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RESEARCH ARTICLE

Biogeographic patterns of blood parasitism in the Aegean wall lizard (*Podarcis erhardii*) across the Cycladic Islands

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Abstract

The biogeography of host-parasite dynamics is an area that has received little attention in studies of island ecology. While a few studies have shed insight on patterns of parasitism in insular host populations, more empirical evidence is needed to ascertain how isolation impacts parasites. Biogeography generally theorizes that the physical size of islands and the duration of each island's isolation can be driving geographic factors controlling species interactions and populations dynamics. To test this, we assessed the effect of island structure and population isolation on the endemic insular lizard *Podarcis erhardii* and its native hemogregarine parasite (Apicomplexa: Adeleorina) in the Cyclades (Aegean Sea). We analyzed the relationships of prevalence and parasitemia of hemogregarine infection with several factors concerning the island (size, time of isolation, spatial isolation, population density) and host (body size) levels using regression and structural equation models, respectively. Regressions indicate that islands with greater host density and islands which have been isolated for shorter timespans tend to have higher hemogregarine prevalences; structural equation models suggest a similar pattern for parasitemia. We hypothesize this may be driven by insular density compensation. Hosts on islands that are more temporally and spatially isolated also tend to have higher prevalence and parasitemia of hemogregarines. Our results indicate that island area, island isolation, and host population density are likely to be significant drivers of changes in host-parasite interactions in fragmented populations.

Highlights

- Land-bridge islands provide a natural study system to explore the effect of island structure and ecology on native host-parasite interactions, such as the relationship of hemogregarine parasites and a lacertid lizard host, *Podarcis erhardii*, in the Cyclades.

- We fit models of prevalence and parasitemia to empirical observational data and demonstrated that biological and geographical factors correlate with parasitism, and that biological and geographical variables are interconnected in this context.

- This work suggests that isolation time and host population density, in particular, are strong predictors of parasitism for native island hosts and that insular density compensation may be a mechanism for this pattern.

- Further exploration of insular host-parasite interactions will not only deepen our understanding of parasite ecology, but may also act as a valuable indicator for how habitat fragmentation may affect host-parasite dynamics in the future.

Introduction

Research in island biogeography has uncovered a variety of ecological patterns and processes that affect fragmented and isolated biotic communities. Many studies focus on the effect of fragmentation and insularity on community structure, physiology, morphology, and predator-prey interactions (MacArthur and Wilson 1967, Adler and Levins 1994, Blondel 2000, Blumstein and Daniel 2005, Hurston et al. 2009, Novosolov et al. 2013), but less is known about the impact of insularity on host-parasite dynamics (i.e., infectious species which decrease host fitness with a pathology that is intensity-dependent, Lafferty and Kuris 2002) (McCallum and Dobson 2002). In a recent survey, biologists determined that

one of island biology's top research priorities was understanding how island isolation and size affected population dynamics (Patiño et al. 2017).

Evidence has shown that isolation can have wide-ranging impacts on host-parasite distributions and interactions (Perkins 2001, Morand and Krasnoz 2010). Hosts may effectively lose parasites when isolated if host populations become small or dispersed (Clay 2003, Torchin et al. 2003), or parasites may evolve to have a wider niche, thereby infecting new additional hosts (Nieberding et al. 2006). Hosts may also be more susceptible to parasitism if hosts are inbred, which could be determined by spatial and temporal isolation trends (Reed and Frankham 2003, Charpentier et al. 2008). Parasitologists and ecologists have developed theory detailing the possibilities of host-parasite evolution following isolation, but there is still much opportunity for empirical and observational study of island biogeography in natural systems (Poulin 2004, Illera et al. 2015).

Generally, insular systems often exhibit higher aggregate population densities on small islands as compared to larger islands and mainland areas, a process known as density compensation (MacArthur et al. 1972, Wright 1980, Rodda and Dean-Bradley 2002). Studies that have investigated the relationships between parasitism and island biogeography suggest that isolation tends to lead smaller islands with denser host populations to harbor higher parasite prevalence and infection intensity, and lower richness of parasite diversity (Gouy de Bellocq et al. 2002, Roca et al. 2009). While density compensation is documented in faunal populations (Wright 1980, Rodda and Dean-Bradley 2002), it is not clear how this process manifests in a host-parasite system when isolation also varies spatially and temporally.

Related biogeography research suggests that more intense isolation lead to a distinct loss of genetic diversity in the parasite and subsequently impoverished parasite populations in hosts (Reed and Frankham 2003, Nieberding et al. 2006, Pérez-Rodríguez et al. 2013, Koop et al. 2014). Two related study found insular parasite populations to be increasingly depauperate the longer the period of isolation from the mainland (Roca et al. 2009, Pérez-Rodríguez et al. 2013). There is also evidence that isolation leads to higher parasitism in inbred populations (Charpentier et al. 2008, Luikart et al. 2008, Chapman et al. 2009). Another study found that island hosts, compared to mainland hosts, had significantly more parasitism as a result of reduced genetic variability and increased host population density (Loiseau et al. 2017). Similarly, spatial isolation of island communities can dramatically alter species composition by affecting immigration and dispersal opportunities (MacArthur and Wilson 1967, Simberloff and Wilson 1969, Simberloff 1976, Whittaker et al. 2007, Rosindell and Harmon 2013), which may affect population size in remaining species. Islands that are more spatially isolated from mainland or other possible "source" land masses are expected to experience lower diversity and more apparent evolutionary divergence than islands that are less isolated but of the same size (Simberloff and Wilson 1969, Simberloff 1976).

The predictions of island biogeography theory have been developed for several decades in the context of free-living species, and little of this work has been extended to consider symbiotic species like parasites, pathogens, and mutualists. Insular communities are, in some cases, depauperate of symbiotic species. Parasites, for example, may be entirely lost in the process of isolation from the host species (Torchin et al. 2003, Tomé et al. 2018). Loss or reduction of symbiotic species may be a result of restrictions in host species abundance, environmental or dispersal limitations, or a consequence of evolutionary changes in hosts, parasites, or both. For parasites infecting isolated host species, we may expect that parasites evolve to be less host specific over time as this may be expected to increase transmission and abundance, however, a depauperate host population or low immigration may reduce or prevent parasite survival. Integrating parasitism into island biogeography theory requires a careful assessment of how free-living species dynamics affect symbiotic species that operate at a finer spatial scale than their hosts. Parasites (and other symbionts) are so intimately associated with hosts that host individuals or populations are functionally equivalent to habitat and patches for parasite species (Kuris et al. 1980, Combes 2001).

The Cycladic islands are an archipelago of land-bridge islands in the Aegean Sea, located off the eastern coast of Greece (Fig. 1). Most of the islands, and the vertebrate populations inhabiting them, became progressively isolated during the gradual rise in sea levels following the last glacial maximum 18000 years ago (Pirazzoli and Pluet 1991, Kapsimalis et al. 2009, Poulos et al. 2009). Duration of island isolation depends on the depth of the underwater saddle between two islands and varies greatly between 25 years and 5.33 million years (Foufopoulos and Ives 1999). Multiple lines of evidence indicate that the Aegean wall lizard (*Podarcis erhardii*, Reptilia: Lacertidae) is a very poor over-water disperser; as a result, the evolutionary history of Cycladic populations reflects the fragmentation history of the islands they inhabit (Foufopoulos and Ives 1999, Hurston et al. 2009). The resulting diversity of habitat and population characteristics of the Cycladic islands present an excellent study system to investigate the long-term effects of habitat fragmentation on the evolutionary ecology of host-parasite interactions.

