Florida)

To test for heterogeneities in transmission and virulence across individual traits and between habitats, we sampled *A. sagrei* in deforested and adjacent hydric forest habitats in Central Florida (Seminole County) in August 2020 and in May–July 2021. Although all sites existed within a suburban metropolitan complex, we defined forested plots as those that were natural ecosystems (primarily hydric hammock and floodplain forest) that featured relatively closed canopies with dense understory vegetation and litter, whereas deforested plots were urban to semi-natural ecosystems characterized by open areas with sparse vegetation, mowed grass, and impervious surfaces such as concrete and asphalt (Chejanovski et al., 2017; Kolbe et al., 2016; Thawley et al., 2019). To reduce confounding variables between plots, we used a paired site selection design with three replicate pairs, including a forested and a sympatric deforested plot (Appendix S1: Figure S1).

We surveyed one site (two plots) per day between the hours of 11:00 AM and 4:00 PM, capturing all lizards seen during a survey. We randomly chose which site to survey on a given day and spent approximately 2.5 h searching for anoles in each habitat plot. We searched for anoles on tree trunks, branches, rocks, ground,

walls, fences, and other natural or man-made perches. Lizards were captured by hand or with a dental-floss lasso, placed individually into cloth bags, and transported to the University of Florida for measurement and diagnostics.

To test for within-habitat heterogeneity in transmission, we supplemented the surveys of one of the plots with local-scale sample localization data. We recorded Global Positioning System (GPS) coordinates at the location where each captured anole was first seen with a Garmin eTrex 22x (accurate to 3 m). Coordinates were only taken at the forested plot of Spring Hammock Preserve in Florida. This plot was selected to best match the characteristics of the plot in Puerto Rico (see below): it spanned 80 × 80 m and was the most undisturbed and isolated from urban development of the three Floridian forested plots. The sampled lizards used for the analysis of within-habitat transmission in Florida were a subset of the samples used for the between-habitat analysis. Therefore—barring the supplementary GPS coordinates for lizards at the Spring Hammock Preserve forested plot—all survey, measurement, and diagnostic methods were identical for sampled lizards used in analyses at both spatial scales.

In the laboratory, we gathered data on individual lizard characteristics that may dictate heterogeneity in transmission or virulence by determining sex and quantifying mass with a 10-g *Pesola* spring scale and snout-vent length (SVL) with a caliper. We extracted blood by tail clipping to be used for diagnosing infection and quantifying certain blood parameters that would act as measures of sublethal virulence. Two drops were collected in i-STAT Alinity CHEM8+ cartridges to obtain lab-quality results of chemical and hematological concentrations in the blood. We measured four chemical concentrations: sodium (Na; in millimoles per liter), potassium (K; in millimoles per liter), ionized calcium (iCa; in millimoles per liter), and glucose (Glu; in milligrams per deciliter). Hematology was measured as hematocrit (% packed cell volume), or the volume percentage of RBCs in the blood. Hematological and blood chemical data from this study are available in Appendix S3 for use as reference values for infected, non-infected, and undiagnosed *A. sagrei*. We made a thin blood smear on a microscope slide using one drop of blood from the tail clipping for microscopy diagnosis and kept additional dried blood samples on filter paper for future molecular analysis. We fixed blood smears with methanol (100%) and stained slides with 10% Giemsa for 50 min at pH 7.0 (Schall, 1996). Because *A. sagrei* is an invasive species, all *A. sagrei* were euthanized after blood collection through injection of a solution of MS-222 into the coelom (Conroy et al., 2009; Leary et al., 2020).

A. gundlachi–Plasmodium spp. (Puerto Rico)

We sampled *A. gundlachi* at the El Verde field station in the Luquillo Experimental Forest in northeastern Puerto Rico during September–October 2015. The site is a subtropical wet forest dominated by tabonuco (*Dacryodes excelsa*) and sierra palms (*Prestoea acuminata* var. *montana*) (Reagan, 1992). It has a closed canopy with dense understory vegetation (Parrotta & Lodge, 1991).

We surveyed an 80 × 80 m^2 plot for 5 h daily, capturing all lizards seen during a survey using the same search and collection methods as in the Florida surveys, except for the use of GPS. The experimental forest is well mapped, with every tree identified and numbered, allowing us to record relative coordinates at the location where each captured anole was first seen (Thompson et al., 2002).

In the laboratory, lizards were sexed and measured for mass and SVL. Blood was extracted by toe clipping (Schall et al., 2000). Toe clipping ensures that individuals are not sampled more than once in a season. We made blood smears on microscope slides and collected dried blood samples on filter paper using the same methods as in the Florida surveys. Captured lizards were released within 24 h after capture in the same areas where they were collected.

Diagnostics

To determine the infection status of *A. sagrei* samples, we scanned stained blood smears using a Nikon Eclipse E200 microscope at 1000× magnification for 8–10 min to identify *P. floridense* parasites based on morphological traits (Telford, 1989). We took pictures of any potential parasites for secondary screening and future reference using a Nikon DS-Fi3 microscope camera attachment.

