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POPULATION PHYSIOLOGY, DEMOGRAPHY, AND GENETICS OF SIDE-  
BLOTCHED LIZARDS (*UTA STANSBURIANA*) RESIDING IN URBAN AND  
NATURAL ENVIRONMENTS

by

Spencer B. Hudson

A dissertation submitted in partial fulfillment  
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Biology and Ecology

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Logan, Utah

2023

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## ABSTRACT

Population physiology, demography, and genetics of side-blotched lizards (*Uta stansburiana*) residing in urban and natural environments

by

Spencer B. Hudson, Doctor of Philosophy

Utah State University, 2023

Major Professor: Dr. Susannah French  
Department: Biology

Urbanization is a major disturbance threatening habitats worldwide, yet there is limited information on how reptile populations respond to rapidly changing landscapes. The novel selective pressures of habitat alteration pose risks for individual health, and in turn, population vital rates. To persist in urban habitats, reptiles may undergo phenotypic shifts in physiological self-maintenance, yielding the potential for evolutionary change. For this dissertation, urban and natural populations of side-blotched lizards (*Uta stansburiana*) were sampled over six years to (i) compare differences in survival rates, (ii) identify physiological traits contributing to survival, (iii) determine the genetic basis of these traits, and (iv) assess their susceptibility to evolutionary change. By

incorporating physiological metrics (metabolic hormone levels, oxidative stress, and immunocompetence) into Capture-Mark-Recapture population models, lizard survival was demonstrated to be lower in urban environments and directly related to immune variation across the landscape. Fitting polygenic Bayesian mapping models to population genomic data yielded low to moderate trait heritability for each trait, which were associated with few measurable effect genetic loci. Population-level genetic comparisons revealed urban patches to be differentiated from those residing in natural areas, though this was owed to isolation by distance. In addition, shared genetic variation and comparable levels of molecular diversity were present across populations. Using an approximate Bayesian computation method on genomic time-series data, differential selection on the traits and their associated genetic loci was not detected, whereas genetic drift was evident across the landscape. Altogether, these findings shed light on the interconnectedness of population demography, physiology, and genetics for reptiles within an urban-natural landscape.

(215 pages)

## PUBLIC ABSTRACT

Population physiology, demography, and genetics of side-blotched lizards (*Uta stansburiana*) residing in natural and urban habitats

Spencer B. Hudson

Wildlife populations across the globe are poised to lose their natural habitat to urbanization, yet there is limited information on how different species handle living in cities. Animals in urban environments are often susceptible to novel stressors, which can threaten their individual health and population viability. The physiological characteristics of animals, such as those related to metabolic hormones, oxidative stress, and immunity, are expected to be important for survival in this context. If so, animals persisting in urban areas may demonstrate physiological differences from their natural counterparts, perhaps due to evolutionary change. These potential outcomes have been documented in birds and mammals, but other taxonomic groups such as reptiles have been studied far less. For this dissertation, lizards were sampled in urban and natural areas for six years to (i) compare annual population survival, (ii) identify physiological traits important for survival, (iii) map the genetic basis of these traits, and (iv) test if and how the physiological traits are evolving in urban environments. Lizard survival was lower in urban environments and related to differences in immunity. Each physiological trait had a low to moderate heritable basis linked to few genetic loci with measurable effects. Population-level genetic comparisons revealed lizards in urban areas to be differentiated from those residing in natural areas, though shared genetic variation was present among populations

along with comparable levels of genetic diversity. Differential selective pressures on the traits and their associated genetic loci were not detected, but indicators of genetic drift were evident across the landscape. Altogether, these findings shed light on the interconnectedness of population demography, physiology, and genetics for reptiles residing in urban environments.

For my late mother, Cynthia Logan



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## CONTENTS

	Page
ABSTRACT.....	ii
PUBLIC ABSTRACT.....	iv
ACKNOWLEDGMENTS.....	vii
LIST OF TABLES.....	xi
LIST OF FIGURES.....	xiii
CHAPTER	
I. INTRODUCTION.....	1
II. POPULATION VITAL RATES OF LIZARDS IN URBAN AND NATURAL HABITATS: LINKS BETWEEN INDIVIDUAL PHYSIOLOGY AND SURVIVAL.....	18
Introduction.....	19
Methods.....	22
Results.....	33
Discussion.....	44
Literature Cited.....	50
III. GENOME-WIDE ASSOCIATION MAPPING OF PHYSIOLOGICAL TRAITS IN WILD LIZARD POPULATIONS.....	64
Introduction.....	65
Methods.....	69
Results.....	81
Discussion.....	102
Literature Cited.....	109

IV.	TIME-SERIES GENOMICS OF WILD LIZARDS IN AN URBAN-NATURAL LANDSCAPE: EVOLUTIONARY IMPLICATIONS FOR PHYSIOLOGICAL TRAITS LINKED TO SURVIVAL.....	134
	Introduction.....	135
	Methods.....	138
	Results.....	151
	Discussion.....	165
	Literature Cited.....	172
V.	SUMMARY & CONCLUSIONS .....	193
	CURRICULUM VITAE.....	195

## LIST OF TABLES

Table	Page	
2.1	Summary of years and number of adult side-blotched lizards sampled at each site within and around St. George Utah. Urban site 2 was demolished for commercial development in late 2015, and as a result, no sampling was conducted there in 2016 and 2017.....	23
2.2	Model assessment and comparison results for the patterns of variation in vital rates associated with habitat type (urban, natural), individual heterogeneity (physiological covariate), and temporal variability (year) of adult side-blotched lizards. The top model is indicated in bold. Predictive ability for each model is based on the Widely Applicable Information Criterion (WAIC), whereby models with a lower score are more supported.....	31
2.3	Summary statistics for the posterior distributions from the top model of demographic parameters based on data collected from adult side-blotched lizards in St. George, UT, USA, 2012–2017. The 95% credible intervals are defined by the 2.5 and 97.5 percentiles.....	33
3.1	Site information and population by year sample sizes for genome-wide association analysis. Urban and natural sites are respectively abbreviated as ‘U’ and ‘N’, followed by their numeric identifier. Urban site 2 was demolished for commercial development in late 2015, and as a result, no sampling was conducted there in 2016 and 2017. Samples were processed in two batches (1 versus 2).....	76
3.2	REML estimates for each trait of the proportion of phenotypic variation found among <i>Uta stansburiana</i> populations (“Prop. var.”). Test statistics (LR = likelihood ratios) and p-values from the null hypothesis test of no population effect are reported.....	82
3.3	Summary of genetic architectures for physiological traits (bactericidal ability, antioxidant capacity, corticosterone) based on the posterior probability distributions from fitting Bayesian sparse linear mixed models. Parameters shown include the percent of phenotypic variation explained by genetic effects (PVE), the percent of PVE attributable to loci with measurable effects (PGE), and the number of loci with measurable effects ( $N_{\text{SNP}}$ ) across populations of <i>Uta stansburiana</i> . Posterior distributions are summarized as median estimates with 95% credible intervals (CrI).....	86

- 3.4 Summary of genetic architectures for physiological traits (bactericidal ability, antioxidant capacity, corticosterone) based on the posterior probability distributions from fitting Bayesian sparse linear mixed models. Parameters shown include the percent of phenotypic variation explained by genetic effects (PVE), the percent of PVE attributable to loci with measurable effects (PGE), and the number of loci with measurable effects ( $N_{\text{SNP}}$ ) within populations of *Uta stansburiana*. Posterior distributions are summarized as median estimates with 95% credible intervals (CRI).....88
- 3.5 Association mapping of SNPs with the ten largest model-average point estimates for each physiological trait in *Uta stansburiana*.....89
- 3.6 Comparison of phenotypic and genetic covariance and correlation matrixes. Included are Random Skewer correlations between mean standardized G- and P-covariance matrixes and PCA similarities between G- and P-correlation matrixes for all physiological traits.....98

## LIST OF FIGURES

Figure	Page
2.1	Approximate locations of urban and natural populations of side-blotched lizards in Washington County, Utah, USA (highlighted orange in the state map). Urban and natural sites are respectively abbreviated as ‘U’ and ‘N’, followed by their numeric identifier.....24
2.2	Average apparent annual survival estimates ( $\Phi$ ) for adult side-blotched lizards from each habitat type. Points represent posterior means with lines spanning 95% credible intervals.....34
2.3	Annual apparent survival estimates ( $\Phi$ ) for adult side-blotched lizards from each habitat type for 2012–2017. Points represent posterior means with lines spanning 95% credible intervals. Solid points indicate urban habitats while hollow points are natural habitats.....35
2.4	Predicted trend in apparent survival ( $\Phi$ ) as a function of bactericidal ability (%) for adult side-blotched lizards across habitat types.....36
2.5	Estimates of annual population size ( $N$ ) for adult side-blotched lizards at each site for 2012–2017. Points represent posterior means with lines at each point spanning 95% credible intervals.....38
2.6	Estimates of population growth rate ( $\lambda$ ) for adult side-blotched lizards at each site for 2012–2016. Points represent posterior means with lines at each point spanning 95% credible intervals.....39
2.7	Estimates of population densities ( $N/ha$ ) for adult side-blotched lizards at each site for 2012–2017. Points represent posterior means with lines at each point spanning 95% credible intervals.....40
2.8	Estimates of local per-capita recruitment for adult side-blotched lizards at each site for 2013–2017. Points represent posterior means with lines at each point spanning 95% credible intervals.....41
2.9	Size estimates for the number of adult side-blotched lizards ever alive at each site during the 2012-2017 study (superpopulation size, $N_s$ ) using restricted occupancy parameterizations. Points represent posterior means with lines at each point spanning 95% credible intervals. Urban and natural sites are respectively abbreviated as ‘U’ and ‘N’, followed by their numeric identifier.....42

- 3.1 Statistical summary of physiological traits in *Uta stansburiana* from a PCA of the phenotypic correlation matrix. Colors denote population while small points represent individual lizards. Dashed ellipses indicate 90% data ellipses generated using the ‘car’ package (v. 2.1.6, Fox & Weisberg, 2011), wherein large dots and solid Ellipses denote mean PC scores (dots) and 95% credible ellipses on those means from a Bayesian fit to a bivariate normal distribution. The bivariate normal model was fit using ‘rjags’ (v. 4.6; Plummer, 2016), with uninformative priors on the means (normal with  $\mu = 0$  and  $\tau = 1e-3$ ) and covariance matrix (inverse Wishart with  $k = 2$  and  $\Sigma = I2$ ). The names ‘Leeds’, ‘Browse’ and ‘Big Hollow’ are synonymous with urban sites 1-3 while the names ‘Bluff’, ‘Dinosaur’, and ‘Manowar’ are synonymous with natural sites 1-3.....83
- 3.2 Violin plots for the posterior distributions taken from Bayesian sparse linear mixed models of physiological traits: bacterial killing ability (BKA), antioxidant capacity (OXY), and corticosterone (CORT). Parameters shown include (A) the percent of phenotypic variation explained by SNPs included in the model (PVE), (B) the percent of PVE attributable to loci with measurable effects (PGE), and (C) the number of SNPs underlying the phenotypic trait ( $N_{SNP}$ ). Horizontal black lines display the quartiles.....87
- 3.3 Estimated effects of each SNP on physiological traits including (a) bacterial killing ability (BKA), (b) antioxidant capacity (OXY), and (c) and corticosterone (CORT). Points denote absolute values of model-averaged effect estimates, that is estimates weighted by the posterior probability of a non-zero effect. In each panel, the effects of the 10 SNPs with the largest estimates are shown in green.....90
- 3.4 Manhattan plots showing posterior inclusions probabilities (PIPs) from Bayesian sparse linear mixed models relating SNPs to physiological traits including: (a) bacterial killing ability or BKA, (b) antioxidant capacity or OXY, and (c) corticosterone or CORT. Each point denotes a SNP, and top ten SNPs are colored green.....91
- 3.5 Scatterplots depicting associations of posterior inclusion probabilities (PIPs) and estimated SNP effects on physiological traits including: (a) bactericidal ability (Pearson  $r = 0.754$ , 95% CI = 0.742 - 0.766,  $p < 0.0001$ ), (b) antioxidant capacity (Pearson  $r = 0.884$ , 95% CI = 0.878 – 0.890,  $p < 0.0001$ ), and (c) corticosterone (Pearson  $r = 0.926$ , 95% CI = 0.922 – 0.929,  $p < 0.0001$ ). Points denote absolute values for model-averaged effect estimates, or estimates weighted by the posterior probability of a non-zero effect, and their associated PIPs. In each panel, the ten largest estimates are shown in green.....92



- 3.6 Scatterplots depicting associations between estimated SNP effects on physiological traits including: (a) bacterial killing ability (BKA) and antioxidant capacity (OXY) (Pearson  $r = 0.199$ , 95% CI = 0.179 - 0.219,  $p < 0.0001$ ), (b) antioxidant capacity (OXY) and corticosterone (CORT) (Pearson  $r = 0.132$ , 95% CI = 0.132 – 0.153,  $p < 0.0001$ ), and (c) corticosterone (CORT) and bacterial killing ability (BKA) (Pearson  $r = 0.295$ , 95% CI = 0.276 – 0.314,  $p < 0.0001$ ). Points denote signed, model-averaged effect estimates, or estimates weighted by the posterior probability of a non-zero effect. In each panel, the effects of the 10 SNPs with the largest estimates are shown in green. Dashed lines in each panel denote no effect.....94
- 3.7 Heat map of pairwise linkage disequilibrium (measured by  $r^2$ ) for the top 10 SNPs with the highest model-averaged point estimates for bactericidal ability (BKA), antioxidant capacity (OXY), and corticosterone (CORT) in *Uta stansburiana*. Included are calculations from inter- and intra-chromosomal positions. Top SNPs are highlighted red for BKA, black for OXY, and green for CORT.....95
- 3.8 Linkage disequilibrium (LD) decay as a function of physical distance across the *Uta stansburiana* genome. The orange line denotes the 95% quantile of LD, and the green line denotes the mean LD ( $r^2 = 0.089$ ).....96
- 3.9 Heat map summarizing the similarity of mean-standardized G-matrixes for the physiological traits. Results are shown for each of the six populations (U1, U2, U3, N1, N2, N3). G-matrix similarity was measured based on random skewer (RS) correlations, which capture similarities in the response to many (in this case 10,000) random directional selection gradients. The dendrograms cluster groups by G-matrix similarity and were computed with the heatmap.2 function in R.....99
- 3.10 Heat maps of phenotypic (left) and genetic (right) correlation matrices for bactericidal ability, antioxidant capacity, and corticosterone levels across local populations of *Uta stansburiana*.....100
- 3.11 Heat maps of phenotypic (left) and genetic (right) correlation matrices for bactericidal ability (BKA), antioxidant capacity (OXY), and corticosterone (CORT) within local population of *Uta stansburiana*.....101
- 4.1 Visual representation of genetic variation based on a principal component analysis of estimated genotypes. The colored symbols denote individuals with colors designating different sites and symbols indicating the sampling year. Urban and natural sites are respectively abbreviated as ‘U’ and ‘N’, followed by their numeric identifier.....149

4.2	Heatmap of pairwise $F_{ST}$ values between urban and natural <i>Uta stansburiana</i> populations. Urban and natural populations are respectively abbreviated as ‘U’ and ‘N’, followed by their numeric identifier.....	150
4.3	Relationship between genetic distance (pairwise $F_{ST}$ ) and inter-site geographic distance (km) for populations of <i>Uta stansburiana</i> (Mantel $r$ -value = 0.4411, $p$ = 0.003).....	151
4.4	Within-population $F_{ST}$ values across generations of <i>Uta stansburiana</i> populations. Points represent means with lines spanning 95% quantiles. Solid points indicate urban habitats while hollow points are natural habitats....	153
4.5	Average allele frequency changes between short time lags (i.e., contiguous generations) and the longest time lags (i.e., 4-5 generations) for urban and natural populations of <i>Uta stansburiana</i> . Points represent means with lines spanning 95% quantiles. Urban and natural populations are respectively abbreviated as ‘U’ and ‘N’, followed by their numeric identifier.....	155
4.6	Overview of contemporary variance effective population sizes and nucleotide diversity levels ( $\pi$ ). Dot plots in panel (A) give Bayesian estimates of variance effective population size based on change from 2012 to 2017 (estimates for urban site 2 are based on change from 2012 to 2015). Points denote the median of the posterior and horizontal lines give the 95% credible intervals. Similarly, dot plots in panel (B) report the median and 95% CRIs for estimates of molecular diversity. Scatterplot in panel (C) depicts the lack of relationship between variance effective population size and population size ( $r^2 = 0.18$ , $P = 0.219$ ) while the scatterplot in panel (D) depicts the lack of relationship between variance effective population size and nucleotide diversity ( $r^2 = -0.03$ , $P = 0.402$ ).....	157
4.7	Probability distributions of the average selection differential (parameter $a$ ) for bacterial killing ability (BKA), antioxidant capacity (OXY), and corticosterone (CORT). Prior distributions are shown in gray and the posterior distributions are in black.....	159
4.8	Probability distributions of how environmental state (urban versus natural) affects the selection for individual loci linked to bacterial killing ability (BKA), antioxidant capacity (OXY), and corticosterone (CORT). Prior distributions are shown in gray and the posterior distributions are in black.....	160
4.9	Observed change in mean polygenic scores calculated from model-averaged effect estimates. Dots denote point estimates, and the shaded gray regions denote 90% credible intervals.....	161

## CHAPTER I

### INTRODUCTION

#### *Landscape effects of urbanization*

Human population growth drives urban development and expansion, causing rapid transformation of natural wildlife habitats (Crane & Kinzig, 2005; McDonald et al., 2013). Urbanization alters both biotic and abiotic environments, affecting ecosystem structure and function (Grimm, 2008). This process leads to habitat loss or fragmentation due to built-up structures (e.g., buildings, roads; Niemelä, 2011), increased vulnerability to physicochemical changes (e.g., hydrology, soil geochemistry, temperature), pollution (e.g., light, noise), non-native species (e.g., predators, competitors), disease transmission, and other anthropogenic disturbances (Lafferty & Kuris, 2005; McKinney, 2006, 2007; Bradley & Altizer, 2007). Consequently, urban landscapes present novel challenges for native species, necessitating assessment of their responses to urbanization and viability in urban environments.

Urbanization is widely recognized to reduce species richness in wildlife conservation studies (McKinney, 2008). However, the underlying mechanisms for these reductions and the exceptional responses of persisting species, especially among animals, remain unclear. Previous research has examined specific urban areas over time and the responses of select species across different urban areas, but findings have been inconsistent (e.g., Fischer et al., 2015). These discrepancies are attributed to the heterogeneity of urban environments, which impose varying selective pressures, and the differential responses of species across taxonomic groups. Here, the

responses of native species to urbanization are influenced by their specific dispersal, habitat, ecology, physiology, and life history. However, most studies have focused on avian and mammalian systems, despite the importance of all local species for ecosystem functioning (Duffy, 2009). Assessing the directionality of responses within less studied taxonomic groups, such as reptiles, can be key to understanding the scope of impact from urbanization for future conservation management (Gibbon et al., 2000; Mitchell et al., 2008).

### *Individual physiological responses*

Physiological responses can provide real-time information about reptile viability in urban environments, yet they remain understudied (Ricklefs & Wikelski, 2002). Nonetheless, a major focus has been recently placed on comparing endocrine function, oxidative status, and immunity across the urban landscape (reviewed in French et al., 2018). Measures of metabolic glucocorticoid hormones, such as corticosterone (CORT) in reptiles, are commonly used to assess energy mobilization for environmental challenges (Sapolsky et al., 2000). Monitoring CORT levels are of importance in this context because of their influence on other physiological systems linked to life history and fitness (Angelier & Wingfield, 2013; Crespi et al., 2013). One of these is the ability to respond to injuries and infections via the immune system, since failing to mount an effective immune response can jeopardize organismal health (McKean & Lazzaro, 2011; Wobeser, 2013). Assays of bacterial killing ability (BKA) are often used here as an integrative measure of constitutive immune function (reviewed in Demas, 2011). Along the same line, the capacity to resist prooxidant chemical species generated for essential cellular functions (e.g., immune function; Nathan & Cunningham-Bussel, 2013) has been recognized as an important measure, since poor antioxidant defenses can undermine longevity via excessive

oxidative stress (e.g., oxidative damage to DNA, proteins, and lipids; Halliwell & Gutteridge, 2015; Sies et al., 2017). Assays measuring both reactive oxygen metabolites and the capacity to bind to and clear those metabolites can therefore be used together as an indicator of oxidative stress for reptiles in urban environments.

When considering these physiological metrics, reptiles have so far been shown to have variable responses to anthropogenic disturbances. Measures of CORT can be lower, higher, or yield no differences in baseline and stress-induced levels across species (e.g., turtles, snakes, lizards; reviewed in French et al., 2018; Injaian et al., 2020). Similarly, urban-induced changes in baseline BKA can be species-dependent (e.g., French et al., 2008; Lucas & French, 2012). While markers of oxidative status have received less attention (Hutton & McGraw, 2016), reptiles in urban settings have been shown to experience elevated oxidative stress (Lucas & French, 2012). Regardless of directionality, these physiological changes can potentially affect individual survival and reproduction, and ultimately population vital rates.

### *Population vital rates*

Linking individual physiological parameters to population viability may provide insights into reptile persistence in urban environments. Studies poised to do so have revealed that elevated physiological stress is associated with decreased survival but increased reproductive output during the breeding season (e.g., Lucas & French, 2012; Hall & Warner, 2017). However, interpreting these measures effectively is challenging due to spatial and temporal variability in survival and reproduction (Holmes et al., 1994; Tilman & Kareiva, 1997). Long-term studies spanning multiple years are therefore necessary to accurately compare reptile population dynamics in urban and natural settings (Rees & Ellner, 2009; Chandler et al., 2018). Accounting

for individual physiology over such periods could reveal their influence over survival and/or reproductive outcomes when responding to urbanization. Any physiological traits that are linked to these fitness components may in turn be implicated in microevolutionary changes across the landscape.

### *Population genetics*

The genetic consequences of urban selective pressures for reptiles remain understudied, leading to uncertainty regarding the evolution or acclimatization of physiological traits in cities (Santangelo et al., 2018; Rivkin et al., 2019). Research has demonstrated genetic drift and restricted gene flow to occur among urban habitat patches, which can reduce genetic diversity and the adaptive potential for physiological traits (Rubin et al., 2001; Delaney et al., 2010; Miles et al., 2019). However, some urban reptiles can maintain genetic diversity, particularly when gene flow is possible (Parham & Papenfuss, 2009; Cureton et al., 2014; Virens et al., 2015; Sunny et al., 2015). Nevertheless, population genetic structure can occur over short geographic and temporal scales for other urban reptiles (Moore et al., 2008; Hoehn et al., 2007, 2013). Emerging studies have corroborated these findings with evidence of divergent phenotypes across the urban-natural landscape (Winchell et al., 2016; 2018), suggesting a potential for reptile populations to acquire physiological adaptations. As such, explicit tests of selection that identify genetically controlled physiological changes should be conducted on various reptile species to appreciate the scope of their responses.

### *Side-blotched lizards in an urban landscape*

Among the reptiles susceptible to urbanization is the side-blotched lizard (*Uta stansburiana*; Baird & Girard, 1852). This species is a small, short-lived, and sexually dimorphic lizard commonly found throughout deserts of western North America (Stebbins & McGinnis, 2018). Their distribution ranges from central Washington to Mexico, though their prevalence is limited by habitat availability and dispersal among localities (individual dispersal beyond 300 m is rare; Doughty & Sinervo, 1994). Within the northern portion of their range, side-blotched lizards inhabit rocky cliffs and side canyons, where embedded lava flows are used for basking and nearby vegetation is used for feeding and refuge (Zani et al., 2009). In the more arid southern regions, these lizards occupy desert scrub, rocky outcroppings, and riparian zones along streams and washes, which offer more insects to eat, moister soil for laying eggs, numerous basking sites, and crevices for seeking shade and escaping predation (Jones & Lovich, 2009). Yet these habitats are being transformed by anthropogenic disturbances, such that *U. stansburiana* populations are increasingly occupying urban areas (e.g., Banville & Bateman, 2012).

This dissertation focused on side-blotched lizards within and around St. George, Utah, which is one of the fastest growing cities in the United States (U.S. Census Bureau, 2022). The lizards herein were located at rocky areas near riparian corridors at the convergence of the Great Basin, Colorado Plateau, and Mojave Desert. However, areas within the city increasingly differ from the surrounding natural landscape due to emerging urban infrastructure including roads, parking lots, buildings, rocky terraces, public parks, and altered waterways. Additionally, regular disturbances from traffic, construction, cycling, walking, and the presence of novel predators (e.g., cats) accompany the increased human presence. Altogether, the rapid habitat alterations to

this area provided an ideal setting to study both individual- and population-level responses to urbanization.

Side-blotched lizards represent an ideal study system to assess population health and vital rates due to their site fidelity, territoriality, large abundance, ease of detection and capture, and the ability to obtain physiological samples in a minimally invasive manner (Scoular et al., 2011). Given their simple diploid genetics (34 chromosomes; consisting of 22 micro- and 12 macrochromosomes) and relatively short generation time (~1-2 years), these lizards are also suitable for studies of population genetics (Pennock et al., 1968; Turner et al., 1970). Previous research has extensively utilized *U. stansburiana* to investigate ecological, demographic, physiological, and evolutionary questions (e.g., Tinkle, 1967, 1969; Sinervo & Licht, 1991). However, the systematics and taxonomy of *Uta* genus have been highly debated, resulting in the description of numerous subspecies or even distinct species across the USA and Mexico (Schmidt, 1921; Mittleman, 1940; Grismer, 1994; Upton & Murphy, 1997). While trait variation among color morphs has been documented in other *U. stansburiana* populations (Sinervo & Lively, 1996; Sinervo et al., 2000), those studied in this dissertation did not exhibit stable color morphs, and thus this aspect of their ecology was not considered.

### *Dissertation overview*

Understanding the physiological characteristics that allow animals to survive and persist in urban landscapes is critical for conservation. Since any factor that affects individual fitness has the potential to affect population vital rates, physiological changes could influence demographic fitness components. How urban and natural lizard populations differ in survival, and which physiological traits significantly contribute to this fitness parameter remains largely



unexplored. Such differences are likely coupled with individual physiology. However, whether trait differences are owed to evolution and/or acclimatization in urban environments also remains undetermined. The following chapters of this dissertation aim to address these questions to better understand the scope of anthropogenic impact on these lizard populations.

In Chapter 2, a six-year capture-mark-recapture (CMR) study was conducted to monitor and compare vital rates of natural and urban *U. stansburiana* populations. With each capture, lizards were sampled for physiological traits including basal measures of innate immunity (i.e., bacterial killing ability or BKA), oxidative resistance (i.e., antioxidant capacity or OXY), and energy-mobilizing glucocorticoid hormone (i.e., corticosterone or CORT) in blood plasma. Open population models were then built and compared using a Bayesian approach so that vital rates (survival, abundance, and local recruitment) could be estimated while accounting for variation in the physiological metrics. High uncertainty in survival was found across both habitat types over time, though survival rates were lower for urban populations overall. There was also strong support for a direct relationship between immune function and survivorship, with survival rates predicted to increase for lizards with greater bactericidal ability. Despite the projected survival differences, estimates of urban population size were either larger or comparable to those at natural sites, while recruitment was more often higher at natural sites. These results not only have wildlife conservation implications for urbanization, but also demonstrate the utility of organismal indicators for understanding individual and population level processes in urban areas and elsewhere.

In Chapter 3, the genetic basis of the physiological traits included in the population demography models was analyzed using genotyping-by-sequencing (GBS) data. The genetic data were derived from red blood cells left over from physiological sampling and were generated as

single nucleotide polymorphisms (SNPs). Multi-locus genome-wide association mapping was then implemented to quantify the proportion of trait variation explained by genetic data (i.e., “chip” heritability), along with the number and contribution of genetic loci with measurable effects. Genomic prediction was included to assess phenotypic and genetic variance-covariance matrices (P- and G-matrices) within versus among populations. Variation in each trait was found to have a polygenic basis with low to moderate heritability and few measurable effect loci. The P- and G-matrices were generally similar, though with slight exceptions across populations. These findings provided a preliminary genetic architecture that should be useful for determining the molecular and cellular functions of candidate regions associated with each trait, well as inferring which mechanisms have shaped their evolution in reptiles.

Chapter 4 builds on Chapters 2 and 3 by estimating the intensity of selection on each physiological trait and their associated genetic loci in urban and natural environments. Here, an approximate Bayesian computation (ABC) method was applied to the genomic time-series data collected across the six-year study. With this method, phenotypic selection was modeled as an explicit function of either an urban or natural environment. The population genomic consequences of selection were then modeled based on estimated genotype–phenotype associations, as summarized by estimated polygenic scores (i.e., genetic breeding values). This allowed for inferences using patterns of change across multiple genetic loci, populations, and generations. In addition, population-level comparisons of genetic structure and differentiation, allele frequency change, variance effective population size, and genetic diversity were tested across environments. Selection was not detected in either urban or natural environments. However, rates of evolution by drift were relatively high across populations, with pronounced allele frequency changes over contiguous generations. Such high rates were consistent with small

estimates of local variance in effective population size, especially at urban sites, despite modest estimates of population size. In contrast, urban and natural populations harbored comparable molecular diversity, which could be owed to periodic gene flow (as indicated by similarities in population genetic structure). These results suggest contemporary evolution by drift may overwhelm selection for physiological traits relevant to these lizards, while other evolutionary processes may help maintain their genetic variation.

In Chapter 5, the concluding chapter, the aims of this dissertation are revisited, and a summary is provided for Chapters 2-4. The results from each study are collectively reconciled in the context of urban ecology and evolution. In doing so, the interconnectedness of population physiology, demography, and genomics are highlighted in support of their use in wildlife conservation studies.

#### LITERATURE CITED

- Angelier, F., & Wingfield, J. C. (2013). Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives. *General and Comparative Endocrinology*, 190, 118-128.
- Banville, M. J., & Bateman, H. L. (2012). Urban and wildland herpetofauna communities and riparian microhabitats along the Salt River, Arizona. *Urban ecosystems*, 15(2), 473-488.
- Bradley, C. A., & Altizer, S. (2007). Urbanization and the ecology of wildlife diseases. *Trends in ecology & evolution*, 22(2), 95-102.
- Bureau, U. S. C. (2022). Over Two-Thirds of the Nation's Counties Had Natural Decrease in 2021. Retrieved from <https://www.census.gov/newsroom/press-releases/2022/population-estimates-counties-decrease.html>

- Chandler, R. B., Hepinstall-Cymerman, J., Merker, S., Abernathy-Conners, H., & Cooper, R. J. (2018). Characterizing spatio-temporal variation in survival and recruitment with integrated population models. *The Auk: Ornithological Advances*, 135(3), 409-426.
- Crane, P., & Kinzig, A. (2005). Nature in the metropolis. In (Vol. 308, pp. 1225-1225): American Association for the Advancement of Science.
- Crespi, E. J., Williams, T. D., Jessop, T. S., & Delehanty, B. (2013). Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? *Functional Ecology*, 27(1), 93-106.
- Cureton, J. C., Janis, M., Lutterschmidt, W. I., Randle, C. P., Ruthven, D. C., & Deaton, R. (2014). Effects of urbanization on genetic diversity, gene flow, and population structure in the ornate box turtle (*Terrapene ornata*). *Amphibia-Reptilia*, 35(1), 87-97.
- Delaney, K. S., Riley, S. P., & Fisher, R. N. (2010). A rapid, strong, and convergent genetic response to urban habitat fragmentation in four divergent and widespread vertebrates. *PloS one*, 5(9), e12767.
- Demas, G. E., Zysling, D. A., Beechler, B. R., Muehlenbein, M. P., & French, S. S. (2011). Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts. *Journal of Animal Ecology*, 80(4), 710-730.
- Doughty, P., Sinervo, B., & Burghardt, G. M. (1994). Sex-biased dispersal in a polygynous lizard, *Uta stansburiana*. *Animal Behaviour*.
- Duffy, J. E. (2009). Why biodiversity is important to the functioning of real-world ecosystems. *Frontiers in Ecology and the Environment*, 7(8), 437-444.

- Fischer, J. D., Schneider, S. C., Ahlers, A. A., & Miller, J. R. (2015). Categorizing wildlife responses to urbanization and conservation implications of terminology. *Conservation Biology*, 29(4), 1246-1248.
- French, S. S., Fokidis, H. B., & Moore, M. C. (2008). Variation in stress and innate immunity in the tree lizard (*Urosaurus ornatus*) across an urban–rural gradient. *Journal of Comparative Physiology B*, 178(8), 997-1005.
- French, S. S., Webb, A. C., Hudson, S. B., & Virgin, E. E. (2018). Town and country reptiles: a review of reptilian responses to urbanization. *Integrative and Comparative Biology*, 58(5), 948-966.
- Gibbons, J. W., Scott, D. E., Ryan, T. J., Buhlmann, K. A., Tuberville, T. D., Metts, B. S., . . . Poppy, S. (2000). The Global Decline of Reptiles, Déjà Vu Amphibians: Reptile species are declining on a global scale. Six significant threats to reptile populations are habitat loss and degradation, introduced invasive species, environmental pollution, disease, unsustainable use, and global climate change. *Bioscience*, 50(8), 653-666.
- Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X., & Briggs, J. M. (2008). Global change and the ecology of cities. *science*, 319(5864), 756-760.
- Grismer, L. L. (1994). Three new species of intertidal side-blotched lizards (genus *Uta*) from the Gulf of California, Mexico. *Herpetologica*, 451-474.
- Hall, J. M., & Warner, D. A. (2017). Body size and reproduction of a non-native lizard are enhanced in an urban environment. *Biological Journal of the Linnean Society*, 122(4), 860-871.
- Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*: Oxford university press, USA.

- Hoehn, M., Dimond, W., Osborne, W., & Sarre, S. D. (2013). Genetic analysis reveals the costs of peri-urban development for the endangered grassland earless dragon. *Conservation Genetics*, *14*(6), 1269-1278.
- Hoehn, M., Sarre, S., & Henle, K. (2007). The tales of two geckos: does dispersal prevent extinction in recently fragmented populations? *Molecular ecology*, *16*(16), 3299-3312.
- Holmes, E. E., Lewis, M. A., Banks, J., & Veit, R. (1994). Partial differential equations in ecology: spatial interactions and population dynamics. *Ecology*, *75*(1), 17-29.
- Hutton, P., & McGraw, K. J. (2016). Urban impacts on oxidative balance and animal signals. *Frontiers in Ecology and Evolution*, *4*, 54.
- Injaian, A. S., Francis, C. D., Ouyang, J. Q., Dominoni, D. M., Donald, J. W., Fuxjager, M. J., . . . Johnson, M. A. (2020). Baseline and stress-induced corticosterone levels across birds and reptiles do not reflect urbanization levels. *Conservation physiology*, *8*(1), coz110.
- Jones, L. L., & Lovich, R. E. (2009). *Lizards of the American Southwest: a photographic field guide*: Rio Nuevo Publishers.
- Lafferty, K. D., & Kuris, A. M. (2005). Parasitism and environmental disturbances. *Parasitism and ecosystems*, 113-123.
- Lucas, L. D., & French, S. S. (2012). Stress-induced tradeoffs in a free-living lizard across a variable landscape: consequences for individuals and populations. *PloS one*, *7*(11), e49895.
- McDonald, R. I., Marcotullio, P. J., & Güneralp, B. (2013). Urbanization and global trends in biodiversity and ecosystem services. In *Urbanization, biodiversity and ecosystem services: Challenges and opportunities* (pp. 31-52): Springer, Dordrecht.

- McKean, K. A., & Lazzaro, B. P. (2011). The costs of immunity and the evolution of immunological defense mechanisms. In (pp. 299-310): Oxford University Press Oxford.
- McKinney, M. L. (2006). Urbanization as a major cause of biotic homogenization. *Biological conservation*, *127*(3), 247-260.
- McKinney, M. L. (2008). Effects of urbanization on species richness: a review of plants and animals. *Urban ecosystems*, *11*(2), 161-176.
- McKinney, M. L., & La Sorte, F. A. (2007). Invasiveness and homogenization: synergism of wide dispersal and high local abundance. *Global Ecology and Biogeography*, *16*(3), 394-400.
- Miles, L. S., Rivkin, L. R., Johnson, M. T., Munshi-South, J., & Verrelli, B. C. (2019). Gene flow and genetic drift in urban environments. *Molecular ecology*, *28*(18), 4138-4151.
- Mitchell, J., Jung Brown, R., & Bartholomew, B. (2008). Urban herpetology. *Herpetological conservation*, *3*, 130Naiman.
- Mittleman, M. B. (1940). Two new lizards of the genus *Uta*. *Herpetologica*, *2*(2), 33-38.
- Moore, J., Miller, H., Daugherty, C., & Nelson, N. (2008). Fine-scale genetic structure of a long-lived reptile reflects recent habitat modification. *Molecular ecology*, *17*(21), 4630-4641.
- Nathan, C., & Cunningham-Bussel, A. (2013). Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nature Reviews Immunology*, *13*(5), 349-361.
- Niemelä, J., Breuste, J. H., Guntenspergen, G., McIntyre, N. E., Elmqvist, T., & James, P. (2011). *Urban ecology: patterns, processes, and applications*: OUP Oxford.
- Parham, J. F., & Papenfuss, T. J. (2009). High genetic diversity among fossorial lizard populations (*Anniella pulchra*) in a rapidly developing landscape (Central California). *Conservation Genetics*, *10*(1), 169-176.

- Pennock, L. A., Tinkle, D. W., & Shaw, M. W. (1968). Chromosome number in the lizard genus *Uta* (family Iguanidae). *Chromosoma*, 24(4), 467-476.
- Rees, M., & Ellner, S. P. (2009). Integral projection models for populations in temporally varying environments. *Ecological Monographs*, 79(4), 575-594.
- Ricklefs, R. E., & Wikelski, M. (2002). The physiology/life-history nexus. *Trends in ecology & evolution*, 17(10), 462-468.
- Rivkin, L. R., Santangelo, J. S., Alberti, M., Aronson, M. F., de Keyser, C. W., Diamond, S. E., . . . Hendry, A. P. (2019). A roadmap for urban evolutionary ecology. *Evolutionary applications*, 12(3), 384-398.
- Santangelo, J. S., Rivkin, L. R., & Johnson, M. T. (2018). The evolution of city life. In (Vol. 285, pp. 20181529): The Royal Society.
- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine reviews*, 21(1), 55-89.
- Schmidt, K. P. (1921). A new name for a subspecies of *Uta stansburiana* Baird and Girard. *American Museum novitates*; no. 15.
- Scoular, K. M., Caffry, W. C., Tillman, J. L., Finan, E. S., Schwartz, S. K., Sinervo, B., & Zani, P. A. (2011). Multiyear home-range ecology of common side-blotched lizards in eastern Oregon with additional analysis of geographic variation in home-range size. *Herpetological Monographs*, 25(1), 52-75.
- Sies, H., Berndt, C., & Jones, D. P. (2017). Oxidative stress. *Annual review of biochemistry*, 86, 715-748.



- Sinervo, B., & Licht, P. (1991). Hormonal and physiological control of clutch size, egg size, and egg shape in side-blotched lizards (*Uta stansburiana*): constraints on the evolution of lizard life histories. *Journal of Experimental Zoology*, 257(2), 252-264.
- Sinervo, B., & Lively, C. M. (1996). The rock–paper–scissors game and the evolution of alternative male strategies. *Nature*, 380(6571), 240-243.
- Sinervo, B., Miles, D. B., Frankino, W. A., Klukowski, M., & DeNardo, D. F. (2000). Testosterone, endurance, and Darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. *Hormones and Behavior*, 38(4), 222-233.
- Stebbins, R. C., & McGinnis, S. M. (2018). *Peterson field guide to western reptiles & amphibians*: Houghton Mifflin.
- Sunny, A., Monroy-Vilchis, O., Zarco-González, M. M., Mendoza-Martínez, G. D., & Martínez-Gómez, D. (2015). Genetic diversity and genetic structure of an endemic Mexican Dusky Rattlesnake (*Crotalus triseriatus*) in a highly modified agricultural landscape: implications for conservation. *Genetica*, 143(6), 705-716.
- Tilman, D., & Kareiva, P. (1997). *Spatial ecology: the role of space in population dynamics and interspecific interactions* (Vol. 30): Princeton University Press.
- Tinkle, D. W. (1967). The life and demography of the side-blotched lizard, *Uta stansburiana*.
- Tinkle, D. W. (1969). The concept of reproductive effort and its relation to the evolution of life histories of lizards. *The American Naturalist*, 103(933), 501-516.
- Turner, F. B., Hoddenbach, G. A., Medica, P. A., & Lannom, J. R. (1970). The demography of the lizard, *Uta stansburiana* Baird and Girard, in southern Nevada. *The Journal of Animal Ecology*, 505-519.

- Upton, D. E., & Murphy, R. W. (1997). Phylogeny of the side-blotched lizards (Phrynosomatidae: *Uta*) based on mtDNA sequences: support for a midpeninsular seaway in Baja California. *Molecular Phylogenetics and Evolution*, 8(1), 104-113.
- Virens, E., Krauss, S. L., Davis, R. A., & Spencer, P. B. (2015). Weak genetic structuring suggests historically high genetic connectivity among recently fragmented urban populations of the scincid lizard, *Ctenotus fallens*. *Australian Journal of Zoology*, 63(4), 279-286.
- Winchell, K. M., Carlen, E. J., Puente-Rolón, A. R., & Revell, L. J. (2018). Divergent habitat use of two urban lizard species. *Ecology and evolution*, 8(1), 25-35.
- Winchell, K. M., Reynolds, R. G., Prado-Irwin, S. R., Puente-Rolón, A. R., & Revell, L. J. (2016). Phenotypic shifts in urban areas in the tropical lizard *Anolis cristatellus*. *Evolution*, 70(5), 1009-1022.
- Wobeser, G. A. (2013). *Essentials of disease in wild animals*: John Wiley & Sons.
- Zani, P., Jones, T., Neuhaus, R., & Milgrom, J. (2009). Effect of refuge distance on escape behavior of side-blotched lizards (*Uta stansburiana*). *Canadian Journal of Zoology*, 87(5), 407-414.

## CHAPTER II

## POPULATION DEMOGRAPHY OF LIZARDS RESIDING IN URBAN AND NATURAL ENVIRONMENTS: LINKS BETWEEN INDIVIDUAL PHYSIOLOGY AND SURVIVAL

The ecological threats imposed by anthropogenic disturbance (e.g., habitat loss and fragmentation, building and road development, light and noise pollution) present risks for individual health, and in turn, population vital rates. It is therefore critical to account for physiological condition when modeling population viability in urban habitats. In this six-year study, capture-mark-recaptures of side-blotched lizards (*Uta stansburiana*) were conducted annually at urban and natural sites within and around the city of St. George, in southwestern Utah, USA. Hierarchical, Bayesian modeling approaches were implemented to estimate population vital rates (survival, abundance, and local recruitment). Individual physiological traits (glucocorticoid hormone levels, oxidative status, and immunocompetence) were accounted for when estimating population-level survival. As a result, survival rates were found to be lower for urban lizard populations overall, though these estimates were highly variable across both habitat types over time. There was also strong support for a direct relationship between immune function and survival, with survival rates predicted to increase for lizards with greater bactericidal ability. Despite the projected survival differences, as well as an observed population crash at one of the urban sites, estimates of urban population size and density were either larger or comparable to those at natural sites, while per-capita recruitment was more often higher at natural sites. This study provides some of the first vital rates of an urban lizard, especially for survival estimates that incorporate individual physiology. The results have implications for conservation

and illustrate the effectiveness of hierarchical Bayesian models for the study of urbanization on wildlife, as well as the importance and utility of organismal indicators for understanding both individual and population level processes.

## INTRODUCTION

Urbanization is a powerful driver of habitat change and has been estimated to be one of the largest threats to reptile biodiversity (Gibbons et al., 2000; McKinney, 2006; McDonald et al., 2008). Anthropogenic disturbances associated with urbanization (e.g., habitat loss and fragmentation; building and road development, light and noise pollution) pose various negative effects for reptiles that could have dire ecological consequences including not only population loss, but also altered patterns of ecosystem structure and function (Hansen et al., 2005). Although some local wildlife exhibit traits that allow them to maintain or expand their populations in human-modified landscapes (i.e., disturbance-tolerant species), the proportion of native species sensitive to urbanization is often greater (McKinney & Lockwood, 1999; Dornelas et al., 2019; Filgueiras et al., 2021). Indeed, this is becoming increasingly evident for reptiles as anthropogenic disturbance continues to cause population declines and even local extirpations (e.g., Endriss et al., 2007; Smolensky & Fitzgerald, 2011; Knapp & Perez-Heydrich, 2012; Wolf et al., 2013; Kolbe et al., 2016). Conservation biologists must therefore use every tool at their disposal to mitigate the threats facing reptiles in this ever-changing world.

Population modeling is an established and useful approach for measuring changes in population vital rates (e.g., survival, fecundity) in this context (e.g., Katz et al., 2013; Wood et al., 2020). Since survival rates often vary over time (e.g., Gaillard & Yoccoz, 2003; Zylstra et al., 2013; Pérez-Mendoza et al., 2014), hierarchical capture–mark–recapture (CMR) models that

share information about the capture and survival process across long periods are necessary to understand natural population dynamics and improve the precision of annual survival estimates (Rees & Ellner, 2009). Rather than treating data from each year as independent, a hierarchical model can adaptively pool information on capture probabilities from each year and use these pooled data to improve survival estimates at each habitat annually (Rivot & Prevost, 2002, Halstead et al., 2012). However, by the time measurable changes at the population level occur, management decisions may be too late to effectively help local wildlife. Beyond accounting for temporal variation, quantifying how survival is influenced by variation in environmental factors or individual traits may be key for predicting future population responses to urbanization (Schofield & Barker, 2011; Frederiksen et al., 2014). Using hierarchical models can better quantify how much of the observed variation in survival is attributable to such covariates versus heterogeneity among habitats and years (Gelman & Hill, 2007; Schaub & Kery, 2012). Doing so will in turn help to identify which environmental features and reptilian traits are crucial to persistence in urban areas.

Currently, there is still a paucity of empirical evidence linking individual-level metrics to survival for reptiles exposed to urbanization (French et al., 2018). Yet, the relevance of functional traits, whether they be morphological, behavioral, or physiological, is becoming increasingly evident in urban areas (e.g., Mitrovich et al., 2009; Putman et al., 2019; Malisch et al., 2020). Accounting for physiological markers of health could be especially informative when monitoring reptiles across human-modified landscapes because they may influence individual survival and ultimately population survival trajectories (Hofer & East, 1998; Wikelski & Cooke, 2006). Insights on which aspects of physiology are most important could in turn reveal how some populations may succeed and others fail under urban pressures. For example, by measuring

immunocompetence (e.g., bactericidal ability) before succumbing to disease, circulating metabolic hormones (e.g., corticosterone) before adverse energetic divestment, or oxidative status before the damaging effects of oxidative stress, managers can include powerful predictors to their models before a population crash occurs (Stevenson et al., 2005). Being able to estimate survival from individual mark–recapture data while accounting for individual physiological parameters, observation error, demographic stochasticity, and environmental stochasticity, is altogether an important step to be taken towards improved predictive ability of population vital rate models for reptile species.