We determined how island geography and host ecology affect the prevalence and intensity (*sensu* Margolis et al. 1982, and hereafter, parasitemia) of parasite infections in the Aegean wall lizard host. *Podarcis erhardii* is a native host for a vector-transmitted hemogregarine parasite (Apicomplexa: Adeleorina). Recent work has shown that hemogregarine parasites on islands are surprisingly host-specific, and that insular parasites tend to reflect the evolutionary history of their hosts (Tome et al. 2018). Consistent with traditional island biogeography theory, island
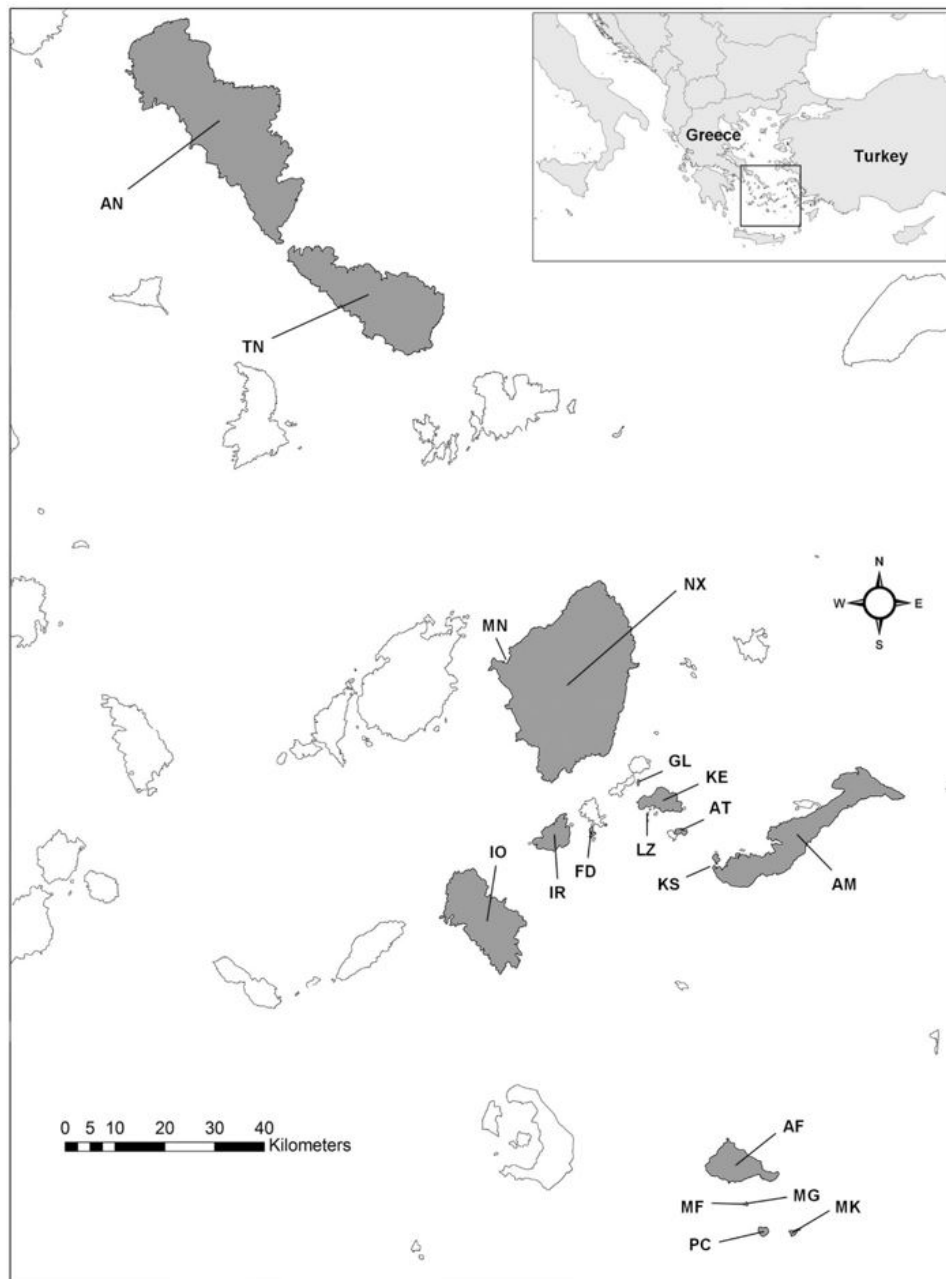
Figure 1. Map of islands sampled for Apicomplexan parasites and their host, *Podarcis erhardii*. Islands in the Cyclades were chosen based on variety of age (in years) and area (in km²) of each island. Islands sampled are shown in grey. Island acronyms: Amorgos (AM), Anafi (AF), Andros (AN), Antikeros (AT), Fidoussa (FD), Glaronissi (GL), Ios (IO), Iraklia (IR), Keros (KE), Kisiri (KS), Lazaros (LZ), Makria (MK), Mando (MN), Megalo Fteno (MG), Mikro Fteno (MF), Naxos (NX), Pacheia (PC), Tinos (TN).

effects on host-parasite interactions should be most apparent in relation to changes in island size and island isolation (*i.e.*, temporal and spatial isolation) (MacArthur and Wilson 1967, Dobson et al. 1992, Galdón et al. 2006). We hypothesized that island geography and biology have significantly influenced the prevalence and parasitemia of hemogregarine parasitism of *P. erhardii* over time. Specifically, we predicted that both prevalence and parasitemia would be negatively associated with island size (km²), island age (years since isolation occured), and spatial isolation

(distance) (km) (cf. MacArthur and Wilson 1967, Hess 1996, Roca et al. 2009), but positively associated with insular host density.

Materials and Methods

Study System

The Cycladic island archipelago comprises several hundred islands and islets. Larger islands are covered by a patchwork of open habitats, ranging from sclerophyllous maquis and coastal heaths to

agricultural areas, riverine thickets, and exposed rock glades. The islands experience typical Mediterranean climate with wet, mild winters and hot, dry summers. In total, we sampled 18 Cycladic islands in this study. Islands sampled were chosen to represent a sufficiently broad range of periods of isolation (25 to 200,000 years) and sizes (0.008 to 379.95 km^2) to reflect the varying conditions in the island system (Table S1). All sampling and data collection was conducted at low elevation (< 500 m above sea level) sites dominated by phrygana or marquis shrubland.