To determine the infection status of *A. gundlachi* samples, we conducted polymerase chain reaction (PCR) testing to identify *Plasmodium* DNA presence in the blood. Due to limited knowledge on the transmittal and virulent differences between the three *Plasmodium* spp. (Schall et al., 2000; Schall & Staats, 2002), the assemblage was combined to identify lizards as infected or non-infected for the purposes of this study. A recent paper studying physiological and environmental drivers of transmission in this same El Verde system scored infection status using the combined assemblage (Otero et al., 2018). Precedent use of this scoring method in a study of the virulent effects of infection in the same El Verde system cites consistency of the relative prevalence of the three species within the same year as justification (Schall et al., 2000; Schall & Pearson, 2000). There is no evidence that anoles

can clear malaria infection; therefore, we assumed infection is chronic (Schall, 1990). For details on PCR protocol, see Supporting Information (Appendix S1).

Diagnosis by a trained observer through slide microscopy rarely, if ever, yields false positives. False negatives can occur when parasitemia is low (mean parasitemia of *P. floridense* in *A. sagrei* in Florida is around 3–6 per 1000 RBCs [Doan et al., 2019; Perkins et al., 2009; Schall, 1996]). Nevertheless, Perkins et al. (2009) found that microscopic examination of *P. floridense* infection in *A. sagrei* was "almost entirely consistent" with molecular screening through PCR.

Analytical framework

We applied a multiple working hypothesis framework for each one of our research questions (Chamberlain, 1890). This approach allowed for the possibility of more than one hypothesis to be simultaneously true (Betini et al., 2017; Elliott & Brook, 2007). We proposed a set of a priori hypotheses for every research question, each of which is represented by a statistical model (Appendix S2: Tables S1–S16).

To answer our questions, we modeled our data using several linear and generalized linear regression models. Each response variable in these models was a quantification of either transmission (i.e., probability of infection) or virulence (i.e., body condition, blood chemical components). Each predictor was an individual or spatial variable that we posited to be a potential source of heterogeneity in the quantified variable of transmission or virulence (i.e., sex, SVL, local-scale host spatial structure, and/or habitat). We also included the sampling site as a predictor factor to control for any inter-site variation stemming from the paired-site design. Due to the limited availability of data from Puerto Rico, we only consider habitat as a source of heterogeneity and blood compositions as measures of virulence for *A. sagrei*. The list of a priori hypotheses for each research question was described as a set of models, each with a unique combination of predictors and their plausible interactions.

All models were fitted using a Bayesian approach to better account for uncertainty in parameter coefficients. We use zero-centered Gaussian vague priors for all parameter values except in the models predicting hematocrit, where we restricted the intercept value to account for the limited reportable range of measurement (15%–75% PCV). Bayes factors are strongly dependent on the width of the parameter priors; therefore, it is not recommended to perform multi-model inference with uninformative priors using Bayes factor weights (Dormann et al., 2018). Instead, we conducted model selection using the widely applicable information criterion, also known as the Watanabe–Akaike information criterion (WAIC), which is the Bayesian analog for AIC when using uninformative priors (Hooten & Hobbs, 2015).

We considered the most parsimonious model to be the one with the lowest WAIC score. In the instances when more than one model in a set had a similarly low WAIC score, we used WAIC weights to assess the relative likelihood of each hypothesis explaining the observed relationship. We used a $\Delta\text{WAIC} < 2$ thresholds for selecting similarly parsimonious models, as it is the widely applied threshold for the analog ΔAIC (Burnham & Anderson, 2004). We consider support for a role of heterogeneity in one of our explained variables if an individual and/or spatial predictor is included in the best model set with little uncertainty in its estimate based on the presence or absence of the null value in the 95% credible intervals and the proportion of the posterior distribution of the same sign as the mean's (Makowski et al., 2019). For the virulence models, we can only consider variation in the explained variables to be a consequence of virulence if infection status is included as a predictor in the most parsimonious models with relative certainty. Only then would any additional predictors in the best set provide evidence of heterogeneous virulence.

To follow, we summarize the details of the model structures, specifications, and compilation information for eight analyses that address our research questions; full details can be found in Appendix S2. To identify potential drivers of heterogeneity in transmission of *Plasmodium* in both *A. sagrei* in Florida and *A. gundlachi* in Puerto Rico, we quantified variation in their probabilities of infection in accordance with host traits and spatial factors. We conducted three separate analyses for transmission based on the scales of the available spatial data: (1) a between-habitat analysis of *P. floridense* infections in *A. sagrei* and within-habitat analyses of (2) *P. floridense* in *A. sagrei* and (3) *Plasmodium* spp. in *A. gundlachi*. Models in the between-habitat analysis (excluding the null model) included combinations of SVL, sex, habitat (i.e., forested vs. deforested), site, and their plausible interactions as covariates. For this and all subsequent analyses, SVL was not included in any model with sex or site due to its strong correlation with these factors.

The two within-habitat analyses compared sets of models that included SVL or sex, as well as a spatial random effect that captures spatial association not measured by the mean function. Using a generalized linear mixed-effects model with a multivariate-*t* distribution spatial Gaussian process allowed us to define correlations between function values based on their spatial coordinates. These models produce a spatial covariance matrix

determined by a spatial variance parameter and a matrix of correlations between function values at any two points, which decay with distance according to a spatial decay rate parameter (Anderson & Ward, 2019; Banerjee et al., 2014; Haftka & Park, 2020). High values of these parameters indicate high spatial correlation, where points close together in space have similar probabilities of infection and the similarity between any two points decreases rapidly with increasing distance. Low values indicate low spatial correlation, where the distance between two points has little effect on the similarity of their probabilities of infection.

Similarly, for virulence, we quantified variability across host traits—and for *A. sagrei*, between habitats—for three indices of pathology in the forms of (4–5) body condition of males of both host species, and the (6) hematocrit and (7–8) chemical composition of the blood of *A. sagrei*.