In this six-year CMR study, six populations of side-blotched lizards (*Uta stansburiana*) were annually sampled for physiological markers of health both within and around St. George, Utah, USA. Bayesian hierarchical, open-population models were constructed to estimate demographic rates (detection, survival, recruitment, population sizes) across sampling occasions and to compare them among habitat types (urban versus natural sites). When estimating detection and survival, sampled measures of baseline immunity (bactericidal ability), oxidative status (reactive oxygen metabolites versus antioxidant capacity), and circulating metabolic hormones (corticosterone) were incorporated as individual covariates into fitted Cormack-Jolly-Seber (CJS) models (Cormack, 1964; Jolly, 1965; Seber, 1965). To estimate local recruitment and population sizes, a Jolly-Seber (JS) model was formulated as a site-specific restricted dynamic occupancy model (Royle & Dorazio, 2008). Overall, population survival, recruitment, and abundance in urban areas were hypothesized to be lower than in natural areas, with variation in physiological health expected to be linked to annual survival across habitat types. Oxidative stress and corticosterone levels were predicted to inversely correspond with survival, while immunocompetence was predicted to correspond positively with survival.

## METHODS

### *Study Species and Area*

Side-blotched lizards are a small, short-lived, and abundant species found within riparian zones of deserts throughout western North America (Stebbins & McGinnis, 2018). They primarily eat arthropods (e.g., insects, spiders, scorpions) and in this region they are often preyed upon by snakes (e.g., *Masticophis flagellum*), larger lizards (e.g., *Cnemidophorus tigris*, *Crotaphytus bicinctores*, *Gambelia wislizenii*), and birds (e.g., *Geococcyx californianus*). At the sampled study sites, their breeding season spans from April to June, with young emerging as early as late May. These lizards represent an ideal model to assess individual health and population vital rates due to their site fidelity, ease of capture and handling, and the rapidity in which physiological samples can be obtained in a minimally invasive manner.

Populations of side-blotched lizards can be observed in habitat patches within the city and sprawl of St. George, Utah, USA, which has been among the fastest growing urban areas in the United States (U.S. Census Bureau, 2022). Here, the lizards are exposed to impervious surfaces, artificial structures, daily human interactions, competition with tree lizards (*Urosaurus ornatus*), as well as predation by cats (*Felis catus*), coachwhip snakes (*M. flagellum*), and roadrunners (*G. californianus*). Lizard populations found in more natural areas lack these pressures or experience them to a lesser degree.

### *Population Sampling*

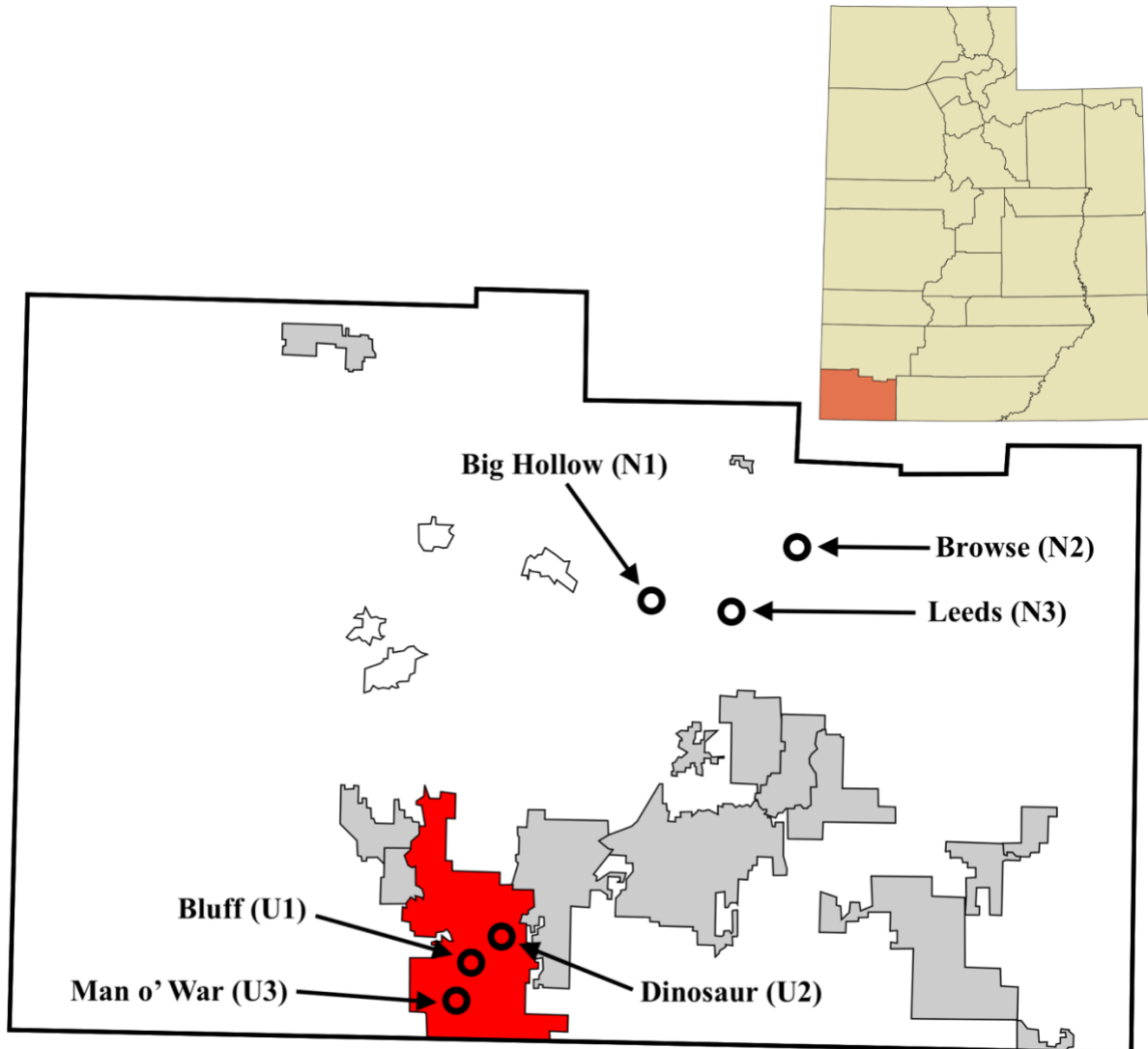
Between May 2012-May 2017, adult side-blotched lizards ( $N = 981$ ) were captured with a snare pole from six study sites within or around the greater St. George area in southwestern Utah (Table 2.1; Fig. 2.1). Sampling was conducted between 0800-1300 to limit potential circadian variation in physiological condition (Choudhury et al., 1982; Dallman et al., 1987; Jones & Gillham, 1988; but see Tyrrell & Cree, 1998; Jones & Bell, 2004). Each sex was selectively snared with similar effort for a balanced sex ratio and therefore unbiased representation of population physiology and vital rates. Upon capture, whole blood was collected from the retro-orbital sinus of each lizard with a heparinized capillary tube within a validated window of time ( $\leq 3$  minutes) to measure basal physiology (MacLean et al., 1973; Romero & Reed, 2005; Tylan et al., 2020). Blood samples were stored on ice until further processing could take place after daily collection, at which time the plasma was isolated via centrifugation and stored at  $-20^{\circ}\text{C}$ .

Each lizard was then toe-clipped (i.e., permanently marked) with a unique 4-digit code for identification and returned for recapture in subsequent years. Here, newly captured lizards were assumed to be a random sample of all unmarked lizards in each population due to the consistency of the sampling protocol (i.e., same capture probability; Williams et al., 2002). Additionally, under the assumption that unbiased demographic rates could be estimated from the median time of capture in one year to that in a subsequent year (Lebreton et al., 1992; White & Burnham, 1999), capture events within years were combined to create simplified individual capture-histories with six sampling occasions (one per year). All procedures were approved by Utah State University IACUC (protocol #2068) and Utah Division of Wildlife Resources (COR #1COLL8382).



**Table 2.1** Summary of years and number of adult side-blotched lizards sampled at each site within and around St. George Utah.

<b>Site</b>	<b>Lat. (°N)</b>	<b>Long. (°W)</b>	<b>Area (ha)</b>	<b>Years sampled</b>	<b>Captures</b>	<b>Lizards</b>
Urban site 1	37.08161	-113.575	0.0136	2012-2017	182	179
Urban site 2	37.09926	-113.532	0.0032	2012-2015	72	69
Urban site 3	37.05262	-113.602	0.0079	2012-2017	195	189
Natural site 1	37.24323	-113.452	0.0180	2012-2017	153	144
Natural site 2	37.33088	-113.28	0.0157	2012-2017	168	153
Natural site 3	37.25966	-113.369	0.01586	2012-2017	211	198



**Figure 2.1** Approximate locations of urban and natural populations of side-blotched lizards in Washington County, Utah, USA (highlighted orange in the state map). Each study site was either within or outside the city of St. George (highlighted red in the county map). All sites consisted of rocky areas  $\leq 1.8$  ha in size near or along riparian corridors close to the intersection of the Great Basin, Colorado Plateau and Mojave Desert. The six sites lay an average of 21 km from one another (minimum = 4 km; maximum = 42 km). Urban and natural sites are respectively abbreviated as ‘U’ and ‘N’, followed by their numeric identifier.

### *Bacterial killing ability*

Bactericidal ability was quantified with a validated volume of blood plasma (6 ul) to assess the relative abundances of circulating immune components (e.g., natural antibodies, antimicrobial peptides). Using the protocol outlined in French and Neuman-Lee (2012), a 1:2 plasma dilution was combined with CO<sub>2</sub>-independent medium (Gibco # 18-045-088, ThermoFisher Scientific, Grand Island, NY, USA), 4 nM l-glutamine, 10<sup>4</sup> colony-producing units of *Escherichia coli* (EPower™ Microorganisms #483-581-1, ATCC 8739, MicroBioLogics, St. Cloud, MN, USA), and agar broth on a 96-well microplate. Included were both positive (i.e., media and bacteria with no plasma) and negative (i.e., media and no plasma or bacteria) controls to account for potential growth and ensure there was no contamination. The plate was incubated for a 12-h period, at which point absorbance per well was measured with a microplate reader at 300 nm (xMark; BioRad Benchmark, Hercules, CA, USA). Bactericidal ability was then calculated as  $(1 - (\text{absorbance of sample} / \text{absorbance of positive controls}) \times 100)$ . Each sample was run in duplicate to generate an average percent score of bactericidal ability. Coefficients of variation were <20% within and among all assays.

### *Oxidative Status*

Two types of colorimetric assays were used on blood plasma to measure both reactive oxygen metabolites and the capacity to bind to and clear those metabolites. Reactive oxygen metabolites were measured using a d-ROMs test that detects variable levels of hydroperoxides (MC435, Diacron International, Italy), which signal lipid and protein oxidative damage. Following Lucas and French (2012), 5 µl of plasma were diluted into 100 µl of the provided acidic buffered solution and ‘end-point mode’ manufacturer instructions were followed thereafter

for use with 96-well microplates and a spectrophotometer (505 nm; xMark, Bio-Rad). Intra-plate variation for the d-ROMs test was 4.0% across samples. Total antioxidant capacity was measured using an OXY-Adsorbent test (MC002, Diacron International, Italy), which determines effectiveness of the blood antioxidant barrier by quantifying tolerance of the oxidant action of hypochlorous acid (HClO; Vassalle et al., 2004). Here, 2  $\mu$ l of plasma were diluted in 100  $\mu$ l of distilled water and manufacturer instructions were followed thereafter for use with 96-well microplates and a spectrophotometer (505 nm; xMark, Bio-Rad). A 5  $\mu$ l subsample of this diluted plasma was then combined with 100  $\mu$ l of the HClO solution provided. Coefficients of variation were <20% within and among all assays.

Values indicating the relative quantity of reactive oxygen metabolites (mg H<sub>2</sub>O<sub>2</sub> / dl) and total non-enzymatic antioxidant capacity (mol HClO / ml) were incorporated into an index of oxidative stress scores (i.e., oxidative index). Each score was calculated by subtracting a standardized OXY-Adsorbent value from a standardized d-ROMs value, representing the relative contribution of reactive oxygen metabolites or antioxidant capacity, with higher values indicating greater oxidative stress (Vassalle, 2008, Vassalle et al., 2008).

### *Corticosterone Levels*

Following a protocol outlined in Neuman-Lee and French (2017), radioimmunoassays were conducted on plasma samples (10  $\mu$ L) for CORT (Ab: MP Biomedicals # 07-120016). Samples were extracted with 30% ethyl acetate:isooctane and run in duplicate for the assay. Individual recoveries were calculated from the extractions to correct for final concentrations with a minimum level of detection of 0.3 ng/ml. Coefficients of variation were <20% within and among all assays.

### *Cormack-Jolly-Seber Models*

Open-population CJS models of the CMR data were developed as state-space models ('alive', 'dead') to estimate detection probability ( $p$ ; the probability of recapturing a marked lizard that is alive in the sampling population) and apparent survival probability ( $\Phi$ ; the probability that a marked lizard is alive in a subsequent sampling occasion and has not permanently emigrated from the study area) at urban versus natural habitats (Gimenez et al., 2007; Royle, 2008). By taking a Bayesian approach to generalized linear mixed modeling (Kery & Schaub, 2011), fixed group effects of habitat type and random time effects of year were combined to estimate apparent survival (hereafter 'survival') and its temporal variability in each habitat separately. Individual heterogeneity was also accounted for by including each of the physiological metrics (bactericidal ability, oxidative status, corticosterone) as continuous covariates in competing models to determine whether survival may be driven by components of physiological health. Candidate models were generated based on biological questions of interest, but statistical power (i.e., limited sample size) was assumed to be insufficient when considering additive effects of multiple individual covariates in each model. The sex of each lizard was also not included as a fixed effect for this reason, and because no significant sex differences in survival were previously shown in these populations (Lucas & French, 2012). Altogether, survival in the full model was specified on a logit scale based on  $\mu_g$  as group-specific means,  $\beta x_i$  as the slope of the individual covariate  $x$ , and  $\epsilon_{g,t}$  as group-specific deviations (temporal residuals) with  $\sigma_g^2$  as group-specific temporal variances:

$$\text{logit}(\phi_{i,t}) = \mu_{g(i)} + \beta x_i + \epsilon_{g(i),t}$$

$$\epsilon_{g,t} \sim \text{Normal}(0, \sigma_g^2).$$

### *Jolly-Seber Model*

An open-population JS model of the CMR data was developed as a restricted dynamic occupancy model (state transitions of ‘not yet entered’, ‘alive’, and ‘dead’) to estimate annual local recruitment ( $B$ ) and population abundance ( $N$ ) at each site (Royle & Dorazio, 2008). Here, complete capture-histories of the marked individuals (i.e., leading capture-history zeros) were included as opposed to conditioning on first capture with the CJS models. Parameter-expanded data augmentation was implemented by adding all-zero capture-histories (200 pseudo-individuals per population), which resulted in a larger data set of a fixed dimension and allowed for a reparametrized version of the model to be analyzed based on an entry-removal process (Royle et al., 2007; Royle & Dorazio, 2012). By again using a hierarchical Bayesian framework (Kery & Schaub, 2011), fixed group effects of site and random time effects of year were combined to estimate survival and its temporal variability at each site separately. Fixed time effects were included for recruitment, along with a constant capture rate. In doing so, population abundance  $N$  at year  $t$  was derived from the latent state variable  $Z_{i,t}$ , where  $i$  is an individual among the total number of individuals  $M$ :

$$N_t = \sum_{i=1}^M Z_{i,t}$$

Estimates of annual population abundance allowed for calculations of population growth rate ( $\lambda$ ) as well as population density ( $N/ha$ ), given the measured areas of each site. Additionally, the number of newly entered individuals ( $B$ ; local recruitment) at each site in year  $t$  was derived as:

$$B_t = \sum_{i=1}^M (1 - Z_{i,t-1})Z_{i,t}$$

This allowed for calculations of per capita recruitment based on annual population abundances. Estimates for the number of lizards ever alive at each site during the study ( $N_s$ ; superpopulation size) were derived from the summation of local recruitment  $B$  as:

$$N_s = \sum B$$

### *Model Fitting*

All models were fit in JAGS (v. 4.2.0; Plummer, 2015) using the ‘R2jags’ package (v. 0.03.08; Su & Yajima, 2015) in the R environment (v. 3.3.1; R Core Team, 2016). For both the CJS and JS models, group-specific detection, mean survival, and standard deviation were assigned uniform priors,  $U(0, 1)$ ,  $U(0, 1)$ , and  $U(0, 10)$ , respectively. Covariate slopes of individual heterogeneity in the CJS models were assigned normal priors,  $N(0, 0.001)$ . Due to blood volume limitations, a population by year mean was calculated for lizards missing a particular physiological covariate value, and each of these were used to complete the data set for analysis. All individual covariates were standardized to have a mean of zero and SD of one to improve the efficiency of the MCMC sampler and make interpretation of model coefficients easier.

For each model, three separate chains were run in parallel with different starting values. Initially, 50,000 sampling iterations were included, with the first 20,000 discarded for adaptation and burn-in, and a thinning rate that kept every sixth sample, resulting in 15,000 samples for inference. To assess model convergence, trace plots were observed to have good mixing of the chains and consistent approximate posterior distributions. Brooks-Gelman-Rubin R statistics were also calculated for all parameters ( $\hat{R} < 1.03$ ; Brooks & Gelman, 1998). Estimates were

reported as posterior means and 95% credible intervals for all parameters, unless specified otherwise.

### *CJS Model Comparison and Assessment*

Widely Applicable Information Criterion (WAIC; Watanabe, 2013) scores were calculated to compare candidate CJS models fitted with the suite of fixed and random effect structures. The WAIC technique is valid in models with random effect structures and is generally recommended for Bayesian ecological analyses (Hooten & Hobbs, 2015). Specifically, WAIC is a function of the likelihood and the estimated number of parameters and provides a close approximation to out-of-sample predictive performance, wherein lower values suggest a better predictive model (i.e., lowest WAIC; Gelman et al., 2014). As such, model specifications and estimates were based on the model most supported by the data. Here, the most competitive model was one with linear effects of bactericidal ability (i.e., immunocompetence) on survival, conflated across habitat types (Table 2.2). Survival estimates were calculated by back-transformation of the logit scale (i.e., the logit function transforms the estimates into survival probabilities constrained between 0 and 1).



**Table 2.2** Model assessment and comparison results for the patterns of variation in vital rates associated with habitat type (urban, natural), individual heterogeneity (physiological covariate), and temporal variability (year) of adult side-blotched lizards. The top model is indicated in bold. Predictive ability for each model is based on the Widely Applicable Information Criterion (WAIC), whereby models with a lower score are more supported.

<b>Model</b>	<b>WAIC</b>	<b><math>\Delta</math>WAIC</b>
<b><math>\Phi</math> (habitat x year + bactericidal ability) <math>p</math> (habitat)</b>	<b>424.4</b>	<b>0</b>
$\Phi$ (habitat x year) $p$ (habitat)	428.0	3.6
$\Phi$ (habitat x year + oxidative status) $p$ (habitat)	428.9	4.5
$\Phi$ (habitat x year + corticosterone) $p$ (habitat)	430.1	5.7

## RESULTS

### *Capture data summary*

There were 981 captures of 932 individuals over the six years of study (Table 2.1). The sex ratio of captured lizards was 1.02 females:1 male; 496 individuals were female and 485 were male. The largest number of individuals were captured at natural site 3, followed by urban site 3 and urban site 1. Of 981 captured individuals, 938 were captured in only one year, 37 were captured in two years, and 6 were captured in three years. The longest interval between the first and last capture of a lizard was three years, but most lizards were recaptured one year (3.8% of recaptures) or two years (0.6%) after their most recent capture.

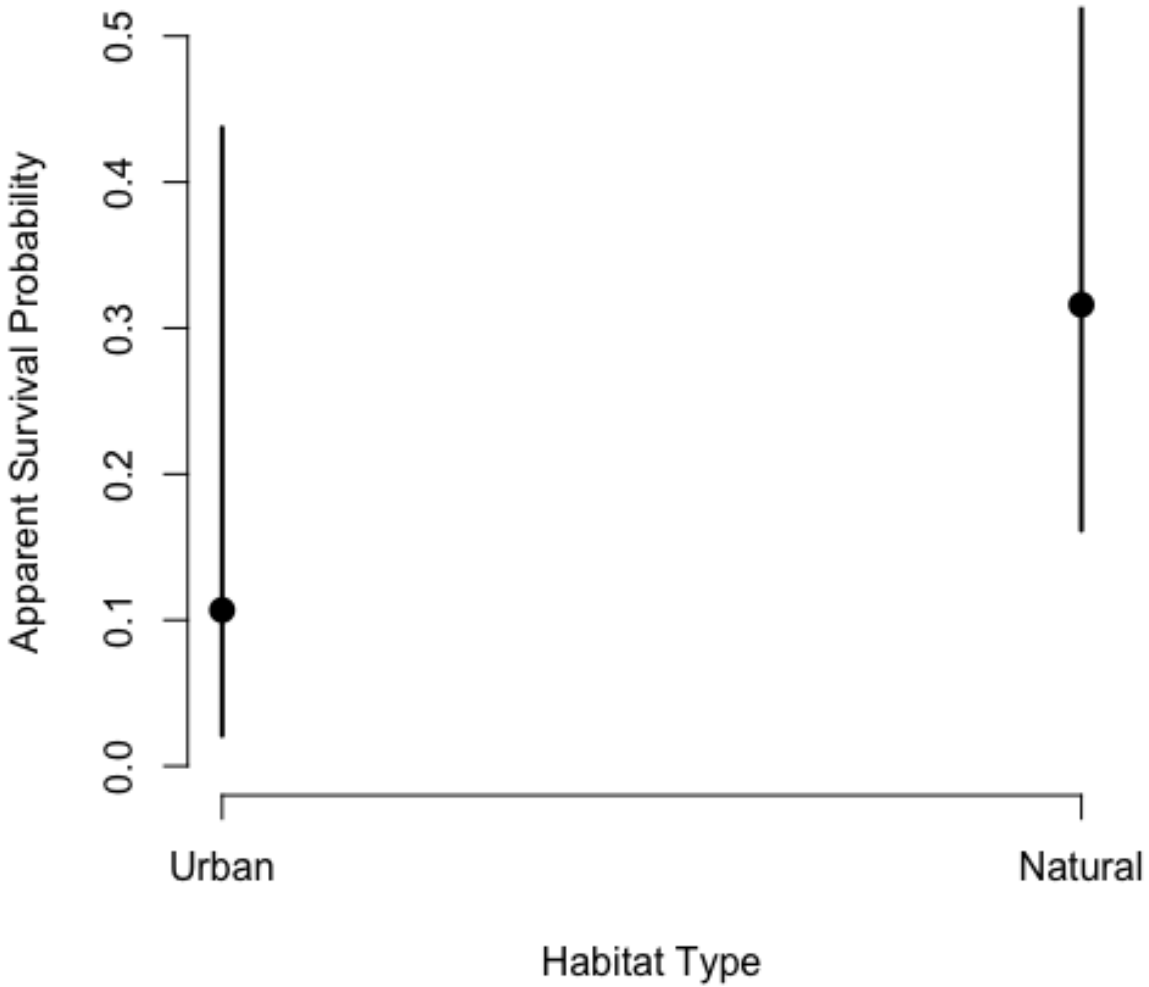
### *Cormack-Jolly-Seber Model*

Mean apparent survival was higher at natural areas outside of St. George ( $\Phi = 0.316$ , 95 % CRI = 0.162-0.596) compared to urban areas within St. George (Fig. 2.2;  $\Phi = 0.107$ , 95 % CRI = 0.021-0.437). The temporal variability in survival was almost twice as great for urban habitats ( $\sigma = 0.883$ , 95 % CRI = 0.031– 3.304) than natural habitats ( $\sigma = 0.481$ , 95 % CRI = 0.016-2.02). Survival rates also increased with bactericidal ability as a covariate, though this immune–survival relationship yielded large uncertainty ( $\beta = 0.181$ , 95 % CRI = -0.121-0.477; Table 2.3; Fig. 2.4). Here, the survival curve showed an increase in survival probability with bactericidal scores, going from a survival rate of <0.2 for lizards with <40% bacterial killing to a peak survival rate of 1.0 for lizards with 100% bactericidal ability.

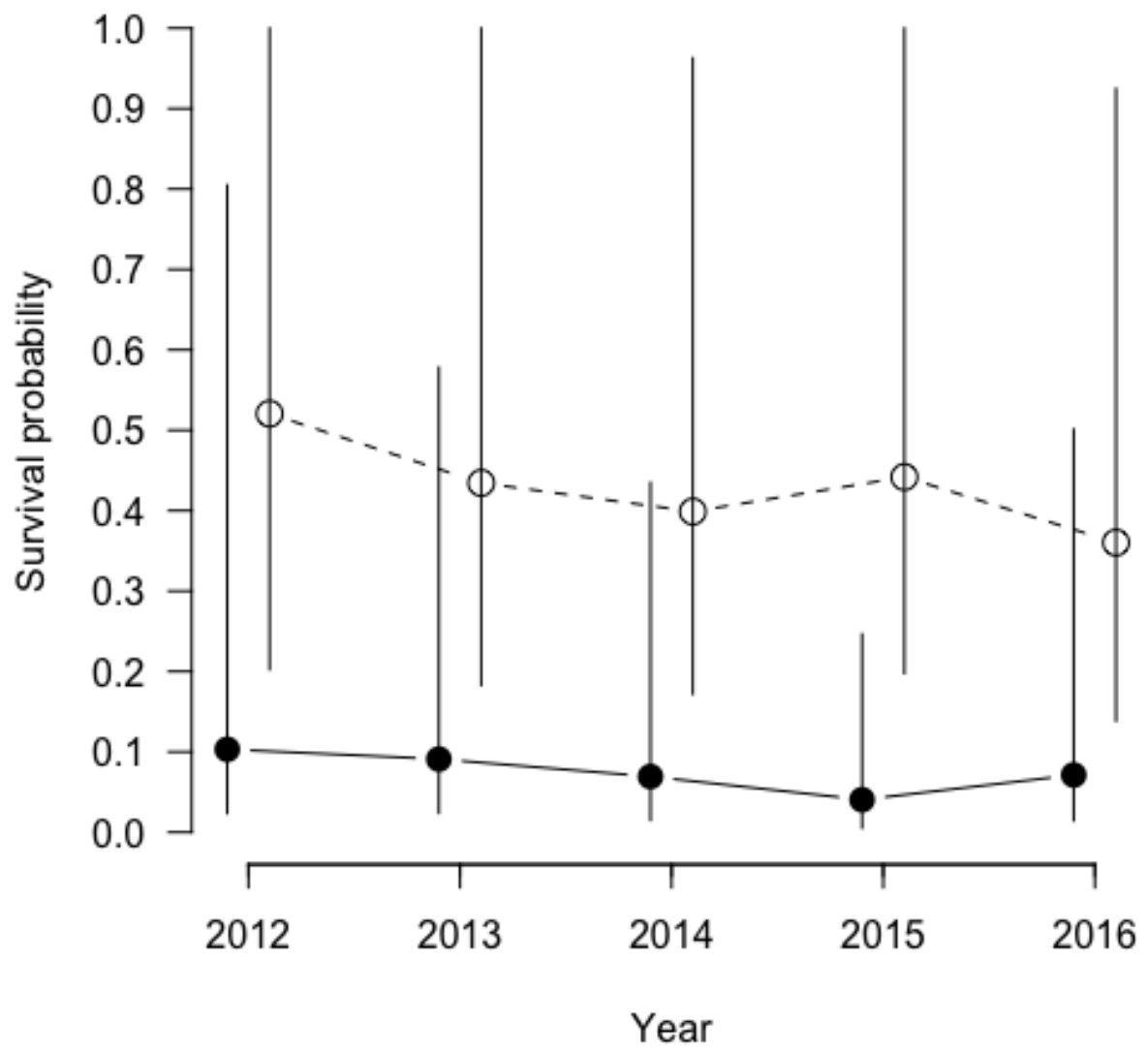
**Table 2.3** Summary statistics for posterior distributions from the top CJS model of demographic parameters based on data collected from urban and natural populations of adult side-blotched lizards between 2012–2017. The 95% credible intervals are defined by the 2.5 and 97.5 percentiles.

Model Component	Parameter	Symbol	Prior distribution	Prior values	Mean	SD	2.5%	97.5%
Survival	Mean apparent survival (urban)	$\Phi$	Uniform	(0, 1)	0.107	0.11	0.021	0.437
	Mean apparent survival (natural)	$\Phi$	Uniform	(0, 1)	0.316	0.11	0.162	0.596
	SD, year random effect on survival (urban)	$\sigma$	Uniform	(0, 10)	0.883	0.923	0.031	3.304
	SD, year random effect on survival (natural)	$\sigma$	Uniform	(0, 10)	0.481	0.614	0.016	2.02
	BKA effect on survival	$\beta$	Normal	(0, 0.001)	0.181	0.153	-0.121	0.477
	Mean capture probability (urban)	$P$	Uniform	(0, 1)	0.502	0.263	0.076	0.97
	Mean capture probability (natural)	$p$	Uniform	(0, 1)	0.252	0.086	0.115	0.451

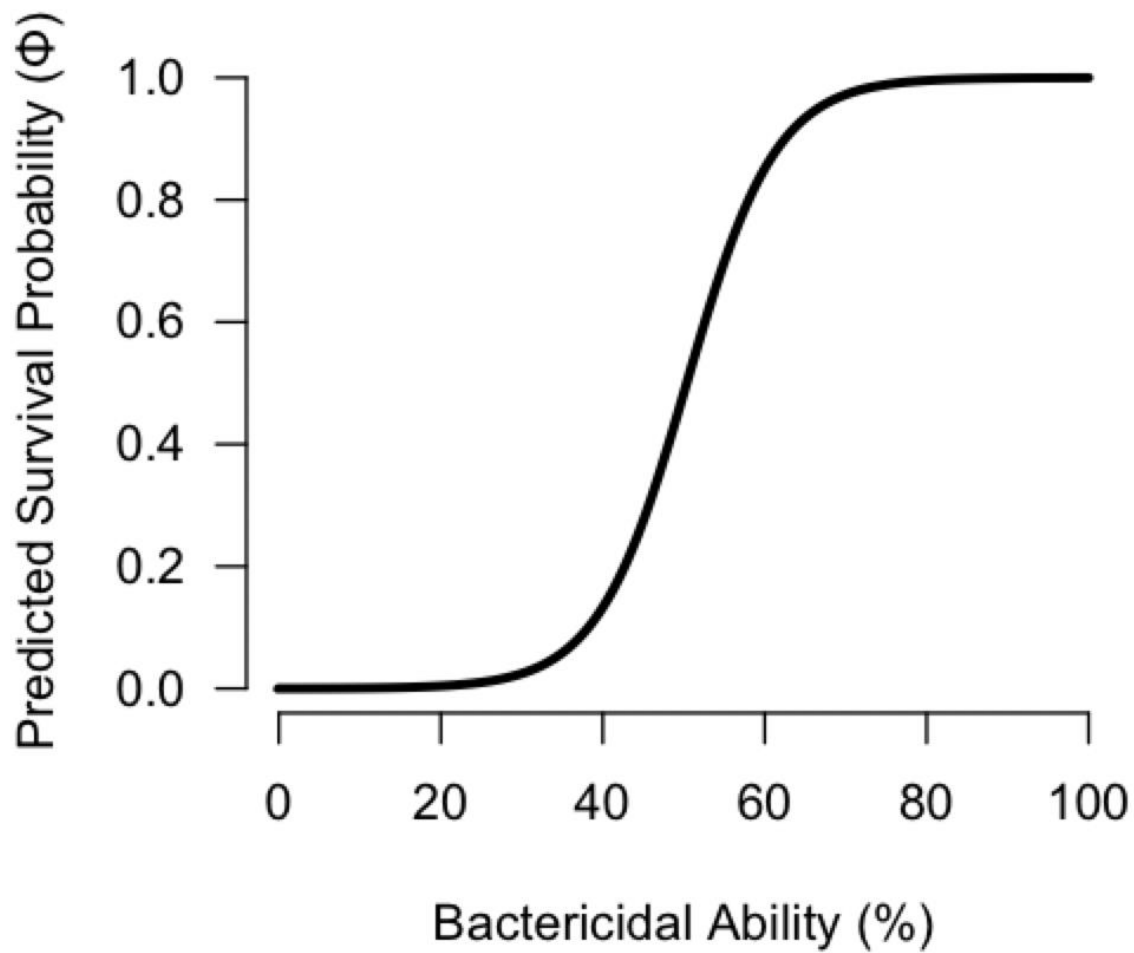
Notes: SD, standard deviation.



**Figure 2.2** Average apparent annual survival estimates ( $\Phi$ ) for adult side-blotched lizards from each habitat type. Points represent posterior means with lines spanning 95% credible intervals.



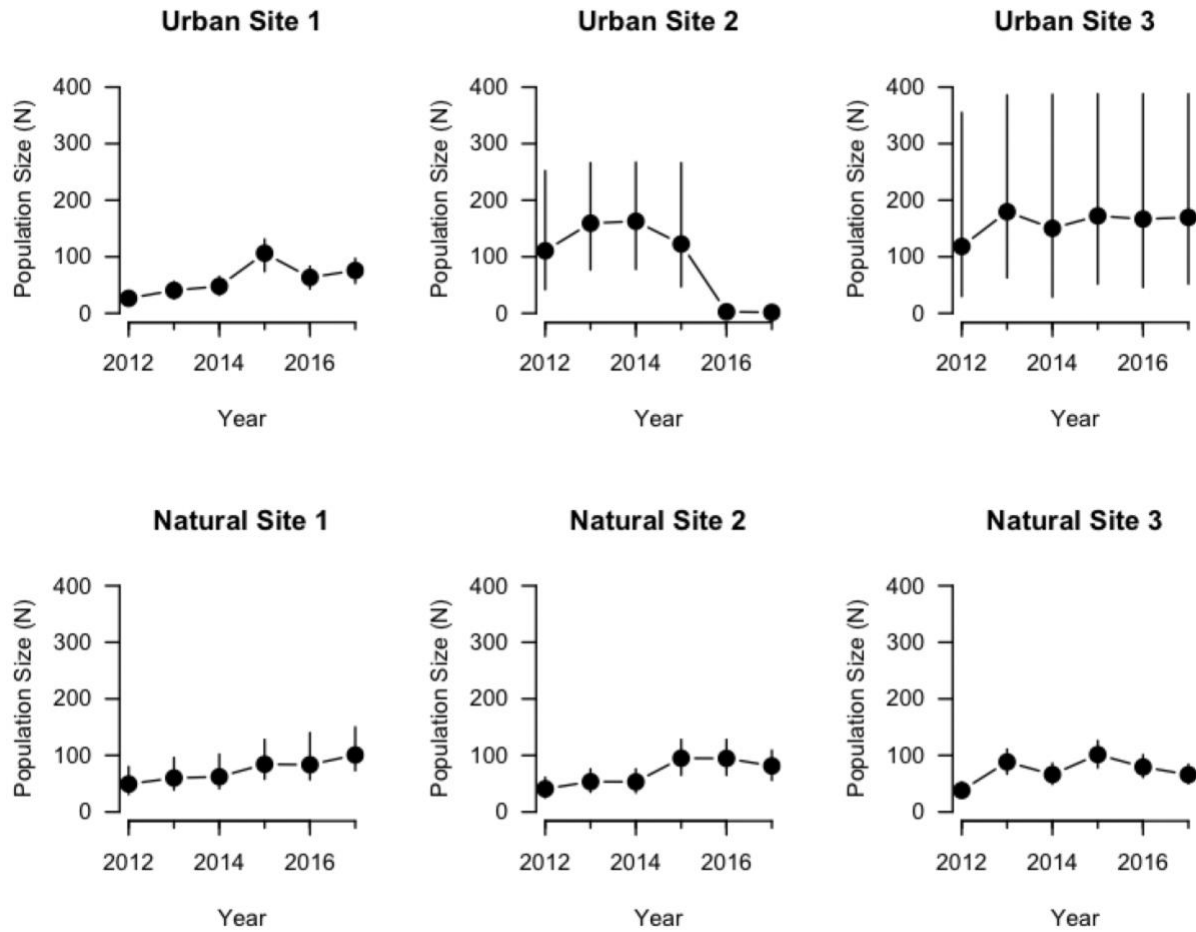
**Figure 2.3** Annual apparent survival ( $\Phi$ ) for adult side-blotched lizards from each habitat type for 2012–2017. Points represent posterior means with lines spanning 95% credible intervals. Solid points indicate urban habitats while hollow points are natural habitats.



**Figure 2.4** Estimated trends in apparent survival ( $\Phi$ ) as a function of bactericidal ability (%) for adult side-blotched lizards across habitat types.

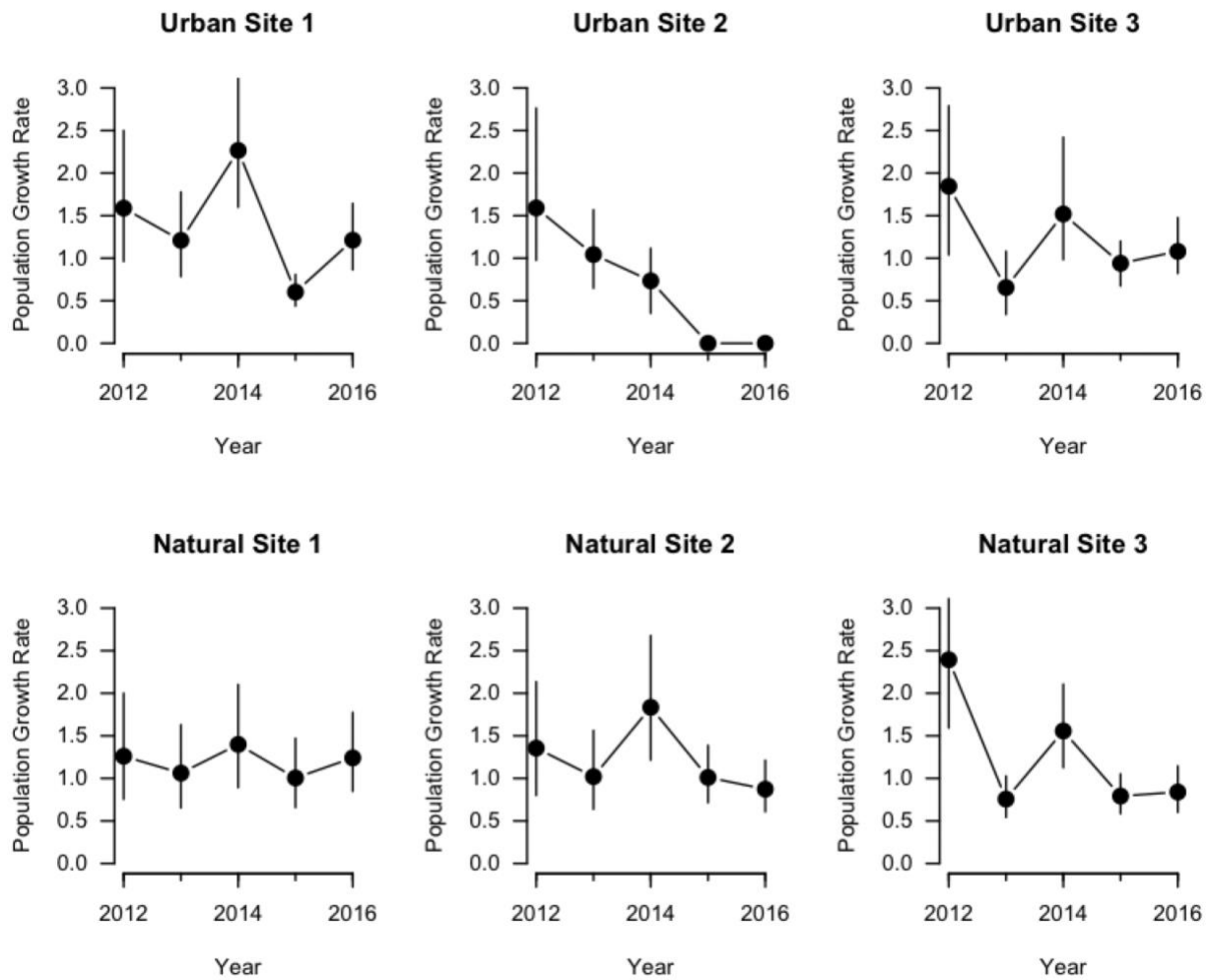
### *Jolly-Seber Model*

Each of the populations were estimated to have increased or remained stable in abundance between 2012-2015 (Table 2.4). By the 2015 sampling occasion, the largest populations were at urban site 2 ( $N = 113.44$ , 95% CRI = 45.0-266.0) and urban site 3 ( $N = 272.13$ , 95% CRI = 57.0-389.0), while the smallest were at natural site 1 ( $N = 83.34$ , 95% CRI = 58.0-124.0) and natural site 2 ( $N = 94.63$ , 95% CRI = 64.0-127.0; Fig. 2.5). Between 2015-2017, most populations decreased but the rate of their trajectories were site-specific (Fig. 2.6), such that in the most extreme case, the population at urban site 2 effectively crashed by 2016 ( $N = 2.66$ , 95% CRI = 0-13) and remained as such in 2017 ( $N = 1.71$ , 95% CRI = 0-9; Fig. 2.5). During the last sampling occasion in 2017, the largest population abundances were at urban site 3 ( $N = 267.10$ , 95% CRI = 58.0-389) and natural site 1 ( $N = 100.01$ , 95% CRI = 72.0-152.0), while the smallest were at urban site 2 ( $N = 1.71$ , 95% CRI = 0-9) and natural site 3 ( $N = 65.87$ , 95% CRI = 49-84; Fig. 2.5). Population density estimates shared similar trends to that of the population abundances (Fig. 2.7), wherein densities by 2015 were largest at urban site 2 ( $N/ha = 345.85$ , 95% CRI = 137.2-810.98) and urban site 3 ( $N/ha = 343.17$ , 95% CRI = 71.88-490.54), while smallest at urban site 2 ( $N/ha = 5.22$ , 95% CRI = 0.0-27.44) and natural site 3 ( $N/ha = 41.43$ , 95% CRI = 30.82-52.83). Per-capita recruitment between 2013-2017 was estimated to be consistently higher at urban site 1 but was otherwise higher at natural sites (Table 2.4; Fig. 2.8). Estimated abundances of the superpopulation (i.e., number of lizards ever alive) at each site during the 2012-2017 period were also comparable (Table 2.4; Fig. 2.9), wherein estimates were highest for natural site 3 ( $N_s = 375.47$ , 95% CRI = 318.0-398.0) but lowest for urban site 2 ( $N_s = 266.94$ , 95% CRI = 253.0-269.0).

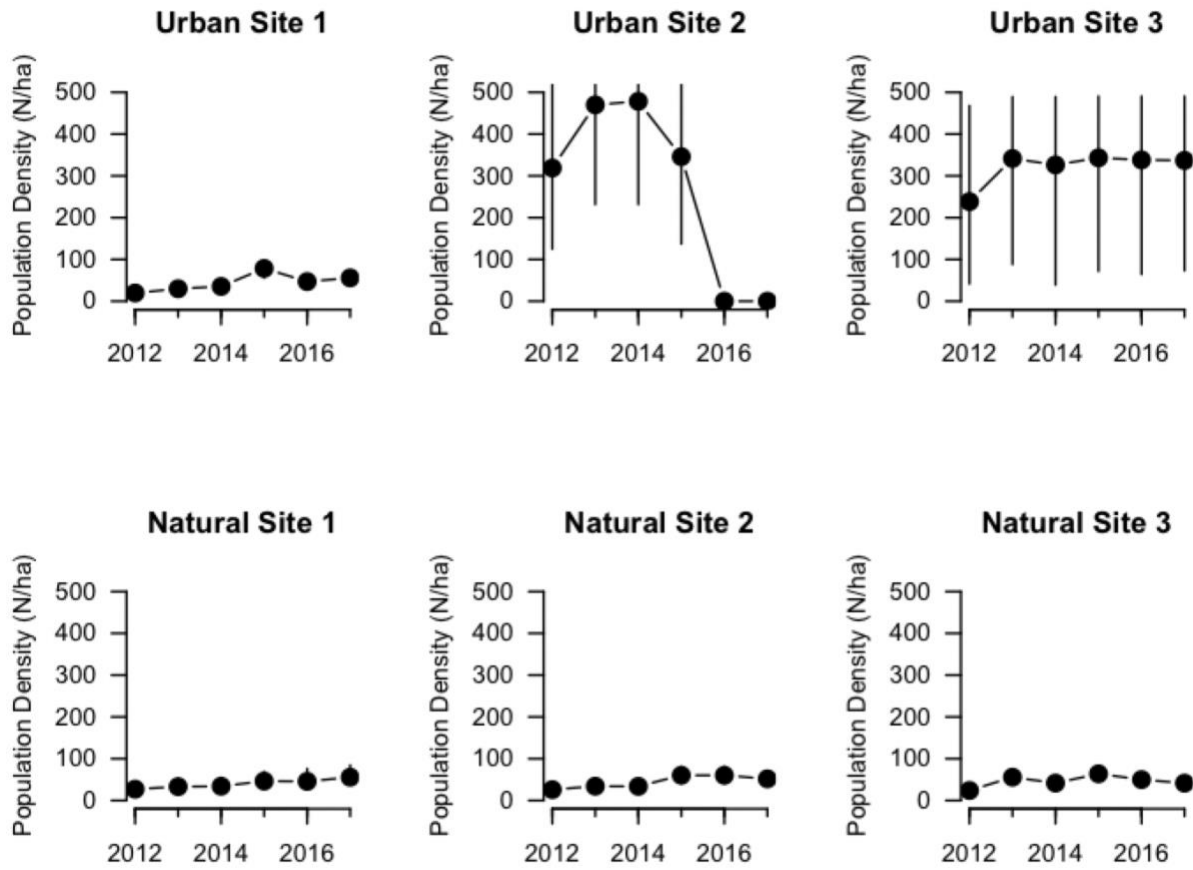


**Figure 2.5** Estimates of annual population abundance ( $N$ ) for adult side-blotched lizards at each site for 2012–2017. Points represent posterior means with lines at each point spanning 95% credible intervals.

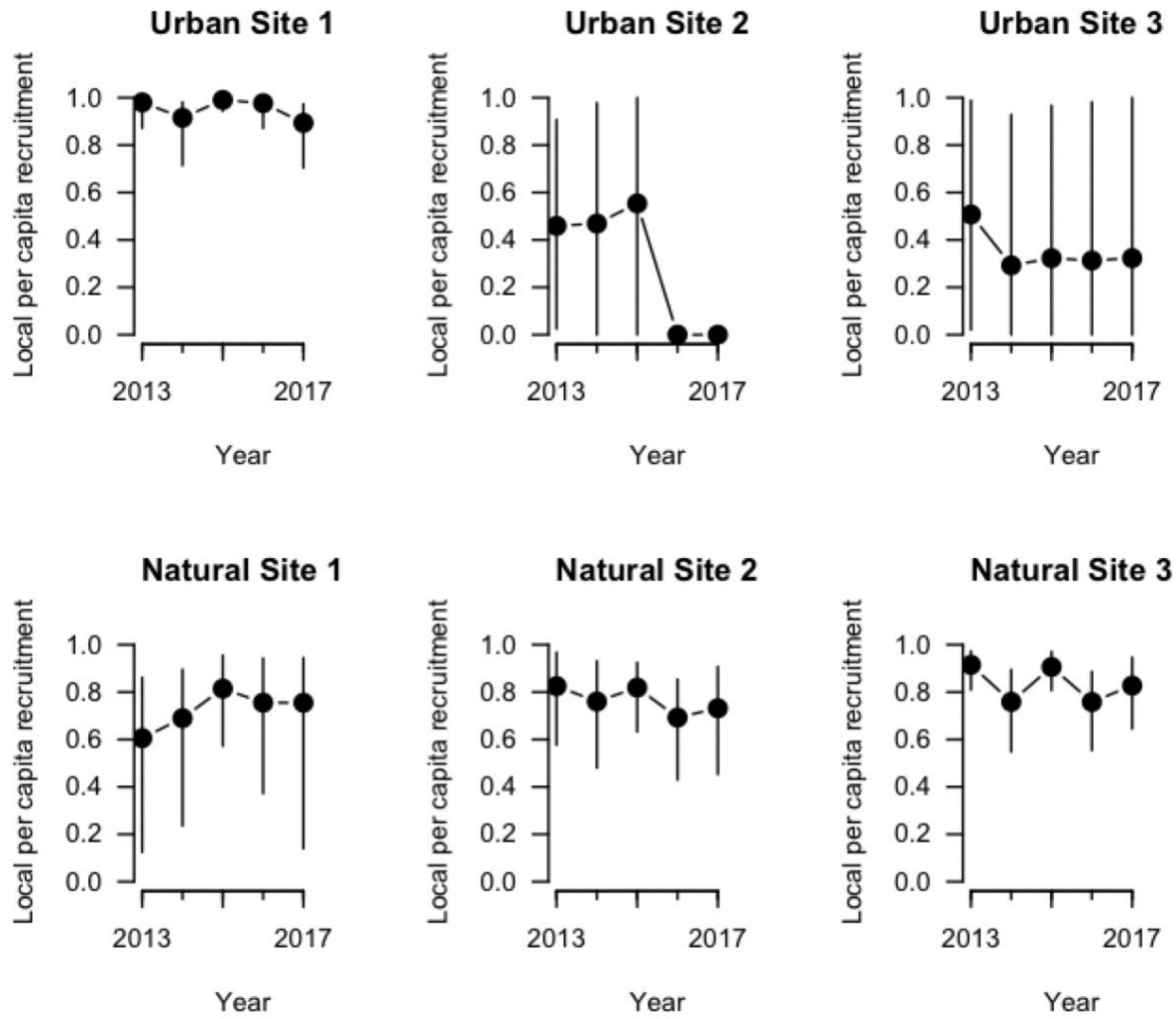




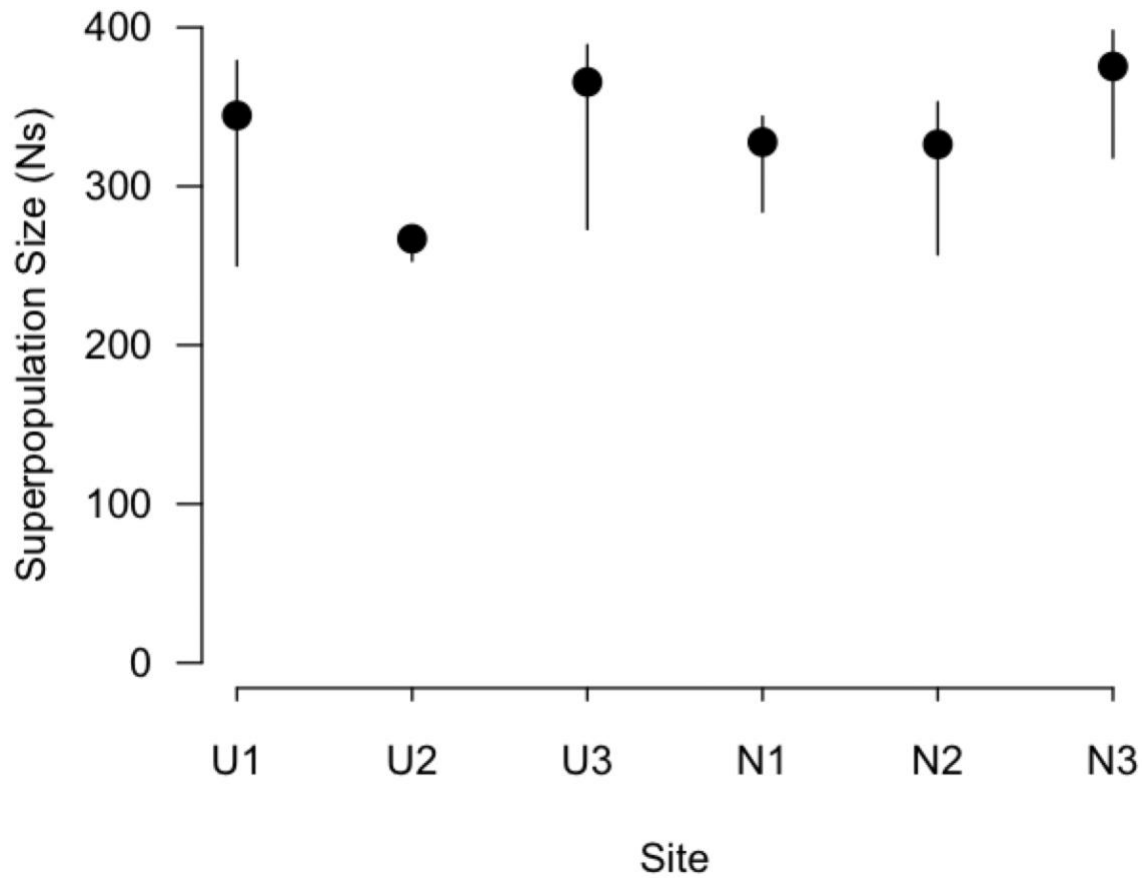
**Figure 2.6** Estimates of population growth rate ( $\lambda$ ) for adult side-blotched lizards at each site for 2012–2016. Points represent posterior means with lines at each point spanning 95% credible intervals.



**Figure 2.7** Estimates of population densities ( $N/ha$ ) for adult side-blotched lizards at each site for 2012–2017. Points represent posterior means with lines at each point spanning 95% credible intervals.



**Figure 2.8** Estimates of local per-capita recruitment for adult side-blotched lizards at each site for 2013–2017. Points represent posterior means with lines at each point spanning 95% credible intervals.



**Figure 2.9** Estimates for the number of adult side-blotched lizards ever alive at each site during the 2012-2017 study (superpopulation size,  $N_s$ ) using restricted occupancy parameterizations. Points represent posterior means with lines at each point spanning 95% credible intervals. Urban and natural sites are respectively abbreviated as ‘U’ and ‘N’, followed by their numeric identifier.

## DISCUSSION

Evidence for urban impacts on wildlife vital rates is amassing, and more studies are revealing the implications of individual physiology when residing in urban habitats (Wikelski & Cooke, 2006). Yet, studies considering both the individual- and population-level responses to urban development are largely underrepresented for reptiles (French et al., 2018). Herein, population vital rates (survival, abundance, and recruitment) of side-blotched lizards residing in urban and natural habitats were assessed with Bayesian hierarchical CMR modeling. Individual physiological traits shaping population-level differences in survival were also identified as a result. Overall, annual survival rates were lower in urban habitats and directly corresponded with individual-level differences in immunocompetence (bactericidal ability). Despite the projected survival differences, as well as an observed population crash at one of the urban sites, estimates of urban population abundance and density were either larger or comparable to those at natural sites, whereas per-capita recruitment was more often higher at natural sites. Altogether, these findings reveal how populations of a widespread lizard species differentially respond to environmental conditions across an urban-natural landscape.

Lower survival estimated at urban habitats is a key indicator that these lizards are negatively impacted by anthropogenic disturbance. The estimates for annual survival herein are consistent with seasonal estimates previously attained for some of these populations (Lucas & French, 2012), suggesting short-term models of survival can, to some extent, predict the negative effect of anthropogenic disturbance on long-term rates. Although survival was comparatively lower at urban habitats, the likelihood of survival was nonetheless estimated to be only moderate for lizards residing in natural habitats. These estimates were unlikely to be overly conflated by movement patterns among populations (i.e., emigration) given the territoriality and small home

range of this species (Wilson, 1991; Scoular et al., 2011). Instead, these findings lend support to side-blotched lizards being generally short-lived in this region (Tinkle, 1967; Gadsden & Castaneda, 2012), prompting the need to monitor other demographic processes (e.g., productivity) in future work. Aside from marked habitat differences in survival, there was also a high degree of uncertainty in the estimates that were likely owed to low recapture rates, and the fate of lizards remaining unknown. Such variability highlights the importance of long-term, multi-population studies for assessing this key vital rate with greater certainty (Sandercock, 2020). Survival estimates from a closely related species, a nearby population of the same species, or from the same population within a narrow period are therefore unlikely to predict accurately the apparent survival in each type of habitat for a focal species. As such, demographic population models should not rely solely on mean estimates, but rather include temporal variation in survival in a stochastic framework, as well as spatial variation (Chandler et al., 2018). Combined, these comparisons suggest that for lizards in urban habitats, there is greater potential for mortality and thus a shorter lifespan, as compared to lizards in undisturbed, natural habitats.

Despite the lower likelihood of urban survival, most of the *U. stansburiana* populations were comparable in abundance at urban and natural areas. Moreover, populations in both habitat types exhibited relatively stable growth rates with some site-specific fluctuations each year, though lower urban survival was consistent with the observed population crash at urban site 2. Apart from this extreme circumstance, the moderate to large urban population abundance indicate *U. stansburiana*, among other lizards (*Phrynosoma* spp., *Sceloporus* spp.) can otherwise tolerate the anthropogenic disturbances that have been normalized in these riparian areas (Doherty et al., 2020; Vesny et al., 2021). The capacity to do so in this species may be attributable to their short generation times (~1 year), which allow surviving hatchlings to reach maturity and

reproduce during subsequent breeding seasons (Tinkle, 1967). In addition, urban *U. stansburiana* can exhibit greater reproductive output (i.e., larger clutch sizes; Lucas & French, 2012), which may be a tradeoff with survival to maintain population viability (Capdevila et al., 2022). This was evident at urban site 1, where population abundance was lower and less dense than at the other urban sites, allowing for higher per-capita recruitment that was on par with that of the natural populations. Here, local recruitment was assumed to be mainly due to reproductive output, in contrast to the natural sites, where recruitment was more likely attributed to both reproduction and immigration from surrounding areas (Le Galliard, 2005). This would help explain how the numbers of lizards ever alive at each site during the study (i.e., superpopulation) were similar, if reproductive output was in fact higher at urban sites. Altogether, the product of these vital rates reconcile how populations of this species can persist in face of anthropogenic disturbances.

The urban riparian zones from which the lizards were sampled host various disturbances that could be compromising survival (personal observation). The underlying facilitator among these is arguably the replacement of nearby native vegetation and rocky areas with built-up infrastructure (e.g., buildings, parking lots, roads, recreational trails, bridges, retention walls). An extreme case of this process was evident when urban site 2 was cleared in 2015 for the development of a parking lot and shopping center, which extirpated the local population (i.e., no lizards were found in subsequent seasons). If any lizards did survive the site demolition, they were likely displaced elsewhere. Urban sites 1 and 3 seem to be less susceptible to this degree of habitat loss as they are both within designated recreation areas. However, these areas have been partially cleared and landscaped for a highly trafficked trail, as well as for nearby commercial and residential structures and lots. These artificial barriers, impervious surfaces, and nearby

human activities likely confine most lizards to the challenges within the remnant patches, while those that leave are potentially threatened by roadside collisions (Koenig et al., 2002; Andrews et al., 2015) and a potential lack of available resources elsewhere (van Heezik & Ludwig, 2012).

Unlike the space available at the natural sites, the limited area allotted for lizards at most urban sites are likely contributing to their higher densities, including those observed for other species with similar habitat requirements (e.g., *Urosaurus ornatus*, *Sceloporus magister*, *Cnemidophorus tigris*). If resources such as food, refuge, and/or mates are ever contested in these densely populated areas (particularly at smaller urban patches), increased agonistic encounters may occur (e.g., Dunham, 1980; Smith, 1981; Vervust et al., 2009; Lancaster et al., 2011), along with injuries that compromise survival (Wilson, 1992; Fox & McCoy, 2000; Baxter-Gilbert & Whiting, 2019). Similarly, the greater prevalence of predators observed in these areas, including cats (*F. catus*), coachwhip snakes (*M. flagellum*), and roadrunners (*G. californianus*), indicates urban *U. stansburiana* are more likely susceptible to injuries and mortality as prey items (Wilson, 1991; Koenig et al., 2002; Audsley et al., 2006; Stobo-Wilson et al., 2021; Mella-Méndez et al., 2022). A greater potential for intra- and interspecies interactions among the urban *U. stansburiana* are corroborated by their higher detection probabilities (i.e., probability of recapture). Frequent contact with competitors and predators also increases the likelihood of disease and parasite transmission, which would also threaten survival (Bradley & Altizer, 2007; Brearley et al., 2013; Becker et al., 2015; Murray et al., 2019). Combined, the intensities of these disturbances at urban versus natural sites are liable to shape the different survival outcomes found for *U. stansburiana*. Yet, since the inferences herein are based solely on observations. Explicit tests linking their survival to these variable urban factors, among others, should be considered in future work. When accounting for individual physiology, survival



estimates were best supported by the data when expressed as a function of bactericidal ability. Here, the likelihood of survival across habitats was greater for lizards with better immunocompetence. These findings corroborate previous within-year survival models that included bactericidal ability as a covariate (Lucas & French, 2012). Taken together, aspects of individual immunity may therefore be a key aspect of population vital rates for *U. stansburiana*. Indeed, immune function has been well documented to be pivotal for lizards recovering from wounds and infections that can occur during environmental challenges (e.g., territorial disputes, predatory encounters). Habitat differences in survival observed in this study may therefore be partly explained by the greater injury rates that can occur for lizards in urban areas (Winchell et al., 2019; Baxter-Gilbert & Whiting, 2019; Balakrishna et al., 2021), coupled with the lower immune function (i.e., wound healing rates, bactericidal ability) previously found for urban populations of this species (Lucas & French, 2012). Given the energetic costs of immune defense and cellular repair (Demas et al., 2011, 2012), lizards in urban areas may be less likely to afford recovery from an immune challenge (Hudson et al., 2021a). For an effective response, tradeoffs between the immune system and other physiological demands are often necessary when resources are limited for lizards (Meylan et al., 2013; Neuman-Lee & French, 2014; Smith et al., 2017; Hudson et al., 2021b). The chances of mortality could therefore be greater for lizards with an insufficient energy budget or one that prioritizes investment in other systems (Lochmiller & Deerenberg, 2000; Graham et al., 2011).

Despite less support for baseline corticosterone or oxidative stress as covariates in estimating survival, these two parameters may still be relevant due to their roles in immune tradeoffs. Given that corticosterone secretion helps to mobilize energy for the demands of daily (e.g., foraging, social interactions) and seasonal routines (e.g., reproductive cycles), as well as

life history responses to predictable and unpredictable events (Wingfield et al., 1998; Sapolsky et al., 2000; Landys et al., 2006; Angelier & Wingfield, 2013; Crespi et al., 2013), investment may be divested from immunity as a result (Cain & Cidlowski, 2017; Bereshchenko et al., 2018). Indeed, elevated baseline corticosterone levels that accompany excessive allostatic load during environmental challenges, whether they be natural or anthropogenic, can suppress lizard immunity and impair survival (Svensson et al., 2002; Comendant et al., 2003; Josseland et al., 2017). Conversely, reactive oxygen species produced by immune cells during an injury or infection can cause oxidative stress that, if not combated, can lead to oxidative damage and reduced longevity (Soneja et al., 2005; Sorci & Faivre, 2009; Nathan & Cunningham-Bussell, 2013; Costantini, 2014). Oxidative stress arising from other physiological processes (e.g., reproduction; Webb et al., 2019; French et al., 2021; Virgin et al., 2022) can alternatively impair immunity by reducing immune cell function and, in turn, compromise recovery from a challenge (Sordillo & Aitken, 2009; Cannizzo et al., 2011; Rahal et al., 2014; Tobler et al., 2015). As such, those that can afford the recurring energetic and oxidative costs of immunity may be more likely to survive. If so, lizards of each sex may be differentially affected due to their respective physiological demands during the breeding season. Since low statistical power (i.e., limited sample size) prevented the inclusion of multiple individual covariates to test for interactive or even additive effects, relationships between circulating corticosterone, oxidative stress, and immunity should be considered for each sex in future work on urban lizard survival.

In conclusion, this research provides important information on population vital rates of lizards residing in urban and natural habitats, which is currently needed for wildlife conservation (Magle et al., 2012). Lower annual survival and population crashes that occur in urban areas occupied by this wide-spread and resilient lizard emphasize the need for management actions to

mitigate effects induced by anthropogenic disturbances, especially for species that are more vulnerable than others. These results also demonstrate the power and flexibility of Bayesian hierarchical models for estimating the vital rates of a lizard species, particularly the effect of individual covariates on survival, such that a researchers' ability to extract the most information from available data could be improved. Physiological characteristics that allow reptiles to survive and persist in variable landscapes, such as immune function for a small lizard species, help elucidate potential fitness and population-level consequences of anthropogenic stressors. In future monitoring of lizard populations, especially those in urban habitats, physiological measures should continue to be taken as predictors of population sustainability. This will be useful for developing sustainable management regimes for populations vulnerable to the effects of anthropogenic disturbance, as well as better understanding the processes that drive observed population changes.

#### LITERATURE CITED

- Andrews, K. M., Langen, T. A., & Struijk, R. P. (2015). Reptiles: overlooked but often at risk from roads. *Handbook of road ecology*, 271-280.
- Angelier, F., & Wingfield, J. C. (2013). Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives. *General and Comparative Endocrinology*, 190, 118-128.
- Audsley, B. W., Bock, C. E., Jones, Z. F., Bock, J. H., & Smith, H. M. (2006). Lizard abundance in an exurban southwestern savanna, and the possible importance of roadrunner predation. *The American midland naturalist*, 155(2), 395-401.

- Balakrishna, S., Amdekar, M. S., & Thaker, M. (2021). Morphological divergence, tail loss, and predation risk in urban lizards. *Urban Ecosystems*, 24(6), 1391-1398.
- Baxter-Gilbert, J. H., & Whiting, M. J. (2019). Street fighters: Bite force, injury rates, and density of urban Australian water dragons (*Intellagama lesueurii*). *Austral Ecology*, 44(2), 255-264.
- Becker, D. J., Streicker, D. G., & Altizer, S. (2015). Linking anthropogenic resources to wildlife–pathogen dynamics: a review and meta-analysis. *Ecology Letters*, 18(5), 483-495.
- Bereshchenko, O., Bruscoli, S., & Riccardi, C. (2018). Glucocorticoids, sex hormones, and immunity. *Frontiers in immunology*, 9, 1332.
- Bradley, C. A., & Altizer, S. (2007). Urbanization and the ecology of wildlife diseases. *Trends in ecology & evolution*, 22(2), 95-102.
- Brearley, G., Rhodes, J., Bradley, A., Baxter, G., Seabrook, L., Lunney, D., Liu, Y., & McAlpine, C. (2013). Wildlife disease prevalence in human-modified landscapes. *Biological Reviews*, 88(2), 427-442.
- Brooks, S. P., & Gelman, A. (1998). General methods for monitoring convergence of iterative simulations. *Journal of computational and graphical statistics*, 7(4), 434-455.
- Bureau, U. S. C. (2022). *Over Two-Thirds of the Nation's Counties Had Natural Decrease in 2021*. <https://www.census.gov/newsroom/press-releases/2022/population-estimates-counties-decrease.html>
- Cain, D. W., & Cidlowski, J. A. (2017). Immune regulation by glucocorticoids. *Nature Reviews Immunology*, 17(4), 233-247.
- Cannizzo, E. S., Clement, C. C., Sahu, R., Follo, C., & Santambrogio, L. (2011). Oxidative stress, inflamm-aging and immunosenescence. *Journal of proteomics*, 74(11), 2313-2323.

- Capdevila, P., Stott, I., Cant, J., Beger, M., Rowlands, G., Grace, M., & Salguero-Gómez, R. (2022). Life history mediates the trade-offs among different components of demographic resilience. *Ecology Letters*, *25*(6), 1566-1579.
- Chandler, R. B., Hepinstall-Cymerman, J., Merker, S., Abernathy-Conners, H., & Cooper, R. J. (2018). Characterizing spatio-temporal variation in survival and recruitment with integrated population models. *The Auk: Ornithological Advances*, *135*(3), 409-426.
- Choudhury, S., De, T. K., Maiti, B., & Ghosh, A. (1982). Circadian rhythm in blood sugar and adrenomedullary hormonal concentrations in an avian and a reptilian species. *General and Comparative Endocrinology*, *46*(1), 110-112.
- Comendant, T., Sinervo, B., Svensson, E. I., & Wingfield, J. (2003). Social competition, corticosterone and survival in female lizard morphs. *Journal of evolutionary biology*, *16*(5), 948-955.
- Cormack, R. M. (1964). Estimates of survival from the sighting of marked animals. *Biometrika*, *51*(3/4), 429-438.
- Costantini, D. (2014). *Oxidative stress and hormesis in evolutionary ecology and physiology: a marriage between mechanistic and evolutionary approaches*. Springer Science & Business Media.
- Crespi, E. J., Williams, T. D., Jessop, T. S., & Delehanty, B. (2013). Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? *Functional Ecology*, *27*(1), 93-106.
- Dallman, M. F., Akana, S. F., Cascio, C. S., Darlington, D. N., Jacobsen, L., & Levin, N. (1987). Regulation of ACTH secretion: variations on a theme of B. Proceedings of the 1986 Laurentian Hormone Conference,
- Demas, G., Nelson, R. J., & Nelson, R. (2012). *Ecoimmunology*. OUP USA.

- Demas, G. E., Zysling, D. A., Beechler, B. R., Muehlenbein, M. P., & French, S. S. (2011). Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts. *Journal of Animal Ecology*, *80*(4), 710-730.
- Doherty, T. S., Balouch, S., Bell, K., Burns, T. J., Feldman, A., Fist, C., Garvey, T. F., Jessop, T. S., Meiri, S., & Driscoll, D. A. (2020). Reptile responses to anthropogenic habitat modification: A global meta-analysis. *Global Ecology and Biogeography*, *29*(7), 1265-1279.
- Dornelas, M., Gotelli, N. J., Shimadzu, H., Moyes, F., Magurran, A. E., & McGill, B. J. (2019). A balance of winners and losers in the Anthropocene. *Ecology Letters*, *22*(5), 847-854.
- Dunham, A. E. (1980). An experimental study of interspecific competition between the iguanid lizards *Sceloporus merriami* and *Urosaurus ornatus*. *Ecological Monographs*, *50*(3), 309-330.
- Endriss, D. A., Hellgren, E. C., Fox, S. F., & Moody, R. W. (2007). Demography of an urban population of the Texas horned lizard (*Phrynosoma cornutum*) in central Oklahoma. *Herpetologica*, *63*(3), 320-331.
- Filgueiras, B. K., Peres, C. A., Melo, F. P., Leal, I. R., & Tabarelli, M. (2021). Winner–loser species replacements in human-modified landscapes. *Trends in ecology & evolution*, *36*(6), 545-555.
- Fox, S. F., & McCoy, J. K. (2000). The effects of tail loss on survival, growth, reproduction, and sex ratio of offspring in the lizard *Uta stansburiana* in the field. *Oecologia*, *122*, 327-334.
- Frederiksen, M., Lebreton, J. D., Pradel, R., Choquet, R., & Gimenez, O. (2014). Identifying links between vital rates and environment: a toolbox for the applied ecologist. *Journal of Applied Ecology*, *51*(1), 71-81.
- French, S. S., & Neuman-Lee, L. A. (2012). Improved ex vivo method for microbiocidal activity across vertebrate species. *Biology Open*, *1*(5), 482-487.

- French, S. S., Virgin, E. E., Ki, K. C., Maryon, D. F., Goode, A. B., & Pasachnik, S. A. (2021). Reproductive stage and clutch size incur energetic and oxidative costs in an endangered iguana, *Ctenosaura oedirhina*. *Journal of Herpetology*, *55*(2), 192-200.
- French, S. S., Webb, A. C., Hudson, S. B., & Virgin, E. E. (2018). Town and country reptiles: a review of reptilian responses to urbanization. *Integrative and Comparative Biology*, *58*(5), 948-966.
- Gadsden, H. c., & Castañeda, G. (2012). Demography of the Side-Blotched Lizard, *Uta stansburiana*, in sand dunes of the central Chihuahuan Desert. *The Southwestern Naturalist*, *57*(3), 292-303.
- Gaillard, J.-M., & Yoccoz, N. G. (2003). Temporal variation in survival of mammals: a case of environmental canalization? *Ecology*, *84*(12), 3294-3306.
- Gelman, A., & Hill, J. (2007). Data analysis using regression and hierarchical/multilevel models. *New York, NY: Cambridge*.
- Gelman, A., Hwang, J., & Vehtari, A. (2014). Understanding predictive information criteria for Bayesian models. *Statistics and computing*, *24*(6), 997-1016.
- Gibbons, J. W., Scott, D. E., Ryan, T. J., Buhlmann, K. A., Tuberville, T. D., Metts, B. S., Greene, J. L., Mills, T., Leiden, Y., & Poppy, S. (2000). The Global Decline of Reptiles, Déjà Vu Amphibians: Reptile species are declining on a global scale. Six significant threats to reptile populations are habitat loss and degradation, introduced invasive species, environmental pollution, disease, unsustainable use, and global climate change. *BioScience*, *50*(8), 653-666.
- Gimenez, O., Rossi, V., Choquet, R., Dehais, C., Doris, B., Varella, H., Vila, J.-P., & Pradel, R. (2007). State-space modelling of data on marked individuals. *ecological modelling*, *206*(3-4), 431-438.
- Graham, A. L., Shuker, D. M., Pollitt, L. C., Auld, S. K., Wilson, A. J., & Little, T. J. (2011). Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. *Functional Ecology*, *25*(1), 5-17.

- Halstead, B., Wylie, G., Coates, P., Valcarcel, P., & Casazza, M. (2012). Bayesian shared frailty models for regional inference about wildlife survival. *Animal Conservation*, *15*(2), 117-124.
- Hansen, A. J., Knight, R. L., Marzluff, J. M., Powell, S., Brown, K., Gude, P. H., & Jones, K. (2005). Effects of exurban development on biodiversity: patterns, mechanisms, and research needs. *Ecological applications*, *15*(6), 1893-1905.
- Hofer, H., & East, M. L. (1998). Biological conservation and stress. *Advances in the Study of Behavior*, *27*, 405-525.
- Hooten, M. B., & Hobbs, N. T. (2015). A guide to Bayesian model selection for ecologists. *Ecological Monographs*, *85*(1), 3-28.
- Hudson, S. B., Virgin, E. E., Brodie, E. D., & French, S. S. (2021b). Recovery from discrete wound severities in side-blotched lizards (*Uta stansburiana*): implications for energy budget, locomotor performance, and oxidative stress. *Journal of Comparative Physiology B*, *191*, 531-543.
- Hudson, S. B., Virgin, E. E., Kepas, M. E., & French, S. S. (2021a). Energy expenditure across immune challenge severities in a lizard: consequences for innate immunity, locomotor performance and oxidative status. *Journal of Experimental Biology*, *224*(17), jeb242608.
- Jolly, G. M. (1965). Explicit estimates from capture-recapture data with both death and immigration-stochastic model. *Biometrika*, *52*(1/2), 225-247.
- Jones, M., & Gillham, B. (1988). Factors involved in the regulation of adrenocorticotrophic hormone/beta-lipotrophic hormone. *Physiological reviews*, *68*(3), 743-818.
- Jones, S. M., & Bell, K. (2004). Plasma corticosterone concentrations in males of the skink *Egernia whitii* during acute and chronic confinement, and over a diel period. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *137*(1), 105-113.



- Josserand, R., Dupoué, A., Agostini, S., Haussy, C., Le Galliard, J.-F., & Meylan, S. (2017). Habitat degradation increases stress-hormone levels during the breeding season, and decreases survival and reproduction in adult common lizards. *Oecologia*, *184*, 75-86.
- Katz, E. M., Tolley, K. A., & Altwegg, R. (2013). Survival and abundance of Cape dwarf chameleons, *Bradypodion pumilum*, inhabiting a transformed, semi-urban wetland. *The Herpetological Journal*, *23*(4), 179-186.
- Kéry, M., & Schaub, M. (2011). *Bayesian population analysis using WinBUGS: a hierarchical perspective*. Academic Press.
- Knapp, C. R., & Perez-Heydrich, C. (2012). Using non-conspicuous metrics to examine selected impacts of disturbance on a long-lived reptile. *Endangered Species Research*, *17*(3), 193-200.
- Koenig, J., Shine, R., & Shea, G. (2002). The dangers of life in the city: patterns of activity, injury and mortality in suburban lizards (*Tiliqua scincoides*). *Journal of Herpetology*, *36*(1), 62-68.
- Kolbe, J. J., VanMiddlesworth, P., Battles, A. C., Stroud, J. T., Buffum, B., Forman, R. T., & Losos, J. B. (2016). Determinants of spread in an urban landscape by an introduced lizard. *Landscape Ecology*, *31*, 1795-1813.
- Lancaster, P., Jessop, T. S., & Stuart-Fox, D. (2011). Testing the independent effects of population and shelter density on behavioural and corticosterone responses of tree skinks. *Australian Journal of Zoology*, *58*(5), 295-302.
- Landys, M. M., Ramenofsky, M., & Wingfield, J. C. (2006). Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *General and Comparative Endocrinology*, *148*(2), 132-149.
- Le Galliard, J. F., Ferrière, R., & Clobert, J. (2005). Effect of patch occupancy on immigration in the common lizard. *Journal of Animal Ecology*, *74*(2), 241-249.

- Lebreton, J.-D., Burnham, K. P., Clobert, J., & Anderson, D. R. (1992). Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. *Ecological Monographs*, 62(1), 67-118.
- Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos*, 88(1), 87-98.
- Lucas, L. D., & French, S. S. (2012). Stress-induced tradeoffs in a free-living lizard across a variable landscape: consequences for individuals and populations. *PLoS One*, 7(11), e49895.
- MacLean, G. S., Lee, A. K., & Wilson, K. J. (1973). A simple method of obtaining blood from lizards. *Copeia*, 1973(2), 338-339.
- Magle, S. B., Hunt, V. M., Vernon, M., & Crooks, K. R. (2012). Urban wildlife research: past, present, and future. *Biological conservation*, 155, 23-32.
- Malisch, J. L., Garland Jr, T., Claggett, L., Stevenson, L., Kohl, E. A., & John-Alder, H. B. (2020). Living on the edge: Glucocorticoid physiology in desert iguanas (*Dipsosaurus dorsalis*) is predicted by distance from an anthropogenic disturbance, body condition, and population density. *General and Comparative Endocrinology*, 294, 113468.
- McDonald, R. I., Kareiva, P., & Forman, R. T. (2008). The implications of current and future urbanization for global protected areas and biodiversity conservation. *Biological conservation*, 141(6), 1695-1703.
- McKinney, M. L. (2006). Urbanization as a major cause of biotic homogenization. *Biological conservation*, 127(3), 247-260.
- McKinney, M. L., & Lockwood, J. L. (1999). Biotic homogenization: a few winners replacing many losers in the next mass extinction. *Trends in ecology & evolution*, 14(11), 450-453.

- Mella-Méndez, I., Flores-Peredo, R., Amaya-Espinel, J. D., Bolívar-Cimé, B., Mac Swiney G, M. C., & Martínez, A. J. (2022). Predation of wildlife by domestic cats in a Neotropical city: a multi-factor issue. *Biological Invasions*, 24(5), 1539-1551.
- Meylan, S., Richard, M., Bauer, S., Haussy, C., & Miles, D. (2013). Costs of mounting an immune response during pregnancy in a lizard. *Physiological and Biochemical Zoology*, 86(1), 127-136.
- Mitrovich, M. J., Diffendorfer, J. E., & Fisher, R. N. (2009). Behavioral response of the coachwhip (*Masticophis flagellum*) to habitat fragment size and isolation in an urban landscape. *Journal of Herpetology*, 43(4), 646-656.
- Murray, M. H., Sánchez, C. A., Becker, D. J., Byers, K. A., Worsley-Tonks, K. E., & Craft, M. E. (2019). City sicker? A meta-analysis of wildlife health and urbanization. *Frontiers in Ecology and the Environment*, 17(10), 575-583.
- Nathan, C., & Cunningham-Bussel, A. (2013). Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nature Reviews Immunology*, 13(5), 349-361.
- Neuman-Lee, L. A., & French, S. S. (2014). Wound healing reduces stress-induced immune changes: evidence for immune prioritization in the side-blotched lizard. *Journal of Comparative Physiology B*, 184, 623-629.
- Neuman-Lee, L. A., & French, S. S. (2017). Endocrine-reproductive-immune interactions in female and male Galápagos marine iguanas. *Hormones and behavior*, 88, 60-69.
- Neuman-Lee, L. A., Hudson, S. B., Webb, A. C., & French, S. S. (2020). Investigating the relationship between corticosterone and glucose in a reptile. *Journal of Experimental Biology*, 223(2).
- Pérez-Mendoza, H. A., Zúñiga-Vega, J. J., Martorell, C., Zurita-Gutiérrez, Y. H., Solano-Zavaleta, I., Hernández-Rosas, A. L., & Molina-Moctezuma, A. (2014). Patterns of spatio-temporal variation

- in the survival rates of a viviparous lizard: the interacting effects of sex, reproductive trade-offs, aridity, and human-induced disturbance. *Population Ecology*, 56, 605-618.
- Plummer, M. (2015). JAGS (Version 4.2. 0). Retrieved from [sourceforge.net/projects/mcmc-jags](http://sourceforge.net/projects/mcmc-jags).
- Putman, B. J., Gasca, M., Blumstein, D. T., & Pauly, G. B. (2019). Downsizing for downtown: limb lengths, toe lengths, and scale counts decrease with urbanization in western fence lizards (*Sceloporus occidentalis*). *Urban Ecosystems*, 22, 1071-1081.
- Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., & Dhama, K. (2014). Oxidative stress, prooxidants, and antioxidants: the interplay. *BioMed research international*, 2014.
- R Core Team, R. (2013). R: A language and environment for statistical computing.
- Rees, M., & Ellner, S. P. (2009). Integral projection models for populations in temporally varying environments. *Ecological Monographs*, 79(4), 575-594.
- Rivot, E., & Prévost, E. (2002). Hierarchical Bayesian analysis of capture mark recapture data. *Canadian Journal of Fisheries and Aquatic Sciences*, 59(11), 1768-1784.
- Romero, L. M., & Reed, J. M. (2005). Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 140(1), 73-79.
- Royle, J. A. (2008). Modeling individual effects in the Cormack–Jolly–Seber model: a state–space formulation. *Biometrics*, 64(2), 364-370.
- Royle, J. A., & Dorazio, R. M. (2008). *Hierarchical modeling and inference in ecology: the analysis of data from populations, metapopulations and communities*. Elsevier.
- Royle, J. A., & Dorazio, R. M. (2012). Parameter-expanded data augmentation for Bayesian analysis of capture–recapture models. *Journal of Ornithology*, 152(Suppl 2), 521-537.

- Royle, J. A., Dorazio, R. M., & Link, W. A. (2007). Analysis of multinomial models with unknown index using data augmentation. *Journal of computational and graphical statistics*, *16*(1), 67-85.
- Sandercock, B. K. (2020). Mark-recapture models for estimation of demographic parameters. *Population ecology in practice*, 157-190.
- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine reviews*, *21*(1), 55-89.
- Schaub, M., & Kéry, M. (2012). Combining information in hierarchical models improves inferences in population ecology and demographic population analyses. *Animal Conservation*, *15*(2), 125-126.
- Schofield, M. R., & Barker, R. J. (2011). Full open population capture–recapture models with individual covariates. *Journal of Agricultural, Biological, and Environmental Statistics*, *16*, 253-268.
- Scoular, K. M., Caffry, W. C., Tillman, J. L., Finan, E. S., Schwartz, S. K., Sinervo, B., & Zani, P. A. (2011). Multiyear home-range ecology of common side-blotched lizards in eastern Oregon with additional analysis of geographic variation in home-range size. *Herpetological Monographs*, *25*(1), 52-75.
- Seber, G. A. (1965). A note on the multiple-recapture census. *Biometrika*, *52*(1/2), 249-259.
- Smith, D. C. (1981). Competitive interactions of the striped plateau lizard (*Sceloporus virgatus*) and the tree lizard (*Urosaurus ornatus*). *Ecology*, *62*(3), 679-687.
- Smith, G. D., Neuman-Lee, L. A., Webb, A. C., Angilletta, M. J., DeNardo, D. F., & French, S. S. (2017). Metabolic responses to different immune challenges and varying resource availability in the side-blotched lizard (*Uta stansburiana*). *Journal of Comparative Physiology B*, *187*, 1173-1182.

- Smolensky, N. L., & Fitzgerald, L. A. (2011). Population variation in dune-dwelling lizards in response to patch size, patch quality, and oil and gas development. *The Southwestern Naturalist*, 56(3), 315-324.
- Soneja, A., Drews, M., & Malinski, T. (2005). Role of nitric oxide, nitroxidative and oxidative stress in wound healing. *Pharmacological reports*, 57, 108.
- Sorci, G., & Faivre, B. (2009). Inflammation and oxidative stress in vertebrate host–parasite systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1513), 71-83.
- Sordillo, L. M., & Aitken, S. L. (2009). Impact of oxidative stress on the health and immune function of dairy cattle. *Veterinary immunology and immunopathology*, 128(1-3), 104-109.
- Stebbins, R. C., & McGinnis, S. M. (2018). *Peterson field guide to western reptiles & amphibians*. Houghton Mifflin.
- Stevenson, R. D., Tuberty, S. R., DeFur, P. L., & Wingfield, J. C. (2005). Ecophysiology and conservation: the contribution of endocrinology and immunology—introduction to the symposium. *Integrative and Comparative Biology*, 45(1), 1-3.
- Stobo-Wilson, A. M., Murphy, B. P., Legge, S. M., Chapple, D. G., Crawford, H. M., Dawson, S. J., Dickman, C. R., Doherty, T. S., Fleming, P. A., & Gentle, M. (2021). Reptiles as food: predation of Australian reptiles by introduced red foxes compounds and complements predation by cats. *Wildlife Research*, 48(5), 470-480.
- Su, Y. S., & Yajima, M. (2015). Package ‘r2jags’. *R package version 0.03-08*.
- Svensson, E., Sinervo, B., & Comendant, T. (2002). Mechanistic and experimental analysis of condition and reproduction in a polymorphic lizard. *Journal of evolutionary biology*, 15(6), 1034-1047.
- Tinkle, D. W. (1967). The life and demography of the side-blotched lizard, *Uta stansburiana*.

- Tobler, M., Ballen, C., Healey, M., Wilson, M., & Olsson, M. (2015). Oxidant trade-offs in immunity: an experimental test in a lizard. *PLoS One*, *10*(5), e0126155.
- Tylan, C., Camacho, K., French, S., Graham, S. P., Herr, M. W., Jones, J., McCormick, G. L., O'Brien, M. A., Tennessen, J. B., & Thawley, C. J. (2020). Obtaining plasma to measure baseline corticosterone concentrations in reptiles: How quick is quick enough? *General and Comparative Endocrinology*, *287*, 113324.
- Tyrrell, C., & Cree, A. (1998). Relationships between corticosterone concentration and season, time of day and confinement in a wild reptile (tuatara, *Sphenodon punctatus*). *General and Comparative Endocrinology*, *110*(2), 97-108.
- van Heezik, Y., & Ludwig, K. (2012). Proximity to source populations and untidy gardens predict occurrence of a small lizard in an urban area. *Landscape and urban planning*, *104*(2), 253-259.
- Vassalle, C. (2008). An easy and reliable automated method to estimate oxidative stress in the clinical setting. *Advanced protocols in oxidative stress I*, 31-39.
- Vassalle, C., Petrozzi, L., Botto, N., Andreassi, M., & Zucchelli, G. (2004). Oxidative stress and its association with coronary artery disease and different atherogenic risk factors. *Journal of internal medicine*, *256*(4), 308-315.
- Vassalle, C., Pratali, L., Boni, C., Mercuri, A., & Ndreu, R. (2008). An oxidative stress score as a combined measure of the pro-oxidant and anti-oxidant counterparts in patients with coronary artery disease. *Clinical biochemistry*, *41*(14-15), 1162-1167.
- Vervust, B., Van Dongen, S., Grbac, I., & Van Damme, R. (2009). The mystery of the missing toes: extreme levels of natural mutilation in island lizard populations. *Functional Ecology*, *23*(5), 996-1003.

- Vesy, M. N., Watters, J. L., Moody, R. W., Schauber, E. M., Mook, J. M., & Siler, C. D. (2021). Survivorship and spatial patterns of an urban population of Texas horned lizards. *The Journal of Wildlife Management*, 85(6), 1267-1279.
- Virgin, E. E., Hudson, S. B., Webb, A. C., & French, S. S. (2022). The Immunological and Oxidative Costs of Reproduction and Associations between Maternal and Egg Yolk Physiology in a Reptile. *Ichthyology & Herpetology*, 110(2), 252-261.
- Watanabe, S. (2013). A widely applicable Bayesian information criterion. *The Journal of Machine Learning Research*, 14(1), 867-897.
- Webb, A. C., Iverson, J. B., Knapp, C. R., DeNardo, D. F., & French, S. S. (2019). Energetic investment associated with vitellogenesis induces an oxidative cost of reproduction. *Journal of Animal Ecology*, 88(3), 461-472.
- White, G. C., & Burnham, K. P. (1999). Program MARK: survival estimation from populations of marked animals. *Bird study*, 46(sup1), S120-S139.
- Wikelski, M., & Cooke, S. J. (2006). Conservation physiology. *Trends in ecology & evolution*, 21(1), 38-46.
- Williams, B. K., Nichols, J. D., & Conroy, M. J. (2002). *Analysis and management of animal populations*. Academic press.
- Wilson, B. S. (1991). Latitudinal variation in activity season mortality rates of the lizard *Uta stansburiana*. *Ecological Monographs*, 61(4), 393-414.
- Wilson, B. S. (1992). Tail injuries increase the risk of mortality in free-living lizards (*Uta stansburiana*). *Oecologia*, 92, 145-152.



- Winchell, K. M., Briggs, D., & Revell, L. J. (2019). The perils of city life: patterns of injury and fluctuating asymmetry in urban lizards. *Biological Journal of the Linnean Society*, *126*(2), 276-288.
- Wingfield, J. C., Maney, D. L., Breuner, C. W., Jacobs, J. D., Lynn, S., Ramenofsky, M., & Richardson, R. D. (1998). Ecological bases of hormone—behavior interactions: the “emergency life history stage”. *American Zoologist*, *38*(1), 191-206.
- Wolf, A. J., Hellgren, E. C., Bogosian, V., & Moody, R. W. (2013). Effects of habitat disturbance on Texas horned lizards: an urban case study. *Herpetologica*, *69*(3), 265-281.
- Wood, D. A., Rose, J. P., Halstead, B. J., Stoelting, R. E., Swaim, K. E., & Vandergast, A. G. (2020). Combining genetic and demographic monitoring better informs conservation of an endangered urban snake. *PLoS One*, *15*(5), e0231744.
- Zylstra, E. R., Steidl, R. J., Jones, C. A., & Averill-Murray, R. C. (2013). Spatial and temporal variation in survival of a rare reptile: a 22-year study of Sonoran desert tortoises. *Oecologia*, *173*(1), 107-116.

## CHAPTER III

GENOME-WIDE ASSOCIATION MAPPING OF PHYSIOLOGICAL TRAITS IN WILD  
LIZARD POPULATIONS

Physiological traits are of evolutionary importance given their roles in optimizing fitness for wild animals. While trait performances or capacities are often reported in the literature, physiological expression can be complex, with relationships that vary across environmental conditions. Understanding the genetic architecture of physiological traits is therefore a necessary step to discerning the sources of individual phenotypic variation. Unfortunately, the genetic contributions for these physiological functions (e.g., additive genetic variation) and their relationships with other traits are not known for many taxa. To help fill this knowledge gap, genotyping-by-sequencing data was generated from wild populations of side-blotched lizards (*Uta stansburiana*). Multi-locus genome-wide association mapping was applied to these data, to quantify genetic variation for physiological traits and to identify single nucleotide polymorphisms (SNPs) associated with their expression. The traits considered included basal measures of innate immunity (i.e., bacterial killing ability or BKA), oxidative resistance (i.e., antioxidant capacity or OXY), and energy-mobilizing glucocorticoid hormone (i.e., corticosterone or CORT) in blood plasma. Here, the proportion of the variation in trait expression explained by the genetic data (i.e., “chip” heritability) was estimated along with the number and contribution of individual loci with measurable effects. Genomic prediction was also implemented to assess phenotypic and genetic variance-covariance matrices (P- and G-matrices) within versus among populations. Variation in each trait was owed to a polygenic basis with low

to moderate heritability and few measurable effect loci. The P- and G-matrices were generally similar, though with slight exceptions across populations. These findings will be useful for determining the molecular and cellular functions of candidate regions associated with each trait and inferring which mechanisms have shaped their evolution in reptiles.

## INTRODUCTION

Physiological systems are crucial for the life history and fitness of animals (Stearns, 1992; Roff, 2002), prompting the need to study how and why they vary across environmental contexts (Ricklefs, & Wikelski, 2002). Variation in physiological performance (or capacity) is now assessed using *in situ* and/or *ex situ* methods to mechanistically understand life history patterns (reviewed in Bennett & Huey, 1990; Garland & Carter, 1994; Feder et al., 2000). Yet, the sources giving rise to variation in physiological traits and their relationships remain largely unexplored, despite this information being instrumental to explaining if and how physiological performance is selected upon within and among populations. Apart from environmental sources (i.e., plasticity; Scheiner, 1993), phenotypic variation is assumed to be largely owed to small contributions from genetic variants segregating in animal populations (Lynch & Walsh, 1998). Identifying the genetic contributions to physiological traits of interest is therefore a necessary step to understand how traits perform and interact, how selection may act on each trait, and how the interactions among traits might influence responses to selection.

The functional capacities of physiological traits have been under ongoing scrutiny regarding their ecological and evolutionary relationships in wildlife populations (reviewed in Williams et al., 2021). Of interest in this context is the ability to respond to injuries and

infections via the immune system, as failing to mount an effective immune response can jeopardize organismal health (McKean & Lazzaro, 2011; Wobeser, 2013). Along the same line, the capacity to resist prooxidant chemical species generated for essential cellular functions, including immunity (e.g., Soneja et al., 2005; Costantini & Moller, 2009; Nathan & Cunningham-Bussel, 2013), has been recognized as important because poor antioxidant defenses can undermine longevity via excessive oxidative stress (e.g., oxidative damage to DNA, proteins, and lipids; Halliwell & Gutteridge, 2015; Sies et al., 2017). Moreover, metabolic hormones that mobilize energy stores for mediating responses to environmental challenges (i.e., glucocorticoids; Sapolsky et al., 2000; Crespi et al., 2013) are of importance because of their influence over physiological systems contributing to fitness (Wingfield et al., 1998; Angelier & Wingfield, 2013). In fact, components of immune function and oxidative resistance have been linked to the regulatory functions of glucocorticoids (Costantini et al., 2011; Cain & Cidlowski, 2017; Bereshchenko et al., 2018; Chainy & Sahoo, 2020; Vagasi et al., 2018; Gormally et al., 2019; Majer et al., 2019) and to each other (Sorci & Faivre, 2009; Hasselquist & Nilsson, 2012; Costantini, 2014; Sebastiano et al., 2018), potentiating complex genetic and phenotypic relationships, which remain unmapped. Components of these physiological systems have so far been shown to be directly encoded in the genome and thus inherited through the germ line, to some extent (Kimbrell & Beutler, 2001; Pamplona & Costantini, 2011; Vettorazzi et al., 2021). As such, estimations of their additive genetic variation, or heritability (Zaitlen & Kraft, 2012), are feasible and promising for making evolutionary inference regarding their patterns of phenotypic variation (i.e., degree to which a trait may evolve; Falconer, 1996).

Quantifying heritability in the wild is beginning to extend beyond traditional paradigms (e.g., experimental breeding, pedigree data; Lynch & Walsh, 1998; Kruuk, 2004), via the

identification of genomic regions that harbor genetic variants for each trait. Genotyping-by-sequencing is a rapid, low-cost, and robust approach for screening breeding populations using single nucleotide polymorphisms (SNPs; Narum et al., 2013; Deakin & Ezaz, 2019).

Systematically capturing genotype-phenotype associations is of fundamental significance to understand the genetic architecture of complex quantitative traits, such as those presumably comprising physiological systems. Polygenic mapping methods can relate trait variation to genetic variants simultaneously, to identify potential major-effect mutations, but also to generate robust estimates of trait genetic variances and covariances, regardless of whether phenotypic variation is owed to few or many genes of large or small effect (e.g., Zhou et al., 2013; Moser et al., 2015; Zeng & Zhou, 2017; Zhu & Zhou, 2020). These methods can also parse the relative contributions of genetic variants with measurable (modest to large) versus near-infinitesimal effects to trait genetic variances and covariances (Lucas et al., 2018; Gompert et al., 2019).

Various mapping approaches have so far been applied to quantitative traits of other wild animal populations (e.g., Comeault et al., 2014; Pallares et al., 2014, 2015; Santure et al., 2015, 2018; Johnston et al., 2016; Riesch et al., 2017). All of those support the notion that the complexity of the genotype-phenotype map determines the speed and trajectory of the response to evolutionary processes (Orr, 2000; Hansen & Houle, 2008; Walsh & Blows, 2009; Wagner & Zhang, 2011). Yet, phenotype mapping studies on complex traits remain rare across taxonomic groups and, as a result, comparatively little is known about the genetic underpinnings of some of the most important physiological systems and their evolution.

When mapping physiological traits of a species, patterns of phenotypic and genetic covariances (as captured by P- and G-matrices) should be considered within and among populations, so that evolutionary and ecological inferences can be made on their genetic

architecture. Various morphological and life history traits have so far been shown to exhibit patterns of covariation attributable to genetic and environmental factors (e.g., Delahaie et al., 2017; Sniegula et al., 2018). Stability in these patterns across populations should reflect a degree of genetic constraint for a given set of traits, whereby evolutionary change directs and inhibits variation in certain directions both across space and over time (Puentes et al., 2016). Covariance may therefore occur for physiological traits linked to immunity, oxidative resistance, and energy balance if their genetic basis diverges across populations in a correlated manner. Here, genetic correlations due to one or several loci regulating multiple traits (i.e., pleiotropy; Stearns, 2010) or alleles being tightly linked by nonrandom association (i.e., linkage disequilibrium or LD; Barton, 2011) may cause physiological traits to evolve together, though as mentioned, among-population covariance can also occur among those that are not genetically correlated. In either case, correlated changes in physiological traits could be directed or inhibited simultaneously by several different evolutionary processes among populations including mutation, selection, migration, and drift (Armbruster & Schwaegerle, 1996). Despite the importance of interpreting their causation, no studies to date have considered phenotypic and genetic covariances in the context of mapping physiological traits of reptiles.

The goal of this study was to quantify the genetic architecture of physiological traits within and among local populations of a wide-spread reptile, the side-blotched lizard (*Uta stansburiana*). Squamate reptiles, the group encompassing this lizard species, serve as important models in ecological and evolutionary physiology (e.g., Seebacher, 2005; Kearney et al., 2009; Poletta et al., 2013; Van Dyke et al., 2014; Andrew et al., 2017), suggesting the utility in understanding the genetic basis of their physiological systems. Here, polygenic mapping and genomic prediction were implemented using Bayesian sparse linear mixed models (BSLMMs) to

associate genetic variation with differences in their bacterial killing ability (BKA), antioxidant capacity (OXY), and levels of the glucocorticoid hormone, corticosterone (CORT). Fitting polygenic Bayesian mapping models to genotypic data derived from SNPs allowed for the (i) estimation of the proportion of phenotypic variance in the trait attributable to additive genetic variance among individuals (narrow-sense heritability) or genetic variation explaining physiological trait variation, (ii) identification of SNPs directly and/or indirectly associated with the physiological pathways under scrutiny, and (iii) the extent that candidate SNPs explain their effects on physiological variation. Genomic prediction provided a means to describe patterns of phenotypic and genetic covariances and quantify the consistency of the genetic variance-covariance matrix (G-matrix), so that evolutionary inferences could be made concerning the direction of genetic covariances within versus among populations. Additive genetic variation underlying each physiological trait was hypothesized to be polygenic, but low to moderate in measure, considering that traits closely linked to fitness are generally less heritable (e.g., Gustafsson, 1986; Mousseau & Roff, 1987). However, heritability was predicted to be relatively consistent within local populations. Further, given the suspected low to moderate heritability for physiological traits (e.g., Martins et al., 2019), their phenotypic correlations were not expected to be strongly reflected at the genetic level (i.e., weak genetic correlations) across populations.

## METHODS

### *Study system*

*Uta stansburiana* are small, short-lived, and abundant lizards found throughout dry regions of western North America (Stebbins & McGinnis, 2018). Their distribution is widespread overall, albeit influenced by the availability of riparian zones within deserts and their limited dispersal among

localities (i.e., dispersal beyond 300 m is rare; Doughty & Sinervo, 1994). Researchers have often used *U. stansburiana* for studies of life history, ecophysiology, and evolution (e.g., Tinkle, 1969; Sinervo & Light, 1991). Due to their ease of capture and sampling, large abundance, simple diploid genetics (34 chromosomes; consisting of 12 macro- and 22 microchromosomes), and generation time (~1-2 years), these lizards are suitable for population genetics (Pennock et al., 1968; Turner et al., 1970). Lizards from this study consisted of six populations located in or around one of the fastest growing urban areas in the United States, St. George, Utah (U.S. Census Bureau, 2022). Three populations reside in fragmented habitats within St. George, where they are exposed to novel substrates (e.g., impervious surfaces), daily human interactions (e.g., recreational, construction, and maintenance activities), competitors (e.g., tree lizards), and novel predators (e.g., cats). The remaining three sites are in a natural area north of St. George and are not exposed to similar human pressures. Each site included rocky areas ( $\leq 1.8$  ha) that were situated near or along a riparian corridor. The six sites lay an average of 21 km from one another (minimum = 4 km; maximum = 42 km; Fig. 2.1). All of which were close to the intersection of the Great Basin, Colorado Plateau and Mojave Desert.

### *Field sampling and processing*

Adult side-blotched lizards were captured with a snare pole during their breeding season (May 2012 - 2017) at the sites within and around St. George, Utah, USA ( $N = 757$ ). Urban site 2 was demolished for commercial development in late 2015, and as a result, no lizards were found there in 2016 and 2017 (Table 3.1). Lizards were collected between 0800 – 1300 to limit circadian variation in physiological condition (Dallman et al., 1987; Jones & Gillham, 1988). Whole blood was collected from the retro-orbital sinus of each lizard with a heparinized capillary tube within a validated window of time ( $\leq 3$  minutes) to measure basal physiology (Romero & Reed, 2005; Tylan et al., 2020). Blood



samples were centrifuged at 6000 RPM for 10 minutes to isolate plasma and erythrocytes. To control for performance in physiological assays (Claunch et al., 2022), samples were kept on ice until they were stored at  $-80^{\circ}\text{C}$  for lab analyses at Utah State University in Logan, Utah. After blood processing, a unique combination of one to four whole toes were removed from each lizard for identification, circumventing pseudo-replication of sampling across years. Lizard sex was also determined based on dorsal color pattern (speckled blue for males, ornate brown for females) and the presence or absence of hemipenes. All lizards were returned to their capture locations after completion. The work presented here was approved under the Department of Wildlife Resources COR #1COLL8382 and Utah State University Institutional Animal Care and Use Committee's protocol #2068.

### *Bacterial killing ability*

Bactericidal ability was quantified with a validated volume of blood plasma (6 ul) to assess the relative abundances of circulating immune components (e.g., natural antibodies, antimicrobial peptides; reviewed in Demas, 2011). Using the protocol outlined in French and Neuman-Lee (2012), a 1:2 plasma dilution was combined with  $\text{CO}_2$ -independent medium (Gibco # 18-045-088, ThermoFisher Scientific, Grand Island, NY, USA), 4 nM l-glutamine,  $10^4$  colony-producing units of *Escherichia coli* (EPower™ Microorganisms #483-581-1, ATCC 8739, MicroBioLogics, St. Cloud, MN, USA), and agar broth on a 96-well microplate. Included were both positive (i.e., media and bacteria with no plasma) and negative (i.e., media and no plasma or bacteria) controls to account for potential growth and ensure there was no contamination. The plate was incubated for a 12-h period, at which point absorbance per well was measured with a microplate reader at 300 nm (xMark; BioRad Benchmark, Hercules, CA, USA). Bactericidal ability was then calculated as  $(1 - (\text{absorbance of sample} / \text{absorbance of positive controls})) \times$

100). Each sample was run in duplicate to generate an average percent score of bactericidal ability. Coefficients of variation were <20% within and among all assays.

#### *Antioxidant capacity*

Total non-enzymatic antioxidant capacity was measured using an OXY-Adsorbent test (MC002, Diacron International, Italy), which determines effectiveness of the blood antioxidant barrier by quantifying tolerance of the oxidant action of hypochlorous acid (HClO). Here, 2  $\mu$ l of plasma were diluted in 100  $\mu$ l of distilled water, and manufacturer instructions were followed thereafter for measuring 96-well microplates with a spectrophotometer at 505 nm (xMark, Bio-Rad). Measures of total antioxidant capacity, or OXY (mol HClO/ml), were acquired with coefficients of variation <20% within and among all assays.

#### *Corticosterone*

Following a protocol outlined in Neuman-Lee and French (2017), radioimmunoassays were conducted on plasma samples (10  $\mu$ L) for CORT (Ab: MP Biomedicals # 07-120016). Samples were extracted with 30% ethyl acetate:isooctane and run in duplicate for the assay. Individual recoveries were calculated from the extractions to correct for final concentrations with a minimum level of detection of 0.3 ng/ml. Coefficients of variation were <20% within and among all assays.

#### *Physiological trait measurements*

Hierarchical models were built to remove the potential effects of sex and population, whereby physiological measurements (bactericidal ability, antioxidant capacity, corticosterone levels) were modeled as a function of population-specific sex effects. Hierarchical normal priors were placed on

these effects with a uniform prior for the variance. Residuals were taken by removing the sex and population means when analyzing across populations, but only sex means were removed for analyzing within populations. Models were fit using Markov chain Monte Carlo (MCMC) with the ‘R2jags’ package (v. 0.03-08; Su & Yajima, 2015) in R (v. 3.3.1; R Core Team, 2016). All physiological measurements were standardized to have a mean of zero and SD of one to improve the efficiency of the MCMC sampler and make interpretation of model coefficients easier. For each model, three separate chains were run in parallel with different starting values. Models included 40,000 sampling iterations, with the first 20,000 discarded for adaptation and burn-in, and a thinning rate that kept every sixth sample. To assess model convergence, trace plots were observed to have good mixing of the chains and consistent approximate posterior distributions. Convergence to the posterior distribution was verified by calculating the Gelman-Rubin potential scale reduction diagnostic ( $\hat{r} \leq 1.03$ ; Brooks & Gelman, 1998) for each measurement, using the R package ‘coda’ (v. 0.19.3; Plummer et al., 2006).

#### *Phenotypic variance partitioning*

Prior to genetic mapping and genomic prediction, the proportion of trait variation found among populations was quantified for each trait. The among-population variance was estimated by fitting linear mixed-effect models via restricted maximum likelihood (REML) in the R package ‘lme4’ (v. 1.1.19; Bates et al., 2015). No fixed effects were included in the models aside from the grand mean. The null hypothesis that the among-population variance was 0 was then tested using an exact restricted likelihood ratio test in the R package ‘rlrsim’ (v. 3.1.3; Scheipl et al., 2008), which was based on 10,000 simulated values to approximate the null distribution (Crainiceanu & Ruppert, 2004; Greven et al., 2008).

A principal components analysis (PCA) was also run with the R function ‘prcomp’ to summarize patterns of physiological variation from the phenotypic correlation matrix (i.e., on the centered and scaled phenotypic covariance matrix or P-matrix) across the six populations. To quantify physiological differences among these groups, Bayesian methods were used to fit the first two PC scores for each population to a bivariate normal distribution with the R package ‘rjags’ (v. 4.6; Plummer, 2016). Noninformative priors were placed on the means (normal with  $\mu = 0$  and  $\tau = 1e^{-3}$ ) and on the covariance matrix (inverse Wishart with  $k = 2$  and  $\Sigma = I_2$ ), and inferences were made from two MCMC runs with a 1,000-iteration burn-in, 5,000 sampling iterations, and a thinning interval of four.

#### *DNA isolation and library preparation*

Total DNA was isolated from each erythrocyte sample using Qiagen’s DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA, USA). Reduced complexity, double-digest restriction-site DNA libraries were prepared following Parchman et al. (2012) with modifications from Gompert et al. (2014). Briefly, DNA was digested with the restriction enzymes EcoRI and MseI (NEB, Inc. Ipswich, MA, USA) and adaptor oligonucleotides were ligated onto the digested DNA fragments. The adaptor oligonucleotides included an Illumina adaptor and unique 8–10 bp identification sequences or barcodes for individual lizard recognition. Fragment libraries were PCR-amplified, pooled together, and fragments between 330 and 475 bp were size-selected and purified using a BluePippin (Sage Science, Beverly, MA, USA) at the USU Center for Integrated Biosystems (Logan, UT, USA). Library preparation and sequencing took place in two batches, with 384 samples sequenced on two lanes of an Illumina HiSeq 2500 in 2016 and 373 samples sequenced on two lanes of a HiSeq 4000 in 2017 (Table 3.1). All DNA libraries ( $N = 2$ ) were sequenced at the University of Texas Genomic Sequencing and

Analysis Facility (Austin, TX, USA), totaling ~870 million single-end 100 bp DNA sequences. As detailed below, care was taken during data processing and analyses to avoid possible confounding batch effects.

**Table 3.1** Site information and population by year sample sizes for genome-wide association analysis.

Samples were processed in two batches (1 versus 2 below).

Site	Lat. (°N)	Long. (°W)	Area (acres)	Batch	Year	N
Urban Site 1	37.08161	-113.575	3.35	1	2012	3
				1	2013	0
				1	2014	4
				1	2015	24
				2	2016	23
				2	2017	19
Urban Site 2	37.09926	-113.532	0.81	1	2012	11
				1	2013	9
				1	2014	13
				1	2015	7
Urban Site 3	37.05262	-113.602	1.96	1	2012	0
				1	2013	27
				1	2014	13
				1	2015	23
				2	2016	16
				2	2017	28
Natural Site 1	37.24323	-113.452	4.44	1	2012	3
				1	2013	9
				1	2014	13
				1	2015	12
				2	2016	17
				2	2017	25
Natural Site 2	37.33088	-113.28	3.87	1	2012	1
				1	2013	0
				1	2014	15
				1	2015	19
				2	2016	24
				2	2017	19
Natural Site 3	37.25966	-113.369	3.92	1	2012	2
				1	2013	18
				1	2014	15
				1	2015	29
				2	2016	24
				2	2017	12

### *DNA sequence alignment and variant calling*

Custom Perl scripts were used to first demultiplex pooled DNA sequences, wherein identifier barcodes served to assign DNA sequences to individual lizards (Gompert et al., 2012). Individuals with few sequences (< 180,000) were removed from subsequent analyses ( $N = 40$ ). Reads from all remaining individuals were assembled and clustered *de novo* with the software ‘vsearch’ (v. 1.11.1; Rognes et al., 2016) to create a pseudo-reference genome. Here, highly similar sequences within individuals (98% identity) were clustered together and outputted as individual centroid files, which were then combined and shuffled. A random subset was taken from the combined centroids (54,631,148 sequences) for additional clustering with a lower similarity (90% identity), a minimum sequence length of 64, and a minimum shorter/longer sequence length ratio of 0.8 to generate consensus sequences. The consensus sequences were then filtered to determine whether any would collapse at an even lower similarity (80% identity), which resulted in a subset of 411,929 distinct (non-collapsed) contigs of at least 8 reads. Contigs were then reformatted, wherein consensus sequences between 72-92 bps in length were retained to total 93,134 contigs. The resulting pseudo-reference genome was indexed, and sequences were aligned using algorithms in ‘bwa’ (v. 0.7.5; Li & Durbin, 2009), where a maximum of five nucleotide differences was allowed, with no more than two mismatches in a 20 bp seed, and a quality threshold for read trimming set to 10.

Sequence alignments were sorted and indexed for calling single nucleotide polymorphisms (i.e., SNPs) using ‘samtools’ and ‘bcftools’ (v. 0.1.19; Li et al., 2009). The coefficient for downgrading the mapping quality of reads with excessive mismatches, and the number of reads per position (max depth), per individual were both set to 50. Bases with a quality score below 15 and reads with a mapping quality below 10 were discarded. The prior for  $\theta$  was set to 0.001, and SNPs where the posterior probability of an invariant nucleotide was below 0.01 were retained (Li, 2011). Sites were

filtered using custom Perl scripts and ‘vcftools’ (v. 0.1.15.6; Danecek et al., 2011), calling for bi-allelic SNPs with a minimum coverage of 700 reads, a maximum mean coverage of 20, a 4-read minimum for the non-reference allele, a minimum mapping quality of 30, and a maximum of 20% missing data across individuals. Individuals with a mean coverage of less than 1 were removed ( $N = 240$ ). These filtering criteria were chosen to ensure sufficient coverage to estimate allele frequencies (while accounting for uncertainty in genotypes) and avoid locus drop-in and drop-out (Buerkle & Gompert, 2013). To reduce bias associated with batch effects (Tom et al., 2017), SNPs with substantial differences in sequence coverage between the two sets (more than half the mean coverage of the combined data) were removed. A final set of 7,442 SNPs and 477 individuals were retained, with a mean coverage of  $\sim 2.5x$  (per individual), for downstream analysis (Table 3.1).

#### *Estimating genotypes and allele frequencies*

As in previous studies (e.g., Gompert et al., 2014, 2015), maximum likelihood estimates of the allele frequencies for each SNP were obtained with the computer program ‘estpem’ (Soria-Carrasco et al., 2014; Riesch et al., 2017). This program implements an expectation-maximization algorithm to provide allele frequency estimates that account for genotype uncertainty (Li et al., 2009; Li, 2011). Compared to methods that call genotypes first, this allowed for the inclusion of individuals with a range of sequence coverage and weighting of their contributions to the allele frequency estimates by the information carried in their sequence data (Buerkle & Gompert, 2013).

An empirical Bayesian approach was taken to estimate individual genotypes for each SNP, wherein allele frequency estimates were used to define prior probabilities for genotypes as  $\Pr(g = 0) = (1-p)^2$ ,  $\Pr(g = 1) = 2p(1-p)$  and  $\Pr(g = 2) = p^2$ . Here,  $g$  denotes counts for the non-reference allele (0, 1 or 2 in diploids) and  $p$  denotes the corresponding allele frequency. Posterior probabilities were then



obtained according to Bayes rule as  $\Pr(g|D, p) = [\Pr(D|g) \Pr(g)] / \Pr(D)$ , where  $\Pr(D|g)$  defines the likelihood of the genotype given the sequence data and quality scores as calculated by ‘samtools’ and ‘bcftools’. Point estimates (posterior means) of genotypes were obtained as  $\Pr(g = 0|D,p) * 0 + \Pr(g = 1|D,p) * 1 + \Pr(g = 2|D,p) * 2$ , which resulted in values between 0 and 2 (copies of the non-reference allele). Pairwise linkage disequilibrium (LD) was calculated from genotype estimates using the ‘geno-r2’ function from ‘vcftools’ (v. 0.1.15; Danecek et al., 2011). Specifically, LD was measured as the squared correlation between genotypes for all pairs of SNPs in a 100 kb window.

### *Genome-wide association mapping and genomic prediction*

Bayesian sparse linear mixed models (BSLMMs) were fit with ‘gemma’ (v. 0.98; Zhou et al., 2013) to (i) characterize physiological genetic architectures, (ii) identify individual trait-associated SNPs, and (iii) infer genomic estimated breeding values (GEBVs), which could then be used to estimate the G-matrix describing the additive genetic covariance among the physiological traits. Unlike traditional mapping methods, this polygenic GWA method considers all SNPs simultaneously while trait values are modeled as a function of a polygenic effect (denoted  $u$ ) and a vector of the potential measurable effects or associations of individual SNPs on the trait (denoted  $\beta$ ; Zhou et al., 2013). In brief, a MCMC algorithm with variable selection was used to estimate posterior inclusion probabilities (PIPs) for each SNP (probability of a zero or non-zero trait effect; Guan & Stephens, 2011), and model-averaged effect estimates were derived by weighting  $\beta$  by the respective PIP (i.e., taking the product of the posterior inclusion probability and effect estimate as a certain, model-averaged effect estimate). Consequently, for each SNP, there is an estimated posterior probability of association (i.e., PIP) and an estimated phenotypic effect conditional on the association. The polygenic term represents expected individual deviations from a grand phenotypic mean based on all SNPs, while accounting for

phenotypic covariances due to overall relatedness or genetic similarity (i.e., observed kinship; Zhou et al., 2013). The kinship matrix also controls for relatedness and population structure when estimating individual SNP effects ( $\beta$ ) and their PIPs. In addition, SNPs in LD with the same causal variant account for each other, since only one needs to be captured by the PIPs for the model.

Derived parameters for estimating genetic architecture included the proportion of the phenotypic variance explained (PVE) by additive genetic effects (based on  $\beta$  and the polygenic term; this should approach the narrow-sense heritability), the proportion of PVE explained by measurable-effect SNPs or those implicated by LD (i.e., PGE; based on  $\beta$  alone), and the number of SNPs with measurable effects or associations that explain phenotypic variance ( $N_{\text{SNP}}$ ). These metrics integrate over effect uncertainty for each of the SNPs, including whether these are nonzero. The expected trait values for an individual based on their additive genetic effects, or genomic estimated breeding values (GEBVs), were obtained from the BSLMMs as captured by both  $\beta$  and  $u$  (i.e., a polygenic score).

BSLMMs were fit for each physiological measurement across all populations (residuals after sex and population effects removed) and within each population (residuals after sex effects removed) using thirty independent MCMC chains each with 100,000 burn-in steps, 1 million sampling steps, and a thinning interval of 10. Convergence to the posterior distribution was verified by calculating the Gelman-Rubin potential scale reduction diagnostic ( $\hat{r} \leq 1.1$ ; Brooks & Gelman, 1998). Brooks-Gelman-Rubin statistics were again calculated for PVE, PGE and  $N_{\text{SNP}}$  ( $\hat{r} < 1.1$ ) with the R package ‘coda’ (v. 0.19.3; Plummer et al., 2006). To reduce bias in estimation, inferences were carried out using the combined values from all iterations across chains (Cowles & Carlin, 1996). Proportional values for PVE and PGE were converted to percentages for interpretation. GEBVs obtained from ‘gemma’ were used to compute the additive genetic variance–covariance matrix (G-matrix; genetic correlations among traits) for the traits within and among the six populations.

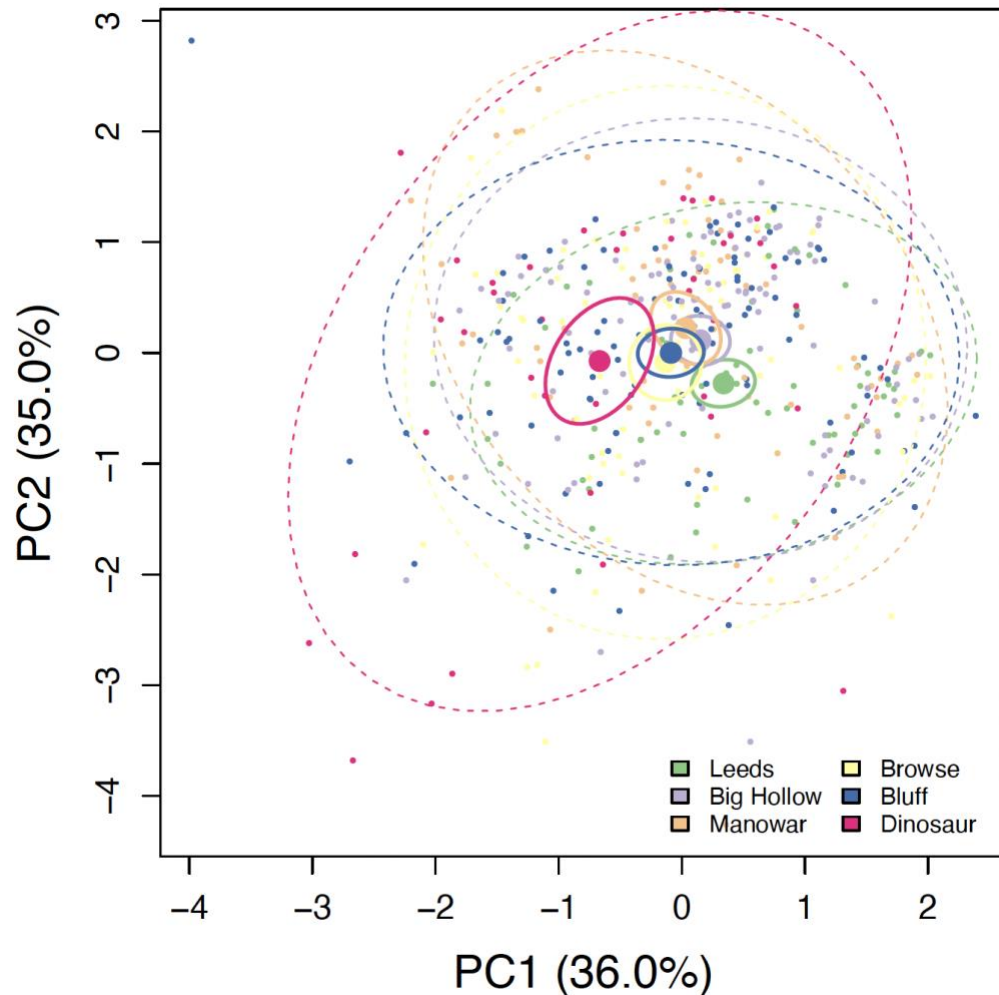
## RESULTS

### *Physiological variation*

Two of the three physiological traits exhibited significant, albeit low among-population variation, as the null model could be confidently rejected for BKA and OXY (restricted likelihood ratio test,  $p < 0.05$ ) but not CORT ( $p = 0.196$ ; Table 3.2). Population explained 5% of the variation for BKA and 11.7% for OXY, but no more than 3% of the variation in CORT. The first two PCs of the phenotypic correlation matrix accounted for 79.5% of the variation among the physiological traits (Fig. 3.1). The combination of mean PC1 and PC2 scores hardly differed across populations (95% credible ellipses on the mean scores were mostly overlapping). Distributions of PC scores also overlapped across populations, though there was considerable variation within populations.

**Table 3.2** REML estimates for each trait of the proportion of phenotypic variation found among *Uta stansburiana* populations (“Prop. var.”). Test statistics (LR = likelihood ratios) and p-values from the null hypothesis test of no population effect are reported.

Traits	Prop. var.	LR	<i>p</i>
Bactericidal Ability	0.050	3.184	< 0.05
Antioxidant Capacity	0.117	16.259	< 0.0005
Corticosterone	0.030	0.196	0.196



**Figure 3.1** Statistical summary of physiological traits in *Uta stansburiana* from a PCA of the phenotypic correlation matrix. Colors denote population while small points represent individual lizards. Dashed ellipses indicate 90% data ellipses generated using the ‘car’ package (v. 2.1.6, Fox & Weisberg, 2011), wherein large dots and solid ellipses denote mean PC scores (dots) and 95% credible ellipses on those means from a Bayesian fit to a bivariate normal distribution. The bivariate normal model was fit using ‘rjags’ (v. 4.6; Plummer, 2016), with uninformative priors on the means (normal with  $\mu = 0$  and  $\tau = 1e^{-3}$ ) and covariance matrix (inverse Wishart with  $k = 2$  and  $\Sigma = I_2$ ). The names ‘Leeds’, ‘Browse’ and ‘Big Hollow’ are synonymous with urban sites 1-3 while the names ‘Bluff’, ‘Dinosaur’, and ‘Manowar’ are synonymous with natural sites 1-3.

### *Genetic basis of physiological variation*

Low PVE estimates were found for BKA across populations (posterior median = 1.9%, CrI = 0.0%–8.4%; Table 3.3; Fig. 3.2A), with an average of 19.0% of phenotypic variation due to genetic effects within populations (median PVEs ranged from 7.3% to 36.4%; Table 3.4). A modest percentage of PVE across populations was due to genetic loci with measurable effects as captured by PGE (posterior median = 35.8%, CrI = 0.0%-93.0%; Fig. 3.2B), with an average of 37.3% within populations (posterior median PGEs ranged from 35.1% to 38.6%). Low  $N_{\text{SNP}}$  estimates were present across populations (posterior median = 9, CrI = 0-184; Fig. 3.2C), with an average of 17 causal loci with measurable phenotypic effects within populations (median  $N_{\text{SNP}}$  ranged from 12 to 23). Consistent with these estimates, all but one SNP associated with bactericidal ability had low PIPs ( $\text{PIP} < 0.05$ ) and correspondingly small model-averaged effect estimates (Table 3.5; Figs. 3-3A, 3-4A). The estimated SNP effects on bactericidal ability and their PIPs were highly correlated (Pearson  $r = 0.754$ , 95% CI = 0.742 - 0.766,  $p < 0.0001$ ; Fig. 3.5A).

Moderate PVE estimates were found for OXY across populations (median = 37.2%, CrI = 20.5%–54.4%; Table 3.3; Fig. 3.2A), with an average of 69.8% of phenotypic variation due to genetic effects within populations (median PVEs ranged from 22.7% to 97.6%; Table 3.4). A modest percentage of PVE was due to genetic loci with measurable effects as captured by PGE antioxidant capacity (posterior median = 39.7%, CrI = 0.0%-92.6%; Fig. 3.2B), with an average of 52.2% attributable to genetic variants with measurable effects within populations (median PGEs ranged from 14.2% to 97.3%). Low  $N_{\text{SNP}}$  estimates were present (posterior median = 59, CrI = 0-246; Fig. 3.2C), with an average of 17 causal loci with measurable phenotypic effects within populations (median  $N_{\text{SNP}}$  ranged from 12 to 23). Several SNPs had moderately larger

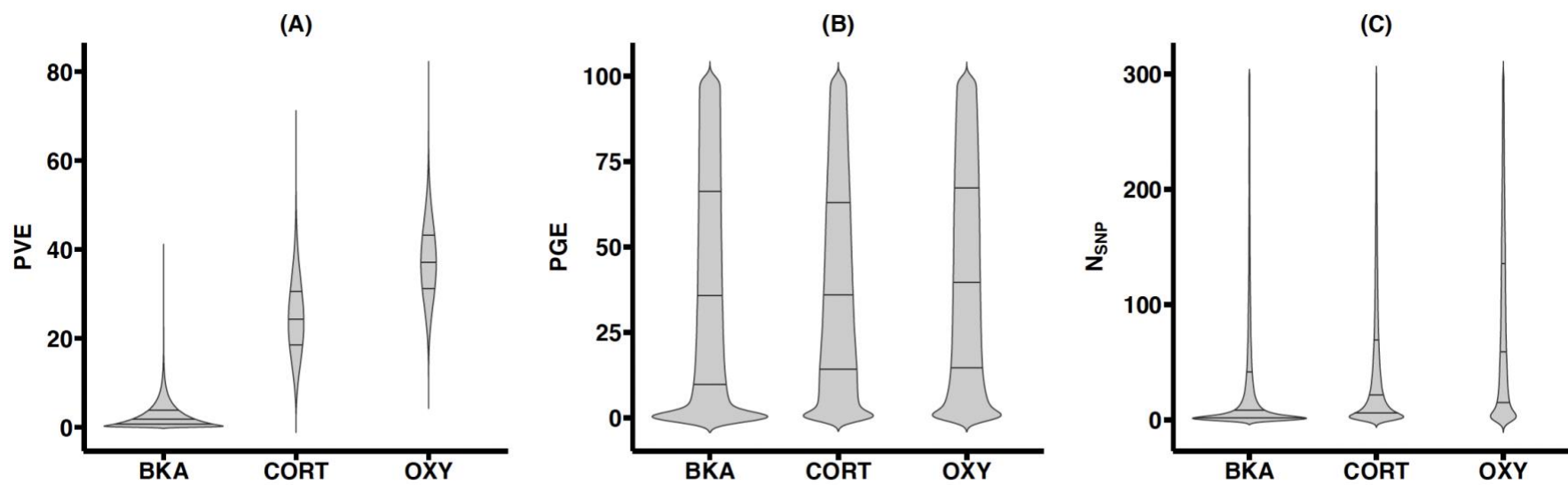
PIPs and model-averaged effect estimates (48 SNPs with PIP > 0.05 and 11 SNPs with PIP > 0.1; Figs. 3-3B, 3-4B), consistent with the larger estimates of heritability. The estimated SNP effects on antioxidant capacity and their PIPs were highly correlated (Pearson  $r = 0.884$ , 95% CI = 0.878 – 0.890,  $p < 0.0001$ ; Fig. 3.5B).

Moderate PVE estimates were also found for CORT across populations (posterior median = 24.4%, CrI = 8.3%–41.8%; Table 3.3; Fig. 3.2A), with an average of 62.5% of phenotypic variation due to genetic effects within populations (median PVEs ranged from 44.6% to 82.1%; Table 3.4). A modest percentage of PVE was due to genetic loci with measurable effects as captured by PGE (posterior median = 36.0%, CrI = 0%–91.2%; Fig. 3.2B), with an average of 53.1% attributable to genetic variants with measurable effects within populations (median PGEs ranged from 37.3% to 97.3%). Low  $N_{\text{SNP}}$  estimates were present (posterior median = 22, CrI = 0–192; Fig. 3.2C), with an average of 12 causal loci with measurable phenotypic effects within populations (median  $N_{\text{SNP}}$  ranged from 5 to 27). Several SNPs had moderately larger PIPs and model-averaged effect estimates (13 SNPs with PIP > 0.05 and 6 SNPs with PIP > 0.1; Figs. 3-3C, 3-4C), once again lending support to larger heritability estimates. The estimated SNP effects on corticosterone and their PIPs were highly correlated (Pearson  $r = 0.926$ , 95% CI = 0.922 – 0.929,  $p < 0.0001$ ; Fig. 3.5C).

**Table 3.3** Summary of genetic architectures for physiological traits (bactericidal ability, antioxidant capacity, corticosterone) based on the posterior probability distributions from fitting Bayesian sparse linear mixed models. Parameters shown include the percent of phenotypic variation explained by genetic effects (PVE), the percent of PVE attributable to loci with measurable effects (PGE), and the number of loci with measurable effects ( $N_{SNPs}$ ) for *Uta stansburiana*. Posterior distributions are summarized as median estimates with 95% credible intervals (CrI).

<b>Phenotype</b>	<b>Median PVE (CrI)</b>	<b>Median PGE (CrI)</b>	<b>Median n-<math>\gamma</math> (CrI)</b>
<i>Bactericidal Ability</i>	1.9 % (0.0 % - 8.4 %)	35.8 % (0.0 % - 93.0 %)	9 (0 - 184)
<i>Antioxidant Capacity</i>	37.2 % (20.5 % - 54.4 %)	39.7 % (0.0 % - 92.6 %)	59 (0 - 246)
<i>Corticosterone</i>	24.4 % (8.3 % - 41.8 %)	36.0 % (0 % - 91.2 %)	22 (0 - 192)





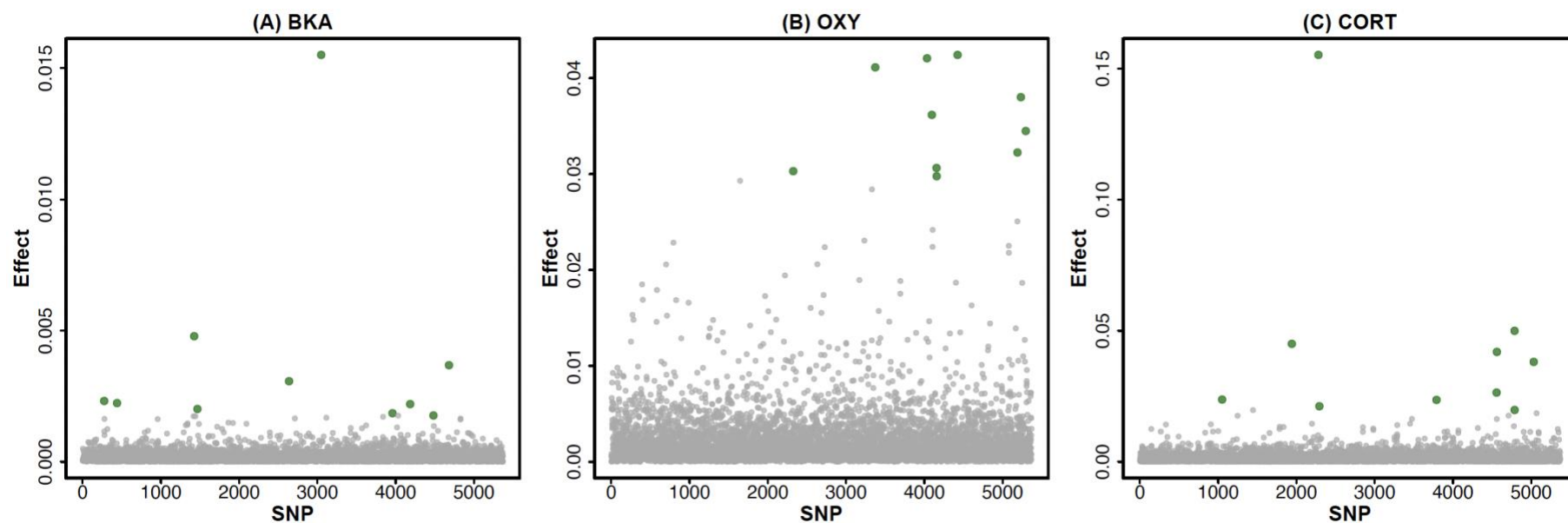
**Figure 3.2** Violin plots for the posterior distributions taken from Bayesian sparse linear mixed models of physiological traits: bacterial killing ability (BKA), antioxidant capacity (OXY), and corticosterone (CORT). Parameters shown include (A) the percent of phenotypic variation explained by SNPs included in the model (PVE), (B) the percent of PVE attributable to loci with measurable effects (PGE), and (C) the number of SNPs underlying the phenotypic trait ( $N_{\text{SNP}}$ ). Horizontal black lines display the quartiles.

**Table 3.4** Summary of genetic architectures for physiological traits (bactericidal ability, antioxidant capacity, corticosterone) based on the posterior probability distributions from fitting Bayesian sparse linear mixed models. Parameters shown include the percent of phenotypic variation explained by genetic effects (PVE), the percent of PVE attributable to loci with measurable effects (PGE), and the number of loci with measurable effects ( $N_{\text{SNP}}$ ) for *Uta stansburiana*. Posterior distributions are summarized as median estimates with 95% credible intervals (CrI).

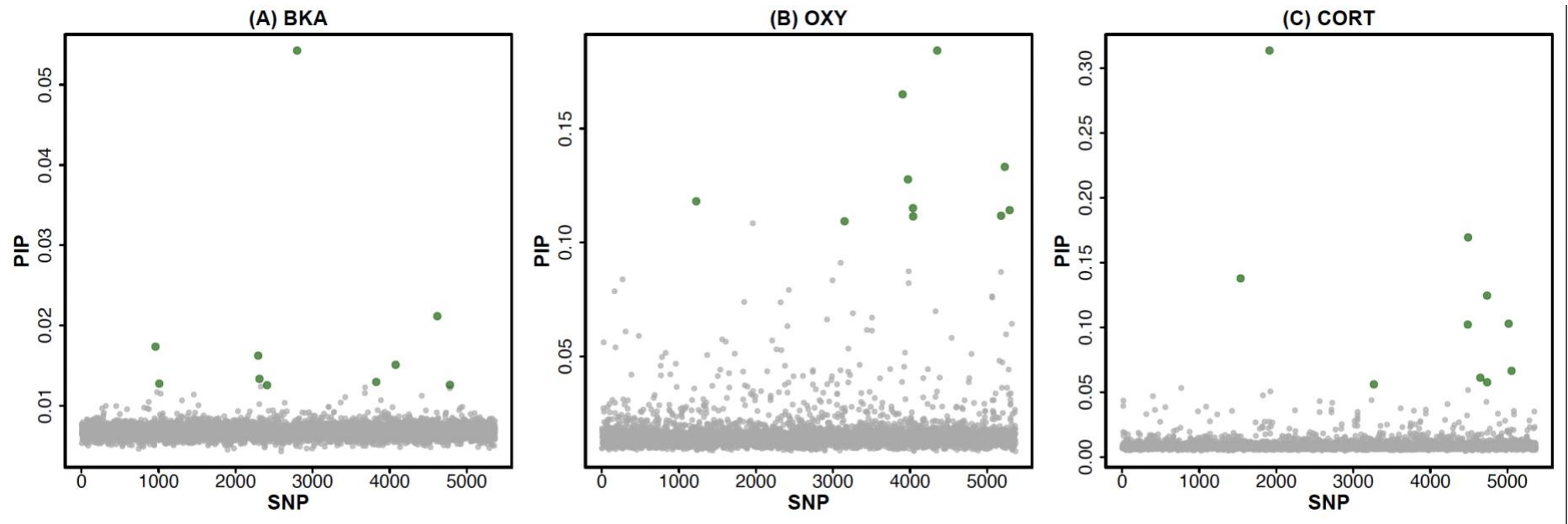
Site	Phenotype	Median PVE (CrI)	Median PGE (CrI)	Median $N_{\text{SNP}}$ (CrI)
Urban Site 1	<i>Bactericidal Ability</i>	8.9% (0.0% - 36.0%)	38.4% (0.0% - 93.5%)	14 (0 - 221)
	<i>Antioxidant Capacity</i>	85.0% (43.9% - 100%)	37.3% (0.0% - 92.6%)	23 (0 - 228)
	<i>Corticosterone</i>	82.1% (31.7% - 100%)	55.7% (7.4% - 99.9%)	6 (0 - 149)
Urban Site 2	<i>Bactericidal Ability</i>	36.4% (3.0% - 100%)	37.1% (0.0% - 92.8%)	23 (0 - 231)
	<i>Antioxidant Capacity</i>	97.6% (93.7% - 99.9%)	97.3% (94.3% - 99.9%)	7 (4 - 10)
	<i>Corticosterone</i>	53.9% (0.6% - 90.4%)	64.8% (0.0% - 98.3%)	7 (0 - 184)
Urban Site 3	<i>Bactericidal Ability</i>	7.3% (0.0% - 31.0%)	36.3% (0.0% - 92.8%)	12 (0 - 199)
	<i>Antioxidant Capacity</i>	51.1% (20.3% - 80.7%)	66.0% (2.8% - 99.9%)	15 (0 - 189)
	<i>Corticosterone</i>	44.6% (10.9% - 75.7%)	58.2% (0.0% - 95.7%)	7 (0 - 179)
Natural Site 1	<i>Bactericidal Ability</i>	20.2% (0.0% - 61.4%)	38.6% (0.0% - 93.2%)	18 (0 - 238)
	<i>Antioxidant Capacity</i>	22.7% (0.0% - 55.4%)	31.0% (0.0% - 91.4%)	19 (0 - 230)
	<i>Corticosterone</i>	62.1% (19.7% - 96.0%)	37.7% (0.0% - 93.5%)	27 (0 - 236)
Natural Site 2	<i>Bactericidal Ability</i>	27.2% (0.0% - 65.7%)	38.0% (0.0% - 93.4%)	17 (0 - 222)
	<i>Antioxidant Capacity</i>	78.0% (43.5% - 100%)	67.3% (4.6% - 99.9%)	12 (0 - 145)
	<i>Corticosterone</i>	54.2% (14.2% - 100%)	38.7% (0.0% - 93.1%)	21 (0 - 230)
Natural Site 3	<i>Bactericidal Ability</i>	13.7% (0.0% - 48.6%)	35.1% (0.0% - 92.4%)	19 (0 - 228)
	<i>Antioxidant Capacity</i>	84.3% (34.3% - 100%)	14.2% (0.0% - 73.6%)	25 (0 - 234)
	<i>Corticosterone</i>	77.8% (54.5% - 100%)	63.3% (35.0% - 95.6%)	5 (1 - 13)

**Table 3.5** Association mapping of SNPs with the ten largest model-average point estimates for each physiological trait in *Uta stansburiana*. Bolded rows indicate positions along shared centroids.

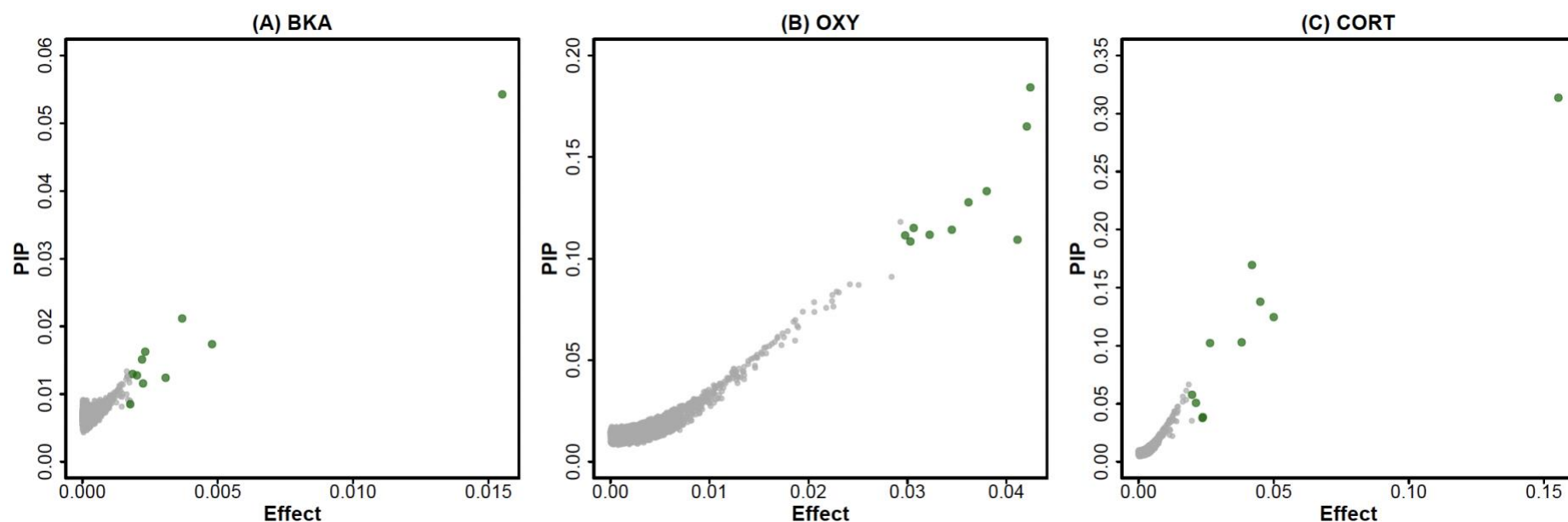
Trait	Centroid	Position	MAPE	PIP
<b>Bactericidal ability</b>	10821486	28	0.0155	0.0543
	12256592	43	0.0037	0.0212
	10227250	38	0.0048	0.0174
	1061550	40	0.0023	0.0163
	11562638	83	0.0022	0.0151
	1062519	77	0.0016	0.0134
	11368417	57	0.0019	0.013
	10235095	40	0.002	0.0128
	12525079	40	0.0016	0.0126
	10666237	25	0.0017	0.0126
<b>Antioxidant capacity</b>	11864101	23	0.0424	0.1843
	11430058	26	0.0421	0.1651
	14293083	27	0.038	0.1332
	11478156	22	0.0362	0.1277
	10298404	61	0.0293	0.1181
	<b>11533605</b>	<b>48</b>	<b>0.0306</b>	<b>0.1151</b>
	14899294	18	0.0345	0.1142
	13945743	21	0.0322	0.1118
	<b>11533605</b>	<b>65</b>	<b>0.0298</b>	<b>0.1115</b>
	10971214	30	0.0411	0.1093
<b>Corticosterone levels</b>	10493224	54	0.1553	0.3136
	<b>12059862</b>	<b>81</b>	<b>0.042</b>	<b>0.1695</b>
	10383061	25	0.045	0.1379
	<b>12407119</b>	<b>32</b>	<b>0.05</b>	<b>0.1246</b>
	13125222	19	0.0381	0.1029
	<b>12059862</b>	<b>50</b>	<b>0.0264</b>	<b>0.1023</b>
	13285589	48	0.0185	0.0666
	12296413	63	0.0176	0.0613
	<b>12407119</b>	<b>38</b>	<b>0.0198</b>	<b>0.0578</b>
	11032277	25	0.0164	0.0562
10493224	54	0.1553	0.3136	



**Figure 3.3** Manhattan plots of SNP effects on physiological traits including (a) bacterial killing ability (BKA), (b) antioxidant capacity (OXY), and (c) corticosterone (CORT). Points denote absolute values of model-averaged effect estimates, that is, estimates weighted by the posterior probability of a non-zero effect. In each panel, the effects of the 10 SNPs with the largest estimates are shown in green.

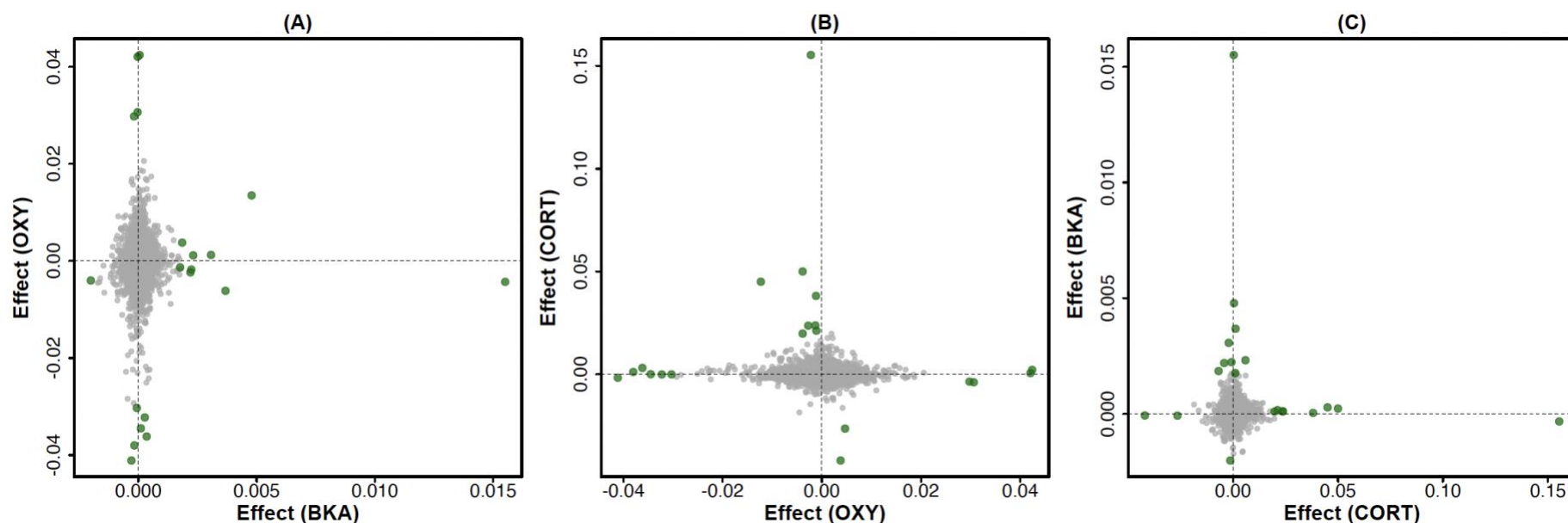


**Figure 3.4** Manhattan plots showing posterior inclusions probabilities (PIPs) from Bayesian sparse linear mixed models relating SNPs to physiological traits including: (a) bacterial killing ability or BKA, (b) antioxidant capacity or OXY, and (c) corticosterone or CORT. Each point denotes a SNP, and top ten SNPs are colored green.



**Figure 3.5** Scatterplots depicting associations of posterior inclusion probabilities (PIPs) and estimated SNP effects on physiological traits including: (a) bactericidal ability (Pearson  $r = 0.754$ , 95% CI = 0.742 - 0.766,  $p < 0.0001$ ), (b) antioxidant capacity (Pearson  $r = 0.884$ , 95% CI = 0.878 - 0.890,  $p < 0.0001$ ), and (c) corticosterone (Pearson  $r = 0.926$ , 95% CI = 0.922 - 0.929,  $p < 0.0001$ ). Points denote absolute values for model-averaged effect estimates, or estimates weighted by the posterior probability of a non-zero effect, and their associated PIPs. In each panel, the ten largest estimates are shown in green.

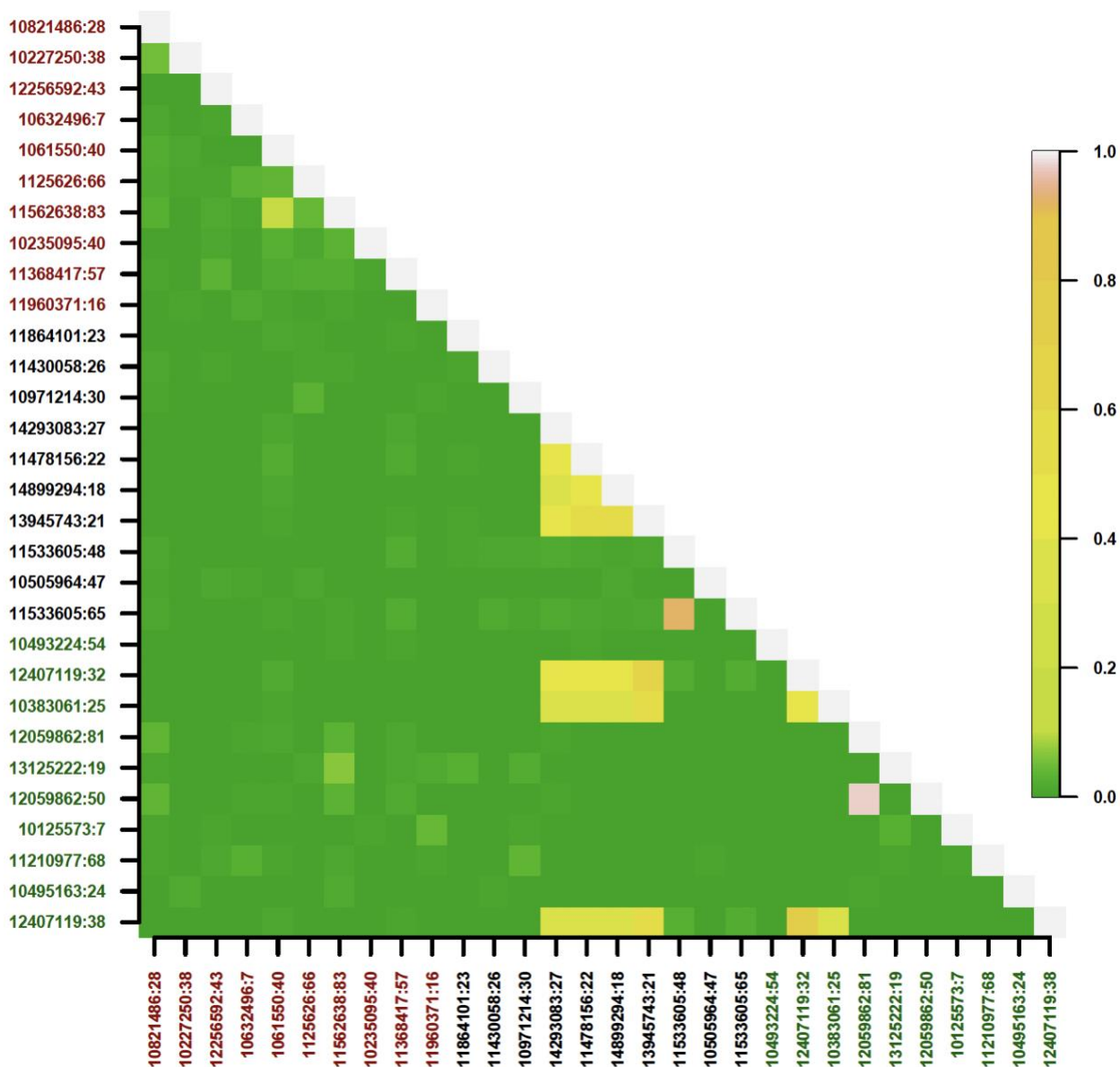
The top-ranking SNPs (i.e., those with the largest PIPs and measurable effects) were mostly spread across the genome (Fig. 3.3) and were independent among traits, such that SNPs most strongly associated with one trait were typically not associated with other traits (Fig. 3.6). However, moderate levels of LD were present among a few pairs of top SNPs contributing to antioxidant capacity and corticosterone (Fig. 3.7). Additionally, a pair of SNPs associated with bactericidal ability yielded moderate LD, while higher LD was present among seven SNP pairs for antioxidant capacity and four SNP pairs for corticosterone. Tight physical linkage was also evident for antioxidant capacity and corticosterone, whereby a few SNP pairs for each trait were near each other on shared contigs (Table 3.5). Overall, linkage disequilibrium decayed with physical genomic distance (Fig. 3.8). Nonetheless, non-trivial LD persisted at a sufficient distance for the SNP markers to likely exhibit LD with at least a reasonable proportion of causal variants. With a marker density of ~2.5 SNP per 1 kb, most causal variants are expected to be within under 1 kb of at least one SNP maker. At the scale of 50 kb, mean LD measured by  $r^2$  was 0.089 (maximum = 1.0).



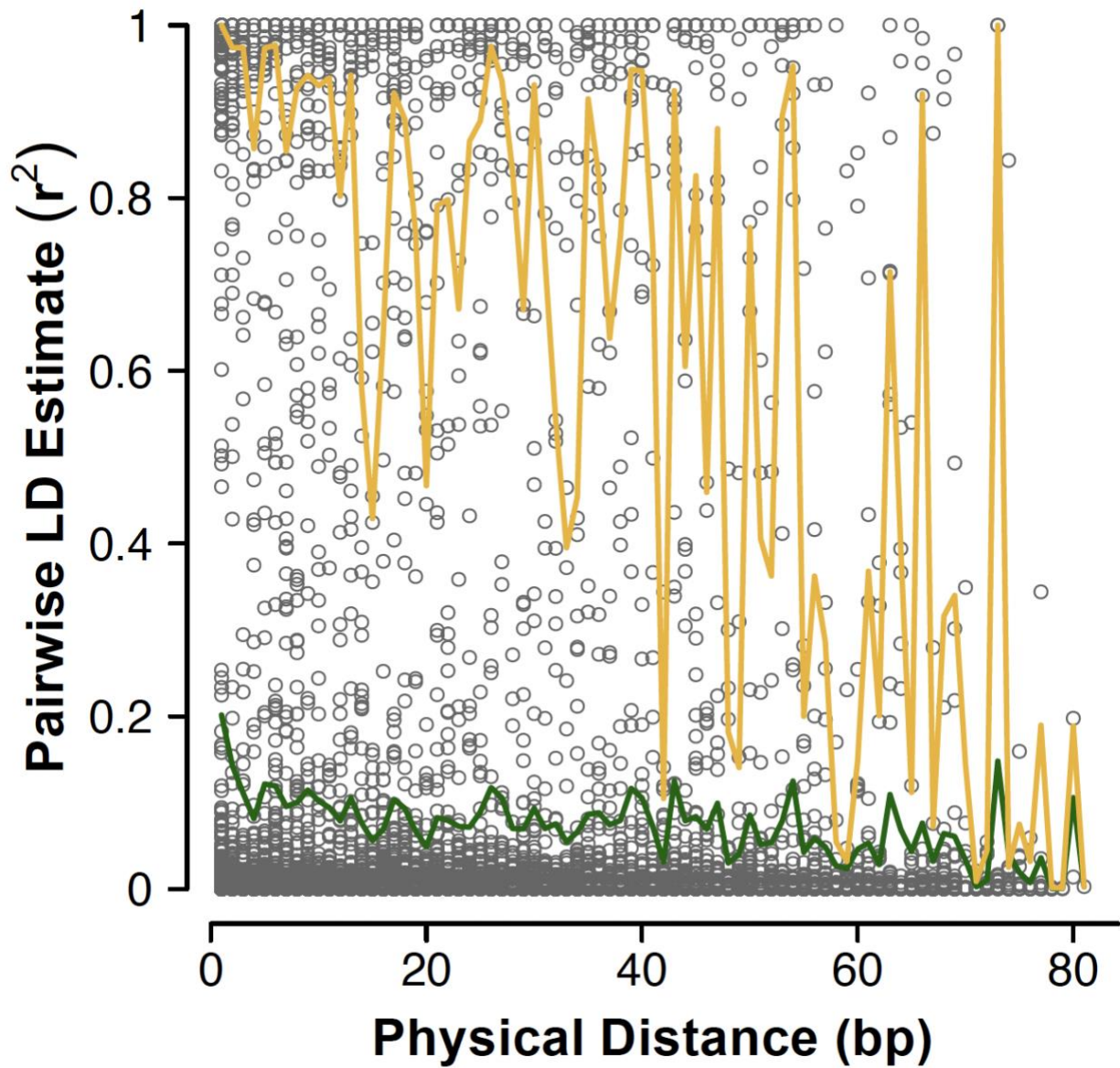
**Figure 3.6** Scatterplots depicting associations between estimated SNP effects on physiological traits including: (a) bacterial killing ability (BKA) and antioxidant capacity (OXY) (Pearson  $r = 0.199$ , 95% CI = 0.179 - 0.219,  $p < 0.0001$ ), (b) antioxidant capacity (OXY) and corticosterone (CORT) (Pearson  $r = 0.132$ , 95% CI = 0.132 – 0.153,  $p < 0.0001$ ), and (c) corticosterone (CORT) and bacterial killing ability (BKA) (Pearson  $r = 0.295$ , 95% CI = 0.276 – 0.314,  $p < 0.0001$ ). Points denote signed, model-averaged effect estimates or estimates weighted by the posterior probability of a non-zero effect. In each panel, the effects of the 10 SNPs with the largest estimates are shown in green. Dashed lines in each panel denote no effect.



## Pairwise LD Estimate ( $r^2$ )



**Figure 3.7** Heat map of pairwise linkage disequilibrium (measured by  $r^2$ ) for the top 10 SNPs with the highest model-averaged point estimates for bactericidal ability (BKA), antioxidant capacity (OXY), and corticosterone (CORT) in *Uta stansburiana*. Included are calculations from inter- and intra-chromosomal positions. Top SNPs are highlighted red for BKA, black for OXY, and green for CORT.



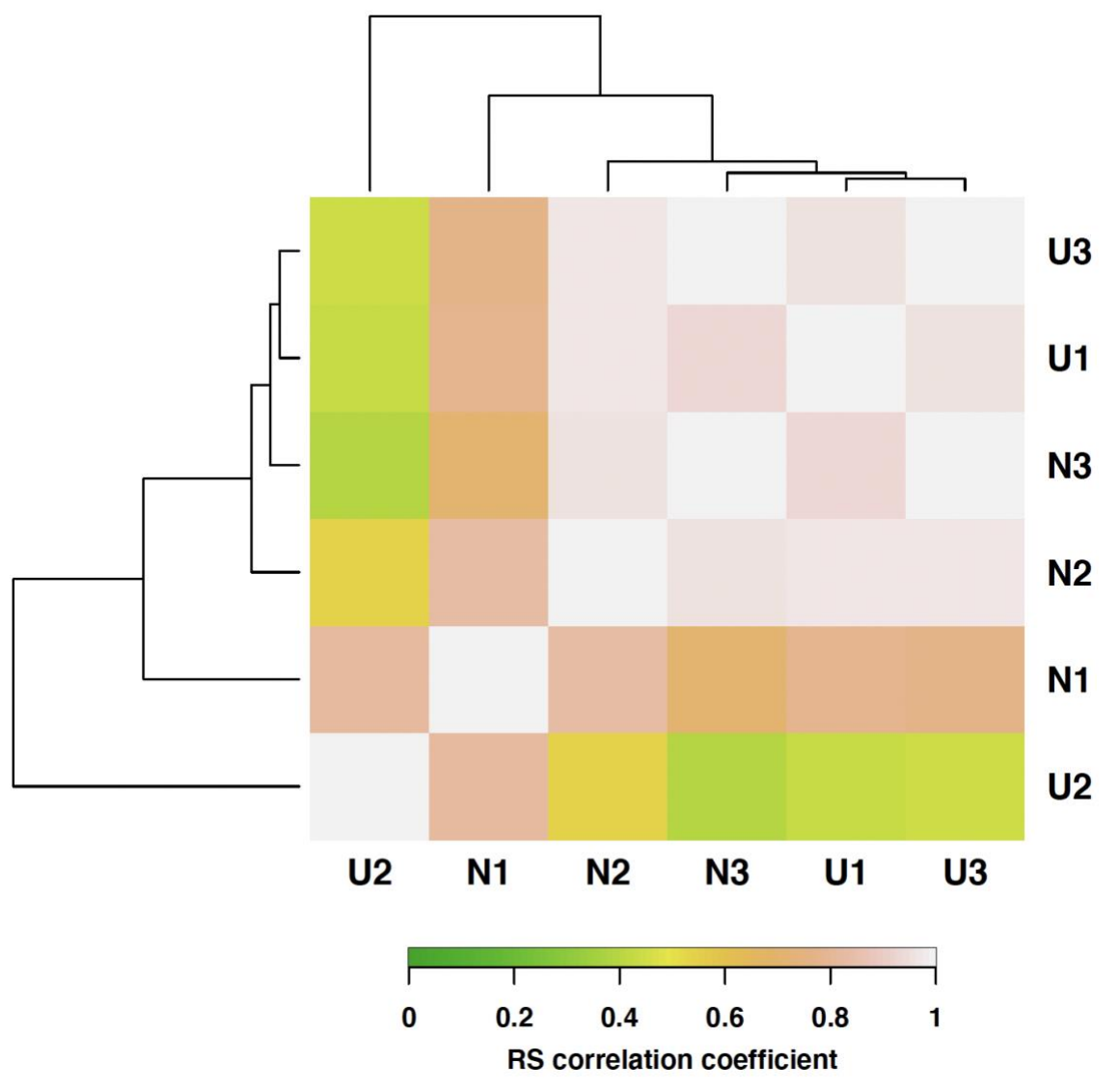
**Figure 3.8** Linkage disequilibrium (LD) decay as a function of physical distance across the *Uta stansburiana* genome. The orange line denotes the 95% quantile of LD, and the green line denotes the mean LD ( $r^2 = 0.089$ ).

*P/G matrix similarity*

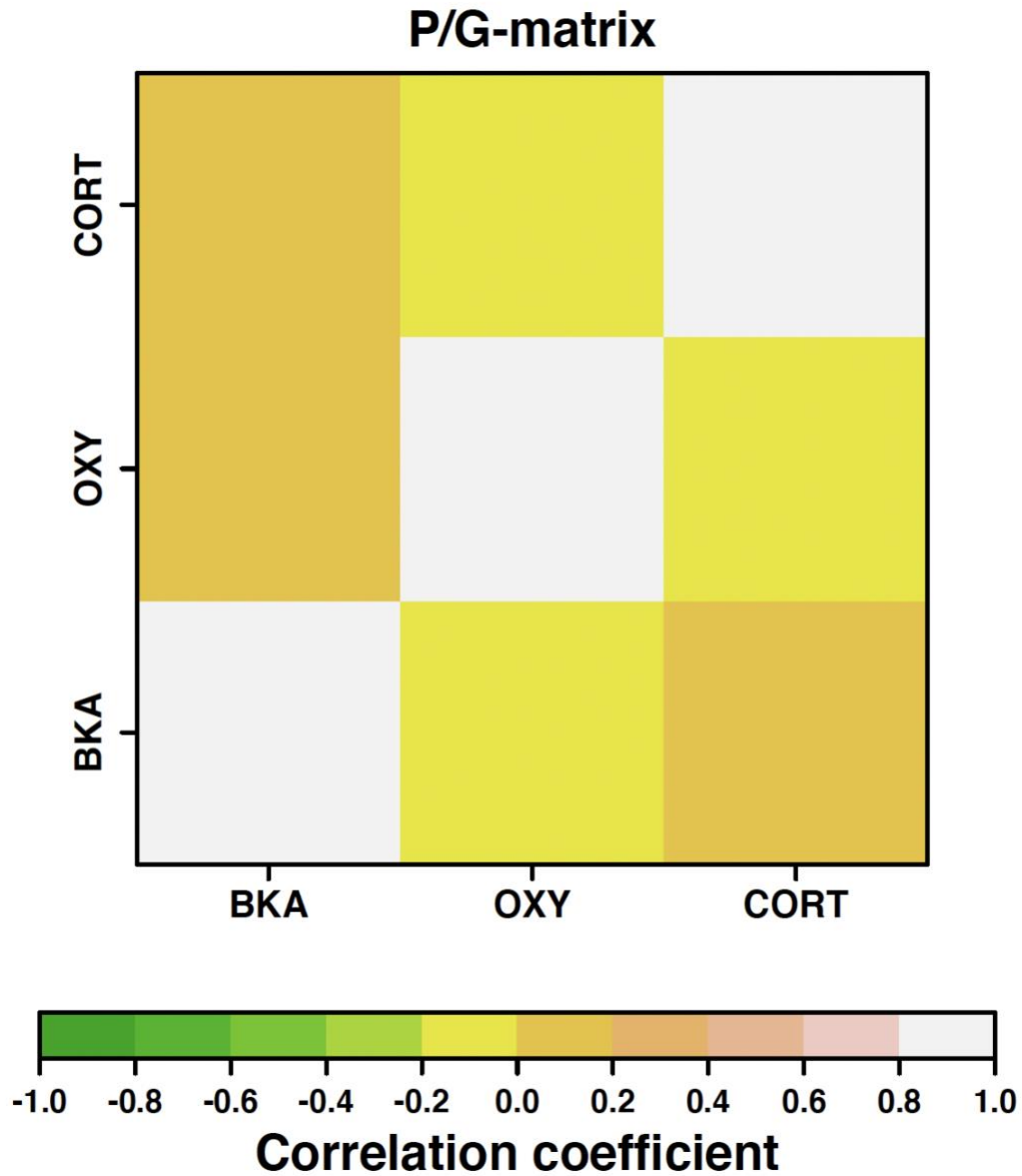
The P- and G-matrices had generally similar structures (i.e., positively correlated) across populations (RS correlation = 0.806, PCAsim = 0.996), although some populations exhibited greater differences between P- and G-matrices than others (Table 3.6; Fig. 3.9). Among-population GEBVs for the physiological traits covaried in a manner that was predicted well from each of the six within-population G-matrices (Fig. 3.10). The G-matrices within populations were also similar, with an average RS correlation of 0.766 and an average PCAsim score of 0.976 (Fig. 3.11). PCA similarity metrics (PCAsim) relating genetic correlations among and within populations were 0.949 for U1, 0.928 for U2, 0.935 for U3, 0.932 for N1, 0.961 for N2, and 0.942 for N3 (Table 3.6). Phenotypic and genetic correlation strength was weak to moderate overall, though correlation directionality depended on population for each trait pair (Fig. 3.9). Within populations, phenotypic and genetic correlations were generally positive for BKA over CORT and OXY, but negative for CORT over OXY. In contrast, the genetic correlations for OXY and BKA was negative, while the phenotypic correlations were positive (Fig. 3.11).

**Table 3.6** Comparison of phenotypic and genetic covariance and correlation matrixes. Included are Random Skewer correlations between mean standardized G- and P-covariance matrixes and PCA similarities between G- and P-correlation matrixes for all physiological traits.

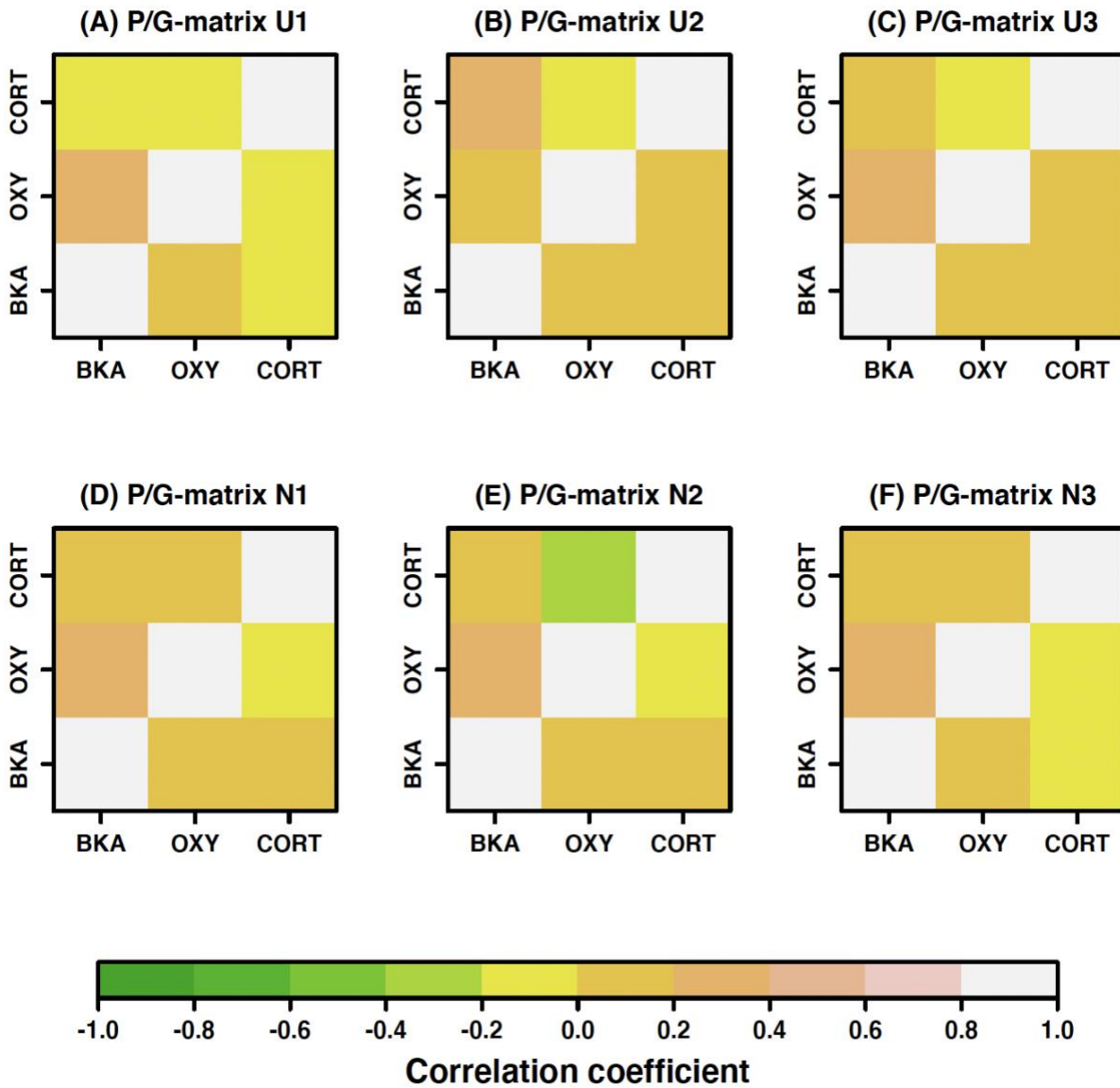
<b>Population</b>	<b>Random Skewer Correlation</b>	<b>PCA Similarity</b>
<b>All sites</b>	0.806	0.996
<b>Urban site 1</b>	0.822	0.934
<b>Urban site 2</b>	0.916	0.902
<b>Urban site 3</b>	0.797	0.815
<b>Natural site 1</b>	0.895	0.840
<b>Natural site 2</b>	0.970	0.985
<b>Natural site 3</b>	0.789	0.802



**Figure 3.9** Heat map summarizing the similarity of mean-standardized G-matrices for the physiological traits. Results are shown for each of the six individual populations (U1, U2, U3, N1, N2, N3). G-matrix similarity was measured based on random skewer (RS) correlations, which capture similarities in the response to many (in this case 10,000) random directional selection gradients. The dendrograms cluster groups by G-matrix similarity and were computed with the heatmap.2 function in R.



**Figure 3.10** Heat maps of phenotypic (left) and genetic (right) correlation matrices for bactericidal ability (BKA), antioxidant capacity (OXY), and corticosterone (CORT) across local population of *Uta stansburiana*.



**Figure 3.11** Heat maps of phenotypic (left) and genetic (right) correlation matrices for bactericidal ability, antioxidant capacity, and corticosterone levels within local populations of *Uta stansburiana*.

## DISCUSSION

Variation in physiological traits and their relationships with one another are central to many long-standing hypotheses regarding evolutionary processes across taxonomic groups. In reptiles, estimates of genetic variation have only been available in the form of broad-sense heritability for a limited number of physiological traits (Garland et al., 1990; Sorci et al., 1995), prompting uncertainty to what extent nonadditive/maternal effects inflate these estimates. For this study, narrow-sense heritability was estimated for measures of innate immune function (BKA), antioxidant capacity (OXY), and the glucocorticoid hormone, corticosterone (CORT), in populations of the lizard species, *U. stansburiana*. Patterns of phenotypic and genetic covariation, along with SNP markers explaining trait variation, were also compared within and among populations. Mapping across populations was critical for parsing the causes of trait covariation, namely, linkage disequilibrium among unlinked loci caused by population differentiation versus pleiotropy or tight physical linkage. With these approaches, all SNPs were incorporated into GEBVs and considered as signals alongside individual SNPs with measurable effects. Genetic differences among populations were also allowed to contribute to phenotypic differences while avoiding redundant SNP and trait associations when capturing the effects of individual SNPs (Zhou et al., 2013). Altogether, evidence for heritability was found for each of the physiological traits, yielding the potential for populations to evolve physiological differences across ecological niches. The results are most consistent with a polygenic basis for trait variation without significant pleiotropic effects or tight linkage. Although SNPs associated with variation were detected for each trait, the genomic regions containing potential candidate genes could not be explored without their annotation from a reference genome, which is currently unavailable.



Estimates of the genetic architecture for each trait and possible implications of the results are discussed below.

Given that traits closely linked to fitness are permanent targets of selection, their additive genetic variance ought to be eroded by environmental pressures at a faster rate and be comparatively lower as a result (Mousseau & Roff, 1987; Roff & Mousseau, 1987). In other words, alleles conferring a higher fitness for physiological traits are liable to be rapidly fixed by natural selection (Roff et al., 1997; Kruuk et al., 2000; Charmantier & Garant, 2005). Therefore, traits necessary for crucial physiological processes are expected to have low to moderate levels of heritability, lying between those of life-history and morphological traits (Orr, 2005). Consistent with this expectation, heritability for BKA was low (PVE posterior median = 1.9%; CrI = 0.0%–8.4%), with only some of that heritability owed to a small number of SNPs (posterior median = 9; CrI = 0–184) with measurable effects (PGE posterior median = 35.8%; CrI = 0.0%–93.0%). Heritability of immune traits (e.g., bactericidal activity, lysozyme activity, alternative complement activity) has previously been estimated to be low or moderate in other taxa (e.g., fish, birds, mammals; Clapperton et al., 2009; Tieleman et al., 2010; Flori et al., 2011; Srisapoomee et al., 2019; Johnston et al., 2020), with certain traits even showing undetectable levels of heritable genetic variation (Brodin et al., 2015). Due to selection on the additive genetic proportion of phenotypic variation, immune traits could be susceptible to accumulating more non-additive genetic variance relative to other traits (e.g., Roff & Emerson, 2006; Ab Ghani et al., 2012; Nespolo et al., 2014). Genotype by environment interactions (i.e., epigenetic effects) may also represent a large portion of variation in innate immunity for reptiles (Begin & Roff, 2004, Zimmerman, 2010, 2020), granted heritability is liable to vary by the species and/or immune pathway in question (Galli et al., 2011; Colonna, 2018; Martin et al., 2021).

Heritability estimates for OXY were instead moderate (posterior median = 37.2%; CrI = 20.5%–54.4%), and to some extent, the genetic underpinnings for such heritability were explained by many SNPs (posterior median = 59; CrI = 0–246) with measurable effects (posterior median = 39.7%; CrI = 0.0%–92.6%). These results corroborate previous studies on the heritability of blood-cell resistance to free-radical attack in other taxa (Costantini & Dell’Omo, 2006; Olsson et al., 2008; Kim et al., 2010a, 2010b; Losdat et al., 2014; Kahar et al., 2016), and are perhaps less likely to be inflated as population effects were removed for variance attributable to the environment (Kruuk & Hadfield, 2007; Pamplona & Costantini, 2011). In concert with previous work, there is thus strong evidence for heritable genetic control of variation in oxidative resistance, which could potentially evolve in response to selection. Here, side-blotched lizard populations may adapt to changing environmental conditions (e.g., temperature, pollutants) that challenge the oxidative balance with fitness consequences. Yet, parsing the environmental and genetic contributions remains necessary to understand the extent that oxidative resistance is affected by evolutionary versus ecological processes (Metcalf & Alonso-Alvarez, 2010; Losdat et al., 2014).

Circulating CORT was also moderately heritable (posterior median = 24.4%; CrI = 8.3%–41.8%), though with a genetic basis owed to fewer SNPs (posterior median = 22; CrI = 0–192) with measurable effects (posterior median = 36.0%; CrI = 0.0%–91.2%). Consistent with these findings, glucocorticoid levels are often moderately heritable in a variety of vertebrate taxa, including birds (e.g., Evans et al., 2006; Jenkins et al., 2014; Béziers et al., 2019) and mammals and fish (e.g., Fevolden et al., 1999; Kadarmideen & Janss, 2007). These heritability estimates are also like those found for other steroid hormones that are key to fitness (e.g., testosterone; Cox et al., 2016; Greives et al., 2017), further suggesting that an appreciable part of the variance in

CORT levels is indeed genetically determined. Genetic contributions to CORT variation may enable selective processes to fine-tune the HPA axis and promote adaptation to specific environments (Partecke et al., 2006; Müller et al., 2007; Fokidis et al., 2009; Atwell et al., 2012; Abolins-Abols et al., 2016). Despite being closely associated with fitness, moderate genetic variance in CORT phenotypes may be maintained by a diversity of selective mechanisms, including fluctuating and disruptive selection, but also frequency-dependent selection (Mousseau & Roff, 1987; Bell, 2010). However, generalizing CORT to be moderately heritable would require more studies on wild populations, which can greatly vary in baseline levels across environmental contexts (Cockrem, 2013; Guindre-Parker, 2020). Interannual environmental differences, for example, could influence the fitness of different glucocorticoid phenotypes and maintain genetic variation if the optimal level of baseline CORT changes from one year to the next (Vitousek et al., 2018). Additional studies investigating the year-effect factors (e.g., food availability, weather conditions) on CORT regulation are needed to better understand how genetic variation in CORT is maintained.

Genetic covariation for the physiological traits at the among population-level was generally consistent with patterns observed within populations, though some exceptions were present. Stability in these patterns reflect a degree of genetic constraint across populations, whereby evolutionary change directs and inhibits variation in certain directions both across space and over time (Puentes et al., 2016). As a result, stability is robust for some suites of traits and weak for others over similar spans of evolutionary time (Arnold et al., 2008). Here, the slight inter-population differences suggest that such constraints are not absolute for the traits under scrutiny, as shown in other taxa (e.g., Renaud et al., 2006; Kelly et al., 2013; Porto et al., 2015). In addition to the effects of genetic drift, differences in the directions of genetic covariation

could be the product of selection favoring different trait combinations for each population (Estes & Arnold, 2007). In other words, if significant evolutionary divergence is occurring among the populations, selection might be acting against inherent genetic constraints for optimal physiological performance. Disentangling the genetic mechanisms behind each trait is likely necessary to understand the nature of the differences in genetic covariation between populations.

The degree to which pleiotropy or tight linkage and high LD contribute to the patterns of phenotypic and genetic covariation (i.e., genetic constraint) observed within and among populations seems to depend on the traits in question. Physiological systems can potentially structure and shape the additive genetic variance-covariance matrix via pleiotropic gene action (e.g., endocrine function; Dantzer & Swanson, 2017; Wittman et al., 2021). Apart from the rapid, non-genomic effects of glucocorticoid hormones (Chen & Qiu, 1999), their pleiotropic effects and influence over gene expression are becoming increasingly evident (MacDougall-Shackleton et al., 2019). The glucocorticoid hormone CORT could constrain the independent evolution of any trait that it mediates, such as immune function and/or oxidative resistance. Glucocorticoid receptor signaling spares or enhances many pathways that are involved in innate immunity, while suppressing those that are involved in adaptive immunity (Cain & Cidlowski, 2017; Shimba et al., 2021). Similarly, glucocorticoids can also act as a modulator of oxidative stress in vertebrates (Costantini et al., 2011). Yet, a large portion of variation for each physiological trait was associated with different major-effect SNPs. Since evolutionary constraint due to pleiotropy would require shared SNPs to generate trait covariance (Estes & Phillips, 2006), any significant pleiotropic effects are therefore either absent or undetectable with the analyzed SNP set and/or physiological metrics. In the latter case, pleiotropic effects could still be relevant since evolution often acts via large numbers of small-effect polygenes (e.g., Rockman, 2012). Further, genetic

effects on multiple traits compared across different genetic backgrounds and environments indicate pleiotropic effects on traits can change (Pavličev & Cheverud, 2015), permitting the potential for context-dependent genetic constraint via pleiotropy.

Although tight physical linkage was present among a few top candidate SNPs for antioxidant capacity and corticosterone levels, none was evident between any pair of traits (i.e., top SNPs were located on different scaffolds). The tightly linked SNPs within each trait are assumedly near causal variants contributing to phenotypic variation if not the causal variants themselves. Yet, a few SNPs associated with antioxidant capacity yielded moderate levels of LD with SNPs associated with corticosterone levels. Only a subset of the genome was sequenced with the GBS approach, and so it is possible and even likely that some causal variants were not in LD with the sequenced SNPs (Catchen et al., 2017; Lowry et al., 2017; McKinney et al., 2017). Moreover, even where SNPs were in LD with causal variants, their effects may have been underestimated because these statistical associations (i.e., LD) were imperfect. Thus, the effects of an unknown subset of contributing alleles were likely missed or underestimated. Although LD among loci and among-trait correlations could arise from modest selection or even drift in geographically isolated population, very strong selection would be needed to maintain such LD within populations in the absence of tight linkage (Gillespie, 2004). Given the polygenic basis of physiological variation, very strong selection at the trait level is particularly unlikely within populations, as this would not necessarily produce strong selection on each genetic locus (e.g., Nosil et al., 2009; Kemper et al., 2014; Tiffin & Ross-Ibarra, 2014).

In summary, this study combined methods (physiological sampling, polygenic modelling, genomic prediction, and evolutionary quantitative genetics) to take a novel approach in identifying genomic regions associated with physiological traits in a lizard species. Variation in

these traits exhibited low to moderate heritability, owing to a polygenic basis, akin to many quantitative traits in model organisms (Mackay et al., 2009). Though the actual effect sizes of causal loci are assumedly larger than estimated here, the degree of underestimation cannot be determined at this time, nor can conclusions about the exact number of loci underlying each trait be made. Additionally, since the SNPs associated with each trait are mostly independent, optimal phenotypes in lizards to environmental conditions may require many SNPs and/or mechanisms. The low genetic covariances present among the traits are assumedly insufficient in constraining (in a broad sense, i.e., to direct or channel) patterns of physiological performance and evolution in this species. More data should be collected to fully discern the genetic and environmental factors shaping hormonal, oxidative, and immune characteristics. Discerning the individual-level processes that shape physiological phenotypes is important not only for understanding how selection has acted, but also for providing predictive power regarding how suites of traits may change in the face of a changing environment (e.g., urbanization, climate change; Visser, 2008). The complexities of these traits, and their interactions with each other, also warrant multiple assays probing different components of each system to further understand their roles in the observed phenotypic and genetic covariation (Sheriff et al., 2011; Pedersen & Babayan, 2011; Demas et al., 2011; Celi, 2011; Downs & Stewart, 2014; Sies et al., 2017; Palme, 2019; Costantini, 2019). With the discovery of genomic regions associated with each trait, the functional roles of candidate genes contributing to phenotypic variation can also be identified and tested for whether they are subject to evolutionary mechanisms affecting fitness across environments.

## LITERATURE CITED

- Ab Ghani, N. I., Herczeg, G., & Merilä, J. (2012). Body size divergence in nine-spined sticklebacks: disentangling additive genetic and maternal effects. *Biological Journal of the Linnean Society*, *107*(3), 521-528.
- Abolins-Abols, M., Hope, S. F., & Ketterson, E. D. (2016). Effect of acute stressor on reproductive behavior differs between urban and rural birds. *Ecology and evolution*, *6*(18), 6546-6555.
- Andrew, A. L., Perry, B. W., Card, D. C., Schield, D. R., Ruggiero, R. P., McGaugh, S. E., Choudhary, A., Secor, S. M., & Castoe, T. A. (2017). Growth and stress response mechanisms underlying post-feeding regenerative organ growth in the Burmese python. *BMC genomics*, *18*(1), 1-18.
- Angelier, F., & Wingfield, J. C. (2013). Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives. *General and Comparative Endocrinology*, *190*, 118-128.
- Armbruster, W., & Schwaegerle, K. (1996). Causes of covariation of phenotypic traits among populations. *Journal of evolutionary biology*, *9*(3), 261-276.
- Arnold, S. J., Bürger, R., Hohenlohe, P. A., Ajie, B. C., & Jones, A. G. (2008). Understanding the evolution and stability of the G-matrix. *Evolution: International Journal of Organic Evolution*, *62*(10), 2451-2461.
- Atwell, J. W., Cardoso, G. C., Whittaker, D. J., Campbell-Nelson, S., Robertson, K. W., & Ketterson, E. D. (2012). Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. *Behavioral Ecology*, *23*(5), 960-969.
- Barton, N. (2011). Estimating linkage disequilibria. *Heredity*, *106*(2), 205.

- Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H., Singmann, H., & Dai, B. (2015). lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1–7. 2014.
- Bégin, M., & Roff, D. A. (2004). From micro-to macroevolution through quantitative genetic variation: positive evidence from field crickets. *Evolution*, *58*(10), 2287-2304.
- Bell, G. (2010). Fluctuating selection: the perpetual renewal of adaptation in variable environments. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1537), 87-97.
- Bennett, A. F., & Huey, R. (1990). Studying the evolution of physiological performance. *Oxford surveys in evolutionary biology*, *7*, 251-284.
- Bereshchenko, O., Bruscoli, S., & Riccardi, C. (2018). Glucocorticoids, sex hormones, and immunity. *Frontiers in Immunology*, *9*, 1332.
- Béziers, P., San-Jose, L. M., Almasi, B., Jenni, L., & Roulin, A. (2019). Baseline and stress-induced corticosterone levels are heritable and genetically correlated in a barn owl population. *Heredity*, *123*(3), 337-348.
- Bollati, V., & Baccarelli, A. (2010). Environmental epigenetics. *Heredity*, *105*(1), 105-112.
- Brodin, P., Jojic, V., Gao, T., Bhattacharya, S., Angel, C. J. L., Furman, D., Shen-Orr, S., Dekker, C. L., Swan, G. E., & Butte, A. J. (2015). Variation in the human immune system is largely driven by non-heritable influences. *Cell*, *160*(1-2), 37-47.
- Brooks, S. P., & Gelman, A. (1998). General methods for monitoring convergence of iterative simulations. *Journal of computational and graphical statistics*, *7*(4), 434-455.
- Buerkle, A., & Gompert, Z. (2013). Population genomics based on low coverage sequencing: how low should we go? *Molecular ecology*, *22*(11), 3028-3035.



- Bureau, U. S. C. (2022). *Over Two-Thirds of the Nation's Counties Had Natural Decrease in 2021*.  
<https://www.census.gov/newsroom/press-releases/2022/population-estimates-counties-decrease.html>
- Cain, D. W., & Cidlowski, J. A. (2017). Immune regulation by glucocorticoids. *Nature Reviews Immunology*, 17(4), 233-247.
- Catchen, J. M., Hohenlohe, P. A., Bernatchez, L., Funk, W. C., Andrews, K. R., & Allendorf, F. W. (2017). Unbroken: RADseq remains a powerful tool for understanding the genetics of adaptation in natural populations. *Molecular ecology resources*, 17(3), 362-365.
- Celi, P. (2011). Biomarkers of oxidative stress in ruminant medicine. *Immunopharmacology and immunotoxicology*, 33(2), 233-240.
- Chainy, G. B., & Sahoo, D. K. (2020). Hormones and oxidative stress: an overview. *Free Radical Research*, 54(1), 1-26.
- Charmantier, A., & Garant, D. (2005). Environmental quality and evolutionary potential: lessons from wild populations. *Proceedings of the Royal Society B: Biological Sciences*, 272(1571), 1415-1425.
- Chen, Y.-Z., & Qiu, J. (1999). Pleiotropic signaling pathways in rapid, nongenomic action of glucocorticoid. *Molecular Cell Biology Research Communications*, 2(3), 145-149.
- Clapperton, M., Diack, A. B., Matika, O., Glass, E. J., Gladney, C. D., Mellencamp, M. A., Hoste, A., & Bishop, S. C. (2009). Traits associated with innate and adaptive immunity in pigs: heritability and associations with performance under different health status conditions. *Genetics Selection Evolution*, 41(1), 1-11.

- Claunch, N. M., Downs, C. J., Schoenle, L. A., Oakey, S. J., Ely, T., Romagosa, C., & Briggs, C. W. (2022). Snap-freezing in the field: effect of sample holding time on performance of bactericidal assays. *Integrative and comparative biology*.
- Cockrem, J. F. (2013). Individual variation in glucocorticoid stress responses in animals. *General and Comparative Endocrinology*, *181*, 45-58.
- Colonna, M. (2018). Innate lymphoid cells: diversity, plasticity, and unique functions in immunity. *Immunity*, *48*(6), 1104-1117.
- Comeault, A. A., Soria-Carrasco, V., Gompert, Z., Farkas, T. E., Buerkle, C. A., Parchman, T. L., & Nosil, P. (2014). Genome-wide association mapping of phenotypic traits subject to a range of intensities of natural selection in *Timema cristinae*. *The American Naturalist*, *183*(5), 711-727.
- Corl, A., Davis, A. R., Kuchta, S. R., & Sinervo, B. (2010). Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation. *Proceedings of the National Academy of Sciences*, *107*(9), 4254-4259.
- Costantini, D. (2014). Oxidative stress and hormesis in evolutionary ecology and physiology. *A marriage between mechanistic and evolutionary approaches*, 362.
- Costantini, D. (2019). Understanding diversity in oxidative status and oxidative stress: the opportunities and challenges ahead. *Journal of Experimental Biology*, *222*(13), jeb194688.
- Costantini, D., Marasco, V., & Møller, A. P. (2011). A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. *Journal of Comparative Physiology B*, *181*(4), 447-456.
- Costantini, D., & Møller, A. P. (2009). Does immune response cause oxidative stress in birds? A meta-analysis. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *153*(3), 339-344.

- Cowles, M. K., & Carlin, B. P. (1996). Markov chain Monte Carlo convergence diagnostics: a comparative review. *Journal of the American Statistical Association*, *91*(434), 883-904.
- Cox, R. M., McGlothlin, J. W., & Bonier, F. (2016). Hormones as mediators of phenotypic and genetic integration: an evolutionary genetics approach. *Integrative and comparative biology*, *56*(2), 126-137.
- Crainiceanu, C. M., & Ruppert, D. (2004). Likelihood ratio tests in linear mixed models with one variance component. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *66*(1), 165-185.
- Crespi, E. J., Williams, T. D., Jessop, T. S., & Delehanty, B. (2013). Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? *Functional Ecology*, *27*(1), 93-106.
- Dallman, M. F., Akana, S. F., Cascio, C. S., Darlington, D. N., Jacobsen, L., & Levin, N. (1987). Regulation of ACTH secretion: variations on a theme of B. Proceedings of the 1986 Laurentian Hormone Conference,
- Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., & Sherry, S. T. (2011). The variant call format and VCFtools. *bioinformatics*, *27*(15), 2156-2158.
- Dantzer, B., & Swanson, E. M. (2017). Does hormonal pleiotropy shape the evolution of performance and life history traits? *Integrative and comparative biology*, *57*(2), 372-384.
- Deakin, J. E., & Ezaz, T. (2019). Understanding the evolution of reptile chromosomes through applications of combined cytogenetics and genomics approaches. *Cytogenetic and Genome Research*, *157*(1-2), 7-20.

- Delahaie, B., Charmantier, A., Chantepie, S., Garant, D., Porlier, M., & Teplitsky, C. (2017). Conserved G-matrices of morphological and life-history traits among continental and island blue tit populations. *Heredity*, *119*(2), 76-87.
- Demas, G. E., Zysling, D. A., Beechler, B. R., Muehlenbein, M. P., & French, S. S. (2011). Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts. *Journal of Animal Ecology*, *80*(4), 710-730.
- Doughty, P., & Sinervo, B. (1994). The effects of habitat, time of hatching, and body size on the dispersal of hatchling *Uta stansburiana*. *Journal of Herpetology*, 485-490.
- Downs, C. J., & Stewart, K. M. (2014). A primer in ecoimmunology and immunology for wildlife research and management. *Calif Fish Game*, *100*, 371-395.
- Estes, S., & Arnold, S. J. (2007). Resolving the paradox of stasis: models with stabilizing selection explain evolutionary divergence on all timescales. *The American Naturalist*, *169*(2), 227-244.
- Estes, S., & Phillips, P. C. (2006). Variation in pleiotropy and the mutational underpinnings of the G-matrix. *Evolution*, *60*(12), 2655-2660.
- Evans, M. R., Roberts, M. L., Buchanan, K. L., & Goldsmith, A. R. (2006). Heritability of corticosterone response and changes in life history traits during selection in the zebra finch. *Journal of evolutionary biology*, *19*(2), 343-352.
- Falconer, D. S. (1996). *Introduction to quantitative genetics*. Pearson Education India.
- Feder, M. E., Bennett, A. P., & Huey, R. B. (2000). Evolutionary Physiology. *Annu, Rev. Ecol. Syst*, *1000*(31), 315-41.
- Fevolden, S. E., Røed, K., Fjalestad, K., & Stien, J. (1999). Poststress levels of lysozyme and cortisol in adult rainbow trout: heritabilities and genetic correlations. *Journal of Fish Biology*, *54*(4), 900-910.

- Flori, L., Gao, Y., Laloë, D., Lemonnier, G., Leplat, J.-J., Teillaud, A., Cossalter, A.-M., Laffitte, J., Pinton, P., & de Vaureix, C. (2011). Immunity traits in pigs: substantial genetic variation and limited covariation. *PloS one*, *6*(7), e22717.
- Fokidis, H. B., Orchinik, M., & Deviche, P. (2009). Corticosterone and corticosteroid binding globulin in birds: relation to urbanization in a desert city. *General and Comparative Endocrinology*, *160*(3), 259-270.
- Foret, S., Kucharski, R., Pittelkow, Y., Lockett, G. A., & Maleszka, R. (2009). Epigenetic regulation of the honey bee transcriptome: unravelling the nature of methylated genes. *BMC genomics*, *10*(1), 1-11.
- Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., & Heilberger, R. (2011). Package “car”: Companion to applied regression. *Wien: www.cran.r-project.org*.
- French, S. S., & Neuman-Lee, L. A. (2012). Improved ex vivo method for microbiocidal activity across vertebrate species. *Biology Open*, *1*(5), 482-487.
- Galli, S. J., Borregaard, N., & Wynn, T. A. (2011). Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. *Nature immunology*, *12*(11), 1035-1044.
- Garland, T., Bennett, A., & Daniels, C. (1990). Heritability of locomotor performance and its correlates in a natural population. *Experientia*, *46*, 530-533.
- Garland, T., & Carter, P. (1994). Evolutionary physiology. *Annual review of physiology*, *56*(1), 579-621.
- Gillespie, J. H. (2004). *Population genetics: a concise guide*. JHU press.
- Gompert, Z., Brady, M., Chalyavi, F., Saley, T. C., Philbin, C. S., Tucker, M. J., Forister, M. L., & Lucas, L. K. (2019). Genomic evidence of genetic variation with pleiotropic effects on caterpillar fitness and plant traits in a model legume. *Molecular ecology*, *28*(12), 2967-2985.

- Gompert, Z., Jahner, J. P., Scholl, C. F., Wilson, J. S., Lucas, L. K., Soria-Carrasco, V., Fordyce, J. A., Nice, C. C., Buerkle, C. A., & Forister, M. L. (2015). The evolution of novel host use is unlikely to be constrained by trade-offs or a lack of genetic variation. *Molecular ecology*, *24*(11), 2777-2793.
- Gompert, Z., Lucas, L. K., Buerkle, C. A., Forister, M. L., Fordyce, J. A., & Nice, C. C. (2014). Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. *Molecular ecology*, *23*(18), 4555-4573.
- Gompert, Z., Lucas, L. K., Nice, C. C., Fordyce, J. A., Forister, M. L., & Buerkle, C. A. (2012). Genomic regions with a history of divergent selection affect fitness of hybrids between two butterfly species. *Evolution: International Journal of Organic Evolution*, *66*(7), 2167-2181.
- Gormally, B. M., Fuller, R., McVey, M., & Romero, L. M. (2019). DNA damage as an indicator of chronic stress: Correlations with corticosterone and uric acid. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *227*, 116-122.
- Greives, T. J., Dochtermann, N. A., & Stewart, E. C. (2017). Estimating heritable genetic contributions to innate immune and endocrine phenotypic correlations: A need to explore repeatability. *Hormones and behavior*, *88*, 106-111.
- Greven, S., Crainiceanu, C. M., Küchenhoff, H., & Peters, A. (2008). Restricted likelihood ratio testing for zero variance components in linear mixed models. *Journal of computational and graphical statistics*, *17*(4), 870-891.
- Guan, Y., & Stephens, M. (2011). Bayesian variable selection regression for genome-wide association studies and other large-scale problems. *The Annals of Applied Statistics*, *5*(3), 1780-1815.
- Guindre-Parker, S. (2020). Individual variation in glucocorticoid plasticity: considerations and future directions. *Integrative and comparative biology*, *60*(1), 79-88.

- Gustafsson, L. (1986). Lifetime reproductive success and heritability: empirical support for Fisher's fundamental theorem. *The American Naturalist*, *128*(5), 761-764.
- Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford university press, USA.
- Hansen, T. F., & Houle, D. (2008). Measuring and comparing evolvability and constraint in multivariate characters. *Journal of evolutionary biology*, *21*(5), 1201-1219.
- Hasselquist, D., & Nilsson, J.-Å. (2012). Physiological mechanisms mediating costs of immune responses: what can we learn from studies of birds? *Animal Behaviour*, *83*(6), 1303-1312.
- Ho, D. H., & Burggren, W. W. (2010). Epigenetics and transgenerational transfer: a physiological perspective. *Journal of Experimental Biology*, *213*(1), 3-16.
- Jenkins, B. R., Vitousek, M. N., Hubbard, J. K., & Safran, R. J. (2014). An experimental analysis of the heritability of variation in glucocorticoid concentrations in a wild avian population. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1790), 20141302.
- Johnson, L. J., & Tricker, P. J. (2010). Epigenomic plasticity within populations: its evolutionary significance and potential. *Heredity*, *105*(1), 113-121.
- Johnston, D., Mukiibi, R., Waters, S. M., McGee, M., Surlis, C., McClure, J. C., McClure, M. C., Todd, C. G., & Earley, B. (2020). Genome wide association study of passive immunity and disease traits in beef-suckler and dairy calves on Irish farms. *Scientific Reports*, *10*(1), 1-10.
- Johnston, S. E., Bérénois, C., Slate, J., & Pemberton, J. M. (2016). Conserved genetic architecture underlying individual recombination rate variation in a wild population of Soay sheep (*Ovis aries*). *Genetics*, *203*(1), 583-598.
- Jones, M., & Gillham, B. (1988). Factors involved in the regulation of adrenocorticotrophic hormone/beta-lipotrophic hormone. *Physiological reviews*, *68*(3), 743-818.

- Kahar, S., Debes, P. V., Vuori, K. A., Vähä, J.-P., & Vasemägi, A. (2016). Heritability, environmental effects, and genetic and phenotypic correlations of oxidative stress resistance-related enzyme activities during early life stages in Atlantic salmon. *Evolutionary Biology*, *43*(2), 215-226.
- Kearney, M., Fujita, M., Ridenour, J., Schön, I., Martens, K., & van Dijk, P. (2009). Lost sex: the evolutionary biology of parthenogenesis.
- Kelly, M. W., Grosberg, R. K., & Sanford, E. (2013). Trade-offs, geography, and limits to thermal adaptation in a tide pool copepod. *The American Naturalist*, *181*(6), 846-854.
- Kemper, K. E., Saxton, S. J., Bolormaa, S., Hayes, B. J., & Goddard, M. E. (2014). Selection for complex traits leaves little or no classic signatures of selection. *BMC genomics*, *15*(1), 1-14.
- Kim, S. Y., Noguera, J., Morales, J., & Velando, A. (2010a). Heritability of resistance to oxidative stress in early life. *Journal of evolutionary biology*, *23*(4), 769-775.
- Kim, S. Y., Velando, A., Sorci, G., & Alonso-Alvarez, C. (2010b). Genetic correlation between resistance to oxidative stress and reproductive life span in a bird species. *Evolution: International Journal of Organic Evolution*, *64*(3), 852-857.
- Kimbrell, D. A., & Beutler, B. (2001). The evolution and genetics of innate immunity. *Nature Reviews Genetics*, *2*(4), 256-267.
- Korneliussen, T. S., Albrechtsen, A., & Nielsen, R. (2014). ANGSD: analysis of next generation sequencing data. *BMC bioinformatics*, *15*(1), 1-13.
- Kruuk, L. E. (2004). Estimating genetic parameters in natural populations using the 'animal model'. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *359*(1446), 873-890.



- Kruuk, L. E., Clutton-Brock, T. H., Slate, J., Pemberton, J. M., Brotherstone, S., & Guinness, F. E. (2000). Heritability of fitness in a wild mammal population. *Proceedings of the National Academy of Sciences*, 97(2), 698-703.
- Kruuk, L. E., & Hadfield, J. D. (2007). How to separate genetic and environmental causes of similarity between relatives. *Journal of evolutionary biology*, 20(5), 1890-1903.
- Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *bioinformatics*, 27(21), 2987-2993.
- Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *bioinformatics*, 25(14), 1754-1760.
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009). The sequence alignment/map format and SAMtools. *bioinformatics*, 25(16), 2078-2079.
- Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos*, 88(1), 87-98.
- Losdat, S., Helfenstein, F., Blount, J. D., & Richner, H. (2014). Resistance to oxidative stress shows low heritability and high common environmental variance in a wild bird. *Journal of evolutionary biology*, 27(9), 1990-2000.
- Love, O. P., McGowan, P. O., & Sheriff, M. J. (2013). Maternal adversity and ecological stressors in natural populations: the role of stress axis programming in individuals, with implications for populations and communities. *Functional Ecology*, 27(1), 81-92.

- Lowry, D. B., Hoban, S., Kelley, J. L., Lotterhos, K. E., Reed, L. K., Antolin, M. F., & Storfer, A. (2017). Breaking RAD: An evaluation of the utility of restriction site-associated DNA sequencing for genome scans of adaptation.
- Lucas, L. K., Nice, C. C., & Gompert, Z. (2018). Genetic constraints on wing pattern variation in *Lycaeides* butterflies: A case study on mapping complex, multifaceted traits in structured populations. *Molecular ecology resources*, *18*(4), 892-907.
- Lynch, M., & Walsh, B. (1998). Genetics and analysis of quantitative traits.
- MacDougall-Shackleton, S. A., Bonier, F., Romero, L. M., & Moore, I. T. (2019). Glucocorticoids and “stress” are not synonymous. *Integrative Organismal Biology*, *1*(1), obz017.
- Mackay, T. F., Stone, E. A., & Ayroles, J. F. (2009). The genetics of quantitative traits: challenges and prospects. *Nature Reviews Genetics*, *10*(8), 565-577.
- Majer, A. D., Fasanello, V. J., Tindle, K., Frenz, B. J., Ziur, A. D., Fischer, C. P., Fletcher, K. L., Seecof, O. M., Gronsky, S., & Vassallo, B. G. (2019). Is there an oxidative cost of acute stress? Characterization, implication of glucocorticoids and modulation by prior stress experience. *Proceedings of the Royal Society B*, *286*(1915), 20191698.
- Martin, L. B., Hanson, H. E., Hauber, M. E., & Ghalambor, C. K. (2021). Genes, environments, and phenotypic plasticity in immunology. *Trends in Immunology*, *42*(3), 198-208.
- Martins, F., Kruuk, L., Llewelyn, J., Moritz, C., & Phillips, B. (2019). Heritability of climate-relevant traits in a rainforest skink. *Heredity*, *122*(1), 41-52.
- McKean, K. A., & Lazzaro, B. P. (2011). The costs of immunity and the evolution of immunological defense mechanisms. *Mechanisms of life history evolution*, 299-310.

- McKinney, G. J., Larson, W. A., Seeb, L. W., & Seeb, J. E. (2017). RAD seq provides unprecedented insights into molecular ecology and evolutionary genetics: comment on Breaking RAD by Lowry et al.(2016). *Molecular ecology resources*, *17*(3), 356-361.
- Metcalfe, N. B., & Alonso-Alvarez, C. (2010). Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Functional Ecology*, *24*(5), 984-996.
- Moser, G., Lee, S. H., Hayes, B. J., Goddard, M. E., Wray, N. R., & Visscher, P. M. (2015). Simultaneous discovery, estimation and prediction analysis of complex traits using a Bayesian mixture model. *PLoS genetics*, *11*(4), e1004969.
- Mousseau, T. A., & Roff, D. A. (1987). Natural selection and the heritability of fitness components. *Heredity*, *59*(2), 181-197.
- Müller, C., Jenni-Eiermann, S., Blondel, J., Perret, P., Caro, S. P., Lambrechts, M. M., & Jenni, L. (2007). Circulating corticosterone levels in breeding blue tits *Parus caeruleus* differ between island and mainland populations and between habitats. *General and Comparative Endocrinology*, *154*(1-3), 128-136.
- Narum, S. R., Buerkle, C. A., Davey, J. W., Miller, M. R., & Hohenlohe, P. A. (2013). Genotyping-by-sequencing in ecological and conservation genomics. *Molecular ecology*, *22*(11), 2841.
- Nathan, C., & Cunningham-Bussel, A. (2013). Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nature Reviews Immunology*, *13*(5), 349-361.
- Nespolo, R. F., Bartheld, J. L., Gonzalez, A., Bruning, A., Roff, D. A., Bacigalupe, L. D., & Gaitán-Espitia, J. D. (2014). The quantitative genetics of physiological and morphological traits in an invasive terrestrial snail: additive vs. non-additive genetic variation. *Functional Ecology*, *28*(3), 682-692.

- Neuman-Lee, L. A., & French, S. S. (2017). Endocrine-reproductive-immune interactions in female and male Galápagos marine iguanas. *Hormones and behavior*, *88*, 60-69.
- Nosil, P., Funk, D. J., & Ortiz-Barrientos, D. (2009). Divergent selection and heterogeneous genomic divergence. *Molecular ecology*, *18*(3), 375-402.
- Olsson, M., Wilson, M., Uller, T., Mott, B., Isaksson, C., Healey, M., & Wanger, T. (2008). Free radicals run in lizard families. *Biology Letters*, *4*(2), 186-188.
- Orr, H. A. (2000). Adaptation and the cost of complexity. *Evolution*, *54*(1), 13-20.
- Orr, H. A. (2005). The genetic theory of adaptation: a brief history. *Nature Reviews Genetics*, *6*(2), 119-127.
- Pallares, L. F., Carbonetto, P., Gopalakrishnan, S., Parker, C. C., Ackert-Bicknell, C. L., Palmer, A. A., & Tautz, D. (2015). Mapping of craniofacial traits in outbred mice identifies major developmental genes involved in shape determination. *PLoS genetics*, *11*(11), e1005607.
- Pallares, L. F., Harr, B., Turner, L. M., & Tautz, D. (2014). Use of a natural hybrid zone for genomewide association mapping of craniofacial traits in the house mouse. *Molecular ecology*, *23*(23), 5756-5770.
- Palme, R. (2019). Non-invasive measurement of glucocorticoids: Advances and problems. *Physiology & behavior*, *199*, 229-243.
- Pamplona, R., & Costantini, D. (2011). Molecular and structural antioxidant defenses against oxidative stress in animals. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *301*(4), R843-R863.
- Parchman, T. L., Gompert, Z., Mudge, J., Schilkey, F. D., Benkman, C. W., & Buerkle, C. A. (2012). Genome-wide association genetics of an adaptive trait in lodgepole pine. *Molecular ecology*, *21*(12), 2991-3005.

- Pavličev, M., & Cheverud, J. M. (2015). Constraints evolve: context dependency of gene effects allows evolution of pleiotropy. *Annual Review of Ecology, Evolution, and Systematics*, 46, 413-434.
- Pedersen, A. B., & Babayan, S. A. (2011). Wild immunology. *Molecular ecology*, 20(5), 872-880.
- Pennock, L. A., Tinkle, D. W., & Shaw, M. W. (1968). Chromosome number in the lizard genus *Uta* (family Iguanidae). *Chromosoma*, 24(4), 467-476.
- Plummer, M., Best, N., Cowles, K., & Vines, K. (2006). CODA: convergence diagnosis and output analysis for MCMC. *R news*, 6(1), 7-11.
- Plummer, M., Stukalov, A., & Denwood, M. (2016). rjags: Bayesian graphical models using MCMC. *R package version*, 4(6).
- Poletta, G. L., Siroski, P. A., Amavet, P. S., Ortega, H. H., & Mudry, M. D. (2013). Reptiles as animal models: examples of their utility in genetics, immunology and toxicology. *Book: Reptile across research field*, 1-39.
- Porto, A., Sebastião, H., Pavan, S. E., VandeBerg, J. L., Marroig, G., & Cheverud, J. M. (2015). Rate of evolutionary change in cranial morphology of the marsupial genus *Monodelphis* is constrained by the availability of additive genetic variation. *Journal of evolutionary biology*, 28(4), 973-985.
- Puentes, A., Granath, G., & Ågren, J. (2016). Similarity in G matrix structure among natural populations of *Arabidopsis lyrata*. *Evolution*, 70(10), 2370-2386.
- R Core Team. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Renaud, S., Auffray, J. C., & Michaux, J. (2006). Conserved phenotypic variation patterns, evolution along lines of least resistance, and departure due to selection in fossil rodents. *Evolution*, 60(8), 1701-1717.

- Ricklefs, R. E., & Wikelski, M. (2002). The physiology/life-history nexus. *Trends in ecology & evolution*, *17*(10), 462-468.
- Riesch, R., Muschick, M., Lindtke, D., Villoutreix, R., Comeault, A. A., Farkas, T. E., Lucek, K., Hellen, E., Soria-Carrasco, V., & Dennis, S. R. (2017). Transitions between phases of genomic differentiation during stick-insect speciation. *Nature ecology & evolution*, *1*(4), 1-13.
- Riggs, A. D., & Xiong, Z. (2004). Methylation and epigenetic fidelity. *Proceedings of the National Academy of Sciences*, *101*(1), 4-5.
- Rockman, M. V. (2012). The QTN program and the alleles that matter for evolution: all that's gold does not glitter. *Evolution: International Journal of Organic Evolution*, *66*(1), 1-17.
- Roff, D. A. (2002). *Life history evolution*.
- Roff, D. A., & Emerson, K. (2006). Epistasis and dominance: evidence for differential effects in life-history versus morphological traits. *Evolution*, *60*(10), 1981-1990.
- Roff, D. A., & Mousseau, T. A. (1987). Quantitative genetics and fitness: lessons from *Drosophila*. *Heredity*, *58*(1), 103-118.
- Roff, D. A., Stirling, G., & Fairbairn, D. J. (1997). The evolution of threshold traits: a quantitative genetic analysis of the physiological and life-history correlates of wing dimorphism in the sand cricket. *Evolution*, *51*(6), 1910-1919.
- Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, *4*, e2584.
- Romero, L. M., & Reed, J. M. (2005). Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *140*(1), 73-79.

- Ryman, N., Laikre, L., & Hössjer, O. (2019). Do estimates of contemporary effective population size tell us what we want to know? *Molecular ecology*, *28*(8), 1904-1918.
- Santure, A. W., & Garant, D. (2018). Wild GWAS—association mapping in natural populations. *Molecular ecology resources*, *18*(4), 729-738.
- Santure, A. W., Poissant, J., De Cauwer, I., Van Oers, K., Robinson, M. R., Quinn, J. L., Groenen, M. A., Visser, M. E., Sheldon, B. C., & Slate, J. (2015). Replicated analysis of the genetic architecture of quantitative traits in two wild great tit populations. *Molecular ecology*, *24*(24), 6148-6162.
- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine reviews*, *21*(1), 55-89.
- Scheiner, S. M. (1993). Genetics and evolution of phenotypic plasticity. *Annual review of ecology and systematics*, *24*(1), 35-68.
- Scheipl, F., Greven, S., & Küchenhoff, H. (2008). Size and power of tests for a zero random effect variance or polynomial regression in additive and linear mixed models. *Computational statistics & data analysis*, *52*(7), 3283-3299.
- Schmidt, K. P. (1921). New species of North American lizards of the genera *Holbrookia* and *Uta*. American Museum novitates; no. 22.
- Sebastiano, M., Eens, M., Messina, S., AbdElgawad, H., Pineau, K., Beemster, G. T., Chastel, O., & Costantini, D. (2018). Resveratrol supplementation reduces oxidative stress and modulates the immune response in free-living animals during a viral infection. *Functional Ecology*, *32*(11), 2509-2519.
- Seebacher, F. (2005). A review of thermoregulation and physiological performance in reptiles: what is the role of phenotypic flexibility? *Journal of Comparative Physiology B*, *175*(7), 453-461.

- Sheriff, M. J., Dantzer, B., Delehanty, B., Palme, R., & Boonstra, R. (2011). Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia*, *166*(4), 869-887.
- Shimba, A., Ejima, A., & Ikuta, K. (2021). Pleiotropic Effects of Glucocorticoids on the Immune System in Circadian Rhythm and Stress. *Frontiers in Immunology*, 4217.
- Sies, H., Berndt, C., & Jones, D. P. (2017). Oxidative stress. *Annual review of biochemistry*, *86*, 715-748.
- Sinervo, B., & Licht, P. (1991). Hormonal and physiological control of clutch size, egg size, and egg shape in side-blotched lizards (*Uta stansburiana*): constraints on the evolution of lizard life histories. *Journal of Experimental Zoology*, *257*(2), 252-264.
- Sniegula, S., Golab, M. J., Drobniak, S. M., & Johansson, F. (2018). The genetic variance but not the genetic covariance of life-history traits changes towards the north in a time-constrained insect. *Journal of evolutionary biology*, *31*(6), 853-865.
- Soneja, A., Drews, M., & Malinski, T. (2005). Role of nitric oxide, nitroxidative and oxidative stress in wound healing. *Pharmacological reports*, *57*, 108.
- Sorci, G., & Faivre, B. (2009). Inflammation and oxidative stress in vertebrate host.
- Sorci, G., Swallow, J. G., Garland Jr, T., & Clobert, J. (1995). Quantitative genetics of locomotor speed and endurance in the lizard *Lacerta vivipara*. *Physiological Zoology*, *68*(4), 698-720.
- Soria-Carrasco, V., Gompert, Z., Comeault, A. A., Farkas, T. E., Parchman, T. L., Johnston, J. S., Buerkle, C. A., Feder, J. L., Bast, J., & Schwander, T. (2014). Stick insect genomes reveal natural selection's role in parallel speciation. *Science*, *344*(6185), 738-742.
- Srisapoome, P., Chatchaiphan, S., Bunnoy, A., Koonawootrittriron, S., & Na-Nakorn, U. (2019). Heritability of immunity traits and disease resistance of bighead catfish, *Clarias macrocephalus* Günther, 1864. *Fish & Shellfish Immunology*, *92*, 209-215.



- Stearns, F. W. (2010). One hundred years of pleiotropy: a retrospective. *Genetics*, *186*(3), 767-773.
- Stearns, S. C. (1992). *The evolution of life histories*.
- Stebbins, R. C., & McGinnis, S. M. (2018). *Peterson field guide to western reptiles & amphibians*.  
Houghton Mifflin.
- Su, Y. S., & Yajima, M. (2015). Package 'r2jags'. *R package version 0.03-08*.
- Sun, W., & Hu, Y. (2013). eQTL mapping using RNA-seq data. *Statistics in biosciences*, *5*(1), 198-219.
- Tieleman, B. I., Croese, E., Helm, B., & Versteegh, M. A. (2010). Repeatability and individual correlates of microbicidal capacity of bird blood. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *156*(4), 537-540.
- Tiffin, P., & Ross-Ibarra, J. (2014). Advances and limits of using population genetics to understand local adaptation. *Trends in ecology & evolution*, *29*(12), 673-680.
- Tinkle, D. W. (1969). The concept of reproductive effort and its relation to the evolution of life histories of lizards. *The American Naturalist*, *103*(933), 501-516.
- Tom, J. A., Reeder, J., Forrest, W. F., Graham, R. R., Hunkapiller, J., Behrens, T. W., & Bhangale, T. R. (2017). Identifying and mitigating batch effects in whole genome sequencing data. *BMC bioinformatics*, *18*(1), 1-12.
- Turner, F. B., Hoddenbach, G. A., Medica, P. A., & Lannon, J. R. (1970). The demography of the lizard, *Uta stansburiana* Baird and Girard, in southern Nevada. *The Journal of Animal Ecology*, 505-519.
- Tylan, C., Camacho, K., French, S., Graham, S. P., Herr, M. W., Jones, J., McCormick, G. L., O'Brien, M. A., Tennessen, J. B., & Thawley, C. J. (2020). Obtaining plasma to measure baseline corticosterone concentrations in reptiles: How quick is quick enough? *General and Comparative Endocrinology*, *287*, 113324.

- Upton, D. E., & Murphy, R. W. (1997). Phylogeny of the side-blotched lizards (Phrynosomatidae: Uta) based on mtDNA sequences: support for a midpeninsular seaway in Baja California. *Molecular Phylogenetics and Evolution*, 8(1), 104-113.
- Vagasi, C. I., Pătraș, L., Pap, P. L., Vincze, O., Mureșan, C., Nemeth, J., & Lendvai, A. Z. (2018). Experimental increase in baseline corticosterone level reduces oxidative damage and enhances innate immune response. *PloS one*, 13(2), e0192701.
- Van Dyke, J. U., Brandley, M. C., & Thompson, M. B. (2014). The evolution of viviparity: molecular and genomic data from squamate reptiles advance understanding of live birth in amniotes. *Reproduction*, 147(1), R15-R26.
- Vettorazzi, S., Nalbantoglu, D., Gebhardt, J. C. M., & Tuckermann, J. (2021). A guide to changing paradigms of glucocorticoid receptor function—A model system for genome regulation and physiology. *The FEBS Journal*.
- Visser, M. E. (2008). Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings of the Royal Society B: Biological Sciences*, 275(1635), 649-659.
- Vitousek, M. N., Taff, C. C., Hallinger, K. K., Zimmer, C., & Winkler, D. W. (2018). Hormones and fitness: evidence for trade-offs in glucocorticoid regulation across contexts. *Frontiers in Ecology and Evolution*, 6, 42.
- Wagner, G. P., & Zhang, J. (2011). The pleiotropic structure of the genotype–phenotype map: the evolvability of complex organisms. *Nature Reviews Genetics*, 12(3), 204-213.
- Walsh, B., & Blows, M. W. (2009). Abundant genetic variation+ strong selection= multivariate genetic constraints: a geometric view of adaptation. *Annual Review of Ecology, Evolution, and Systematics*, 40, 41-59.
- Walsh, B., & Lynch, M. (2018). *Evolution and selection of quantitative traits*. Oxford University Press.

- Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews Genetics*, *10*(1), 57-63.
- Waples, R. S. (1989). A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics*, *121*(2), 379-391.
- Watson, H., Videvall, E., Andersson, M. N., & Isaksson, C. (2017). Transcriptome analysis of a wild bird reveals physiological responses to the urban environment. *Scientific Reports*, *7*(1), 1-10.
- Williams, H. J., Shipley, J. R., Rutz, C., Wikelski, M., Wilkes, M., & Hawkes, L. (2021). Future trends in measuring physiology in free-living animals. *Philosophical Transactions of the Royal Society B*, *376*(1831), 20200230.
- Wingfield, J. C., Maney, D. L., Breuner, C. W., Jacobs, J. D., Lynn, S., Ramenofsky, M., & Richardson, R. D. (1998). Ecological bases of hormone—behavior interactions: the “emergency life history stage”. *American Zoologist*, *38*(1), 191-206.
- Wittman, T. N., Robinson, C. D., McGlothlin, J. W., & Cox, R. M. (2021). Hormonal pleiotropy structures genetic covariance. *Evolution Letters*, *5*(4), 397-407.
- Wobeser, G. A. (2013). *Essentials of disease in wild animals*. John Wiley & Sons.
- Zaitlen, N., & Kraft, P. (2012). Heritability in the genome-wide association era. *Human genetics*, *131*(10), 1655-1664.
- Zeng, P., & Zhou, X. (2017). Non-parametric genetic prediction of complex traits with latent Dirichlet process regression models. *Nature communications*, *8*(1), 1-11.
- Zhou, X., Carbonetto, P., & Stephens, M. (2013). Polygenic modeling with Bayesian sparse linear mixed models. *PLoS genetics*, *9*(2), e1003264.
- Zhu, H., & Zhou, X. (2020). Statistical methods for SNP heritability estimation and partition: a review. *Computational and Structural Biotechnology Journal*, *18*, 1557-1568.

Zimmerman, L., Vogel, L., & Bowden, R. (2010). Understanding the vertebrate immune system: insights from the reptilian perspective. *Journal of Experimental Biology*, 213(5), 661-671.

Zimmerman, L. M. (2020). The reptilian perspective on vertebrate immunity: 10 years of progress. *Journal of Experimental Biology*, 223(21), jeb214171.

## CHAPTER IV

TIME-SERIES GENOMICS OF WILD LIZARDS IN AN URBAN-NATURAL LANDSCAPE:  
EVOLUTIONARY IMPLICATIONS FOR PHYSIOLOGICAL TRAITS LINKED TO  
SURVIVAL

The pervasive challenges of urbanization on wildlife prompt consideration of the evolutionary potential of traits relevant to survival in cities. Despite past research identifying patterns of phenotypic divergence across the urban-natural landscape, few studies have provided genetic evidence of trait evolution owed to adaptive and nonadaptive processes in this context. In this study, population genomic time-series data of a widespread reptile, the side-blotched lizard (*Uta stansburiana*), were used to test for environmental differences (urban versus natural) in genetic variation and selection on functionally relevant physiological traits (bacterial killing ability, antioxidant capacity, and corticosterone) and their associated genetic loci. Additionally, population-level comparisons of genetic structure (PCA) and differentiation ( $F_{ST}$ ), allele frequency change ( $|\Delta p|$ ), variance effective population size ( $N_e V$ ), and molecular diversity ( $\pi$ ) were compared across environments. Side-blotched lizards inhabiting urban patches were genetically differentiated from those residing in natural areas (mean  $F_{ST} = 0.03$ ), but this was largely owed to isolation by distance. Subtle genetic similarities and comparable levels of molecular diversity (mean  $\pi = 0.333$ ) were also evident across populations. Moreover, selection was not detected for any of the physiological traits or associated genetic loci, while indicators of drift were evident across the landscape (mean  $N_e V = 31$ ), especially for urban populations ( $N_e V \geq 10$ ). These results suggest that contemporary evolution by drift can overwhelm selection on

physiological traits for *U. stansburiana*, while genetic variation for trait expression seems to be maintained by other evolutionary processes. These factors contribute to the growing knowledge of contemporary evolution and genetic viability for lizards occupying urban-natural landscapes.

## INTRODUCTION

Urbanization represents one of the most pervasive drivers of evolutionary change, as species worldwide are threatened by the rapid transformation of their landscape (Crane & Kinzig, 2005; McDonald et al., 2013). Both the biotic and abiotic environment undergo numerous changes that alter the structure and function of ecosystems and reduce biodiversity via extirpation (Gibbons et al., 2000; Cushman, 2006; Grimm et al., 2008; McKinney, 2008). Oftentimes, urban habitats are either lost or fragmented due to built-up structures (e.g., buildings, roads, other impermeable surfaces; Niemelä et al., 2011), while being susceptible to physicochemical changes (e.g., hydrology, soil geochemistry, nutrient cycling, temperature), pollution (e.g., light, noise), non-native species (e.g., predators, competitors), novel disease transmission, and other anthropogenic activities (Lafferty & Kuris, 2005; Mckinney, 2006; Bradley & Altizer, 2007). Combined, these novel pressures make urban areas challenging environments for native wildlife, with potential evolutionary implications for their phenotypic responses (Donihue & Lambert, 2015; Hendry et al., 2017; Johnson & Munshi-South, 2017; Schell et al., 2021). Indeed, patterns of phenotypic divergence are becoming increasingly evident across the urban-natural landscape, indicating functionally relevant traits may be undergoing rapid evolutionary change among persisting species (Alberti et al., 2017; Sanderson et al., 2022). Yet, population genomic evidence of local adaptation remains largely absent for animals residing in urban areas (Messer et al., 2016; Santangelo et al., 2018; Rivkin et al., 2019).

Traits encompassing life history, morphology, physiology, and behavior are now posited to be selected upon to produce urban ecotypes over short time periods (e.g., Reid et al., 2016; Winchell et al., 2016; Diamond et al., 2017, 2018; Brans & De Meester, 2018; Brans et al., 2018). Alleles beneficial to traits with a heritable basis (i.e., additive genetic variation) could shift in frequency under intense urban selective pressures, resulting in phenotypic responses that yield improved fitness outcomes. However, the capacity of genotypes to produce variable phenotypes (i.e., trait plasticity) could be mediating the selective responses to urbanization, and selection could even be acting on plasticity itself (Price et al., 2003; Lande, 2009; Chevin et al., 2010). In addition, changes in allele frequencies may be compounded by genetic drift in urban environments, particularly for populations with reduced sizes or greater isolation due to habitat fragmentation and bottleneck effects (reviewed in Miles et al., 2019). While drift can be partially counteracted by gene flow (Crispo et al., 2011; Theodorou et al., 2018), genetic connectivity can be impeded by urban features for animals with specific habitat requirements and low vagility (i.e., unable to disperse across the urban matrix; Frankham et al., 2019). In turn, a large continuous population is liable to become several smaller, genetically differentiated populations susceptible to reductions in genetic diversity and an accumulation of deleterious mutations via inbreeding depression (Higgins & Lynch, 2001; Couvet, 2002; Noël et al., 2007; Munshi-South et al., 2016; Lourenço et al., 2017). Combined, these evolutionary processes prompt uncertainty over the extent that urban ecotypes arise from local adaptations (Reed et al., 2003), though they could be the difference between whether a species persists or vanishes from urban areas (Frankham, 1995a). Granted even that selection on relevant traits themselves could be tested (e.g., fitness associations that vary by environment), instances where selection occurs intermittently within a study period could be overlooked. Identifying the strength, form, and

targets of selection with population genomic data should therefore help reveal the adaptive potential of phenotypic responses to urbanization (Buffalo & Coop, 2019, 2020).

Among the traits that affect the likelihood of persistence in urban wildlife, those linked to physiological systems have been underrepresented from a population genomic perspective (e.g., Campbell-Staton et al., 2020; Green et al., 2023). Of relevance in this context is the ability to respond to injuries and infections via the immune system, since failing to mount an effective immune response can jeopardize organismal health and survival (McKean & Lazzaro, 2011; Wobeser, 2013). Along the same line, the capacity to resist prooxidant chemical species generated for essential cellular functions, including immunity (e.g., Soneja et al., 2005; Sorci & Faivre, 2009; Costantini & Moller, 2009; Nathan & Cunningham-Bussel, 2013), has been recognized as important, because poor antioxidant defenses can undermine longevity via excessive oxidative stress (e.g., oxidative damage to DNA, proteins, and lipids; Halliwell & Gutteridge, 2015; Sies et al., 2017). Moreover, metabolic hormones that mobilize energy stores (i.e., glucocorticoids; Sapolsky et al., 2000; Crespi et al., 2013) are often consequential because of their role in mediating physiological responses to environmental challenges (Wingfield et al., 1998; Angelier & Wingfield, 2013). Along with other heritable traits, these physiological parameters can shift in urban wildlife, perhaps caused by selection for alternative life history strategies under novel environmental conditions. Yet the degree to which evolution, phenotypic plasticity, or a combination thereof may underlie differences in physiology remains unclear. Testing for selection on genetic variants should help reveal the extent that these traits and their associated loci are adaptively evolving in urban systems (Barrett & Hoekstra, 2011; Losos et al., 2013; Rivkin et al., 2019).



In this study, evolutionary changes were assessed in urban and natural populations of a widespread reptile, the side-blotched lizard (*Uta stansburiana*), to help resolve the fundamental causes of physiological differences previously found across their landscape (Lucas & French, 2012). An approximate Bayesian computation (ABC) method was used to detect and quantify selection on physiological traits (bacterial killing ability, antioxidant capacity, and corticosterone) mapped to genomic time-series data. With this method, phenotypic selection was modeled as an explicit function of either an urban or natural environment. The population genomic consequences of selection were then modeled based on estimated genotype–phenotype associations, allowing for inferences using patterns of change across multiple genetic loci, populations, and generations. Additionally, population-level comparisons of genetic structure and differentiation, variance effective population size, and genetic diversity were tested between environments. Populations of *U. stansburiana* inhabiting fragmented urban patches were hypothesized to be genetically differentiated from those residing in natural riparian zones. Urban populations were also expected to have greater susceptibility to genetic drift (i.e., smaller effective population sizes), and thus lower molecular diversity levels. Given the suspected polygenic basis and plasticity of physiological traits, strong selection was hypothesized to be unlikely, though fluctuating selection was still expected to be detectable among populations.

## METHODS

### *Study system*

*Uta stansburiana* are small, short-lived, and abundant lizards found throughout dry regions of western North America (Stebbins & McGinnis, 2018). Their distribution is widespread overall, albeit influenced by the availability of riparian zones within deserts and their limited dispersal among

localities (i.e., dispersal beyond 300 m is rare; Doughty & Sinervo, 1994). Researchers have often used *U. stansburiana* for studies of life history, ecophysiology, and evolution (e.g., Tinkle, 1969; Sinervo & Licht, 1991). Due to their ease of capture and sampling, large abundance, simple diploid genetics (34 chromosomes; consisting of 12 macro- and 22 microchromosomes), and generation time (~1-2 years), these lizards are suitable for population genetics (Pennock et al., 1968; Turner et al., 1970). Lizards from this study consisted of six populations located in or around one of the fastest growing urban areas in the United States, St. George, Utah (U.S. Census Bureau, 2022). Three populations reside in fragmented habitats within St. George, where they are exposed to novel substrates (e.g., impervious surfaces), daily human interactions (e.g., recreational, construction, and maintenance activities), competitors (e.g., tree lizards), and novel predators (e.g., cats). The remaining three sites are in a natural area north of St. George and are not exposed to similar human pressures. Each site included rocky areas ( $\leq 1.8$  ha) that were situated near or along a riparian corridor. The six sites lay an average of 21 km from one another (minimum = 4 km; maximum = 42 km; Fig. 2.1). All of which were close to the intersection of the Great Basin, Colorado Plateau and Mojave Desert.

### *Field sampling and processing*

Adult side-blotched lizards were captured with a snare pole during their breeding season (May 2012 - 2017) at the sites within and around St. George, Utah, USA ( $N = 757$ ). Urban site 2 was demolished for commercial development in late 2015, and as a result, no lizards were found there in 2016 and 2017 (Table 3.1). Lizards were collected between 0800 – 1300 to limit circadian variation in physiological condition (Dallman et al., 1987; Jones & Gillham, 1988). Whole blood was collected from the retro-orbital sinus of each lizard with a heparinized capillary tube within a validated window of time ( $\leq 3$  minutes) to measure basal physiology (Romero & Reed, 2005; Tylan et al., 2020). Blood

samples were centrifuged at 6000 RPM for 10 minutes to isolate plasma and erythrocytes. To control for performance in physiological assays (Claunch et al., 2022), samples were kept on ice until they were stored at  $-80^{\circ}\text{C}$  for lab analyses at Utah State University in Logan, Utah. After blood processing, a unique combination of one to four whole toes were removed from each lizard for identification, circumventing pseudo-replication of sampling across years. Lizard sex was also determined based on dorsal color pattern (speckled blue for males, ornate brown for females) and the presence or absence of hemipenes. All lizards were returned to their capture locations after completion. The work presented here was approved under the Department of Wildlife Resources COR #1COLL8382 and Utah State University Institutional Animal Care and Use Committee's protocol #2068.

### *Bacterial killing ability*

Bactericidal assays serve as an integrative measure of constitutive immune function, in which a known concentration of bacteria is added to animal plasma containing important immunological components (e.g., natural antibodies, phagocytes, complement, antimicrobial peptides) that kill the bacteria (reviewed in Demas, 2011). Measuring the bacterial growth outcomes translates to the bactericidal capacity of the plasma, and in turn, the immunocompetence of the animal. Here, a validated volume of blood plasma (6  $\mu\text{l}$ ) was used to assess the relative abundances of circulating immune components. Using the protocol outlined in French and Neuman-Lee (2012), a 1:2 plasma dilution was combined with  $\text{CO}_2$ -independent medium (Gibco # 18-045-088, ThermoFisher Scientific, Grand Island, NY, USA), 4 nM l-glutamine,  $10^4$  colony-producing units of *Escherichia coli* (EPower™ Microorganisms #483-581-1, ATCC 8739, MicroBioLogics, St. Cloud, MN, USA), and agar broth on a 96-well microplate. Included were both positive (i.e., media and bacteria with no plasma) and negative (i.e., media and no plasma or bacteria) controls to account for potential growth

and ensure there was no contamination. The plate was incubated for a 12-h period, at which point absorbance per well was measured with a microplate reader at 300 nm (xMark; BioRad Benchmark, Hercules, CA, USA). Bacterial killing ability, or BKA (%), was then calculated as  $(1 - (\text{absorbance of sample} / \text{absorbance of positive controls}) \times 100)$ . Each sample was run in duplicate to generate an average percent score with coefficients of variation <20% within and among all assays.

#### *Antioxidant capacity*

Total non-enzymatic antioxidant capacity was measured using an OXY-Adsorbent test (MC002, Diacron International, Italy), which determines effectiveness of the blood antioxidant barrier by quantifying tolerance of the oxidant action of hypochlorous acid (HClO). Here, 2  $\mu$ l of plasma were diluted in 100  $\mu$ l of distilled water, and manufacturer instructions were followed thereafter for measuring 96-well microplates with a spectrophotometer at 505 nm (xMark, Bio-Rad). Measures of total antioxidant capacity, or OXY (mol HClO/ml), were acquired with coefficients of variation <20% within and among all assays.

#### *Corticosterone*

Following a protocol outlined in Neuman-Lee and French (2017), radioimmunoassays were conducted on plasma samples (10  $\mu$ L) for CORT (Ab: MP Biomedicals # 07-120016). Samples were extracted with 30% ethyl acetate:isooctane and run in duplicate for the assay. Individual recoveries were calculated from the extractions, to correct for final concentrations with a minimum level of detection of 0.3 ng/ml. Coefficients of variation were <20% within and among all assays.

### *Physiological trait measurements*

Hierarchical models were built to remove the potential effects of sex and population, whereby physiological measurements (bactericidal ability, antioxidant capacity, corticosterone levels) were modeled as a function of population-specific sex effects. Hierarchical normal priors were placed on these effects with a uniform prior for the variance. Residuals were taken by removing the sex and population means when analyzing across populations, but only sex means were removed for analyzing within populations. Models were fit using Markov chain Monte Carlo (MCMC) with the ‘R2jags’ package (v. 0.03-08; Su & Yajima, 2015) in R (v. 3.3.1; R Core Team, 2016). All physiological measurements were standardized to have a mean of zero and SD of one to improve the efficiency of the MCMC sampler and make interpretation of model coefficients easier. For each model, three separate chains were run in parallel with different starting values. Models included 40,000 sampling iterations, with the first 20,000 discarded for adaptation and burn-in, and a thinning rate that kept every sixth sample. To assess model convergence, trace plots were observed to have good mixing of the chains and consistent approximate posterior distributions. Convergence to the posterior distribution was verified by calculating the Gelman-Rubin potential scale reduction diagnostic ( $\hat{r} \leq 1.03$ ; Brooks & Gelman, 1998) for each measurement with the R package ‘coda’ (v. 0.19.3; Plummer et al., 2006).

### *DNA isolation and library preparation*

Total DNA was isolated from each erythrocyte sample using Qiagen’s DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA, USA). Reduced complexity, double-digest restriction-site DNA libraries were prepared following Parchman et al. (2012) with modifications from Gompert et al. (2014). Briefly, DNA was digested with the restriction enzymes EcoRI and MseI (NEB, Inc. Ipswich, MA, USA) and adaptor oligonucleotides were ligated onto the digested DNA fragments. The adaptor

oligonucleotides included an Illumina adaptor and unique 8–10 bp identification sequences or barcodes for individual lizard recognition. Fragment libraries were PCR-amplified, pooled together, and fragments between 330 and 475 bp were size-selected and purified using a BluePippin (Sage Science, Beverly, MA, USA) at the USU Center for Integrated Biosystems (Logan, UT, USA). Library preparation and sequencing took place in two batches, with 384 samples sequenced on two lanes of an Illumina HiSeq 2500 in 2016 and 373 samples sequenced on two lanes of a HiSeq 4000 in 2017 (Table 3.1). All DNA libraries ( $N = 2$ ) were sequenced at the University of Texas Genomic Sequencing and Analysis Facility (Austin, TX, USA), totaling ~870 million single-end 100 bp DNA sequences. As detailed below, care was taken during data processing and analyses to avoid possible confounding batch effects.

#### *DNA sequence alignment and variant calling*

Custom Perl scripts were used to first demultiplex pooled DNA sequences, wherein identifier barcodes served to assign DNA sequences to individual lizards (Gompert et al., 2012). Individuals with few sequences ( $< 180,000$ ) were removed from subsequent analyses ( $N = 40$ ). Reads from all remaining individuals were assembled and clustered *de novo* with the software ‘vsearch’ (v. 1.11.1; Rognes et al., 2016) to create a pseudo-reference genome. Here, highly similar sequences within individuals (98% identity) were clustered together and outputted as individual centroid files, which were then combined and shuffled. A random subset was taken from the combined centroids (54,631,148 sequences) for additional clustering with a lower similarity (90% identity), a minimum sequence length of 64, and a minimum shorter/longer sequence length ratio of 0.8 to generate consensus sequences. The consensus sequences were then filtered to determine whether any would collapse at an even lower similarity (80% identity), which resulted in a subset of 411,929 distinct (non-

collapsed) contigs of at least 8 reads. Contigs were then reformatted, wherein consensus sequences between 72-92 bps in length were retained to total 93,134 contigs. The resulting pseudo-reference genome was indexed, and sequences were aligned using algorithms in 'bwa' (v. 0.7.5; Li & Durbin, 2009), where a maximum of five nucleotide differences was allowed, with no more than two mismatches in a 20 bp seed, and a quality threshold for read trimming set to 10.

Sequence alignments were sorted and indexed for calling single nucleotide polymorphisms (i.e., SNPs) using 'samtools' and 'bcftools' (v. 0.1.19; Li et al., 2009). The coefficient for downgrading the mapping quality of reads with excessive mismatches, and the number of reads per position (max depth) per individual were both set to 50. Bases with a quality score below 15 and reads with a mapping quality below 10 were discarded. The prior for  $\theta$  was set to 0.001, and SNPs where the posterior probability of an invariant nucleotide was below 0.01 were retained (Li, 2011). Sites were filtered using custom Perl scripts and 'vcftools' (v. 0.1.15.6; Danecek et al., 2011), calling for bi-allelic SNPs with a minimum coverage of 700 reads, a maximum mean coverage of 20, a 4-read minimum for the non-reference allele, a minimum mapping quality of 30, and a maximum of 20% missing data across individuals. Individuals with a mean coverage of less than 1 were removed ( $N = 240$ ). These filtering criteria were chosen to ensure sufficient coverage to estimate allele frequencies (while accounting for uncertainty in genotypes) and avoid locus drop-in and drop-out (Buerkle & Gompert, 2013). To reduce bias associated with batch effects (Tom et al., 2017), SNPs with substantial differences in sequence coverage between the two sets (more than half the mean coverage of the combined data) were removed. A final set of 7,442 SNPs and 477 individuals were retained with a mean coverage of  $\sim 2.5x$  (per individual) for downstream analysis (Table 3.1).

### *Estimating genotypes and allele frequencies*

As in previous studies (e.g., Gompert et al., 2014, 2015), maximum likelihood estimates of the allele frequencies for each SNP were obtained with the computer program ‘estpem’ (Soria-Carrasco et al., 2014; Riesch et al., 2017). This program implements an expectation-maximization algorithm to provide allele frequency estimates that account for genotype uncertainty (Li et al., 2009; Li, 2011). Compared to methods that call genotypes first, this allowed for the inclusion of individuals with a range of sequence coverage and weighting of their contributions to the allele frequency estimates by the information carried in their sequence data (Buerkle & Gompert, 2013).

An empirical Bayesian approach was taken to estimate individual genotypes for each SNP, wherein allele frequency estimates were used to define prior probabilities for genotypes as  $\Pr(g = 0) = (1-p)^2$ ,  $\Pr(g = 1) = 2p(1-p)$  and  $\Pr(g = 2) = p^2$ . Here,  $g$  denotes counts for the non-reference allele (0, 1 or 2 in diploids) and  $p$  denotes the corresponding allele frequency. Posterior probabilities were then obtained according to Bayes rule as  $\Pr(g|D, p) = [\Pr(D|g) \Pr(g)] / \Pr(D)$ , where  $\Pr(D|g)$  defines the likelihood of the genotype given the sequence data and quality scores as calculated by ‘samtools’ and ‘bcftools’. Point estimates (posterior means) of genotypes were obtained as  $\Pr(g = 0|D, p) * 0 + \Pr(g = 1|D, p) * 1 + \Pr(g = 2|D, p) * 2$ , which resulted in values between 0 and 2 (copies of the non-reference allele).

### *Genome-wide association mapping and genomic prediction*

Bayesian sparse linear mixed models (BSLMMs) were fit with ‘gemma’ (v. 0.98; Zhou et al., 2013) to (i) characterize physiological genetic architectures, (ii) identify individual trait-associated SNPs, and (iii) infer genomic estimated breeding values (GEBVs), which could then be used to estimate the G-matrix describing the additive genetic covariance among the physiological traits. Unlike



traditional mapping methods, this polygenic GWA method considers all SNPs simultaneously while trait values are modeled as a function of a polygenic effect (denoted  $u$ ) and a vector of the potential measurable effects or associations of individual SNPs on the trait (denoted  $\beta$ ; Zhou et al., 2013). In brief, a MCMC algorithm with variable selection was used to estimate posterior inclusion probabilities (PIPs) for each SNP (probability of a zero or non-zero trait effect; Guan & Stephens, 2011), and model-averaged effect estimates were derived by weighting  $\beta$  by the respective PIP (i.e., taking the product of the posterior inclusion probability and effect estimate as a certain, model-averaged effect estimate). Consequently, for each SNP, there is an estimated posterior probability of association (i.e., PIP) and an estimated phenotypic effect conditional on the association. The polygenic term represents expected individual deviations from a grand phenotypic mean based on all SNPs, while accounting for phenotypic covariances due to overall relatedness or genetic similarity (i.e., observed kinship; Zhou et al., 2013). The kinship matrix also controls for relatedness and population structure when estimating individual SNP effects ( $\beta$ ) and their PIPs. In addition, SNPs in LD with the same causal variant account for each other since only one needs to be captured by the PIPs for the model.

Derived parameters for estimating genetic architecture included the proportion of the phenotypic variance explained (PVE) by additive genetic effects (based on  $\beta$  and the polygenic term; this should approach the narrow-sense heritability), the proportion of PVE explained by measurable-effect SNPs or those implicated by LD (i.e., PGE; based on  $\beta$  alone), and the number of SNPs with measurable effects or associations that explain phenotypic variance ( $N_{\text{SNP}}$ ). These metrics integrate over effect uncertainty for each of the SNPs, including whether these are nonzero. The expected trait values for an individual based on their additive genetic effects, or genomic estimated breeding values (GEBVs), were obtained from the BSLMMs as captured by both  $\beta$  and  $u$  (i.e., a polygenic score).

BSLMMs were fit for each physiological measurement across all populations (residuals after sex and population effects removed) and within each population (residuals after sex effects removed) using 30 independent MCMC chains each with 100,000 burn-in steps, 1 million sampling steps, and a thinning interval of 10. Convergence to the posterior distribution was verified by calculating the Gelman-Rubin potential scale reduction diagnostic ( $\hat{r} \leq 1.1$ ; Brooks & Gelman, 1998). Brooks-Gelman-Rubin statistics were again calculated for PVE, PGE and  $N_{\text{SNP}}$  ( $\hat{r} < 1.1$ ) with the R package ‘coda’ (v. 0.19.3; Plummer et al., 2006). To reduce bias in estimation, inferences were carried out using the combined values from all iterations across chains (Cowles & Carlin, 1996). Proportional values for PVE and PGE were converted to percentages for interpretation.

#### *Genetic variation and population structure*

To visualize patterns of genetic variation (based on genetic similarity), a principal component analysis (PCA) was performed in R using the ‘prcomp’ function. Here, centered but not scaled genotype estimates were used as input (i.e., the covariance matrix). Genetic differentiation between populations and years was quantified based on pairwise  $F_{\text{ST}}$  in R (v. 3.3.1; R Core Team, 2016). Comparisons were computed as  $F_{\text{ST}} = (1/L \sum_L (H_T - H_S)) / (1/L \sum_L H_T)$ , where  $H_T$  and  $H_S$  are the expected heterozygosities for the combined and individual population or generation pairs and the sums are over the  $L$  SNPs. Additionally, change in time for individual SNPs was quantified by calculating the differences in allele frequencies between years. These calculations were done in R.

To test for isolation by distance, a Mantel test on pairwise genetic distances ( $F_{\text{ST}}$  estimates) and geographic distances (km) among populations was performed in R using the ‘ecodist’ package (v. 2.0.9, Goslee et al., 2020), in which significance was assessed with 10,000 permutations. A partial

Mantel test was also implemented to control for effects of geographic distance in assessing whether genetic distances significantly varied by site types (urban versus natural).

*Population abundances, Contemporary  $N_eV$ , and diversity levels*

As described in Chapter 2, annual population abundances ( $N$ ) were estimated from capture-mark-recapture data using an open-population Jolly-Seber model that was developed as a restricted dynamic occupancy model. Here, site-specific abundances estimates were averaged across sampling year intervals that were applicable to the genetic analyses.

Variance effective population size ( $N_eV$ ), when based on the magnitude of allele frequency change in a population, serves as a reliable indicator of random genetic drift (Waples, 1989; Ryman et al., 2019). Both contemporary variance effective population sizes and genetic diversity levels (i.e.,  $\theta$ ; a proxy for long-term effective population size) were estimated for comparison across sites. Estimates of contemporary  $N_eV$  were obtained from the C++ software ‘VarNe’ (v. 0.9) using a Bayesian bootstrap method (see Gompert & Messina, 2016). Input to run this program included allele frequencies from the first and last sampling year available for each site (2012 and 2017 for U1; 2012 and 2015 for U2; 2013 and 2017 for U3, N1, N3; 2014 and 2017 for N2), number of genetic loci (7442 SNPs), number of generations (i.e., sampling year interval), and average population abundance estimates ( $N_{U1} = 60$ ,  $N_{U2} = 124$ ,  $N_{U3} = 68$ ,  $N_{N1} = 293$ ,  $N_{N2} = 81$ ,  $N_{N3} = 172$ ). Change over the largest time interval per site was prioritized, as this is less sensitive to uncertainty associated with sampling error (i.e., change by drift compounds across generations, whereas sampling error is unique to each sample). Evolution was assumed to occur solely by genetic drift with this approach, considering selection on a modest number of

SNPs should not be reflected in  $N_eV$  estimates. Point estimates (posterior medians) of  $N_eV$  were summarized from 1000 Bayesian bootstrap replicates.

Genetic diversity levels were estimated in each population and generation with the neutral theory expectation that  $\theta = 4N_e\mu$ . Here,  $N_e$  is the long-term effective population size,  $\mu$  is the mutation rate, and  $\theta = \pi$  (i.e., nucleotide diversity). Using the C++ software ‘ANGSD’ (v. 0.933-71), diversity levels were estimated with bam alignment files as input, while accounting for uncertainty in genotypes and whether individual nucleotides are variable (Korneliussen et al., 2014). Each population was analyzed with genotype likelihoods computed in the same way as ‘samtools’. A minimum mapping quality of 30 and bases with minimum quality of 20 were required for reads, and computations were based on the folded site-frequency spectrum.

#### *ABC time-series*

As described in Gompert (2021), an approximate Bayesian computation (ABC) method was implemented in the open-source computer software ‘fsabc’ (v. 0.2.1) and the R package ‘abc’ (v. 2.1; Csillery et al., 2012) to estimate the intensity and variability of selection on each physiological trait and associated genetic loci. With this method, phenotypic selection was modeled as an explicit function of environmental state, and the population genomic consequences of selection were modeled based on the estimated genotype–phenotype associations from the polygenic genome-wide association mapping models previously described. This allowed inferences to be informed by patterns of change across multiple genetic loci, populations, and generations. Here, environment-dependent (urban versus natural) directional phenotypic selection was assumed for each physiological trait. The model included drift based on the  $N_eV$  estimates but assumed negligible effects of gene flow and mutation.

For the model, phenotypic selection was related to the environment as a linear function using the equation  $S_{jk} = a + bx_{jk}$ , where for population  $j$  and generation  $k$ ,  $x$  is environmental state,  $S$  is the selection differential (i.e., trait mean before and after selection), and  $a$  and  $b$  are coefficients that determine the effect of the environment on the selection differential. Spike-and-slab priors for these coefficients were specified, such that  $\text{Pr}(a) \sim \pi\delta_0 + (1 - \pi)U(l, u)$ . Here,  $\pi$  is the probability of a zero coefficient,  $U(l, u)$  denotes a uniform probability density function with lower bound  $l$  and upper bound  $u$ , and  $\delta_0$  denotes a point mass at zero. Phenotypic selection was then related to expected allele frequency change based on the trait-genotype associations (Walsh & Lynch, 2018; Gompert, 2021).

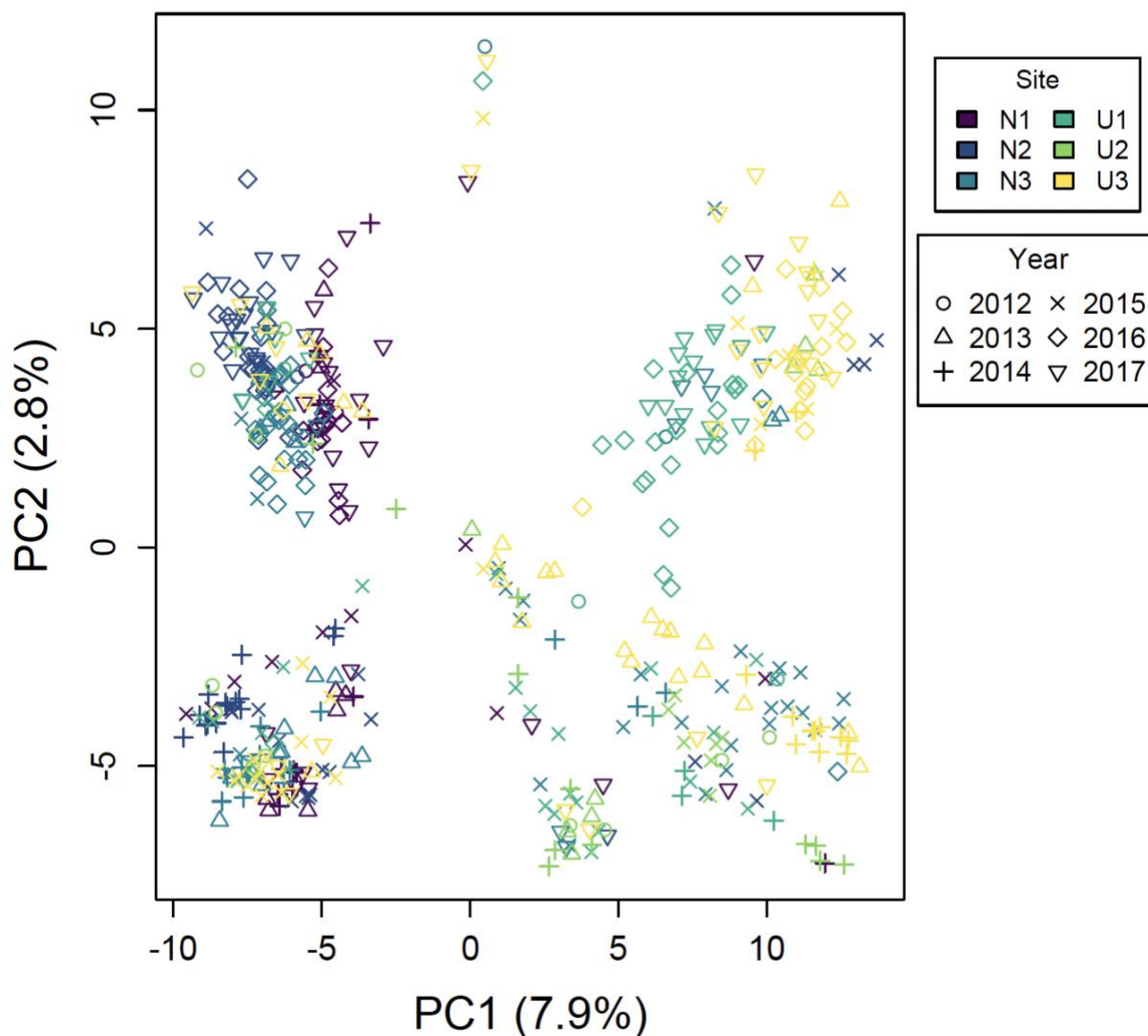
The model was fit using the model-averaged effect estimates calculated from the genome-wide association mapping. The priors on the selection model parameters ( $a$  and  $b$ ) were bounded by uninformative values, -10 and 10. The analysis was based on one million simulations. Inferences were based on 1000 samples from the posterior after applying the rejection sampling adjustment in ‘abc’ (Beaumont et al., 2002; Csillery et al., 2012). The data inputted to ‘fsabc’ included population-by-generation minor allele frequency estimates from ‘estpem’ (v. 0.1; Soria-Carrasco et al., 2014; Riesch et al., 2017), probabilistic estimates of the SNP effects (genotype associations) for each trait from ‘gemma’ (v. 0.98; Zhou et al., 2013), point estimates (posterior medians) of variance effective population size from ‘varNe’, population-by-generation sample sizes, and a binary environmental covariate (1 = urban site, 0 = natural site) expected to influence the phenotype–fitness relationship (i.e., the selection differential) across generations. Here, the maximum number of populations ( $j = 4$ ; U1, U3, N1, N3) with the longest interval of contiguous generations ( $k = 5$ ; 2013-2017) were included in the

analysis. Since each of the trait measurements were standardized to have SD of 1 prior to mapping, estimates of phenotypic variance were kept at the default model values (i.e.,  $\sigma^2 = 1$ ).

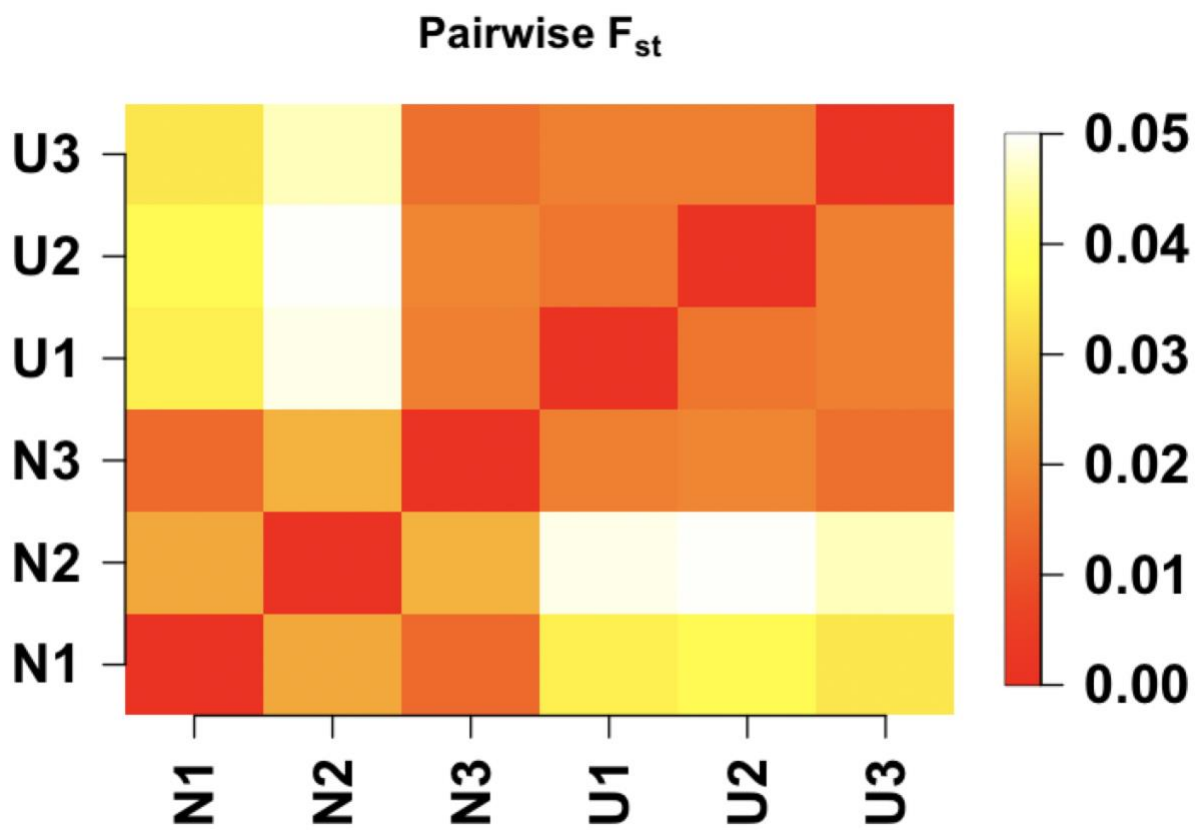
## RESULTS

### *Patterns of genetic variation in space and time*

Genetic differentiation among lizard populations was low to moderate and did not exceed genetic differentiation through time. Although most lizards clustered by population in a PCA ordination, each cluster included genetic similarities from other populations (Fig. 4.1). The first principal component (PC1) explained 7.9% of the variance in the genotype data and the second component (PC2) explained 2.8% of this variance. Between-population  $F_{ST}$  values varied from 0.012 to 0.043 (mean = 0.024; Fig. 4.2), and urban-natural  $F_{ST}$  values varied from 0.013 to = 0.043 (mean = 0.030). Here, sequencing batch data from 2013-2015 included between-population  $F_{ST}$  values varying from 0.017 to 0.067 (mean = 0.036), while batch data from 2016-2017 yielded comparable  $F_{ST}$  values (mean = 0.044, range = 0.018-0.075). A significant correlation between genetic distance ( $F_{ST}$ ) and inter-site geographic distance (km) was present (Mantel  $r$ -value = 0.4411,  $p = 0.003$ ), suggesting a pattern of IBD (Fig 4-3). When accounting for IBD effects, genetic distance did not significantly vary by site type (Mantel  $r$ -value = -0.468,  $p = 0.266$ ).

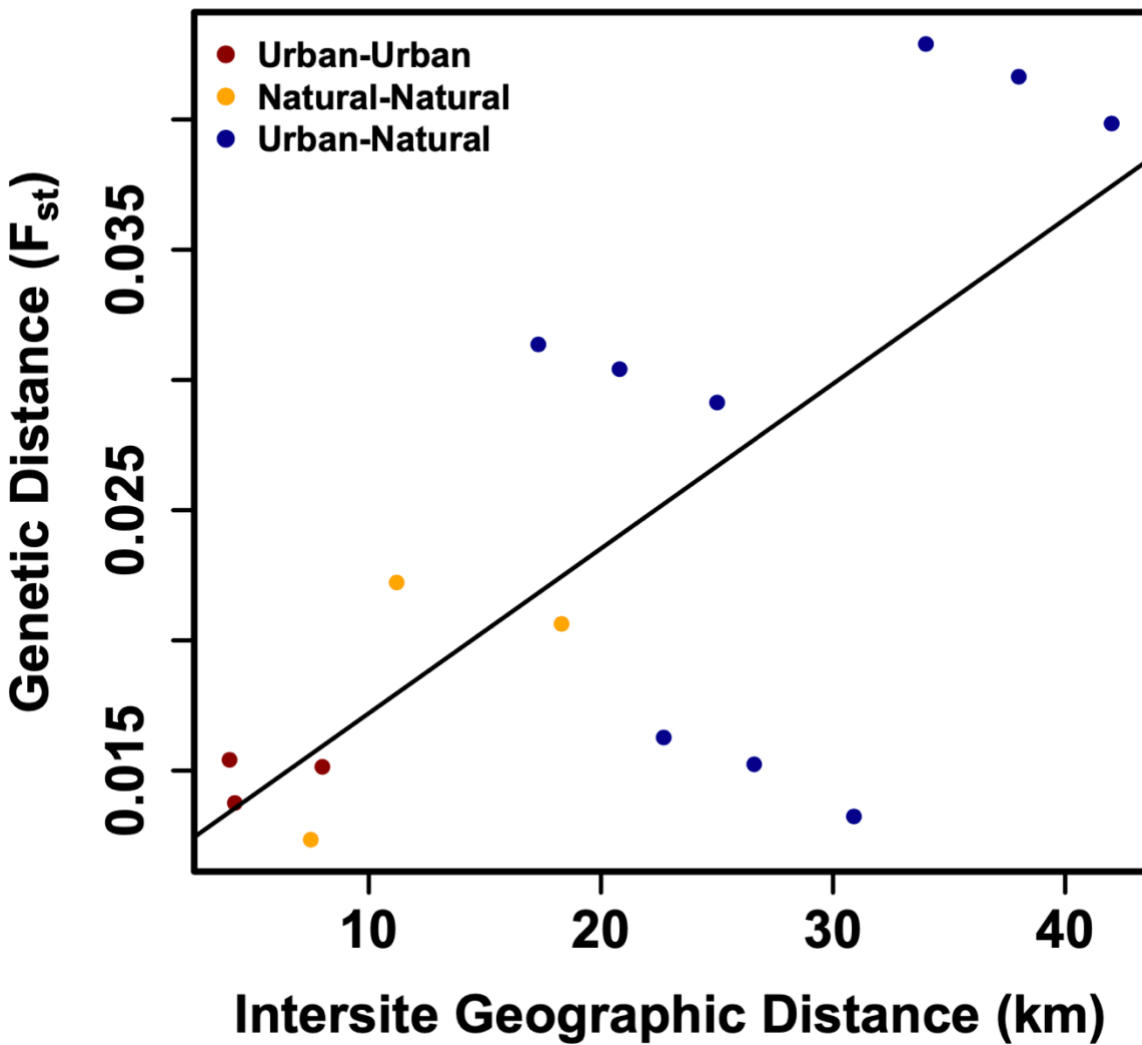


**Figure 4.1** Visual representation of genetic variation based on a principal component analysis of estimated genotypes. The colored symbols denote individuals with colors designating different sites and symbols indicating the sampling year. Urban and natural sites are respectively abbreviated as ‘U’ and ‘N’, followed by their numeric identifier.



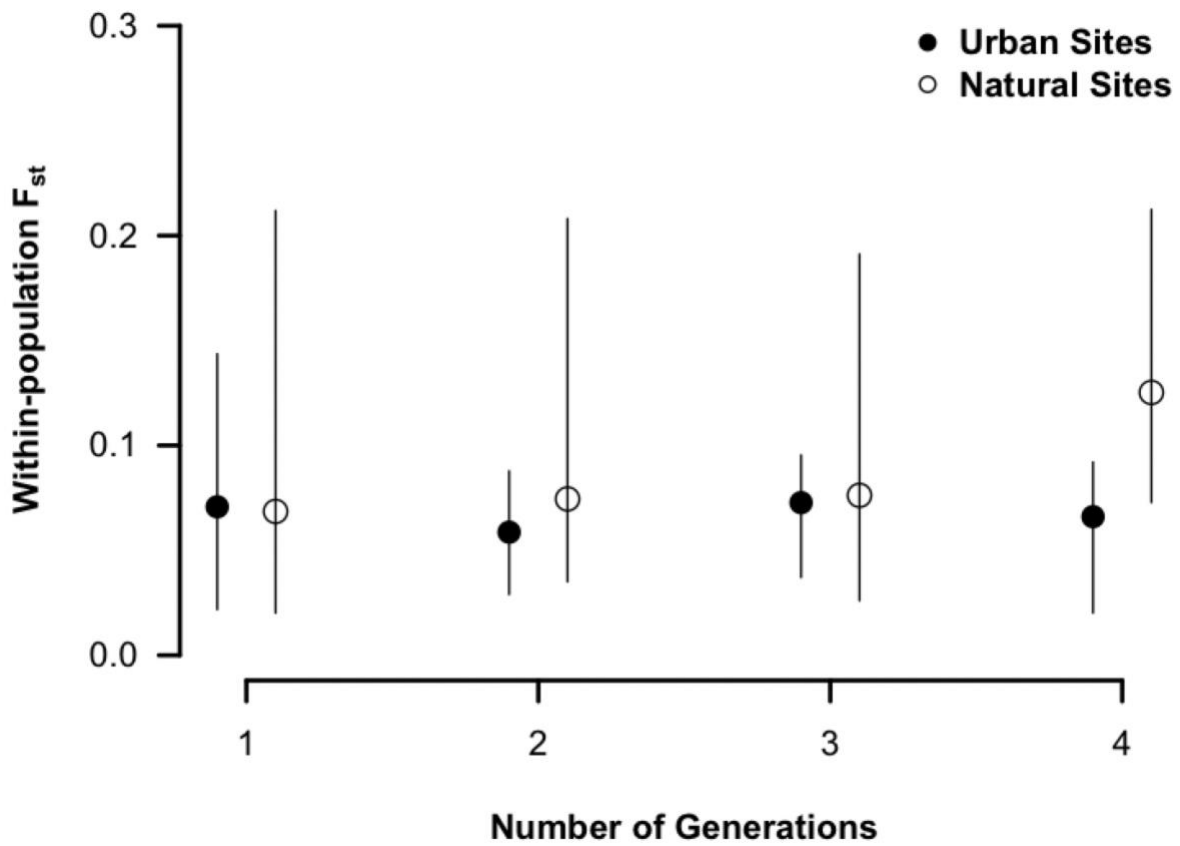
**Figure 4.2** Heatmap of pairwise  $F_{ST}$  values between urban and natural *Uta stansburiana* populations. Urban and natural populations are respectively abbreviated as ‘U’ and ‘N’, followed by their numeric identifier.





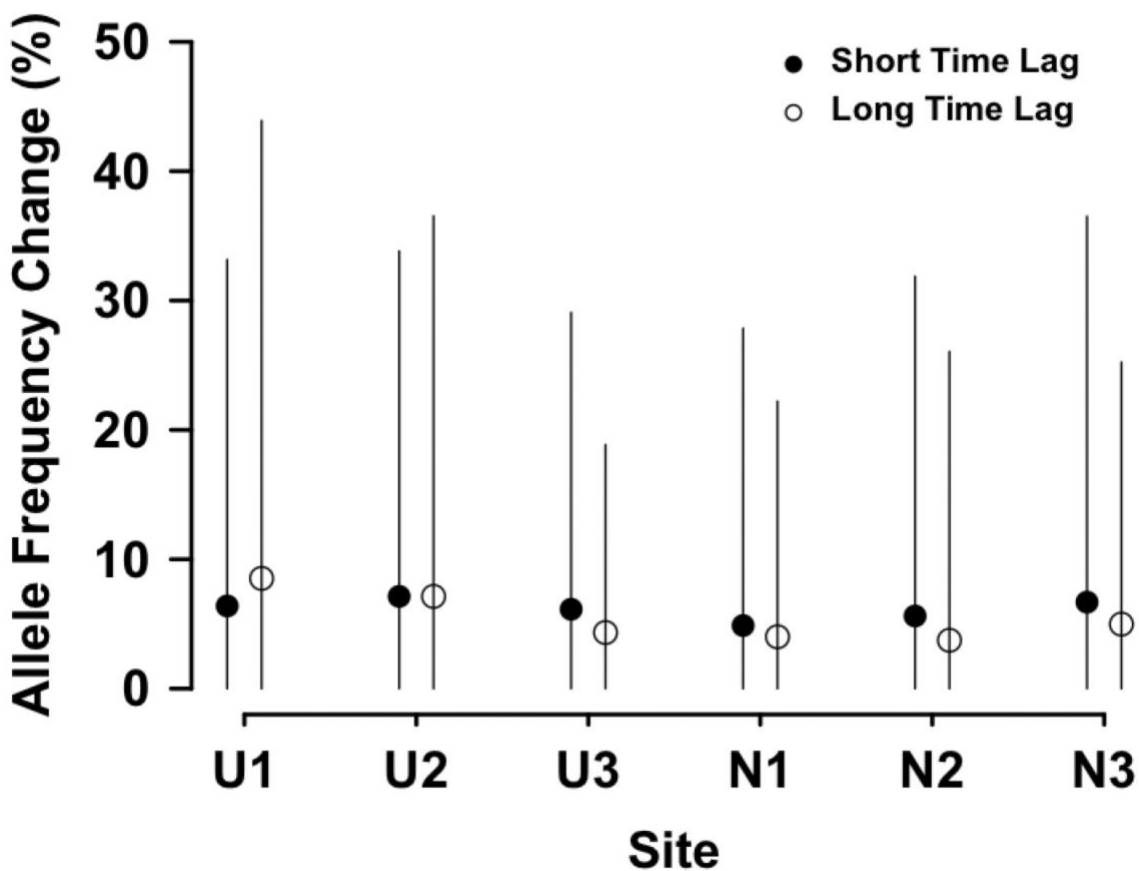
**Figure 4.3** Relationship between genetic distance (pairwise  $F_{ST}$ ) and geographic distance (km) among sites (Mantel  $r$ -value = 0.4411,  $p$  = 0.003).

Within-population  $F_{ST}$  values were higher than between-population  $F_{ST}$  values, ranging from 0.045 to 0.120 (mean across generations = 0.074). Here, within-population  $F_{ST}$  values during the shortest time lags (i.e., contiguous generations; mean = 0.070; range = 0.019-0.258) were comparable to those at the longest time lags (i.e., 4-5 generations; mean = 0.096, range = 0.017-0.219; Fig. 4.4). Within-population  $F_{ST}$  values across contiguous generations were relatively similar between urban (mean = 0.071, range = 0.019-0.158) and natural populations (mean = 0.069, range = 0.019-0.212). However, during the longest time lag,  $F_{ST}$  values within natural populations (mean = 0.125, range = 0.072-0.219) were notably higher than urban populations (mean = 0.066, range = 0.017-0.092).



**Figure 4.4** Within-population  $F_{ST}$  values across generations of *Uta stansburiana* populations. Points represent means with lines spanning 95% quantiles. Solid points indicate urban habitats while hollow points are natural habitats.

Consistent with the moderate levels of temporal genetic structure, average allele frequency changes between contiguous generations varied from 5.7% to 7.1% (mean across all populations = 6.1%). Here, average change within populations was slightly higher at urban sites (mean = 7.1%) than natural sites (mean = 5.7%; Fig. 4.5). Numerous loci exhibited much larger changes within a generation, as the 97.5th quantile of change varied among populations from 27.9% to 36.5% (mean = 32.1%) and the maximum change was very high (mean = 68.6%, range = 65.8–71.7%). Across the longest time lag, the mean change in each population varied from 3.7 to 8.5% (mean = 5.5%). Here, average change within populations was slightly higher at urban sites (mean = 6.7%) than natural sites (mean = 4.2%; Fig. 4.5). Again, numerous loci exhibited much larger allele frequency changes, where for example, the 97.5th quantile of change varied among populations from 18.9 to 43.9% (mean = 28.8%) and the maximum change was very high (mean = 66.4%, range = 39.2–99.8%).

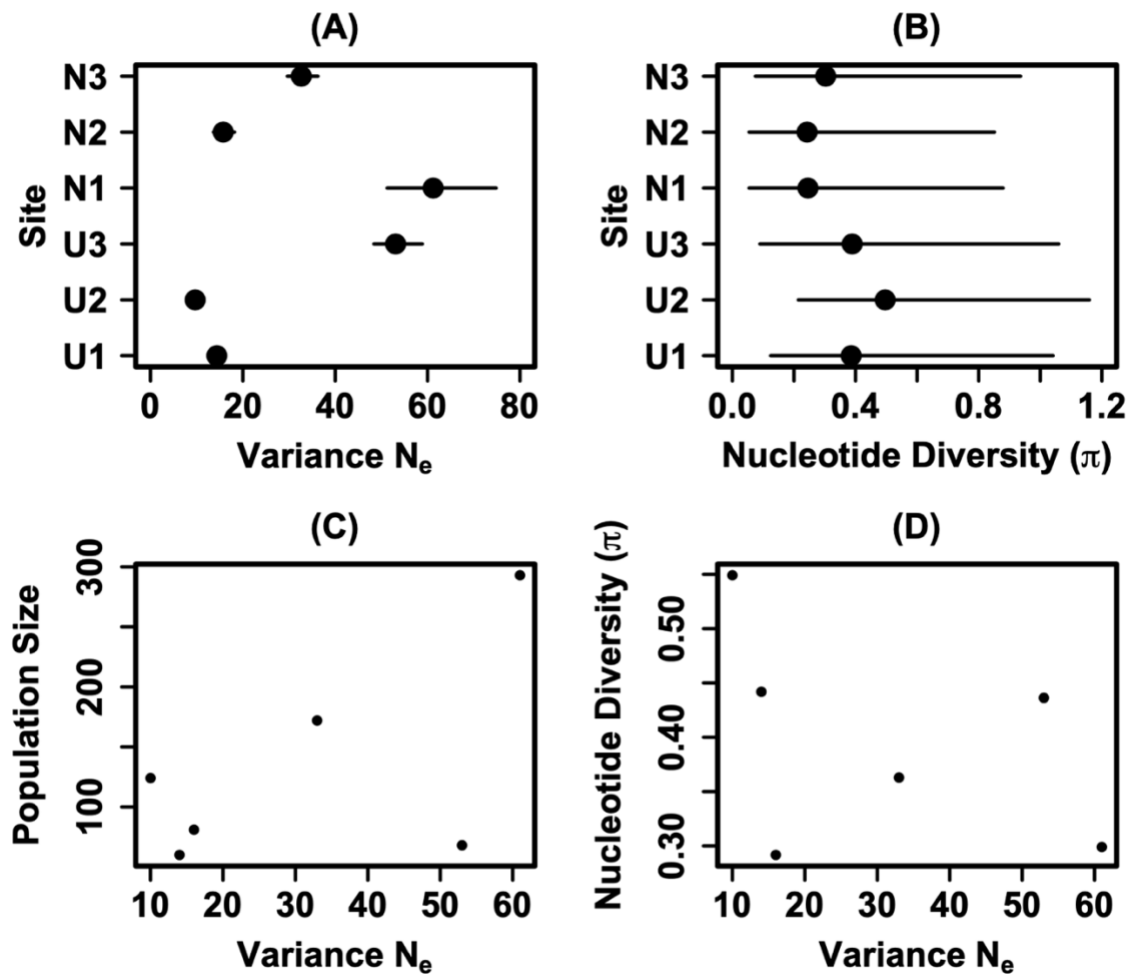


**Figure 4.5** Average allele frequency changes between short time lags (i.e., contiguous generations) and the longest time lags (i.e., 4-5 generations) for urban and natural populations of *Uta stansburiana*. Points represent means with lines spanning 95% quantiles. Urban and natural populations are respectively abbreviated as ‘U’ and ‘N’, followed by their numeric identifier.

*Variance effective population size and molecular diversity*

Variance  $N_e$  estimates based on allele frequency change were substantially lower, with an average of 31 individuals across sites (Fig. 6A). The lowest  $N_eV$  was at urban site 2 (median = 10, 95% CRI = 9-10) and the highest  $N_eV$  was at natural site 1 (median = 61, 95% CRI = 51-75; Fig. 5A). No relationship was present for estimates of population size and  $N_eV$  (Pearson  $r = 0.59$ , 95% CIs = -0.43–0.95,  $P = 0.222$ ; Fig. 6B). Consistent with general expectations from other studies, the ratio of  $N_eV$  to population size was 0.28 across sites (see Frankham, 1995b). Comparatively, the  $N_eV$  to population size ratio was higher at urban sites (mean = 0.36) than at natural sites (mean = 0.20).

Estimates of genetic diversity levels ( $\pi$ ) at urban sites (mean = 0.48, range = 0.44-0.55) were higher than at natural sites (mean = 0.32, range = 0.29-0.36; mean across populations = 0.40; Fig. 6C). Given these estimates and assuming  $\theta = 4N_e\mu$ , a very high mutation rate of  $3.2 \times 10^{-3}$  would be needed to obtain an estimate of effective population size that approximates the mean of 31 reported herein. Moreover, diversity levels varied less among populations than  $N_eV$  estimates (coefficient of variation, diversity = 0.248,  $N_eV = 0.699$ ). No relationship was detected for diversity levels and  $N_eV$  estimates (Pearson  $r = -0.42$ , 95% CI = -0.92–0.59,  $P = 0.40$ ; Fig. 6D).

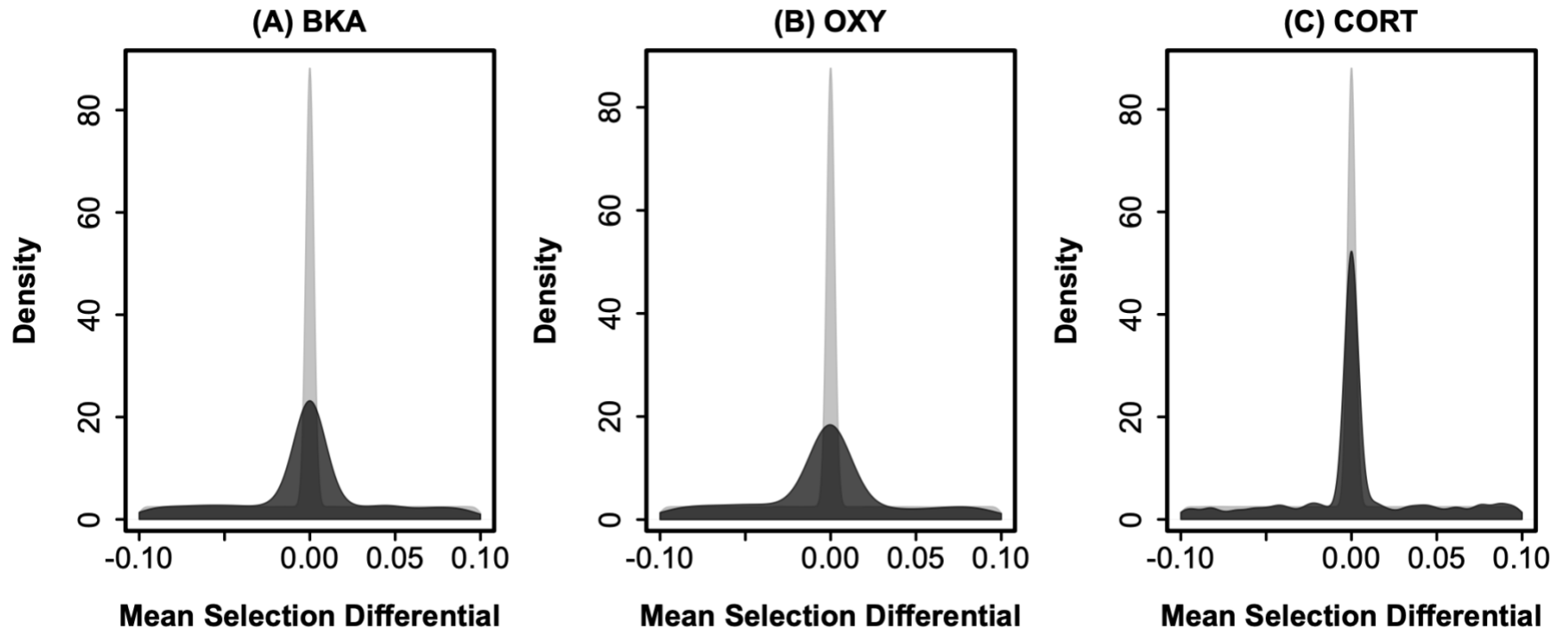


**Figure 4.6** Overview of contemporary variance effective population sizes and nucleotide diversity levels ( $\pi$ ). Dot plots in panel (A) give Bayesian estimates of variance effective population size based on change from 2012 to 2017 (estimates for urban site 2 are based on change from 2012 to 2015). Points denote the median of the posterior and horizontal lines give the 95% credible intervals. Similarly, dot plots in panel (B) report the median and 95% CRIs for estimates of molecular diversity. Scatterplot in panel (C) depicts the lack of relationship between variance effective population size and population size ( $r^2 = 0.18$ ,  $P = 0.219$ ) while the scatterplot in panel (D) depicts the lack of relationship between variance effective population size and nucleotide diversity ( $r^2 = -0.03$ ,  $P = 0.402$ ).

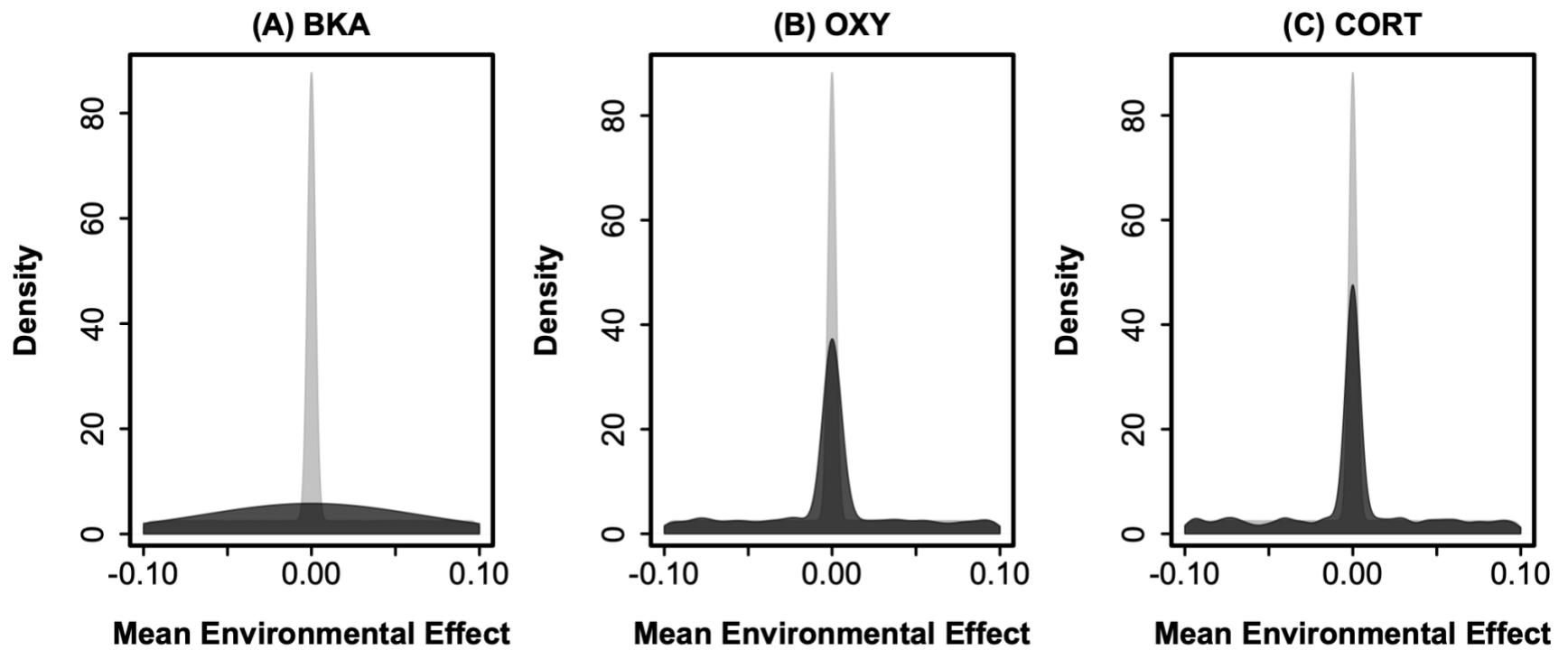
*Tests of selection*

There was no evidence of selection on any of the physiological traits (Fig. 4.7). Estimates of the mean selection differential, parameter ( $a$ ), were equal to -0.002 for BKA (95% CRI = -0.091-0.088), -0.002 for OXY (95% CRI = -0.09-0.09) and 0.002 for CORT (95% CRI = -0.090-0.091). There was also no evidence that selection varied as a function of the binary covariate of urbanization (Fig. 4.8). Estimates of the mean environmental effect, parameter ( $b$ ), were equal to -0.001 for BKA (95% CRI = -0.091-0.091), -0.001 for OXY (95% CRI = -0.092-0.091), and -0.0004 for CORT (95% CRI = -0.093-0.090). Moreover, calculations of mean breeding values or polygenic scores across populations revealed no indication of consistent directional selection, as these values fluctuated over generations instead (Fig. 4.9).

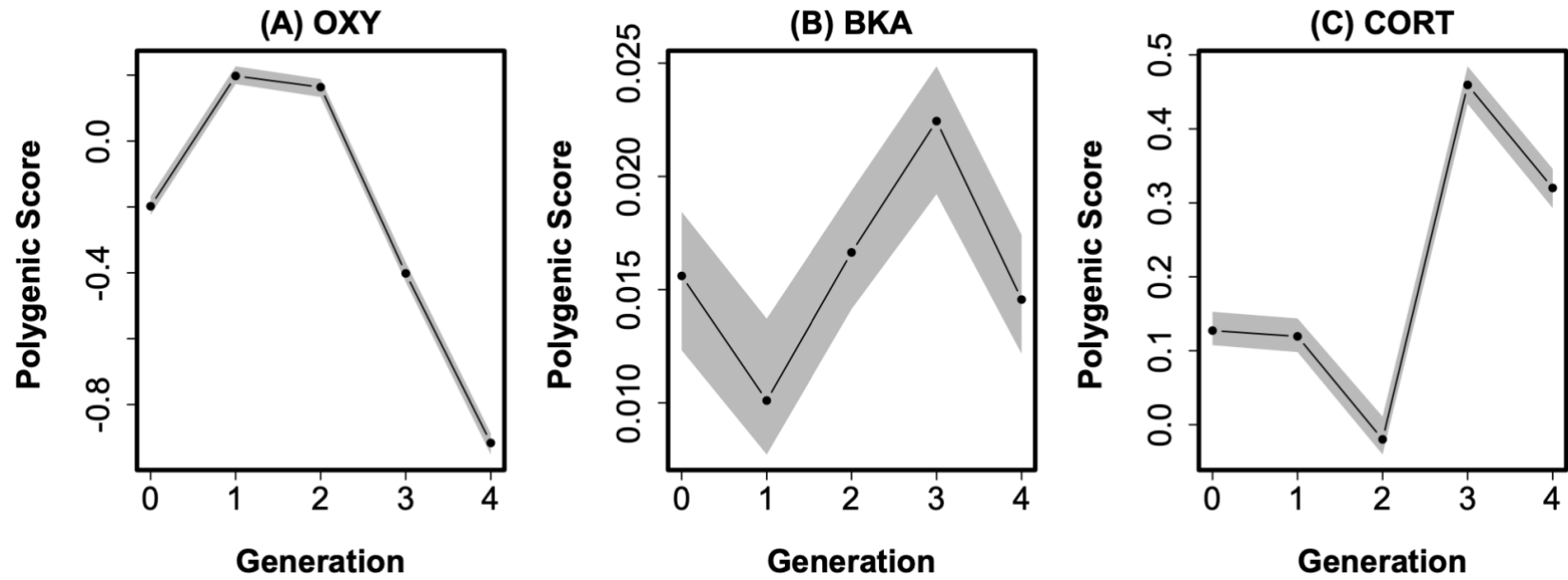




**Figure 4.7** Probability distributions of the average selection differential for bacterial killing ability (BKA), antioxidant capacity (OXY), and corticosterone (CORT). Prior distributions are shown in gray and the posterior distributions are in black.



**Figure 4.8** Probability distributions of how environmental state (urban versus natural) affects the selection for individual loci linked to bacterial killing ability (BKA), antioxidant capacity (OXY), and corticosterone (CORT). Prior distributions are shown in gray and the posterior distributions are in black.



**Figure 4.9** The observed change in mean polygenic scores calculated from model-averaged effect estimates, which includes uncertainty in allele frequencies. Dots denote point estimates, and the shaded gray regions denote 90% credible intervals.

## DISCUSSION

Despite concerns for the adaptive potential of urban wildlife, studies linking their phenotypic and genetic variation to evolutionary processes are lacking (Magle et al., 2012). Making these connections is necessary to better ascertain whether functionally relevant traits are undergoing adaptive evolutionary changes (e.g., Miranda et al., 2013; Miranda, 2017). This study sought to do so in a widespread reptile, the side-blotched lizard, by estimating selection on physiological traits (bacterial killing ability, antioxidant capacity, corticosterone levels) and their associated genetic loci across an urban-natural landscape. Selection was not detected as a result, nor did it differ between urban and natural environments. However, rates of evolution by drift were relatively high across populations, with pronounced changes in allele frequencies over successive generations. Such high rates were consistent with low estimates of local variance effective population size, especially at urban sites, despite modest estimates of population size. In contrast, urban and natural populations harbored comparable molecular diversity, which could be owed to periodic gene flow (as indicated by similarities in population genetic structure). These results suggest contemporary evolution by drift may predominate over selection for physiological traits relevant to these lizards, while other evolutionary processes may help maintain their genetic variation.

Although genetic divergence was evident among populations, as expected, this was largely due to isolation by distance, rather than habitat differences. In addition, the genetic similarities present at urban and natural sites differ from the degree of population structuring documented in other urban lizard species (Delaney, 2010; Beninde et al., 2018; Richmond et al., 2016; but see Maldonado et al., 2012; Virens et al., 2015). Even previous genetic analyses of *U.*

*stansburiana* populations in other urban areas are inconsistent with the degree of differentiation found among the urban populations herein (Delaney, 2010). One explanation for these differences is historically high genetic connectivity among the recently fragmented urban populations, with perhaps the greatest genetic divergence where patch isolation via development is oldest, farthest, and most intensive (e.g., Richmond et al., 2009). Alternatively, the urban matrix may still be permeable enough to permit gene flow among the populations. Indeed, a degree of continuity between populations is possible, considering the urban lizards were all sampled along the same waterway, which includes adjacent, albeit sparse patches of remnant habitat and bridges between sites. However, these explanations do not fully account for the similarities among urban and natural lizards, unless select individuals from other local populations (perhaps located between the sampled populations) are dispersing to, and interbreeding at, both areas. This species would seem incapable of doing so when considering their short dispersal distances (typically < 300 m) and small home range sizes (beyond 1,200 m<sup>2</sup> is rare; Doughty & Sinervo, 1994; Scoular et al., 2011). Yet, since the habitat suitable for *U. stansburiana* is so widespread in the greater region, there remains a potential for gene flow up to hundreds of kilometers from the sample sites (Hague & Routman, 2016).

Population structure could also have been influenced by the color polymorphism of this species, as each morph can have distinct patterns of mating and dispersal based on genetic similarity (Sinervo & Clobert, 2003; Sinervo et al., 2006; Bleay & Sinervo, 2007). When accounting for color morphs, however, there were no notable differences in the observed clustering patterns (but see Yewers et al., 2019). Perhaps the most plausible explanation, although untestable within the limits of this study, may be that genetic structure is confounded by the location of these populations within an intergrade zone of up to three nominal subspecies (*U.*

*s. stansburiana*, *U. s. uniformis*, *U. s. elegans*; McKinney, 1971; Corl et al., 2010). It is increasingly evident that population admixture in lizards is increasingly being facilitated by anthropogenic habitat disturbance, as the alteration or removal of natural barriers can permit novel intra- and interspecific interactions (reviewed in Grabenstein & Taylor, 2018). If lizards belonging to populations that previously had been genetically distinct are now interbreeding and producing offspring of mixed ancestry, hybridization events could be shaping their resulting genetic variation (Schulte et al., 2012; Abbott et al., 2013). With other studies corroborating this possibility (e.g., Leache, 2011; Beninde et al., 2018), comprehensive species sampling and genomic sequencing in neighboring areas may better account for any underlying gene flow and introgression.

In addition to the patterns of genetic variation across geographic space, appreciable genome-wide changes were observed across populations on a generation-to-generation time scale. A growing number of studies of genomic time series have reported similar findings (e.g., Campbell-Staton et al., 2017; Couto Alves et al., 2019; Bi et al., 2019; Gompert, 2021), lending further support to the rapidity of evolution over short periods (Hendry & Kinnison, 1999; Gingerich, 2019). The large changes in allele frequencies and low  $N_eV$  estimates, particularly with respect to population sizes, indicate that both urban and natural lizards are impacted by genetic drift. Greater drift at most of the urban sites could be owed to environmental stochasticity, such as local bottleneck events linked to habitat loss and alteration from human development. Susceptibility to drift, coupled with putative isolation in urban habitat patches (i.e., little to no gene flow, potential inbreeding), prompted the expectation that levels of diversity would be low (Kimura, 1983; Charlesworth, 2009). This could have diminished the adaptive potential required to respond to urban challenges and have promoted a vulnerability to

extirpation (Reed et al., 2003; Reed, 2004). However, estimates shown here and in a previous study were instead comparable across the landscape (Delaney et al., 2010), indicating that genetic diversity for this lizard species and others may be robust to various degrees of urbanization (Parham & Papenfuss, 2009; Richmond et al., 2009). The potential for gene flow to offset reductions in genetic diversity, even when rare on an individual basis, remains plausible (i.e., rescue effect; Ferriere & Legendre, 2013; Gonzalez et al., 2013; Bell et al., 2019). For these populations, perhaps at least one immigrant per generation is helping to maintain genetic viability (Mills & Allendorf, 1996).

Diversity levels may still be at risk for this species and others as reductions in urban patch size can increase their population relatedness via inbreeding (Delaney et al., 2010; Cureton et al., 2014). The effects of inbreeding may not immediately threaten these populations, however, considering that several other lizard species exhibit preferential selection of genetically different or genetically diverse mates (e.g., Stow & Sunnucks, 2004; Smith et al., 2009; Remon et al., 2012). Nonetheless, inbreeding depression and loss of fitness could ultimately occur if habitat conditions worsen (Keller & Waller, 2002; Reed & Frankham, 2003; DeWoody et al., 2021; García-Dorado & Caballero, 2021; but see Teixeira & Huber, 2021). Losses in genetic variation would be expected to arise quickly under such circumstances, considering the short generation time for this species. In the meantime, multiple components of the urban habitat (e.g., size, configuration, and age), as well as species-typical demographic attributes, may explain the genetic maintenance observed in these populations.

Short-term evolutionary changes owed to drift, gene flow, and even selection on other traits, rather than on the measured physiological traits, are not necessarily unexpected when considering the heritable basis of physiological characteristics and their phenotypic sensitivity to

environmental heterogeneity among reptiles (Warner et al., 2018; Jonsson et al., 2022). As shown in Chapter 3 and in previous studies, physiological traits often have relatively low to moderate heritability, due to their close fitness associations (Mousseau & Roff, 1987). Without a large amount of heritable variation, selective responses to urbanization could be limited for baseline immunity, oxidative resistance, and glucocorticoid release (Zera et al., 2007; McGlothlin & Ketterson, 2008; Adkins-Regan, 2008). Constraints on their adaptive evolution may be necessary so that important functions and interactions (e.g., molecular interdependence, hormonal regulation) can be conserved across environmental conditions (Cohen et al., 2012; Vitousek et al. 2019). Physiological adaptations may therefore be uncommon for urban lizards, as opposed to more heritable traits that can undergo rapid microevolutionary changes (e.g., morphological characteristics; Winchell et al. 2016, 2023; Falvey et al., 2020).

Urban selective pressures relevant to these traits may instead be mitigated by plasticity, which could either hinder or facilitate their evolution (Price et al., 2003; Lande, 2009; Chevin et al., 2010). Indeed, plastic responses for these physiological parameters are becoming increasingly evident for reptiles experiencing environmental change (e.g., Palacios et al., 2011; Treidel et al., 2016; Holden et al., 2022), though the effects of their developmental plasticity on adult phenotypes in this context remain poorly understood (Mitchell et al., 2018). Nonetheless, plasticity may be key to the differences in immunity, oxidative stress, and circulating corticosterone underlying life history strategies in these populations (see Lucas & French, 2012). If plasticity is more heritable than each trait in themselves, then the primary evolutionary responses to urbanization could alternatively be shifts in plasticity that are partially independent of the response to selection on the mean of each trait (Scheiner & Lyman, 1991; Thompson et al., 2021). Here, selection may shape plastic responses in adaptive directions via genetic



accommodation (i.e., increases or decreases in plasticity), which could enable physiological phenotypes to differ with variable conditions previously experienced in evolutionary history (DeWitt & Scheiner, 2004; Van Buskirk, 2017; Lafuente & Beldade, 2019). Yet, plasticity could also evolve in nonadaptive ways due to physiological constraints (Ghalambor et al., 2007, 2015; Murren et al., 2015), and in rapidly changing environments plasticity could even be maladaptive (Urban et al., 2014).

In any case, local selective pressures in urban areas must overcome the high susceptibility to drift associated with these populations to have adaptive potential. Regional adaptations for these traits may be more likely if facilitated by higher metapopulation or species  $N_eV$  and the spread of adaptive alleles via greater gene flow. Any effects of selection versus drift on the urban populations could be diluting adaptive alleles, even if they arise through limited gene flow or novel mutations (Lanfear et al., 2014; but see Bell et al., 2019). Considering how readily drift can facilitate stochastic population decline, extirpation may even occur before adaptive alleles are introduced (Willi et al., 2006; Ferriere & Legendre, 2013; Gonzalez et al., 2013). However, substantial levels of genetic diversity have so far been maintained in each population, indicating that neither selection nor drift may be as impactful as initially expected, and that local gene flow or mutation rates could instead play major roles in the observed patterns of genetic variation. Nonetheless, the balance of these processes may affect the geographic scale and extent of physiological adaptation to urban environments.

Future work investigating phenotypic divergence and local adaptation in this species and other urban-dwelling reptiles remains necessary for other functionally relevant traits, whether they be physiological, morphological, or behavioral (Lande & Arnold, 1983; Rausher, 1992; Schell, 2018). As pursued in this study, testing for genome-wide selection linked to such traits

would help explain the patterns of phenotypic variation observed across urban landscapes. If genomic targets are successfully identified, estimating standardized selection gradients and differentials would be instrumental to determining local adaptations (Kingsolver et al., 2001). Complementary field or laboratory experiments (e.g., reciprocal transplant experiments) would be necessary to parse out the influence of phenotypic plasticity, followed by tests manipulating specific urban features that have putatively driven adaptive evolution (Thomassen et al. 2018). Discerning the contributions of gene expression would provide further insight into the plasticity of genes linked to phenotypic variation in urban environments (Wang et al., 2009; Sun & Hu, 2013; Watson et al., 2017; Lafuente & Beldade, 2019), as may be the case for the physiological traits tested in this study. Epigenetic changes, which can be horizontally inherited via somatic cells or inherited trans-generationally via stable germ line changes (e.g., DNA methylation; Ho & Burggren, 2010), are relevant in this context, given their potential roles in altering gene expression for environmental acclimatization (Riggs & Xiong, 2004). Specifically, urban stressors experienced during organismal development (e.g., nutrition, pollution; Foret et al., 2009; Murgatroyd et al., 2009; Bollati & Baccarelli, 2010) are liable to have epigenetic consequences, granted that the extent to which phenotypes are altered remains largely unknown. Modifications to epigenetic mechanisms that occur during such environmentally sensitive periods may mediate gene regulation for physiological acclimatization. If stressors are excessive, lizards may become more resistant or susceptible to environmental challenges, which could yield fitness consequences within populations (Love et al., 2013). Epigenetic changes across generations may, in turn, have implications for evolutionary processes (Johnson & Tricker, 2010), either facilitating or constraining physiological adaptation to local urban environments.

In conclusion, urban selective pressures may not be as prevalent or intense for physiological parameters as more heritable traits that are functionally relevant to urban lizards. Appreciable short-term evolutionary changes may instead be largely owed to drift (as indicated by  $N_eV$ ) and other processes (e.g., gene flow, novel mutations), which could be contributing to admixture and maintaining genetic diversity across populations. Local adaptation for the physiological traits (i.e., bactericidal ability, antioxidant capacity, corticosterone levels) and their associated genetic loci now seems less likely than differential trait expression within the natural range of plasticity. Continuing tests of selection on genome-wide variation, particularly when accounting for habitat quality and population demography, will help to understand the causes and consequences of urban trait divergence. This will also shed light on the susceptibility of urban wildlife populations to other evolutionary forces. With a greater understanding of how different species evolve in cities, conservation and management can be better addressed when establishing more resilient and sustainable urban ecosystems.

#### LITERATURE CITED

- Abbott, R., Albach, D., Ansell, S., Arntzen, J. W., Baird, S. J., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C. A., & Buggs, R. (2013). Hybridization and speciation. *Journal of evolutionary biology*, 26(2), 229-246.
- Adkins-Regan, E. (2008). Do hormonal control systems produce evolutionary inertia? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1497), 1599-1609.

- Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K. M., Hunt, V. M., Apgar, T. M., & Zhou, Y. (2017). Global urban signatures of phenotypic change in animal and plant populations. *Proceedings of the National Academy of Sciences*, *114*(34), 8951-8956.
- Buerkle, A., & Gompert, Z. (2013). Population genomics based on low coverage sequencing: how low should we go? *Molecular Ecology*, *22*(11), 3028-3035.
- Angelier, F., & Wingfield, J. C. (2013). Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives. *General and Comparative Endocrinology*, *190*, 118-128.
- Barrett, R. D., & Hoekstra, H. E. (2011). Molecular spandrels: tests of adaptation at the genetic level. *Nature Reviews Genetics*, *12*(11), 767-780.
- Beaumont, M. A., Zhang, W., & Balding, D. J. (2002). Approximate Bayesian computation in population genetics. *Genetics*, *162*(4), 2025-2035.
- Bell, D. A., Robinson, Z. L., Funk, W. C., Fitzpatrick, S. W., Allendorf, F. W., Tallmon, D. A., & Whiteley, A. R. (2019). The exciting potential and remaining uncertainties of genetic rescue. *Trends in Ecology & Evolution*, *34*(12), 1070-1079.
- Beninde, J., Feldmeier, S., Veith, M., & Hochkirch, A. (2018). Admixture of hybrid swarms of native and introduced lizards in cities is determined by the cityscape structure and invasion history. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1883), 20180143.
- Bi, K., Linderoth, T., Singhal, S., Vanderpool, D., Patton, J. L., Nielsen, R., Moritz, C., & Good, J. M. (2019). Temporal genomic contrasts reveal rapid evolutionary responses in an alpine mammal during recent climate change. *PLoS genetics*, *15*(5), e1008119.

- Bleay, C., & Sinervo, B. (2007). Discrete genetic variation in mate choice and a condition-dependent preference function in the side-blotched lizard: implications for the formation and maintenance of coadapted gene complexes. *Behavioral Ecology*, *18*(2), 304-310.
- Bollati, V., & Baccarelli, A. (2010). Environmental epigenetics. *Heredity*, *105*(1), 105-112.
- Bradley, C. A., & Altizer, S. (2007). Urbanization and the ecology of wildlife diseases. *Trends in Ecology & Evolution*, *22*(2), 95-102.
- Brans, K. I., & De Meester, L. (2018). City life on fast lanes: Urbanization induces an evolutionary shift towards a faster lifestyle in the water flea *Daphnia*. *Functional Ecology*, *32*(9), 2225-2240.
- Brans, K. I., Stoks, R., & De Meester, L. (2018). Urbanization drives genetic differentiation in physiology and structures the evolution of pace-of-life syndromes in the water flea *Daphnia magna*. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1883), 20180169.
- Brooks, S. P., & Gelman, A. (1998). General methods for monitoring convergence of iterative simulations. *Journal of computational and graphical statistics*, *7*(4), 434-455.
- Buffalo, V., & Coop, G. (2019). The linked selection signature of rapid adaptation in temporal genomic data. *Genetics*, *213*(3), 1007-1045.
- Buffalo, V., & Coop, G. (2020). Estimating the genome-wide contribution of selection to temporal allele frequency change. *Proceedings of the National Academy of Sciences*, *117*(34), 20672-20680.
- Bureau, U. S. C. (2022). *Over Two-Thirds of the Nation's Counties Had Natural Decrease in 2021*. <https://www.census.gov/newsroom/press-releases/2022/population-estimates-counties-decrease.html>
- Campbell-Staton, S. C., Cheviron, Z. A., Rochette, N., Catchen, J., Losos, J. B., & Edwards, S. V. (2017). Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole lizard. *Science*, *357*(6350), 495-498.

- Campbell-Staton, S. C., Winchell, K. M., Rochette, N. C., Fredette, J., Maayan, I., Schweizer, R. M., & Catchen, J. (2020). Parallel selection on thermal physiology facilitates repeated adaptation of city lizards to urban heat islands. *Nature ecology & evolution*, *4*(4), 652-658.
- Charlesworth, B. (2009). Effective population size and patterns of molecular evolution and variation. *Nature Reviews Genetics*, *10*(3), 195-205.
- Chevin, L.-M., Lande, R., & Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS biology*, *8*(4), e1000357.
- Claunch, N. M., Downs, C. J., Schoenle, L. A., Oakey, S. J., Ely, T., Romagosa, C., & Briggs, C. W. (2022). Snap-freezing in the field: effect of sample holding time on performance of bactericidal assays. *Integrative and Comparative Biology*.
- Cohen, A. A., Martin, L. B., Wingfield, J. C., McWilliams, S. R., & Dunne, J. A. (2012). Physiological regulatory networks: ecological roles and evolutionary constraints. *Trends in Ecology & Evolution*, *27*(8), 428-435.
- Corl, A., Davis, A. R., Kuchta, S. R., & Sinervo, B. (2010). Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation. *Proceedings of the National Academy of Sciences*, *107*(9), 4254-4259.
- Costantini, D., & Møller, A. P. (2009). Does immune response cause oxidative stress in birds? A meta-analysis. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *153*(3), 339-344.
- Couto Alves, A., De Silva, N. M. G., Karhunen, V., Sovio, U., Das, S., Taal, H. R., Warrington, N. M., Lewin, A. M., Kaakinen, M., & Cousminer, D. L. (2019). GWAS on longitudinal growth traits reveals different genetic factors influencing infant, child, and adult BMI. *Science Advances*, *5*(9), eaaw3095.

- Couvet, D. (2002). Deleterious effects of restricted gene flow in fragmented populations. *Conservation Biology*, 16(2), 369-376.
- Cowles, M. K., & Carlin, B. P. (1996). Markov chain Monte Carlo convergence diagnostics: a comparative review. *Journal of the American Statistical Association*, 91(434), 883-904.
- Crane, P., & Kinzig, A. (2005). Nature in the metropolis. *Science*, 308(5726), 1225-1225.
- Crispo, E., Moore, J. S., Lee-Yaw, J. A., Gray, S. M., & Haller, B. C. (2011). Broken barriers: Human-induced changes to gene flow and introgression in animals: An examination of the ways in which humans increase genetic exchange among populations and species and the consequences for biodiversity. *BioEssays*, 33(7), 508-518.
- Csilléry, K., François, O., & Blum, M. G. (2012). abc: an R package for approximate Bayesian computation (ABC). *Methods in ecology and evolution*, 3(3), 475-479.
- Cureton, J. C., Janis, M., Lutterschmidt, W. I., Randle, C. P., Ruthven, D. C., & Deaton, R. (2014). Effects of urbanization on genetic diversity, gene flow, and population structure in the ornate box turtle (*Terrapene ornata*). *Amphibia-Reptilia*, 35(1), 87-97.
- Cushman, S. A. (2006). Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological conservation*, 128(2), 231-240.
- Dallman, M. F., Akana, S. F., Cascio, C. S., Darlington, D. N., Jacobsen, L., & Levin, N. (1987). Regulation of ACTH secretion: variations on a theme of B. Proceedings of the 1986 Laurentian Hormone Conference,
- Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., & Sherry, S. T. (2011). The variant call format and VCFtools. *bioinformatics*, 27(15), 2156-2158.

- Delaney, K. S., Riley, S. P., & Fisher, R. N. (2010). A rapid, strong, and convergent genetic response to urban habitat fragmentation in four divergent and widespread vertebrates. *Plos one*, 5(9), e12767.
- Demas, G. E., Zysling, D. A., Beechler, B. R., Muehlenbein, M. P., & French, S. S. (2011). Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts. *Journal of Animal Ecology*, 80(4), 710-730.
- DeWitt, T. J., & Scheiner, S. M. (2004). *Phenotypic plasticity: functional and conceptual approaches*. Oxford University Press.
- DeWoody, J. A., Harder, A. M., Mathur, S., & Willoughby, J. R. (2021). The long-standing significance of genetic diversity in conservation. *Molecular Ecology*, 30(17), 4147-4154.
- Diamond, S. E., Chick, L., Perez, A., Strickler, S. A., & Martin, R. A. (2017). Rapid evolution of ant thermal tolerance across an urban-rural temperature cline. *Biological Journal of the Linnean Society*, 121(2), 248-257.
- Diamond, S. E., Chick, L. D., Perez, A., Strickler, S. A., & Martin, R. A. (2018). Evolution of thermal tolerance and its fitness consequences: parallel and non-parallel responses to urban heat islands across three cities. *Proceedings of the Royal Society B: Biological Sciences*, 285(1882), 20180036.
- Donihue, C. M., & Lambert, M. R. (2015). Adaptive evolution in urban ecosystems. *Ambio*, 44(3), 194-203.
- Doughty, P., & Sinervo, B. (1994). The effects of habitat, time of hatching, and body size on the dispersal of hatchling *Uta stansburiana*. *Journal of Herpetology*, 485-490.



- Falvey, C. H., Aviles-Rodriguez, K. J., Hagey, T. J., & Winchell, K. M. (2020). The finer points of urban adaptation: intraspecific variation in lizard claw morphology. *Biological Journal of the Linnean Society*, *131*(2), 304-318.
- Ferriere, R., & Legendre, S. (2013). Eco-evolutionary feedbacks, adaptive dynamics and evolutionary rescue theory. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1610), 20120081.
- Foret, S., Kucharski, R., Pittelkow, Y., Lockett, G. A., & Maleszka, R. (2009). Epigenetic regulation of the honey bee transcriptome: unravelling the nature of methylated genes. *BMC genomics*, *10*, 1-11.
- Frankham, R. (1995a). Conservation genetics. *Annual review of genetics*, *29*(1), 305-327.
- Frankham, R. (1995b). Effective population size/adult population size ratios in wildlife: a review. *Genetics Research*, *66*(2), 95-107.
- Frankham, R., Ballou, J., Ralls, K., Eldridge, M., Dudash, M., Fenster, C., Lacy, R., & Sunnucks, P. (2019). Population fragmentation causes inadequate gene flow and increases extinction risk. *A practical guide for genetic management of fragmented animal and plant populations*. Oxford University Press, New York.
- French, S. S., & Neuman-Lee, L. A. (2012). Improved ex vivo method for microbiocidal activity across vertebrate species. *Biology Open*, *1*(5), 482-487.
- García-Dorado, A., & Caballero, A. (2021). Neutral genetic diversity as a useful tool for conservation biology. *Conservation Genetics*, *22*(4), 541-545.
- Ghalambor, C. K., Hoke, K. L., Ruell, E. W., Fischer, E. K., Reznick, D. N., & Hughes, K. A. (2015). Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature*, *525*(7569), 372-375.

- Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, *21*(3), 394-407.
- Gibbons, J. W., Scott, D. E., Ryan, T. J., Buhlmann, K. A., Tuberville, T. D., Metts, B. S., Greene, J. L., Mills, T., Leiden, Y., & Poppy, S. (2000). The Global Decline of Reptiles, Déjà Vu Amphibians: Reptile species are declining on a global scale. Six significant threats to reptile populations are habitat loss and degradation, introduced invasive species, environmental pollution, disease, unsustainable use, and global climate change. *BioScience*, *50*(8), 653-666.
- Gingerich, P. D. (2019). *Rates of evolution: a quantitative synthesis*. Cambridge University Press.
- Gompert, Z. (2016). Bayesian inference of selection in a heterogeneous environment from genetic time-series data. *Molecular Ecology*, *25*(1), 121-134.
- Gompert, Z. (2021). A population-genomic approach for estimating selection on polygenic traits in heterogeneous environments. *Molecular ecology resources*, *21*(5), 1529-1546.
- Gompert, Z., Jahner, J. P., Scholl, C. F., Wilson, J. S., Lucas, L. K., Soria-Carrasco, V., Fordyce, J. A., Nice, C. C., Buerkle, C. A., & Forister, M. L. (2015). The evolution of novel host use is unlikely to be constrained by trade-offs or a lack of genetic variation. *Molecular Ecology*, *24*(11), 2777-2793.
- Gompert, Z., Lucas, L. K., Buerkle, C. A., Forister, M. L., Fordyce, J. A., & Nice, C. C. (2014). Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. *Molecular Ecology*, *23*(18), 4555-4573.
- Gompert, Z., Lucas, L. K., Nice, C. C., Fordyce, J. A., Forister, M. L., & Buerkle, C. A. (2012). Genomic regions with a history of divergent selection affect fitness of hybrids between two butterfly species. *Evolution: International Journal of Organic Evolution*, *66*(7), 2167-2181.

- Gompert, Z., & Messina, F. J. (2016). Genomic evidence that resource-based trade-offs limit host-range expansion in a seed beetle. *Evolution*, *70*(6), 1249-1264.
- Gonzalez, A., Ronce, O., Ferriere, R., & Hochberg, M. E. (2013). Evolutionary rescue: an emerging focus at the intersection between ecology and evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1610), 20120404.
- Goslee, S., Urban, D., & Goslee, M. S. (2020). Package 'ecodist'. *Package 'ecodist'*.
- Grabenstein, K. C., & Taylor, S. A. (2018). Breaking barriers: causes, consequences, and experimental utility of human-mediated hybridization. *Trends in Ecology & Evolution*, *33*(3), 198-212.
- Green, L., Faust, E., Hinchcliffe, J., Brijs, J., Holmes, A., Englund Örn, F., Svensson, O., Roques, J. A., Leder, E. H., & Sandblom, E. (2023). Invader at the edge—Genomic origins and physiological differences of round gobies across a steep urban salinity gradient. *Evolutionary applications*, *16*(2), 321-337.
- Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X., & Briggs, J. M. (2008). Global change and the ecology of cities. *Science*, *319*(5864), 756-760.
- Guan, Y., & Stephens, M. (2011). Bayesian variable selection regression for genome-wide association studies and other large-scale problems. *The Annals of Applied Statistics*, *5*(3), 1780-1815.
- Hague, M. T., & Routman, E. J. (2016). Does population size affect genetic diversity? A test with sympatric lizard species. *Heredity*, *116*(1), 92-98.
- Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford university press, USA.
- Hendry, A. P., Gotanda, K. M., & Svensson, E. I. (2017). Human influences on evolution, and the ecological and societal consequences. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1712), 20160028.

- Hendry, A. P., & Kinnison, M. T. (1999). Perspective: the pace of modern life: measuring rates of contemporary microevolution. *Evolution*, *53*(6), 1637-1653.
- Higgins, K., & Lynch, M. (2001). Metapopulation extinction caused by mutation accumulation. *Proceedings of the National Academy of Sciences*, *98*(5), 2928-2933.
- Ho, D. H., & Burggren, W. W. (2010). Epigenetics and transgenerational transfer: a physiological perspective. *Journal of Experimental Biology*, *213*(1), 3-16.
- Holden, K. G., Gangloff, E. J., Miller, D. A., Hedrick, A. R., Dinsmore, C., Basel, A., Kutz, G., & Bronikowski, A. M. (2022). Over a decade of field physiology reveals life-history specific strategies to drought in garter snakes (*Thamnophis elegans*). *Proceedings of the Royal Society B*, *289*(1967), 20212187.
- Johnson, L. J., & Tricker, P. J. (2010). Epigenomic plasticity within populations: its evolutionary significance and potential. *Heredity*, *105*(1), 113-121.
- Johnson, M. T., & Munshi-South, J. (2017). Evolution of life in urban environments. *Science*, *358*(6363), eaam8327.
- Jones, M., & Gillham, B. (1988). Factors involved in the regulation of adrenocorticotrophic hormone/beta-lipotrophic hormone. *Physiological reviews*, *68*(3), 743-818.
- Jonsson, B., Jonsson, N., & Hansen, M. M. (2022). Knock-on effects of environmental influences during embryonic development of ectothermic vertebrates. *The Quarterly Review of Biology*, *97*(2), 95-139.
- Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, *17*(5), 230-241.
- Kimura, M. (1983). *The neutral theory of molecular evolution*. Cambridge University Press.

- Kingsolver, J. G., Gomulkiewicz, R., & Carter, P. A. (2001). Variation, selection and evolution of function-valued traits. *Microevolution rate, pattern, process*, 87-104.
- Korneliussen, T. S., Albrechtsen, A., & Nielsen, R. (2014). ANGSD: analysis of next generation sequencing data. *BMC bioinformatics*, 15(1), 1-13.
- Lafferty, K. D., & Kuris, A. M. (2005). Parasitism and environmental disturbances. *Parasitism and ecosystems*, 113-123.
- Lafuente, E., & Beldade, P. (2019). Genomics of developmental plasticity in animals. *Frontiers in genetics*, 10, 720.
- Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of evolutionary biology*, 22(7), 1435-1446.
- Lande, R., & Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evolution*, 1210-1226.
- Lanfear, R., Kokko, H., & Eyre-Walker, A. (2014). Population size and the rate of evolution. *Trends in Ecology & Evolution*, 29(1), 33-41.
- Leache, A. D. (2011). Multi-locus estimates of population structure and migration in a fence lizard hybrid zone. *Plos one*, 6(9), e25827.
- Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *bioinformatics*, 27(21), 2987-2993.
- Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *bioinformatics*, 25(14), 1754-1760.

- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009). The sequence alignment/map format and SAMtools. *bioinformatics*, *25*(16), 2078-2079.
- Losos, J. B., Arnold, S. J., Bejerano, G., Brodie III, E., Hibbett, D., Hoekstra, H. E., Mindell, D. P., Monteiro, A., Moritz, C., & Orr, H. A. (2013). Evolutionary biology for the 21st century. *PLoS biology*, *11*(1), e1001466.
- Lourenço, A., Álvarez, D., Wang, I. J., & Velo-Antón, G. (2017). Trapped within the city: Integrating demography, time since isolation and population-specific traits to assess the genetic effects of urbanization. *Molecular Ecology*, *26*(6), 1498-1514.
- Love, O. P., McGowan, P. O., & Sheriff, M. J. (2013). Maternal adversity and ecological stressors in natural populations: the role of stress axis programming in individuals, with implications for populations and communities. *Functional Ecology*, *27*(1), 81-92.
- Lucas, L. D., & French, S. S. (2012). Stress-induced tradeoffs in a free-living lizard across a variable landscape: consequences for individuals and populations. *Plos one*, *7*(11), e49895.
- Maldonado, S. P., Melville, J., Peterson, G. N., & Sumner, J. (2012). Human-induced versus historical habitat shifts: identifying the processes that shaped the genetic structure of the threatened grassland legless lizard, *Delma impar*. *Conservation Genetics*, *13*, 1329-1342.
- McDonald, R. I., Marcotullio, P. J., & Güneralp, B. (2013). Urbanization and global trends in biodiversity and ecosystem services. In *Urbanization, biodiversity and ecosystem services: Challenges and opportunities* (pp. 31-52). Springer, Dordrecht.
- McGlothlin, J. W., & Ketterson, E. D. (2008). Hormone-mediated suites as adaptations and evolutionary constraints. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1497), 1611-1620.

- McKean, K. A., & Lazzaro, B. P. (2011). The costs of immunity and the evolution of immunological defense mechanisms. *Mechanisms of life history evolution*, 299-310.
- McKinney, C. O. (1971). An analysis of zones of intergradation in the side-blotched lizard, *Uta stansburiana* (Sauria: Iguanidae). *Copeia*, 596-613.
- McKinney, M. L. (2006). Urbanization as a major cause of biotic homogenization. *Biological conservation*, 127(3), 247-260.
- McKinney, M. L. (2008). Effects of urbanization on species richness: a review of plants and animals. *Urban ecosystems*, 11(2), 161-176.
- Messer, P. W., Ellner, S. P., & Hairston Jr, N. G. (2016). Can population genetics adapt to rapid evolution? *Trends in Genetics*, 32(7), 408-418.
- Miles, L. S., Rivkin, L. R., Johnson, M. T., Munshi-South, J., & Verrelli, B. C. (2019). Gene flow and genetic drift in urban environments. *Molecular Ecology*, 28(18), 4138-4151.
- Mills, L. S., & Allendorf, F. W. (1996). The one-migrant-per-generation rule in conservation and management. *Conservation Biology*, 10(6), 1509-1518.
- Miranda, A. C. (2017). Mechanisms of behavioural change in urban animals: the role of microevolution and phenotypic plasticity. In *Ecology and conservation of birds in urban environments* (pp. 113-132). Springer.
- Miranda, A. C., Schielzeth, H., Sonntag, T., & Partecke, J. (2013). Urbanization and its effects on personality traits: a result of microevolution or phenotypic plasticity? *Global change biology*, 19(9), 2634-2644.

- Mitchell, T. S., Janzen, F. J., & Warner, D. A. (2018). Quantifying the effects of embryonic phenotypic plasticity on adult phenotypes in reptiles: a review of current knowledge and major gaps. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 329(4-5), 203-214.
- Mousseau, T. A., & Roff, D. A. (1987). Natural selection and the heritability of fitness components. *Heredity*, 59(2), 181-197.
- Munshi-South, J., Zolnik, C. P., & Harris, S. E. (2016). Population genomics of the Anthropocene: Urbanization is negatively associated with genome-wide variation in white-footed mouse populations. *Evolutionary applications*, 9(4), 546-564.
- Murgatroyd, C., Patchev, A. V., Wu, Y., Micale, V., Bockmühl, Y., Fischer, D., Holsboer, F., Wotjak, C. T., Almeida, O. F., & Spengler, D. (2009). Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nature neuroscience*, 12(12), 1559-1566.
- Murren, C. J., Auld, J. R., Callahan, H., Ghalambor, C. K., Handelsman, C. A., Heskell, M. A., Kingsolver, J., Maclean, H. J., Masel, J., & Maughan, H. (2015). Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. *Heredity*, 115(4), 293-301.
- Nathan, C., & Cunningham-Bussell, A. (2013). Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nature Reviews Immunology*, 13(5), 349-361.
- Neuman-Lee, L. A., & French, S. S. (2017). Endocrine-reproductive-immune interactions in female and male Galápagos marine iguanas. *Hormones and behavior*, 88, 60-69.
- Niemelä, J., Breuste, J. H., Guntenspergen, G., McIntyre, N. E., Elmqvist, T., & James, P. (2011). *Urban ecology: patterns, processes, and applications*. OUP Oxford.
- Noël, S., Ouellet, M., Galois, P., & Lapointe, F.-J. (2007). Impact of urban fragmentation on the genetic structure of the eastern red-backed salamander. *Conservation Genetics*, 8(3), 599-606.



- Palacios, M. G., Sparkman, A. M., & Bronikowski, A. M. (2011). Developmental plasticity of immune defence in two life-history ecotypes of the garter snake, *Thamnophis elegans*—a common-environment experiment. *Journal of Animal Ecology*, *80*(2), 431-437.
- Parchman, T. L., Gompert, Z., Mudge, J., Schilkey, F. D., Benkman, C. W., & Buerkle, C. A. (2012). Genome-wide association genetics of an adaptive trait in lodgepole pine. *Molecular Ecology*, *21*(12), 2991-3005.
- Parham, J. F., & Papenfuss, T. J. (2009). High genetic diversity among fossorial lizard populations (*Anniella pulchra*) in a rapidly developing landscape (Central California). *Conservation Genetics*, *10*(1), 169-176.
- Pennock, L. A., Tinkle, D. W., & Shaw, M. W. (1968). Chromosome number in the lizard genus *Uta* (family Iguanidae). *Chromosoma*, *24*(4), 467-476.
- Plummer, M., Best, N., Cowles, K., & Vines, K. (2006). CODA: convergence diagnosis and output analysis for MCMC. *R news*, *6*(1), 7-11.
- Price, T. D., Qvarnström, A., & Irwin, D. E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1523), 1433-1440.
- Rausher, M. D. (1992). The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution*, *46*(3), 616-626.
- Reed, D. H. (2004). Extinction risk in fragmented habitats. *Animal Conservation forum*,
- Reed, D. H., & Frankham, R. (2003). Correlation between fitness and genetic diversity. *Conservation Biology*, *17*(1), 230-237.
- Reed, D. H., Lowe, E. H., Briscoe, D. A., & Frankham, R. (2003). Fitness and adaptation in a novel environment: effect of inbreeding, prior environment, and lineage. *Evolution*, *57*(8), 1822-1828.

- Remón, N., Galán, P., & Naveira, H. (2012). Chronicle of an extinction foretold: genetic properties of an extremely small population of *Iberolacerta monticola*. *Conservation Genetics*, *13*, 131-142.
- Richmond, J. Q., Reid, D. T., Ashton, K. G., & Zamudio, K. R. (2009). Delayed genetic effects of habitat fragmentation on the ecologically specialized Florida sand skink (*Plestiodon reynoldsi*). *Conservation Genetics*, *10*(5), 1281-1297.
- Richmond, J. Q., Wood, D. A., Swaim, K. E., Fisher, R. N., & Vandergast, A. G. (2016). Historical habitat barriers prevent ring-like genetic continuity throughout the distribution of threatened alameda striped racers (*Coluber lateralis euryxanthus*). *Herpetologica*, *72*(3), 202-213.
- Riesch, R., Muschick, M., Lindtke, D., Villoutreix, R., Comeault, A. A., Farkas, T. E., Lucek, K., Hellen, E., Soria-Carrasco, V., & Dennis, S. R. (2017). Transitions between phases of genomic differentiation during stick-insect speciation. *Nature ecology & evolution*, *1*(4), 1-13.
- Riggs, A. D., & Xiong, Z. (2004). Methylation and epigenetic fidelity. *Proceedings of the National Academy of Sciences*, *101*(1), 4-5.
- Rivkin, L. R., Santangelo, J. S., Alberti, M., Aronson, M. F., de Keyzer, C. W., Diamond, S. E., Fortin, M. J., Frazee, L. J., Gorton, A. J., & Hendry, A. P. (2019). A roadmap for urban evolutionary ecology. *Evolutionary applications*, *12*(3), 384-398.
- Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, *4*, e2584.
- Romero, L. M., & Reed, J. M. (2005). Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *140*(1), 73-79.
- Ryman, N., Laikre, L., & Hössjer, O. (2019). Do estimates of contemporary effective population size tell us what we want to know? *Molecular Ecology*, *28*(8), 1904-1918.

- Sanderson, S., Beausoleil, M. O., O'Dea, R. E., Wood, Z. T., Correa, C., Frankel, V., Gorné, L. D., Haines, G. E., Kinnison, M. T., & Oke, K. B. (2022). The pace of modern life, revisited. *Molecular Ecology*, *31*(4), 1028-1043.
- Santangelo, J. S., Rivkin, L. R., & Johnson, M. T. (2018). The evolution of city life. *Proceedings of the Royal Society B*, *285*(1884), 20181529.
- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine reviews*, *21*(1), 55-89.
- Scheiner, S. M., & Lyman, R. F. (1989). The genetics of phenotypic plasticity I. Heritability. *Journal of evolutionary biology*, *2*(2), 95-107.
- Scheiner, S. M., & Lyman, R. F. (1991). The genetics of phenotypic plasticity. II. Response to selection. *Journal of evolutionary biology*, *4*(1), 23-50.
- Schell, C. J. (2018). Urban evolutionary ecology and the potential benefits of implementing genomics. *Journal of Heredity*, *109*(2), 138-151.
- Schell, C. J., Stanton, L. A., Young, J. K., Angeloni, L. M., Lambert, J. E., Breck, S. W., & Murray, M. H. (2021). The evolutionary consequences of human-wildlife conflict in cities. *Evolutionary applications*, *14*(1), 178-197.
- Schulte, U., Veith, M., & Hochkirch, A. (2012). Rapid genetic assimilation of native wall lizard populations (*Podarcis muralis*) through extensive hybridization with introduced lineages. *Molecular Ecology*, *21*(17), 4313-4326.
- Scoular, K. M., Caffry, W. C., Tillman, J. L., Finan, E. S., Schwartz, S. K., Sinervo, B., & Zani, P. A. (2011). Multiyear home-range ecology of common side-blotched lizards in eastern Oregon with

- additional analysis of geographic variation in home-range size. *Herpetological Monographs*, 25(1), 52-75.
- Sies, H., Berndt, C., & Jones, D. P. (2017). Oxidative stress. *Annual review of biochemistry*, 86, 715-748.
- Sinervo, B., Calsbeek, R., Comendant, T., Both, C., Adamopoulou, C., & Clobert, J. (2006). Genetic and maternal determinants of effective dispersal: the effect of sire genotype and size at birth in side-blotched lizards. *The American Naturalist*, 168(1), 88-99.
- Sinervo, B., & Clobert, J. (2003). Morphs, dispersal behavior, genetic similarity, and the evolution of cooperation. *Science*, 300(5627), 1949-1951.
- Sinervo, B., & Licht, P. (1991). Hormonal and physiological control of clutch size, egg size, and egg shape in side-blotched lizards (*Uta stansburiana*): constraints on the evolution of lizard life histories. *Journal of Experimental Zoology*, 257(2), 252-264.
- Smith, A. L., Gardner, M. G., Fenner, A. L., & Bull, C. M. (2009). Restricted gene flow in the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) in a fragmented agricultural landscape. *Wildlife Research*, 36(6), 466-478.
- