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RESEARCH ARTICLE

Urbanization predicts infection risk by a protozoan parasite in non-migratory populations of monarch butterflies from the southern coastal U.S. and Hawaii

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Abstract

Context Urbanization can affect the density of hosts, altering patterns of infection risk in wildlife. Most studies examining associations between urbanization and host-parasite interactions have focused on vertebrate wildlife that carry zoonotic pathogens, and less is known about responses of other host taxa, including insects.

Objectives Here we ask whether urban development predicts infection by a protozoan, *Ophyrocystis elektroscirrha*, in three populations of monarchs (*Danaus plexippus*): migratory monarchs in northeastern U.S., non-migratory monarchs in southeastern coastal U.S., and non-migratory monarchs in Hawaii.

Methods We used impervious surface and developed land cover classes from the National Land Cover Database to derive proportional measures of urban development and an index of land cover aggregation at two spatial scales. Parasite data were from previous field sampling (Hawaii) and a citizen science project focused on monarch infection in North America.

Results Proportional measures of urban development predicted greater infection prevalence for non-migratory monarchs sampled in the southern coastal U.S. and Hawaii, but not in the northern U.S. Aggregations of low intensity development, dominated by single-family housing, predicted greater infection prevalence in monarchs from the northern and southern coastal U.S. populations, but predicted lower infection prevalence in Hawaii.

Conclusions Because natural habitats have been reduced by land-use change, plantings for monarchs in residential areas and urban gardens has become popular among the public. Mechanisms that underlie higher infection prevalence in urban landscapes remain unknown. Further monitoring and experimental studies are needed to inform strategies for habitat management to lower infection risk for monarchs.

Keywords *Danaus plexippus* · *Ophryocystis elektroscirrha* · Host–parasite interaction · Gardens · Tropical milkweed · Pollinator

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Introduction

Habitat changes associated with urbanization can influence wildlife–pathogen interactions through multiple mechanisms, including changes in host density, altered host resistance or tolerance to infection, and shifts in host community composition (Prange et al. 2003; Bradley and Altizer 2007). While numerous studies have examined how urbanization predicts pathogen changes in vertebrate wildlife (especially birds, mammals, and reptiles; Giraudeau et al. 2014; Dugarov et al. 2018; Páez et al. 2018), more attention is needed for pathogens of urban invertebrates. Understanding how urbanization modifies infection patterns could be particularly important for insect pollinators, given the evidence of pollinators' frequent use of urban habitats (including parks, gardens, and vacant lots; Baldock et al. 2015) and the extensive dependence on pollination services for agriculture and food production (Gallai et al. 2009; Potts et al. 2010). Further, pollinators are facing widespread declines (Potts et al. 2010; Goulson et al. 2015), and parasites and infectious diseases are major contributors to pollinator losses, with substantial evidence coming from populations of honeybees and bumblebees (Fürst et al. 2014). Urbanization might intensify infection risks to pollinators if they experience crowding that increases contact rates (Goulson et al. 2012; Theodorou et al. 2016), or changes in the quantity and quality of forage and reproductive resources that alter pollinator susceptibility or tolerance to infection (Alaux et al. 2010; Dolezal and Toth 2018). Pollinators could also experience restricted movements in a fragmented landscape with isolated habitat patches, remaining in locations for longer durations. This might support the build-up of infectious stages of pathogens in the environment.

Evidence of how urbanization impacts pollinators and their pathogens originates primarily from bees (Hymenoptera), with past work showing positive association with pathogen prevalence due to higher local host abundance. Urban parks, gardens, and vacant lots can offer shelter, forage and reproductive resources for pollinators (Bhattacharya et al. 2003; Harrison and Winfree 2015); however, green spaces tend to be isolated within the urban landscape (Wen et al. 2013; de la Barrera et al. 2016) and offer limited diversity of useful and attractive plants (Garbuzov et al. 2015). Fragmentation, small patch sizes, and limited resources provided by urban green spaces likely concentrate pollinators into small habitat patches and increase the risk for pathogen transmission. For example, increased infection rates by several intestinal parasites in the buff-tailed bumblebee (*Bombus terrestris*) in urban parks and gardens, as compared to rural gardens, was attributed to higher local host abundance in urban sites (Goulson et al. 2012). Similarly, urbanization increased pathogen loads and transmission in honeybees (*Apis mellifera*; Youngsteadt et al. 2015) and several bumblebee species (*Bombus* spp.; Theodorou et al. 2016) due to the sharing of flowers which can act as vectors for numerous pathogens (Singh et al. 2010; McArt et al. 2014). It remains unknown whether urbanization has similar consequences for pathogen pressure in other pollinator guilds. Because pollinator mobility may be impacted by roads and buildings, examining the spatial scale at which urban development influences infection risk is crucially needed.