Body condition is commonly used as an index for the energetic well-being of an individual, where individuals with relatively high indices of condition are assumed to be in a better energetic or physiological state (Ardia, 2005; Peig & Green, 2009; Schulte-Hostedde et al., 2005). Evidence for the correlation between anole body condition and *Plasmodium* infection is mixed (Bonneaud et al., 2017; Cruz et al., 2022; Otero et al., 2018; Sánchez et al., 2018; Schall & Pearson, 2000). We calculated the body condition of male *A. sagrei* and *A. gundlachi* separately as residuals from an ordinary least-squares regression of body mass on SVL (Ardia, 2005; Schall & Pearson, 2000; Schulte-Hostedde et al., 2005). We only consider males in our analysis of body condition because females are often gravid, and the additional mass of eggs violates the assumption that mass increases linearly with body length (Green, 2001). Furthermore, we controlled for differences in mass due to tail break condition by analyzing only individuals with complete original tails. To assess effects by *Plasmodium* infection on body condition, we compared two sets of linear regression models. The *A. sagrei* model set included combinations of habitat (i.e., forested vs. deforested), site, and infection status as covariates. For *A. gundlachi*, the models are similar except that habitat was not included as a predictor because habitat was homogeneous in the Puerto Rican data set.

We quantify hematological changes with a hematocrit test that measures the volume proportion of erythrocytes (RBCs). RBCs distribute oxygen throughout the body, so hematocrit can be an index of an individual's capability of delivering oxygen to its body tissues, with low hematocrit levels signaling low hemoglobin, also referred to as anemia. Anemia can also occur with increased ratios of immature RBCs, which house less hemoglobin than mature cells. This increase in immature cells is a well-documented effect of *Plasmodium* infection in lizards, resulting in reduced hemoglobin in *Sceloporus* and *Agama* lizards but not *Anolis*—a disparity likely due to substantially greater changes in cell maturity in the non-anole species (Schall, 1982, 2002). Although RBC maturity and hemoglobin have been measured for *P. floridense* infections of other *Anolis* species, our study provides the first measure of hematocrit in *Plasmodium–Anolis* infections and the first measure of erythrocytic hematology in *P. floridense–A. sagrei* infections. To assess the effects of *P. floridense* infection on hematocrit in the blood of *A. sagrei*, we compared a set of generalized linear regression models which included combinations of infection status, SVL, sex, habitat, site, and their plausible interactions as covariates.

We also measured individuals' metabolisms and chemical balances through a metabolic panel blood test. We measure glucose—the body's main source of energy—and electrolytes including sodium, potassium, and ionized calcium—electrically charged minerals that control fluids and pH balance in the body, which is critical for the proper functioning of nerve and muscle cells. *Plasmodium* parasites' preferred energy substrate is glucose, so parasitized erythrocytes, including those of Western fence lizards infected with *P. mexicanum*, show increased consumption of glucose compared to uninfected cells that create high metabolic demand when parasites proliferate, reflecting low glucose levels in the blood (hypoglycemia) (Planche et al., 2005; Sherman, 1979). Electrolyte alterations, including high sodium (hypernatremia), low potassium (hypokalemia), and low calcium levels (hypocalcemia), are also common complications of malaria and can be indicators of the severity of the disease (Sitprija, 2008). We found no instances of electrolytes used as measures of pathology in past studies of lizard–malaria.

We quantified chemical composition by combining the measurements of four substances (sodium, potassium, ionized calcium, and glucose) into integrative components through a principal components analysis (PCA). PCA reduces the dimensionality of these multivariate data by combining these four blood measurements into abstract linear combinations of the original variables called principal components (PCs) that maximize the explained variance of each blood measurement (Digby & Kempton, 1987). The first two PCs (PC1 and PC2) explain 61.9% of the variation in the four blood concentrations (Figure 1). To assess the effects of *P. floridense* infection on the chemical composition of the blood of *A. sagrei*, we compared two sets of linear regression models—one for each of these two PCs—which included combinations of