Podarcis erhardii is native to the southern Balkans, ranging as far north as Bulgaria and west into Albania; it is found throughout mainland Greece and the Aegean islands, including the Cyclades archipelago (Valakos et al. 1999). The complex geologic history of the Cycladic islands has driven notable divergence in Podarcis morphology (Brock et al. 2015, Donihue 2016), behavior (Pérez-Mellado and Casas 1997, Deem and Hedman 2014, Donihue et al. 2015), and genetic diversity (Poulakakis et al. 2003, Hurston et al. 2009). In natural habitats, P. erhardii is commonly infected with mites (Ophionyssus sp., Acari: Trombiculidae), as well as with ticks (Haemaphysalis sp., Acari: Ixodoidea) on islands with domestic livestock (Pafilis et al. 2013). Podarcis lizards are also commonly infected with hemogregarine parasites in native populations (Harris et al. 2011, Garrido and Pérez-Mellado 2013).

Hemogregarine parasites (Apicomplexa: Adeleorina) infect red blood cells (RBCs) of their hosts and are found in a wide range of vertebrate species, including all orders of Reptilia (Telford, 2008) and several Podarcis species (Roca and Galdón 2009). Hemogregarines are vectored by a diverse group of invertebrates, including mites (Acari), fleas (Siphonaptera), and sandflies (Phlebotominae) (Smith 1996). The effects of hemogregarine infection on reptile hosts, including Podarcis lizards, are not widely understood.

Data Collection

We determined island age using bathymetric data and historic estimates of rising sea levels in the region (from the Pleistocene until present) (see Brock et al. 2015). Island area is represented as the size of islands in square kilometers. Host population density was determined as the number of P. erhardii individuals seen across a 100-meter transect (individuals/100-m) on each island at the site of sampling. Along the same transect at each study site, we estimated vegetation cover as the percent ground covered by plants in 1-squared meter quadrat. Spatial isolation of each island from the mainland was measured as the distance (in kilometers) separating the focal island to the nearest larger land mass (i.e. a potential source land mass for overwater dispersal). All field sampling was conducted in 2016.

Resident P. erhardii populations at a single representative site on each island (Fig. 1) were sampled in habitat patches at low elevation dominated by phrygana or maquis shrubland. Sites were non-adjacent to roads, towns, agriculture, or others forms of development, and were comparable across islands.

We collected between 18 and 34 adult individuals from each island, using hand-held nooses. All collections were conducted from May to July, during the main reproductive period of P. erhardii. Caught lizards were sexed and measured for body size; snout-vent length (cm) was noted as the length from snout tip to base of the tail and body mass was taken using a digital scale (grams). Blood samples were taken from each lizard via toe-clipping (see Schall 1990, Langkilde et al. 2006). Blood smears were prepared on microscope slides and dried at ambient temperatures, then fixed using methanol. Blood smears were subsequently stained using a hematoxylin and eosin stain (Fisher HealthCare™ Hema 3™ Manual Staining System). We then released each lizard at the site of capture.

Blood parasites were identified based on morphological characteristics (Fig. S1), as per Telford (2008). Across blood samples from Podarcis hosts, hemogregarines exhibited consistent morphology and thus are expected to be the same species. Based on morphology, it is suspected that the hemogregarine parasite studied here is a species of Hepatozoon (Telford 2008, Telford, pers. comm.). We infer from natural history and studies of other Podarcis lizards that the blood parasite we study here is a native parasite for this host (Harris et al. 2011). We determined presence of hemogregarines and parasitemia in infected hosts by scoring blood smears for the presence of infected mature erythrocytes. Each smear was examined until 10000 mature RBCs had been evaluated. We calculated infection prevalence as percent of infected individuals out of total sampled from each island population (per Margolis et al. 1982). Parasitemia per individual was calculated as the number of infected RBCs out of 10000 RBCs. No additional blood parasites were found during microscopy of blood samples.

Statistical Analyses

All statistical analyses were conducted in R (version 3.6.1, R Core Team 2019). We used multiple linear regressions to assess the relationship between parasite prevalence, island-level variables (island age, island area, island distance, vegetation cover, and host density), and averaged host-level variables per island population. Averaged host-level variables were calculated by taking the average of each variable per island sample, and this was applied to measures of snout-vent length and body mass. We present two of the regression models which were assessed based on model fit. Assumptions of linearity were confirmed using the 'gvlma' package in R (Pena and Slate 2006). Both models included island-level variables and host-level variables (averaged per island) (Table 1). In the first model, we included all five island-level predictors to explain the prevalence (percent infection) of parasitism per island. In the second model we removed insignificant terms from the regression to see if it improved model fit.