Here we examine whether urbanization at two spatial scales predicts parasite infection in three populations of monarch butterflies (*Danaus plexippus*) that differ in migratory behavior and breeding phenology. Monarchs are commonly found in rural agricultural and natural landscapes, and also occur in urban and suburban environments (Oberhauser et al. 2001; Shapiro 2002). In eastern North America, monarchs migrate annually from breeding sites as far north as southern Canada to overwintering sites in central Mexico (Urquhart and Urquhart 1978). Monarchs also form non-migratory populations that breed year-round in tropical and sub-tropical locations, including Hawaii (Ackery and Vane-Wright 1984; Zalucki and Clarke 2004), and in the southeastern U.S., particularly along the gulf coast, at latitudes below approximately 32°N where a humid subtropical climate dominates and winter freezes are infrequent (Howard et al. 2010; Satterfield et al. 2015). Monarchs are commonly infected by a specialist protozoan, *Ophryocystis elektroscirrha* (hereafter, OE), which occurs in all monarch populations examined to date, and is transmitted from adults to caterpillars via spores scattered onto eggs and larval milkweed host plants (McLaughlin and Myers 1970). Infections decrease monarch lifespan, reproductive success, size, flight performance and migratory success (Altizer and Oberhauser 1999; Bradley and Altizer 2005; De Roode et al. 2006; Altizer et al. 2015) and likely

reduce monarch population sizes, although quantitative estimates of population-level impacts are lacking. Previous work comparing OE infections within and across populations showed that OE prevalence increases with both larval density and the duration of occupancy of breeding habitats (Bartel et al. 2011), and decreases with migratory distance travelled (Altizer et al. 2000, 2015). In particular, continuous use of the same habitats allows for the accumulation of parasite spores, which can persist on monarch larval host plants for extended periods of time (Satterfield et al. 2016, 2017). Both migratory and non-migratory monarch populations demonstrate temporal and spatial variation in OE infection prevalence, suggesting additional environmental factors are influencing infection risk.

Like many other pollinators, monarchs have experienced population declines coincident with habitat loss and other factors (Brower et al. 2012; Flockhart et al. 2015). As a result, the general public is interested in providing monarch habitat by planting nectar sources (i.e., flowering plant species) and larval host plants. One particularly attractive and easy to grow larval host plant sold by nurseries is an exotic species, tropical milkweed (*Asclepias curassavica*) (Satterfield et al. 2015). Unlike most native milkweeds in U.S. that enter dormancy in the fall, tropical milkweed persists, even growing year-round in mild climates (Batalden and Oberhauser 2015; Satterfield et al. 2015). High concentrations of cardenolides (secondary compounds) in tropical milkweed and several other milkweed species are attractive to ovipositing monarchs (Oyeyele and Zalucki 1990; Zalucki et al. 1990) and can reduce the virulence of OE infections, potentially allowing infected monarchs to live longer and transmit more parasites (De Roode et al. 2008; Sternberg et al. 2012). In the southern coastal U.S., recently-formed sedentary populations now commonly appear, likely in response to the year-round availability of tropical milkweed in residential areas; OE prevalence can reach high levels at these sites (Satterfield et al. 2015).

In this study, we pair citizen science and field data on parasite infection in monarchs with land cover data to ask whether the amount, type and aggregation of urban development in the landscape predicts OE infection prevalence in (i) migratory monarchs sampled in the northern U.S., and non-migratory monarchs sampled in (ii) the southern coastal U.S. and (ii) Hawaii. We expected that monarchs from sites surrounded by more urbanization and more aggregated developed areas would experience a greater risk of OE infection, as might occur if monarchs are crowded in small resources patches (primarily garden habitats). We further expected that infection risk would be highest in areas dominated by low intensity developed land cover (i.e., residential areas). Residential areas tend to contain landscape enhancements including gardens with flowering plants that attract monarchs and other butterflies. Additionally, the public has been encouraged to plant milkweed habitat for monarchs in residential areas (e.g., Monarch Watch Waystation Program; Oberhauser et al. 2008; Taylor 2018), rendering this land cover particularly important to examine in the context of OE infection. Because monarchs can move both within and between habitat patches during the breeding season (Zalucki and Kitching 1982; Miller et al. 2012; Zalucki et al. 2016), we tested our predictions at two spatial scales to explore possible relationships between infection and urbanization.

Methods

Study sites and infection data

Monarchs in the southern coastal U.S. (non-migratory winter breeders) and the northern U.S. (migratory summer breeders) were sampled for parasites during 2011–2013 as part of the ongoing citizen science program Project Monarch Health (monarchparasites.org). Sites were sampled by volunteer participants during periods of monarch breeding, which in the northern U.S. occurred during spring and summer (Jun–Sep), and in the southern U.S. occurred year-round (although southern samples were restricted to Dec–Feb to avoid sampling migratory monarchs passing through these areas in spring and early fall). The winter timing of sampling in the southern coastal U.S. coincided with the general absence of native milkweeds, such that monarchs sampled during this time predominantly used exotic tropical milkweed, which is known to sustain non-migratory winter-breeding monarchs (Satterfield et al. 2015, 2016, 2018). A total of 68 sampling locations from U.S. (Fig. 1a) were included in the analyses, with 2807 individual monarchs sampled across both years.