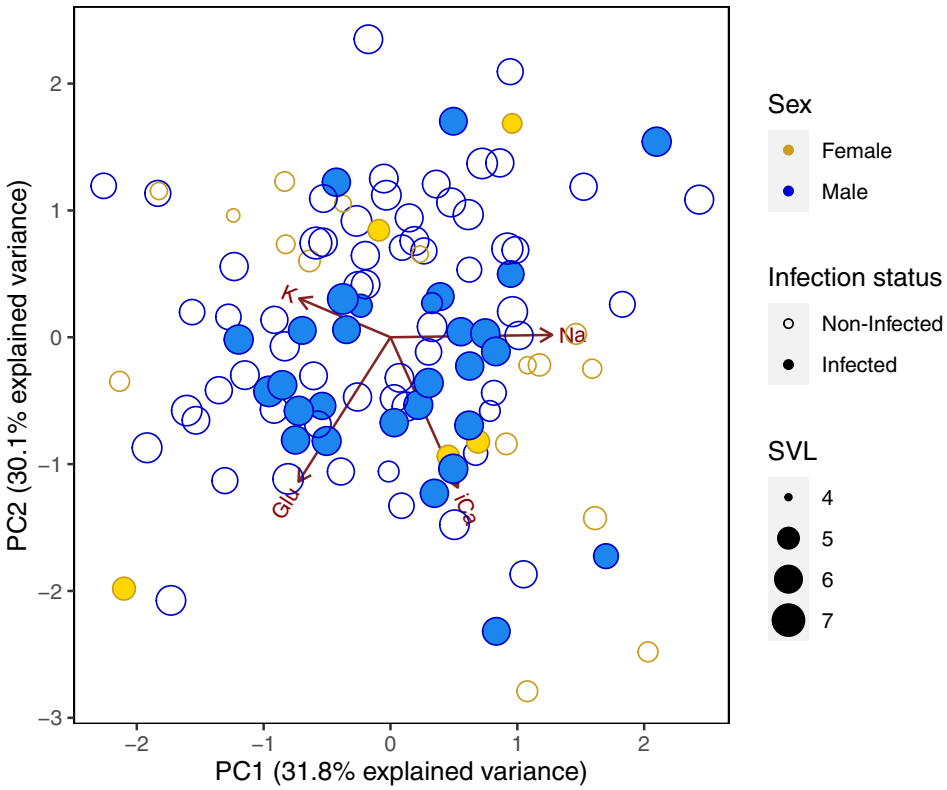

FIGURE 1 The plot shows principal components analysis (PCA) results on four blood parameters—sodium (Na), potassium (K), glucose (Glu), and ionized calcium (iCa)—for 110 *Anolis sagrei*. Points correspond to the Principal Component 1 (PC1) and Principal Component 2 (PC2) scores of each individual and are colored by sex (yellow = females and blue = males), shaded by infection status (empty = non-infected and filled = infected), and sized in proportion to snout-vent length (SVL). Arrows represent the correlation of each blood parameter with PC1 and PC2. PC1 and PC2 together describe over 60% of the variation in the blood parameters. Note that neither principal component separates the individuals by sex, infection status, or size.

infection status, SVL, sex, habitat, and site, and their plausible interactions as covariates.

RESULTS

We sampled 305 *A. sagrei* in Florida (230 males and 75 females; 131 in hydric forest habitat and 174 in deforested habitat) and 124 *A. gundlachi* in Puerto Rico (72 males and 52 females). Overall, we found an infection prevalence of 33% in Florida, while the prevalence in Puerto Rico was 35%.

Transmission

A. sagrei–P. floridense (Florida)

The two most parsimonious models explaining the probability of infection at a broad spatial scale included SVL and habitat (forested vs. deforested), with the interactive model ($WAIC_{wt} = 0.46$; Appendix S2: Table S1) performing slightly better than the additive model ($WAIC_{wt} = 0.45$). Note that below all quantities between brackets represent 95% credible intervals. The interactive model predicted that the probability of infection was 2.12 (1.30, 3.46) times $(\Pr(\beta_F > 1|\text{data}) = 0.998)$ higher in hydric forests than in deforested habitats, while the additive model predicted a similar increase of 2.33 (1.35, 3.74) times $(\Pr(\beta_F > 1|\text{data}) = 0.999)$. With the interaction, the probability of infection was also predicted to increase 1.09 (0.96, 1.25) times $\left(\Pr\left(\beta_{SVL,F} > 1|\text{data}\right) = 0.998\right)$ with a millimeter increase in SVL in forested habitat and 1.03 (0.98, 1.08) times $\left(\Pr\left(\beta_{SVL,DF} > 1|\text{data}\right) = 0.885\right)$ in deforested habitat (Figure 2). In the model without the interaction, the probability of infection increased 1.06 (1.02, 1.09) times $\left(\Pr\left(\beta_{SVL,F} > 1|\text{data}\right) = 0.998\right)$ with a millimeter increase in SVL.

Transmission is also spatially heterogeneous within habitats. The two best-ranked models explaining the probability of infection at a local spatial scale included the spatial Gaussian process ($\sum WAIC_{wt} = 0.90$; Appendix S2: Table S3), suggesting a prominent role of spatial heterogeneity in explaining transmission patterns

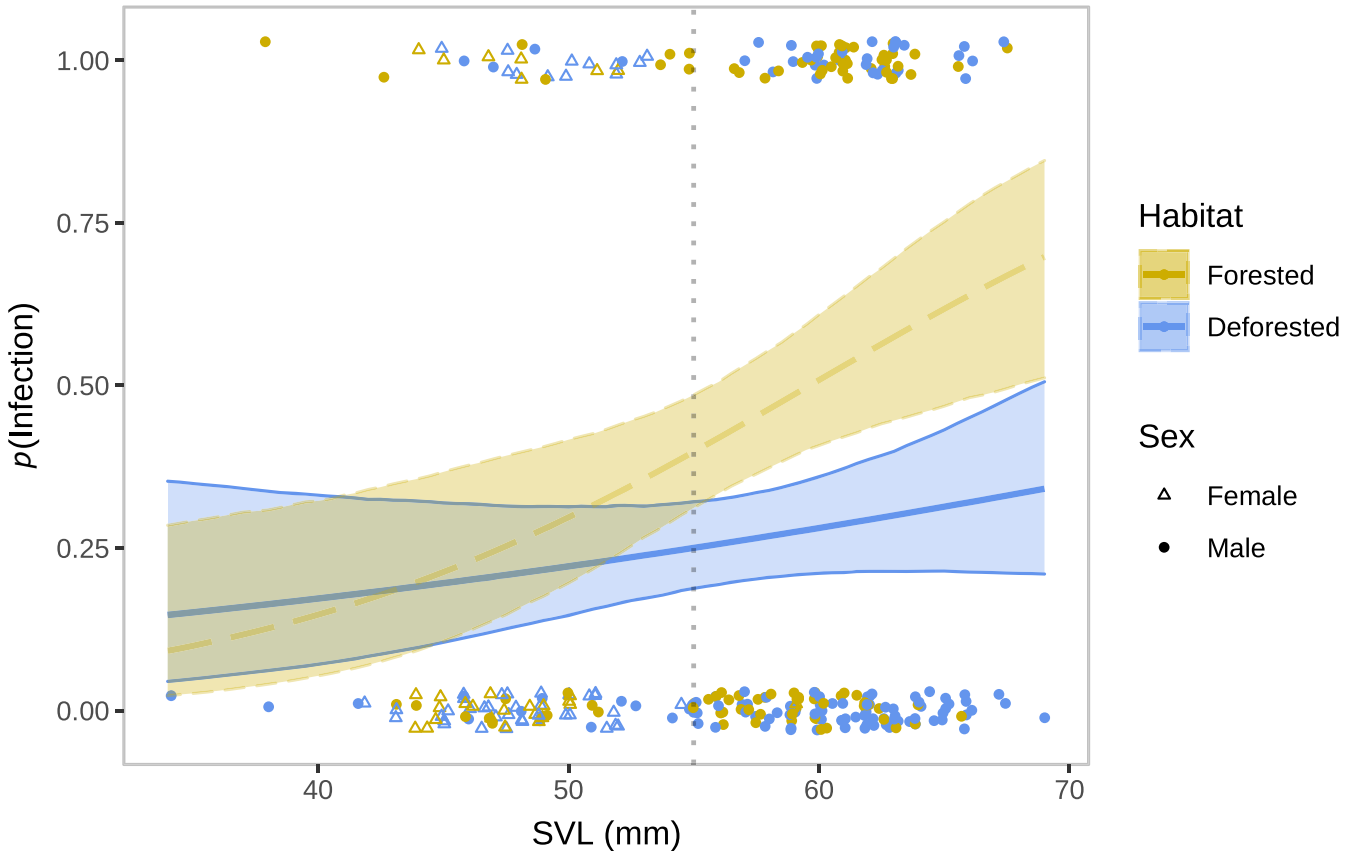

FIGURE 2 The figure shows the predicted relationship between the probability of infection of *Anolis sagrei* by *Plasmodium floridense* and snout-vent length (SVL) in Floridian hydric forests and deforested habitats. The model predicts that the probability of infection increases with increasing body size. The probability of infection is higher in forested habitats for anoles >55 mm: a threshold denoted by the vertical dotted line. Lines represent mean estimates and ribbons represent 95% credible intervals. Points represent anole samples; their sexes are shown to highlight the lack of females above the 55-mm threshold.

in this system. The top-ranked model ($WAIC_{wt} = 0.49$) predicted a mean spatial decay rate of 19.61 (6.44, 36.44), the amplitude of which was further scaled up by a spatial deviation parameter of 3.36 (1.23, 7.26). These estimates indicate a rapid decay in the correlation of any two points as spatial separation increases—in other words, the probabilities of infection of any two points become increasingly dissimilar with distance (Figure 3b). The second-best model ($WAIC_{wt} = 0.41$) estimated a similar spatial decay rate of 19.68 (6.22, 36.24) and spatial deviation parameter of 3.45 (1.29, 7.37), while also predicting males to be 3.06 (0.48, 22.65) ($\Pr(\beta_{Sex} > 1|\text{data}) = 0.88$) times more likely to be infected than females.

A. gundlachi–Plasmodium spp. (Puerto Rico)

Local-scale results from Puerto Rico are less clear as to the benefit of including a spatial random effect in transmission models. Two models stood out as the most parsimonious ($\sum WAIC_{wt} = 0.88$; Appendix S2: Table S5), both predicting the probability of infection to increase 1.09 (1.04, 1.15) ($\Pr(\beta_{SVL} > 1|\text{data}) = (0.99, 0.99)$) times with each millimeter increase in SVL. While the most parsimonious model included the spatial process, its estimated mean spatial decay rate of 8.62 (0.71, 22.36), the amplitude of which was scaled down by a spatial deviation parameter of 0.87 (0.11, 2.15), corresponds to a fairly slowly decaying spatial correlation with distance (Figure 3d). This model only slightly outperformed the next best model, which excluded the spatial process ($\Delta WAIC = 0.19$).

Virulence

Body condition

We found no clear evidence of an effect of *P. floridense* infection on the body condition of *A. sagrei*. While the body condition of male *A. sagrei* appears to be better predicted by models that include a negative effect of

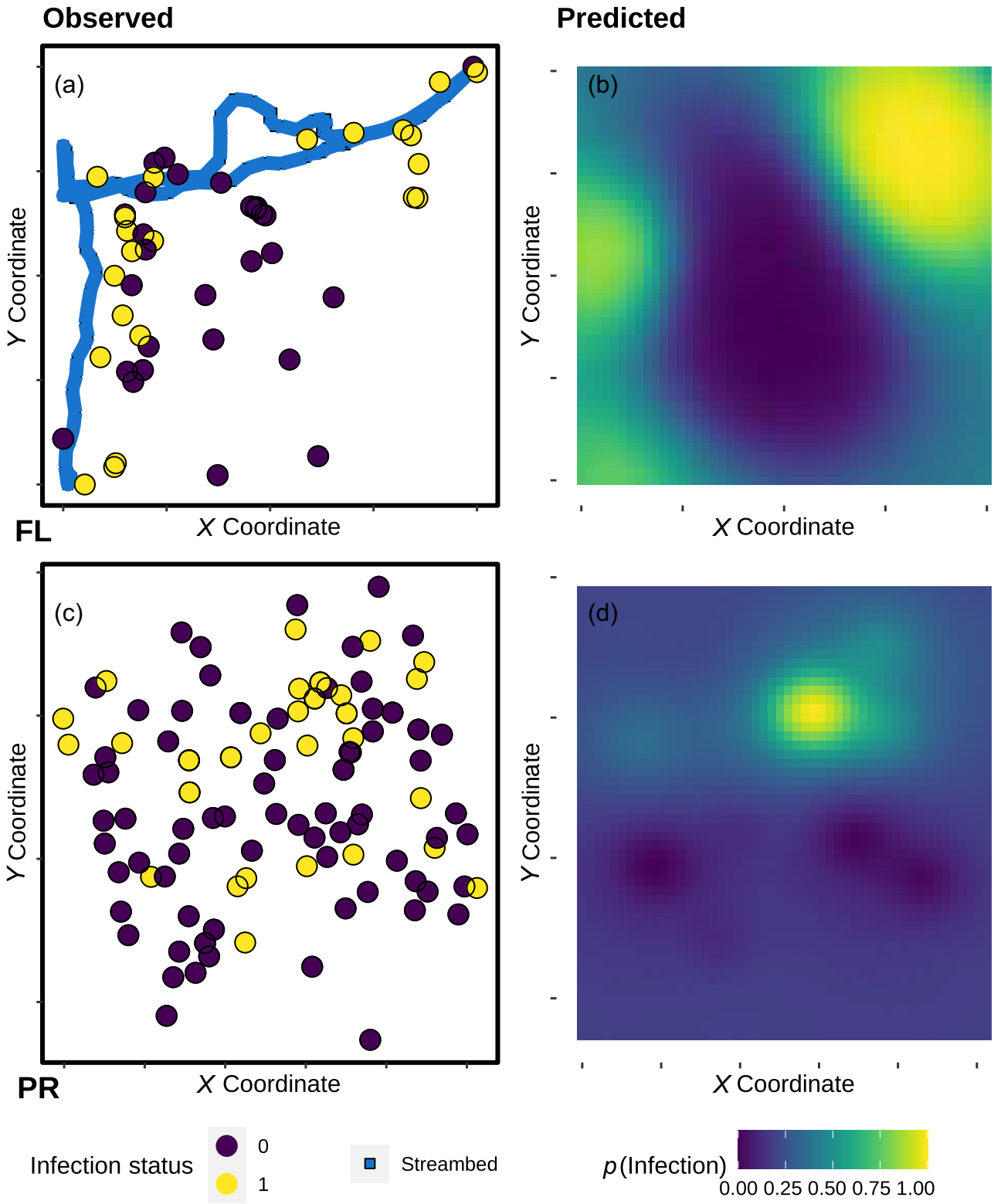

FIGURE 3 The figure shows the spatial distribution of (a and c) *Plasmodium floridense* infection in *Anolis sagrei* in a forested plot in Florida (FL) and (b and d) *Plasmodium* spp. infection in *Anolis gundlachi* in a plot of Puerto Rican (PR) forest. (a and c) Plots show observed infection status of surveyed individuals geopositioned at their location of capture. A geopositioned streambed is included in (a) for the sake of contextualizing the observations. (b and d) Plots show spatial predictions of the probability of infection from the best-ranked models from each site. (b) and (d) show hotspots of infection (yellow) corresponding to clusters of infected individuals in (a) and (c), and cold spots (blue) corresponding to clusters of non-infected individuals in (a) and (c). Predictions in (b) are for male *A. sagrei*; predictions in (d) are for *A. gundlachi* of mean snout-vent length.

infection in three of the four most parsimonious models, the null model was still the third-best model ($WAIC_{wt} = 0.13$), suggesting uncertainty as to whether differences in body condition can be explained by any of the proposed variables (Appendix S2: Table S7). Similarly, we found no clear evidence of an effect of *Plasmodium* infection on the body condition of *A. gundlachi*, with both the infection and null models performing similarly at explaining the observed body condition of male *A. gundlachi* ($\Delta WAIC = 0.24$; Appendix S2: Table S9). More detailed descriptions of results for all the analyses of virulence can be found in Appendix S2.

Hematocrit

We found no clear evidence that infection status affected hematocrit. Six of the 30 models were selected as the best approximators of hematocrit ($\sum WAIC_{wt} = 0.59$; Appendix S2: Table S11), four of which did not include an effect of infection ($\sum WAIC_{wt} = 0.39$). The null model (intercept-only) was the best model in the set ($WAIC_{wt} = 0.16$), providing further uncertainty as to the existence of a relationship between infection status and hematocrit.

Chemical composition of blood

We found no clear relationship between chemical composition and infection status. Four models performed similarly in predicting PC1 ($\sum WAIC_{wt} = 0.49$), the best of which was the null model ($WAIC_{wt} = 0.21$). Out of these four parsimonious models, only the second-best ($WAIC_{wt} = 0.11$) included infection status as a predictor, and it was uncertain as to the direction of this effect ($\beta_{Infection} = 0.18\,[-0.27, 0.63]$; $Pr(\beta_{Infection} > 0|data) = 78.7$). For PC2, no models stood out during model selection, with seven models having $\Delta WAIC < 2$ ($\sum WAIC_{wt} = 0.60$). Four of these models included infection status as a covariate ($\sum WAIC_{wt} = 0.32$). Still, the similar performances of the null model ($WAIC_{wt} = 0.11$) and other models excluding infection status, along with the uncertainty in the estimates of its effect by those that do, make any relationship between infection status and PC2 unclear.

DISCUSSION

While homogeneous mixing is a prevalent assumption in classic vector-borne disease theory, heterogeneity is common in nature and understanding how this variability mediates transmission and virulence is key to improving our understanding of vector-borne disease ecology and evolution in more realistic settings. Here, we showed the utility of incorporating spatial heterogeneity and variability in host body size and, to a lesser extent, sex into transmission models for two separate Caribbean lizard–malaria systems. Still, we found no clear evidence of pathology in the health measurements assessed and hence no grounds for making inferences about heterogeneous virulence.

Spatial variability in transmission was evident between and within habitats. In Florida, we found habitat to be a key determinant of infection probability, with probabilities in hydric forests within the urban matrix more than double those in deforested habitats. This suggests that Floridian hydric forests provide better conditions than deforested habitats for vectors and/or their interactions with hosts, including vertical heterogeneity, lower temperatures, or accessibility to standing water critical for vector reproduction. *Culex* mosquito spatial distribution in Southeastern United States shows increased abundance with increasing canopy cover and in floodplain forests like those of our forested plots in Florida (Burkett-Cadena et al., 2013). In addition, our forested habitats contain seasonal tributaries of the St. John's River basin: fresh water sources that have been found in previous niche models to be strong indicators of *P. floridense* infection to *A. sagrei* (Perkins et al., 2007). In contrast, elevated temperatures typical of deforested urban heat islands (Oke, 1982) limit vector populations in regions with warm climates (Huey et al., 2012; Paaijmans et al., 2012). Also, previous long-term studies on lizard malaria in the Caribbean have shown that the probability of infection peaks at intermediate temperatures, suggesting that increased temperatures reduce vector density, vector susceptibility, and/or parasite survival (Adelman et al., 2013; Murdock et al., 2012; Otero et al., 2018). As a result, our findings imply that not all urban environments are the same, and even within urban environments, there can be heterogeneity in vector-borne disease transmission intensity driven likely by microclimatic variability.

Transmission was also spatially heterogeneous within habitats, but the importance of this spatial correlation to predicting infection is clearer in the *A. sagrei* system than for *A. gundlachi* as both top models in Florida included the spatial process. A visual assessment of Figure 3 might suggest that anoles have a clustered distribution in the Floridian plot, with areas of high density occurring near stream banks where water concentrates after rain at this site. In this informal context, the spatial process parameter estimates suggest that individuals making up these

clusters are likely to share the same infection status given the short distances between them. This is supported by the occurrence of the predicted hotspots of infection in these areas of high anole density and the low infection probabilities where individuals were sparser—a pattern shared by other avian and mammalian malaria systems (Isaksson et al., 2013; Wolfe et al., 2002). Thus, our models suggest a prominent role of host spatial distribution in driving spatially heterogeneous transmission, but this may only be part of the equation. The presence of infections among these clustered individuals possibly stems from their proximity to fresh water—a necessary resource for mosquito breeding—which has been found to be an important variable in predicting the probability of *P. floridense* infection in *A. sagrei* in Florida (Perkins et al., 2007). This could point to vector habitat as a key determinant of infection prevalence at this smaller scale as well, and it promotes the roles of vector and host densities as complementary mechanisms of transmission dynamics (Tipping & Weber, 2008).

While Puerto Rican infection patterns were best explained by the body size model that incorporated spatial correlation, the similar performance of the body size model that did not include the spatial process as well as the weaker spatial correlation compared to the Florida plot suggest that capturing unmeasured spatial association may not substantially improve transmission models in this system. The less-pronounced nature of the spatial association in this system could be a consequence of differences in the transmission dynamics of the multiple parasite species, the existence of more than one vector, or environmental conditions that might vary throughout the site. It is more challenging to speculate on the mechanisms driving the heterogeneity in this system because we know less about the transmission ecology of this parasite assemblage. Despite this, our transmission results ultimately encourage accounting for between- and within-population spatial heterogeneity in models of *Plasmodium–Anolis* transmission, although further experimental research is necessary to draw a distinction between the roles of vector and host densities as causative agents of spatial heterogeneity in infection.

At the individual level, host size was an important source of heterogeneity in the probabilities of infection in both systems. This result is in line with most anole–malaria studies, showing increases in infection probability with SVL (Otero et al., 2018; Schall, 1996). The strong support for this effect in our broad-scale models of *A. sagrei* infection brings clarity to the findings of Doan et al. (2019), which gave substantial—yet only partial—weight to the inclusion of SVL in modeling the probability of *P. floridense* infection in anoles in the same county in Florida. This pattern is most likely driven by increased susceptibility in larger lizards, as physiological and behavioral differences between sizes can influence a lizards' exposure to vectors. To start, anole size is often correlated with age and thus cumulative exposure to chronic infection. Day to day, larger anoles may be prone to more mosquito bites because increased skin surface results in larger available areas for vector biting (Port et al., 1980), and increased carbon dioxide emanation may attract more mosquitoes (Takken, 1999). Moreover, larger anoles occupy larger territories, exposing them to more vector habitat, and expend more energy defending them, reducing their immune efficiency via resource trade-offs (Cook, 2019; Evans, 1938; Tokarz, 1985).

It follows that male anoles, which are larger and defend wider territories than females, should experience higher rates of infection. Increased malaria prevalence in males has been observed in many lizard populations, including *A. gundlachi* at El Verde, although this pattern has not been supported in previous studies of *A. sagrei* in Florida (Doan et al., 2019; Perkins et al., 2009; Schall, 1996; Schall et al., 2000; Schall & Marghoob, 1995; Schall & Vogt, 1993). While post-hoc Fisher's exact tests do show marginal to strong evidence of higher prevalence in males than females in Florida (36% vs. 25%; $p = 0.09$) and Puerto Rico (44% vs. 23%; $p = 0.02$), none of our transmission analyses showed clear support for including sex in models of infection probability. It is most likely that any variation in the probability of infection between sexes in our data was better captured by SVL and host spatial structure. This is evident in the rankings of the local-scale transmission models in Florida, where the additional variation captured by including sex in the spatial model was not substantial enough to justify an additional degree of freedom (Appendix S2: Table S3). Similarly, the selection of broad-scale transmission models in Florida favors the interactive effects of habitat and SVL over sex, despite the predictions of this model revealing a clear disparity in prevalence patterns between sexes: the probability of infection is nearly double in forested habitats for lizards >55 mm in SVL—a threshold only 1 of 75 females surpasses and 33 of 230 males fall below—and essentially the same in both habitats for lizards <55 mm (Figure 2). While sexually dimorphic physiology and behaviors in anoles likely drive some heterogeneity in transmission, other variables correlated to these underlying mechanisms, such as body size and spatial structure, explain more of the heterogeneity and prove to be more useful predictors of transmission in our study systems than the binary factor of sex.

We found no clear evidence of pathology from *Plasmodium* infection in either host species. Therefore, making any inferences regarding potential sources of heterogeneous virulence in these systems would be

inappropriate. Although comparative data of lizard–malaria systems in California and Africa suggest that malaria tends to be virulent to some lizard hosts, *P. floridense*, *P. azurophilum*, and *P. leucocytica* infections in *Anolis* hosts in the Caribbean appear to be nearly benign (Schall, 2002). The inconsistent inclusion of infection status in our top models offers no support to refute these previous findings. Positive, negative, and null body condition–infection relationships have all been found in *Plasmodium–Anolis* systems (Bonneaud et al., 2017; Cruz et al., 2022; Otero et al., 2018; Sánchez et al., 2018; Schall & Pearson, 2000). There is also a large variability in body condition's relation to fitness, with more than one study finding no correlation with survival in *A. sagrei* (Bonneaud et al., 2017; Cox & Calsbeek, 2015; Sánchez et al., 2018). Our inconclusive assessments in both study systems of body condition as a pathological consequence of infection are consistent with these findings. Increased immaturity of RBCs has been shown in *P. floridense* and *P. azurophilum* infections of Caribbean *Anolis* species not studied here (Schall, 2002; Schall & Staats, 2002), but this was not coupled with significant changes in hemoglobin. Our examination of hematocrit—the first for a *Plasmodium–Anolis* system—did not regard infection status as an important predictor, adding to the evidence that *Plasmodium* infection is not correlated with anemia in anoles. This study was also the first to investigate potential electrolyte and metabolite alterations in *Plasmodium–Anolis* infections, but neither sodium, potassium, calcium, nor glucose was well-predicted by infection status in either preliminary separate analyses of each component or the combinatory PCA.

A growing literature of theoretical and mathematical models on the evolution of virulence has posited many hypotheses to explain the benign nature of these infections, but the rarity and inconsistency of tests of these hypotheses leave this yet unsolved. More data on the cost of parasitism for natural *Plasmodium–Anolis* systems are needed to better understand the evolutionary and ecological mechanisms driving this apparent lack of virulence (Schall, 2002). The novel measures of pathology from this study are a valuable contribution in this sense. What is more, recent studies have discovered novel costs of *Plasmodium* infection in *A. sagrei* and *A. gundlachi*, including increases in leukocyte counts relative to erythrocytes (Bessa et al., 2020; Doan et al., 2019) and higher degrees of erythrocyte size variation within individual infected lizards than in non-infected lizards (Cruz et al., 2022), although further research is necessary to determine whether these are ultimately related to a reduction in fitness and if these patterns are geographically or individually heterogeneous.

All in all, the consistency of our results across systems suggests prominent roles of individual and spatial heterogeneities as driving factors in the transmission of vector-borne diseases. These findings fill key gaps in our understanding of the spatial dynamics of transmission at different scales and add new perspectives to the mounting evidence of how individuals differ in their probabilities of infection. The mechanistic explanation of the lack of strong evidence of pathological effects of *Plasmodium* on these anoles remains an interesting open question that carries implications for the theoretical link between transmission and virulence. Therefore, our results suggest that vector-borne disease dynamics in the wild are more complex than what classic theoretical models capture. Understanding this complexity should become a priority as the field evolves.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Toohey, 2022a) are available from Figshare: https://doi.org/10.6084/m9.figshare.20775475. Code (Toohey, 2022b) is available from Zenodo: https://doi.org/10.5281/zenodo.7041846.

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SUPPORTING INFORMATION

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