We used structural equation models (SEM) to test the relationship between parasitemia and island- and host-level variables. SEM combines confirmatory factor analysis with multiple regression and provides a

Table 1. Results of multiple linear regressions on prevalence of hemogregarine infection in insular *Podarcis erhardii* populations in the Cyclades (Hlavac 2018). Both models included island-level variables and host-level variables (averaged per island: average snout-vent length and average body mass). For each model, the coefficient is given and standard error is in parentheses, and the p-value range is indicated by asterisks.

|	Results of multiple regressions on prevalence of hemogregarine infection in insular *P. erhardii* populations	
	Dependent variable:	
	Prevalence (% infection)	
	(1)	**(2)**
Island Age	-0.075*	-0.075**
	(0.036)	(0.027)
Island Area	-0.050	
	(0.054)	
Host Density	0.201**	0.222***
	(0.073)	(0.059)
Island Distance	0.072	
	(0.099)	
Vegetation Cover	-0.074	
	(0.130)	
Average Snout-Vent Length	5.307**	4.234**
	(2.349)	(1.817)
Average Body Mass	-1.868**	-1.737**
	(0.695)	(0.616)
Intercept	-17.284*	-13.356*
	(8.573)	(6.549)
Observations	18	18
R²	0.717	0.690
Adjusted R²	0.519	0.594
Residual Std. Error	0.202 (df = 10)	0.185 (df = 13)
F Statistic	3.624** (df = 7; 10)	7.219*** (df = 4; 13)

Note: *$p<0.1$ **$p<0.05$ ***$p<0.01$

framework to explicitly test hypotheses on both direct and indirect causal relationships among data (Ullman 2001, Arhonditsis et al. 2006, Schreiber et al. 2006). Unlike other methods used in hypothesis testing, SEM is a robust technique for assessing relationships among data with complex interrelationships and interactions (Arhonditsis et al. 2006) making it ideal for ecological data collected at two scales that are expected to be correlated. SEM models were generated using the 'lavaan' package (Rosseel 2012). The first hypothesized SEM (Model A) is based on two latent variables that we predicted to be related to parasitism of *Podarcis* hosts: 'island' is defined by direct paths to island area (km²), time of isolation of each island population (age in years), the spatial isolation, *i.e.* distance of each island to the nearest larger land mass (km), vegetation cover (percent cover averaged on a 100-m transect) and host density (indiv/100-m) (Fig. 2). 'Host' was defined by direct paths to body mass (g), snout-vent length (cm), age class (adult or juvenile), and sex. 'Island' and 'host' latent variables were predicted to have direct connections to parasitemia (at the host-individual scale). We used modification indices to explore

additional links in the data that may not have been included in the original conceptualization of Model A. Of the relationships suggested by modification indices, four additional indirect paths were included in the second SEM (Model B) of parasitemia data, which represent interaction terms. An indirect path was created between host age class and snout-vent length, between host sex and host body mass, and between vegetation cover and both island area and host density. We also included indirect paths between island area and host density in both Model A and B, because a large amount of island research has found significant correlation between island size and population density (MacArthur et al. 1972, Wright 1980, Rooda and Dean-Bradley 2002, Keesing et al. 2006).

Among the measurements of island characteristics, some variables violated normality assumptions (even when transformed) used for maximum likelihood estimation procedures in traditional SEMs, so we used diagonally-weighted least squares estimation for these models (Mîndrilă 2010). Model fit was assessed using chi-square tests, root-mean square error of approximation, and comparative fit index.
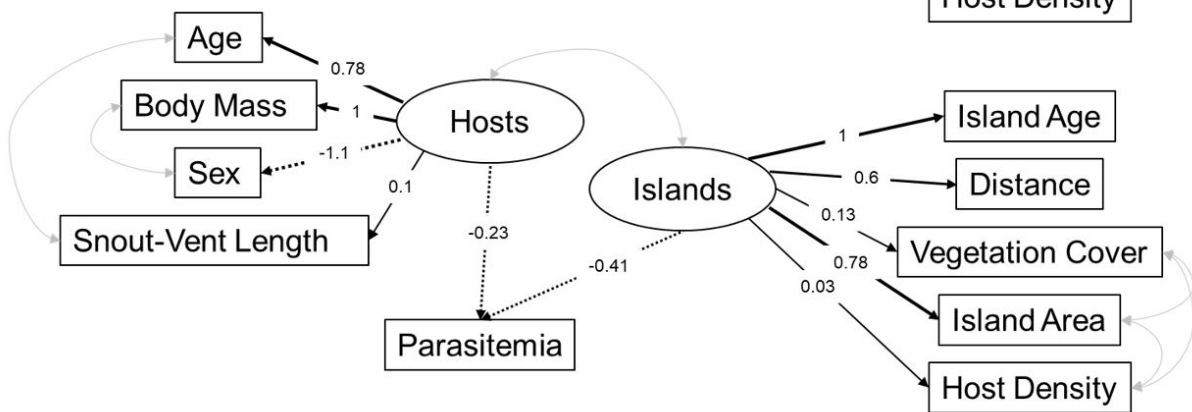
Model A
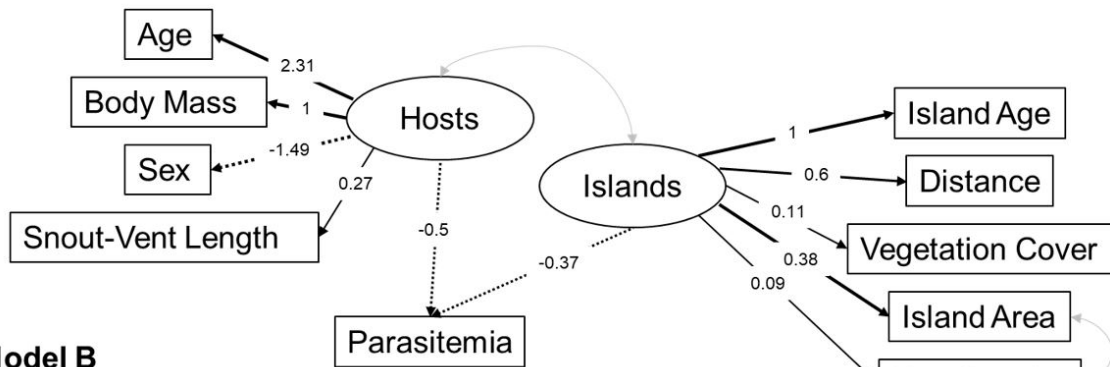
Model B

Figure 2. Structural equation model of the *Podarcis erhardii*-hemogregarine system. Goodness-of-fit metrics for the final model are: chi-square = 89.321, df = 28, RMSEA = 0.067, CFI = 0.964. Path coefficients between variables are shown next to arrows and are unstandardized partial regression coefficients. Arrow widths are proportional to the regression coefficients – note that categorical variables in SEM models are assessed using a reference level, therefore the effect of each value is relative to the reference level (Lefcheck 2021). Negative relationships are represented by dashed arrows and positive relationships by solid arrows. The hypothesized model is based on two latent variables, shown in circles, that we predicted to be related to parasitism of *Podarcis* hosts: 'island' is defined by direct paths to island area (km^2), island age (years), spatial isolation, i.e. distance of each island to the nearest larger land mass (km), vegetation cover (percent cover) and host density (indiv/100-m). 'Host' was defined by body mass (g), snout-vent length (cm), age class (adult or juvenile), and sex. 'Island' and 'host' latent variables were predicted to have direct relationship with parasitemia. Indirect paths, shown in grey arrows, were included between the latent 'island' and 'host' terms, as well as between island area and host density. Model A and B differed in that Model B also included four additional indirect paths between host age class and snout-vent length, between host sex and host body mass, and between vegetation cover and both island area and host density. Model B was determined to be the best fitting model in this system.

Fig. 2 illustrates the causal relationships that were hypothesized for this host-parasite system.

Results

Hemogregarine infections were ubiquitous across all 18 study islands, but prevalence varied widely. Infection prevalence ranged between 7% in the island population on Antikeros to 100% on the island Mando; on average, 68% of all lizards were infected (of 511 total individuals sampled: see Table S1). Overall parasitemia ranged from 0.0001 to 0.1211% infected RBCs (per 10000 RBCs). The average parasitemia for all infected *P. erhardii* hosts was 0.0044% infected RBCs; average parasitemia for *P. erhardii* populations ranged from 0.0002 to 0.027% infected RBCs.

Prevalence Among Islands

Our first model indicated that vegetation cover, distance to mainland, and island area were not significant predictors for parasite prevalence (adjusted R^2 = 0.52, p = 0.032; Table 1). Removing these terms from our regression did not dramatically improve our model fit (adjusted R^2 = 0.59, p = 0.0028; Table 1). This model indicates that prevalence is negatively correlated with island age and average host body mass, and positively correlated with host density and snout-vent length. These models also suggest that prevalence is non-significantly negatively correlated with island area and vegetation cover, and non-significantly positively correlated with distance to mainland. This suggests that islands with more dense host populations and

more recent (younger) islands tend to have higher hemogregarine prevalences.

Structural Equation Models of Parasitemia

Model A did not meet criteria for model fitting parameters (chi-square = 187.383, df = 32, RMSEA = 0.100, CFI = 0.909). We rejected this model because the root-mean square error of approximation suggests a poor fit (Browne and Cudeck 1993). The second SEM of parasitemia among individual hosts (Model B) provided a better fit to the data (chi-square = 89.321, df = 28, RMSEA = 0.067, CFI = 0.964) than did Model A. Following Browne and Cudeck (1993), the root-mean square error of approximation falls in the bounds of a 'close' fit (< 0.08). The comparative fit index 0.968 also suggests a close fit (CFI should be close to 1) for this model (Bentler, 1990), and the chi-square of Model B (89.321) is nearly half that of Model A (187.383).

Discussion

Fragmentation and isolation of landscapes have complex effects on the ecology of host-parasite interactions; they are often mediated through a variety of ecological processes that may operate at different spatial scales. The natural history of land-bridge islands reflects the natural history of insular populations and can ultimately inform their evolutionary responses to isolation (Foufopoulos and Ives 1999, Hurston et al. 2009) and thus provide a suitable model system to examine the factors driving ecological processes at multiple scales. Our results support that island geography can shape not only the ecology of insular host populations, but also the interactions between hosts and their parasites.

Our analyses show that habitat fragmentation and insularity can significantly alter prevalence and parasitemia of hemogregarine infection in *Podarcis* hosts over evolutionary time, and this effect of insularity on parasitism likely occurs through two primary ecological mechanisms. The first mechanism concerns change in host population density initiated, in part, by changing island size (*i.e.*, density compensation); the second is the effect of isolation time, which likely leads to changes in genetic variability (Whittaker and Fernández-Palacios 2007, Hidasi-Neto et al. 2018).

Specifically, the regression and SEM indicate that increasing island area reduces prevalence of hemogregarine infection, as well as parasitemia in the host *P. erhardii* in Aegean island populations, although this yielded a statistically non-significant p-value. We interpret the output as potential evidence that smaller islands harbor more infected individuals, and infected individuals harbor more parasites. Two of the smallest islands sampled, Kisiri and Mando, also had particularly high prevalence (96% and 100%, respectively) and average parasitemia (0.027% and 0.0053% infected RBCs, respectively) of all islands sampled. While this finding was not statistically significant (α = 0.05) we argue it provides enough correlational evidence to suggest that island area is

an important factor to consider in future explorations of island host-parasite communities.

We also found significant positive relationships between host density and prevalence and parasitemia. Lizard populations are significantly denser on smaller Cycladic islands, consistent with the operation of density compensation, by which islands which are smaller in area support higher density of insular species compared to mainland populations (MacArthur et al. 1972, Wright 1980, Rooda and Dean-Bradley 2002, Keesing et al. 2006). The resulting associations between hemogregarine infection and island area and host density, taken together, support that density compensation for *P. erhardii* populations effectively extended to its native blood parasite. To this end, host density is greater on smaller islands and infection is subsequently more prevalent and intense. This indicates that biogeography in the region has shaped the distribution and intensity of hemogregarine parasitism. For example, on the small island Megalo Fteno (area: 0.06 km^2, host density: 20 indiv./100-m) prevalence (96%) and average parasitemia (0.00325% infected RBCs) were markedly higher than on the larger, less dense island Antikeros (area: 1.05 km^2, host density: 1 indiv./100-m), where prevalence was only 7%, and average parasitemia was 0.0002% infected RBCs.

It is also worth noting that our SEM analyses on parasitemia indicated that our models (Model B) better fit empirical data when including interaction terms among variables (see *Results*). The complex interrelationships of these island- and host-level variables illustrate the value of robust tools like SEM for testing ecological data (Ullman 2001, Arhonditsis et al. 2006) because it allows flexibility to consider interacting ecological variables. Some of the interaction terms that were added to fit Model B represent biological realism, e.g. the well-studied relationship between an organism's age and sex with individual body size (Hill et al. 2016). More notable, however, is the interaction terms between vegetation cover and both island area and host density, as well as an interaction term between island area and host density which was included in both model A and B (Fig. 2). We interpret the interaction of these terms as evidence for potentially important trophic relationships among species in this system, and the role of resource availability on parasitism of *P. erhardii*. Hemogregarines are trophically-transmitted parasites, with invertebrates often serving as vectors (Telford 2008). Therefore, we might expect that differences in vegetation and variation in *P. erhardii* population size between islands may be drivers of heterogeneity in invertebrate (*i.e.*, potential vectors) distribution (Wu et al. 2019). Hematophagous ectoparasites were not explicitly explored in this study, but we did observe common (and occasionally heavy) occurrence of mites (*Ophionyssus* sp.) on *P. erhardii*. *Ophionyssus* sp. may be another possible vector of hemogregarines (Allison and Desser 1981, Haklová-Kočíková et al. 2014). This would be a valuable area of future research to understand the transmission

biology and biogeography of the entire life cycle of this hemogregarine parasite.

The time since isolation of each island was negatively related to hemogregarine prevalence and parasitemia, while spatial isolation (distance to mainland) was positively (though non-significantly) related to prevalence and parasitemia. Island populations that have been isolated for increasingly long periods of time have lower prevalence of hemogregarines and lower levels of parasitemia. One possibility is that reduced genetic diversity of isolated populations of hemogregarine parasites leads to impoverished parasite populations in insular hosts that are isolated for long periods (Pérez-Rodríguez et al. 2013, but see also Illera et al. 2015). However, other research on isolation of hosts and their parasites has demonstrated that host may be more susceptible to parasitism due to inbreeding over time (Acevedo-Whitehouse and Cunningham 2006, Chapman et al. 2009, Luikart et al. 2008). Genetic diversity is reduced over time following isolation of a population, and the implications of reduced genetic variability in hosts as well as in parasites has cascading impacts on host-parasite interactions (Nieberding et al. 2006, Plaisance et al. 2008, Ishtiaq et al. 2009). In cases where hosts are inbred, genetic inbreeding may lead to immunosuppression and thereby increase parasitism (Reed and Frankham 2003, Charpentier et al. 2008). This study suggests that isolation leads to lower parasitism prevalence and lower parasitemia in host populations isolated for longer periods. A possible mechanism for this pattern is a loss of parasite genetic diversity over evolutionary time, thereby reducing the ability of parasites to maintain transmission cycles. Spatial isolation, however, was positively (though not significantly) related to prevalence and parasitemia. Islands that are spatially isolated likely have less colonization events (MacArthur and Wilson 1967, Valente et al. 2020). This suggests that the causative factor for increased or decreased parasitism in this system is connected to the prevalence of opportunities for inputs (of hosts) from other land masses, rather than temporal isolation *per se* (Ishtiaq et al. 2009).

The significance of island area, host density, and island spatial and temporal isolation on the distribution and intensity of hemogregarine infections in *P. erhardii* populations demonstrate several important ecological changes that occur following isolation of insular populations. The onset of density compensation following restriction of habitat size leads to notable changes in the ecology of insular species (Blumstein and Daniel 2005, Keesing et al. 2006) and their parasites, as shown here. The influence these changes have on host-parasite interactions has not previously received much attention in disease ecology literature or in studies on the biogeography of parasites.

This study provides a model of how host-parasite interactions can change over evolutionary time when populations become isolated following fragmentation. We found that changes in amount of available habitat and density of populations can effectively change the prevalence and intensity of native infections. Our analyses also suggest that reduction in genetic diversity, a function in part of how long a population has been isolated, could be involved in reducing prevalence and parasitemia, although this remains to be formally tested. This in turn suggests that spatial fragmentation can drive changes in host-parasite dynamics over time. In particular, the size of island habitats and the density of populations on those islands may significantly alter the interactions between hosts and hemogregarine parasites through the mechanism of density compensation following isolation (MacArthur et al. 1972, Wright 1980, Keesing et al. 2006). The temporal and spatial isolation of islands also affects these interactions over evolutionary time, potentially by impacting the genetic variability of hosts and parasites, however, further exploration is this area is necessary for understanding genetic responses to geographic isolation in host-parasite island systems (Illera et al. 2015). The influence of these island characteristics on host-parasite interactions implies that parasitism can be significantly altered by the characteristics of the islands or island-like fragments. Such implications are important for when predicting how fragmentation may alter host-parasite ecology in natural systems, and by what mechanism.

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Author Contributions

JF and SM contributed to field work planning, data collection, manuscript drafting and editing, and approval of the final manuscript and associated figures and materials. JF also conducted project design, laboratory work, and data analysis.

Data Accessibility

The datasets generated and/or analyzed during the current study are available in the figshare repository at 10.6084/m9.figshare.5673421 and 10.6084/m9.figshare.5673415. The authors will provide any additional data or analyses upon reasonable request.

Supplementary Material

The following materials are available as part of the online article from
https://escholarship.org/uc/fb

Table S1. Measures of island geographic characteristics and aggregate results of host biology, parasite prevalence, and parasitemia per island population of Podarcis erhardii.

Figure S1. Image of infected mature erythrocytes from a blood smear of Podarcis erhardii.

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