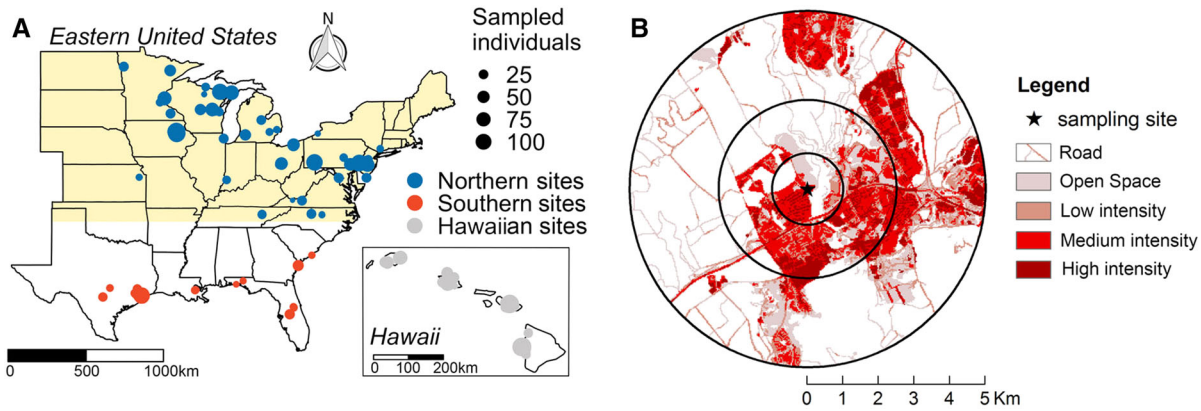

Fig. 1 **a** Sampling locations for three monarch populations examined in this study. The summer breeding range of eastern North American migratory monarchs is highlighted in yellow, and locations where migratory monarchs were sampled appear as blue circles (N = 47 sites). Winter-breeding non-migratory monarchs were sampled in southern coastal U.S. (red circles; N = 21 sites), and year-round breeding monarchs were sampled in Hawaii (gray circles; N = 17 sites). **b** Example site with four developed land cover classes surrounding a sampling location in Hawaii at two buffer sizes (1 and 2.5 km; two innermost circles). (Color figure online)

Butterflies were tested for OE infection non-destructively by pressing clear tape (approximately 1 cm^2) against adult abdomens; samples were scored for infection status at a laboratory at the University of Georgia. Samples with $>$ 100 spores were scored as infected (following Bartel et al. 2011; Satterfield et al. 2015), indicating an infection was acquired as a larva. Samples with $<$ 100 spores were scored as uninfected and include monarchs with no spores, and those with low numbers of spores that can result from passive spore transfer between adult monarchs or transfer during the capture and sampling process. The full data set for the northern and the southern coastal U.S. is provided in Supplementary materials in Satterfield et al. (2015).

Monarchs sampled in Hawaii (non-migratory year-round breeders) were captured by researchers as adults at 17 sites across four islands in 2007, 2009, 2010 (Fig. 1a). A total of 885 monarchs were scored for infection status in Hawaii using methods described above. Hawaii infection data were previously analyzed in Pierce et al. (2014), and are made available in main text and supporting information of the study. In both continental U.S. and Hawaii data sets, sites with fewer than five samples were excluded from analyses due to low probability of detecting infected individuals if fewer than five monarchs were sampled per site. Data from the three regions (northern U.S., southern coastal U.S., and Hawaii) were aggregated per site and year, and sample sizes ranged from 5 to 148 monarchs. Data from each region were analyzed separately owing to differences in monarch migratory behavior and average OE prevalence. We calculated infection prevalence as the number of infected individuals divided by the total number of individuals sampled per site or region.

Land cover data and urbanization metrics

To quantify urbanization at each sampling location, we used ArcGIS 10.2.2 (ESRI 2011) to examine land cover and impervious surface data for the mainland U.S. from the most recently available, 2011, National Land Cover Database, at a scale of 30 m $\times$ 30 m (hereafter, NLCD; Homer et al. 2015; Xian et al. 2011), and for Hawaii from the 2001 NLCD, at a scale of 30 m $\times$ 30 m (Homer et al. 2007). While a more recent NLCD (i.e., closer to the years of OE sampling) for Hawaii would be ideal for this study, the 2001 edition was the only one available for all four islands examined here. We calculated two metrics of urbanization. First, we quantified the proportion of land area with impervious surface (e.g., human-made structures through which water cannot infiltrate, such as roads, parking lots, rooftops) around each sampling location. Second, we calculated proportion of land area with four distinct developed land cover intensities around sampling sites as classified in the NLCD (Xian et al. 2011; Homer et al. 2015). The four distinct land cover classes reflect the degree to which the landscape is

dominated by residential areas and modified by urbanization (NLCD values 21–24; Fig. 1b; for detailed description of each class see Table 1 and Supplementary material). Finally, we calculated a metric termed "clumpiness index" (CLUMPY), which estimates the degree of aggregation of land cover classes around each sampling site using FRAGSTATS (McGarigal et al. 2012). Specifically, the clumpiness index measures clustering of a given land cover class relative to a spatially random distribution, ranges from − 1 (maximal disaggregation) to 1 (maximal aggregation), and is independent of the class area (Neel et al. 2004).

Because the transmission of OE infection occurs at the plant level, it is unclear at what scale urban development could influence infection risk. However, previous work suggests that monarchs are a highly mobile species, with moderate site fidelity to larval host plant (milkweed) patches, and that adults can move on the order of 12 km during the breeding season in fragmented landscapes (Zalucki 1983; Zalucki et al. 2016). For this reason, we calculated urbanization metrics within two circular buffers around each sampling site with radii of 1 and 2.5 km, capturing a circumference of 6 and 16 km, respectively (Fig. 1b). The areas encompassed by these two circular buffers around sampling sites likely capture the short-term movements of a breeding monarch. Preliminary analysis revealed that low-, medium- and high-intensity developed land covers tended to be highly correlated at the two spatial scales (see Supplementary material for correlation matrices); therefore we focused on low intensity developed land cover in our final analyses to avoid multicollinearity (Zuur et al. 2009).

Statistical analysis

We used R programming software (version 3.4.4) for statistical analyses (R Core Team 2018). To account for spatial autocorrelation in infection prevalence, latitude and longitude were included as covariates in all statistical models. We ran separate analyses for each of the three monarch populations (i.e., regions) and tested for relationships between OE prevalence and (1) proportion of impervious surface, (2) proportion of two land covers (open and low intensity developed) and (3) aggregation (clumpiness index) of land cover types. We used generalized linear mixed models (GLMM) with binomial error distribution, weights as sample size, with year and site as random factors (package lme4; Bates et al. 2017). We rescaled the explanatory variables to have a mean of 0 and a standard deviation of 1, to facilitate coefficient comparisons (Zuur et al. 2009).

Because impervious surface was correlated with the two land cover intensities, we ran separate models for impervious surface and for land covers at each scale. Similarly, because aggregation index was correlated with the proportion of land cover for one of the

Table 1 Urbanization variables along with descriptions used in the study

Variable	Description
Impervious surface	Landscape that is impervious to water and mainly consists of human-made structures, such as rooftops and pavement
Open space	The lowest intensity of urbanization, dominated by vegetation planted for recreation including lawn grasses, parks, and golf courses, with impervious surface accounting for less than 20% of total cover. NLCD class 21
Low intensity developed	A mix of single-family housing units and vegetation, with impervious surface accounting for 20–49% of total cover. NLCD class 22
Medium intensity developed	Single-family housing units, with 50–79% of the total cover consisting of impervious surface. NLCD class 23
High intensity developed	Highly urbanized areas dominated by buildings, with 80–100% of the land cover as impervious surface. NLCD class 24
Clumpiness index	Spatial clustering of land cover classes relative to a spatially random distribution; ranges from − 1 (maximal disaggregation) to 1 (maximal aggregation)

Variables are as defined in National Land Cover Data (NLCD) by Homer et al. (2007) and Xian et al. (2011), and in McGarigal et al. (2012)

regions, we ran separate models for aggregation index. Next, to test which of the two scales best explain patterns between urbanization and monarch infection, we compared the models using Akaike's information criteria corrected for small sample size (AICc). Models with the lowest AIC value were accepted as the most parsimonious model. Models within two AICc of the top model were considered to have equivalent levels of support and therefore competitive (Burnham and Anderson 2003). We used quantile comparison plots and Grubbs tests to identify outliers in infection prevalence (i.e., sites; Grubbs 1950; Zuur et al. 2010). Three outliers were identified in the northern U.S. (prevalence = 0.40, 0.49 and 0.63). Outlier removal improved quantile comparison plots, and Grubbs test detected no further outliers. We present results of statistical analyses without outliers, but include the outliers in figures of raw data (Figs. 2, 3; Zuur et al. 2010).

Results

General results

Infection prevalence differed among regions (ANOVA, $F_{2,110} = 50.67$, $p < 0.001$; Tukey's HSD, $p < 0.001$): at seasonal breeding sites the northern U.S., average infection prevalence was lowest at 0.10 ± 0.01 (mean $\pm$ SE; N = 2032 monarchs; excluding outliers). At year-round breeding sites in the southern coastal U.S., infection prevalence averaged 0.59 ± 0.06 (N = 635 monarchs). Average infection prevalence in Hawaii was intermediate at 0.34 ± 0.04 (N = 885 monarchs).

When examining infection data, Moran's I-tests of spatial autocorrelation in OE prevalence suggested significant autocorrelation within Hawaii (I = 0.174, $p = 0.026$), marginally significant autocorrelation within the southern coastal U.S. sites (I = 0.185, $p = 0.087$) and no autocorrelation in the northern U.S. (I = 0.022, $p = 0.530$). Spatial autocorrelation was not detected in residuals of the models, suggesting that including latitude and longitude in each model adequately corrected for spatial correlations.

Sites sampled for this study ranged in development from rural to highly urban. Specifically, the proportion of impervious surface surrounding sampling sites ranged from 0.01 to 0.90 (Figs. 2a, S1). Rural sites, where the proportion of impervious surface is low (< 10%) comprised approximately one-third of the data. The proportion of impervious surface was, on average, lower within the northern U.S. than Hawaii and the southern coastal U.S. at both 1 km and 2.5 km scales (ANOVA, 1 km: $F_{2,82} = 10.39$, $p < 0.001$; 2.5 km: $F_{2,82} = 12.26$, $p < 0.001$; Tukey's HSD, $p < 0.05$; Fig. S2a). The proportion of open land cover per site varied between 0 and 0.53 and was similar across regions and scales (1 km: $F_{2,82} = 0.91$, $p = 0.41$; 2.5 km: $F_{2,82} = 1.95$, $p = 0.15$; Tukey's HSD, $p > 0.05$; Fig. S2b). The proportion of low developed land cover per site varied between 0 and 0.72 (Fig. S2b) and was similar across regions at the 1 km scale ($F_{2,82} = 2.45$, $p = 0.09$; Tukey's HSD, $p > 0.05$). At the 2.5 km scale, the southern coastal U.S. and the northern U.S. had similar proportions of

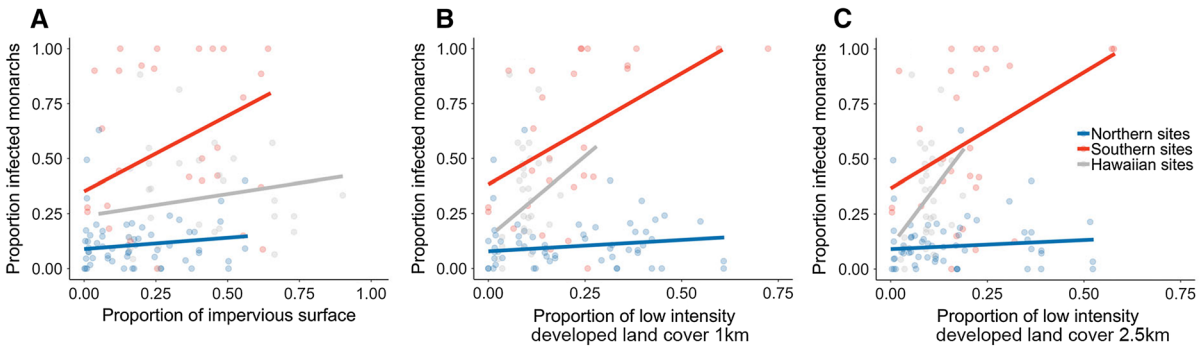

Fig. 2 Proportion of infected monarchs at each sampling location for Hawaii (gray), northern U.S. (blue) and southern coastal U.S. (red) in relation to the proportion of **a** impervious surface at 1 km scale, and of low intensity developed land cover (NLCD class 22) at **b** 1 km scale, and at **c** 2.5 km scale. Data points represent individual sites and lines show predictions of the best-supported GLM models. (Color figure online)

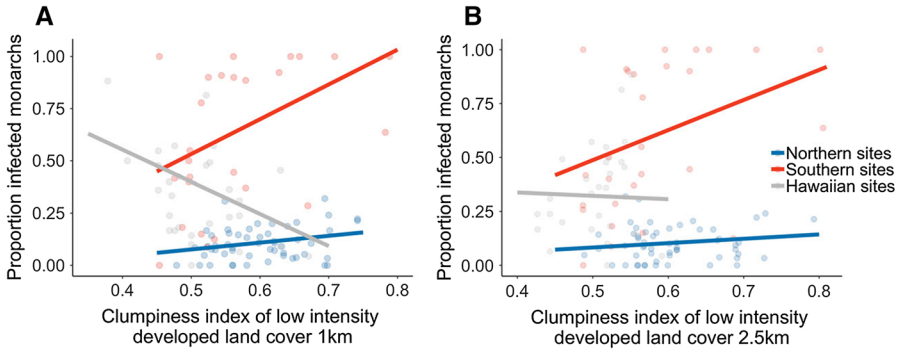

Fig. 3 Proportion of infected monarchs at each sampling location for Hawaii (gray), northern U.S. (blue) and southern coastal U.S. (red) in relation to clumpiness index (as defined in Table 1) of low intensity developed land cover (NLCD class 22) at **a** 1 km scale, and at **b** 2.5 km scale. Data points represent individual sites and lines show predictions of the results of the GLM models. (Color figure online)

low intensity land cover, which was, on average, higher than the proportion of low intensity land cover in Hawaii ($F_{2,82} = 4.82$, $p = 0.011$; Tukey's HSD, $p < 0.05$; Fig. S2b). Aggregation indices of open land cover per site varied between 0.14 and 0.80 and were lower in Hawaii than in the northern and southern coastal U.S. (1 km: $F_{2,76} = 6.55$, $p < 0.01$; 2.5 km: $F_{2,76} = 10.55$, $p < 0.001$; Tukey's HSD, $p < 0.05$; Fig. S2c). Aggregation of low developed land cover per site varied between 0.38 and 0.81 across the two scales (Fig. S2c), with Hawaii showing the lowest average values at both scales scale (1 km: $F_{2,76} = 7.03$, $p < 0.01$; 2.5 km: $F_{2,76} = 6.76$, $p < 0.01$; Tukey's HSD, $p < 0.05$).

Associations between infection and urban land use

Analyses for all three regions and at both spatial scales showed positive relationships between OE prevalence and the proportion of impervious surface, although this pattern was significant only for the southern coastal U.S. (at 1 km scale; Table 2). The most parsimonious model (i.e., lowest AIC) showed that within the southern U.S., infection prevalence was best predicted by the proportion of impervious surface at the 1 km scale, and this positive relationship was moderately strong ($z = 2.26$, $p = 0.02$; Table 2; Fig. 2a). For the northern U.S., the most parsimonious model indicated that infection prevalence was again best predicted by impervious surface at the 1 km scale, although the relationship was not significant ($p > 0.05$; Table 2; Fig. 2a). For Hawaii, the most parsimonious model showed a non-significant but positive relationship at the 2.5 km scale between infection prevalence and the proportion of impervious surface (Table 2).

Table 2 Summary of GLMMs for predicting monarch infection prevalence based on impervious surface land cover in the northern U.S., southern coastal U.S., and Hawaii at two spatial scales

Region	Proportion impervious surface			AIC	Weight
	Estimate (SE)	Z	*P*		
Hawaii					
1 km	0.45 (0.47)	0.96	0.34	189.1	0.49
2.5 km	0.49 (0.48)	1.02	0.31	189	0.51
Northern U.S.					
1 km	0.31 (0.24)	1.29	0.20	249.1	0.67
2.5 km	0.11 (0.27)	0.39	0.69	250.5	0.33
Southern coastal U.S.					
1 km	**1.89 (0.84)**	**2.26**	**0.02**	200.2	0.75
2.5 km	1.51 (0.89)	1.70	0.09	202.4	0.25

Significant terms are presented in bold, $p < 0.05$. Full model outputs are available in Supplementary material (Tables S4–S6)

Analyses of OE prevalence and land cover classes (open and low intensity, NLCD classes 21 and 22 respectively) showed a significant positive influence of the proportion of low intensity land cover in the southern U.S. and Hawaii. The proportion of open land cover had no association with infection prevalence in any region ($p > 0.05$; Table 3). Higher OE prevalence was associated with more low intensity development in the southern coastal U.S. at both scales (1 km:

Table 3 Summary of GLMMs for predicting monarch infection prevalence in the northern U.S., southern coastal U.S., and Hawaii at two spatial scales

Significant terms are presented in bold, $p < 0.05$. Full model outputs are available in Supplementary material (Tables S7–S9)

Region	Proportion open			Proportion low intensity			AIC	Weight
	Estimate (SE)	Z	*P*	Estimate (SE)	Z	*P*		
Hawaii								
1 km	0.37 (0.45)	0.83	0.41	0.81 (0.44)	1.82	0.07	188.3	0.32
2.5 km	0.20 (0.44)	0.46	0.65	**0.87 (0.40)**	**2.20**	**0.03**	186.7	0.68
Northern U.S.								
1 km	− 0.10 (0.23)	− 0.42	0.68	0.38 (0.23)	1.65	0.10	250.2	0.70
2.5 km	− 0.17 (0.30)	− 0.57	0.57	0.24 (0.28)	0.86	0.39	251.9	0.30
Southern coastal U.S.								
1 km	− 0.98 (0.55)	− 1.79	0.07	**2.72 (0.68)**	**4.02**	**< 0.001**	187.3	1
2.5 km	− 1.13 (0.72)	− 1.60	0.12	**1.76 (0.76)**	**2.32**	**0.02**	198	0

z = 4.02, $p < 0.001$; 2.5 km: z = 2.32, $p = 0.02$) and in Hawaii at the 2.5 km scale (z = 2.20, $p = 0.03$; Table 3; Fig. 2b, c). The relationship was not significant for the northern U.S. ($p = 0.1$, Table 3). Model selection indicated that OE is best predicted by land cover classes at the 1 km scale in the northern and the southern U.S., and at the 2.5 km scale in Hawaii (Table 3; Fig. 2b, c).

Analyses of land cover aggregation indices showed that OE prevalence increased with aggregation of low intensity development in the southern coastal U.S. at both spatial scales (1 km: z = 2.53, $p = 0.01$; 2.5 km: z = 2.19, $p = 0.03$) and in the northern US at the 1 km scale only (z = 2.03, $p = 0.04$; Table 4; Fig. 3a). In Hawaii, aggregation of low intensity developed land cover at the 1 km scale predicted lower OE prevalence, although the relationship was marginally significant (z = − 1.92, $p = 0.06$; Fig. 3a). Model selection indicated that OE is best predicted by aggregation of land cover classes at the1 km scale. Full model outputs are reported in Supplementary material (Tables S4–12).

Discussion

This study provides the first evidence that urbanization predicts higher prevalence of the protozoan *O. elektroscirrha* in non-migratory monarch butterflies inhabiting the southern coastal U.S., and adds to the growing body of literature suggesting that pollinators can experience increased pathogen pressure in urban environments (e.g., honeybees and bumblebees; Goulson et al. 2012; Youngsteadt et al. 2015; Theodorou

Table 4 Summary of GLMMs for aggregation (clumpiness index) of developed land covers describing monarch infection prevalence in northern U.S., southern U.S., and Hawaii at two spatial scales

Region	Aggregation of open			Aggregation of low intensity			AIC	Weight
	Estimate (SE)	Z	*P*	Estimate (SE)	Z	*P*		
Hawaii								
1 km	0.52 (0.42)	1.23	0.22	− 1.01 (0.53)	− 1.92	0.06	187.3	0.89
2.5 km	0.35 (0.54)	0.66	0.51	− 0.07 (0.5)	− 0.14	0.89	191.4	0.11
Northern U.S.								
1 km	− 0.10 (0.30)	− 0.34	0.73	**0.45 (0.22)**	**2.03**	**0.04**	242.2	0.99
2.5 km	− 0.09 (0.24)	− 0.37	0.71	0.27 (0.24)	1.16	0.25	251.3	0.01
Southern U.S.								
1 km	− 0.91 (0.71)	− 1.28	0.20	**2.25 (0.89)**	**2.53**	**0.01**	180.6	1
2.5 km	− 0.28 (0.67)	− 0.42	0.67	**1.55 (0.71)**	**2.19**	**0.03**	201.5	0

Significant terms are presented in bold, $p < 0.05$. Full model outputs are available in Supplementary material (Tables S10–S12)

et al. 2016; Cohen et al. 2017; McArt et al. 2017). The positive association between urbanization metrics and parasitism in non-migratory monarchs could be due to two related factors. First, monarchs could aggregate in garden habitats in residential landscapes. Naturally occurring larval host plants, which are necessary for reproduction and larval development, tend to be scarce during the winter months (when sampling was conducted for this study), because native milkweed plants enter dormancy during the fall. During winter months, larval host plants in the southern U.S. might be more commonly associated with garden habitats that are relatively isolated in the landscape. Thus, non-migratory monarchs breeding during cooler winter months (rather than migrating to Mexico to overwinter) might be forced to concentrate in available habitats, resulting in crowding of adult and immature stages. Indeed, densities of eggs at southern U.S. sites can reach an average of 1.49 eggs/plant, which is at least 30 times higher than egg densities in the northern U.S. (Nail et al. 2015; Majewska et al. 2018). Such immature stage crowding could increase the probability of acquiring OE infection (Lindsey et al. 2009; Bartel et al. 2011) leading to the pattern we observed here with higher infection prevalence in more urbanized habitats (with isolated patches of larval host plants).

Second, the presence of an exotic larval host plant, tropical milkweed (*Asclepias curassavica*) at most of the winter sampling locations in the southern U.S. could contribute, in part, to the relationship between higher infection prevalence and urbanization metrics. Tropical milkweed is associated with human-dominated landscapes because it primarily exists where it is planted in urban parks and gardens. Tropical milkweed may exacerbate host densities because it is highly attractive to monarch butterflies (Majewska et al. 2018), likely for its high concentration and diversity of cardenolide secondary compounds, which provide monarchs with some protection from natural enemies (Brower et al. 1968; Lefèvre et al. 2010). Monarchs that feed on high-cardenolide milkweeds as larvae emerge with lower spore loads and live longer as adults, which might explain why adult female monarchs infected with OE preferentially lay eggs on tropical milkweed versus other milkweeds (an example of trans-generational medication; Lefèvre et al. 2010). Cardenolides from tropical milkweed, however, do not cure monarchs of infection, and could increase opportunities for parasite transmission by lengthening infected individuals' lifespans (De Roode et al. 2008; Lefèvre et al. 2010). As an additional factor, prolonged breeding phenology of monarchs in tropical milkweed patches in the southern U.S. allows the long-lived OE spores to accumulate on milkweeds and cause high infection rates (Satterfield et al. 2015, 2016). Altogether, the attractiveness of tropical milkweed to monarchs and its association with parasite accumulation could intensify the positive relationship between infection and urbanization in non-migratory monarchs.

Relationships between infection and urbanization showed different patterns in Hawaii, where monarchs breed year-round. At Hawaiian sites, OE infection increased with the proportion of low intensity developed land cover, but decreased with an aggregation index of low intensity developed land cover. It is important to note that the absence of a relationship between infection and the proportion of impervious surface in Hawaii might be due to the high occurrence of lava surfaces in Hawaii. Thus, impervious surface in Hawaii might represent a less accurate measure of urbanization than developed land cover classes (Homer et al. 2015).

Reasons why OE infection prevalence in Hawaii tended to increase with the proportion of low intensity developed land cover but decrease with aggregation of low intensity land cover are not straightforward. Past work showed that infection prevalence of OE in Hawaii varies widely among sites and islands, ranging from 0 to nearly 100%, and this variation is not predicted by host population genetic structure (Pierce et al. 2014). Monarchs themselves are also not native to Hawaii, and colonized the islands in mid-1800s following the introduction of their larval host plants (Zalucki and Clarke 2004). In addition to tropical milkweed, exotic giant milkweed (*Calotropis gigantea* and *C. procera*) and exotic balloon-plant (*Gomphocarpus physocarpus*) are currently present in Hawaii (Motooka et al. 2003). Each of these species has high toxicity, produces foliage year-round, and is associated with human habitation in Hawaii. Particularly, because the larval host plant, giant milkweed, is commonly found in residential areas of Hawaii and associated managed landscapes (R. Harrison, pers. obs.), monarch densities are likely highest near residential areas, relative to rural sites. On the other hand, if developed areas of the island are well-

connected (greater aggregation index), monarchs might be able to disperse further and spread more evenly throughout the islands, thereby experiencing lower infection risk.

Interestingly, OE infection in Hawaii was best predicted by the proportion of developed land cover at the 2.5 km scale (although the 1 km scale model had equivalent support; Table 2), and by aggregation at the 1 km scale. One plausible explanation for this finding is that different mechanisms are operating at different spatial scales. Multiple non-native milkweed species that are connected in the landscape might dilute the risk of infection on smaller scales, although more work is needed to elucidate the distribution and connectivity of different host plant species in Hawaii. Another possibility is that frequent colonization and local extinction of monarch populations in patches of larval host plants generates spatial variation in infection prevalence (McCallum and Dobson 2002). Future work examining the patch dynamics of Hawaii monarch sampling sites in the context of metapopulation models could offer insights into mechanisms affecting among-site variation in infection prevalence.

In contrast to areas with non-migratory monarchs, we generally did not find significant relationships between OE infection and urbanization metrics for migratory monarchs in the northern U.S., although aggregation of low intensity development was positively related to infection prevalence. Average OE prevalence tended to be low (0.10) in the northern U.S., which might limit the power of our analyses to detect predictors of prevalence variation. Moreover, the high abundance and diversity of wild native milkweed plants (Pleasants 2017; Pocius et al. 2018) that are commonly found in disturbed habitats, such as roadsides and agricultural areas, might distribute monarchs more evenly across the landscape mosaic, away from urbanized areas.

In conclusion, we found positive associations between urbanization metrics and parasite prevalence in non-migratory monarch populations, where exotic larval host plants commonly occur in proximity to human-inhabited areas. Our results agree with recent work on honeybees and bumblebees (Goulson et al. 2012; Youngsteadt et al. 2015; Theodorou et al. 2016; Cohen et al. 2017; McArt et al. 2017) as well as numerous vertebrate studies (reviewed in Bradley and Altizer 2007), showing that wildlife can experience higher pathogen pressure in urban landscapes. Our study indicates that certain types of urbanized landscapes, particularly residential development, might favor parasite transmission among monarchs in some regions. Further, the results suggest that urban development at the smaller 1 km scale, compared to the 2.5 km scale, better predicts infection risk in the monarch-OE system.

Our study underscores the value of large-scale milkweed restoration outside of urban areas for lowering infection risk, and emphasizes the importance of monitoring OE infection across the rural–urban gradient. We show that infection prevalence is not universally high at developed sites, and continued involvement of volunteer participants in monitoring will be important in examining practices that could influence variation in prevalence within sites. Detailed studies that examine both rural and urban milkweed patches will be key to providing recommendations for how urban sites can be managed to limit infection risk. For example, it is possible that recommendations could be made regarding the patch size and distribution of milkweed plants at a site to limit aggregation. Given that urban development is projected to increase globally (Seto et al. 2011), we might expect that monarch OE incidence will increase in regions altered by expanding human development and the planting of exotic larval host plant species. Understanding the consequences of parasitism for monarch populations, and strategies for lowering infection risk, may be particularly important in the mainland U.S., given recent declines in migratory monarch numbers in both eastern and western North America (Schultz et al. 2017; Thogmartin et al. 2017).

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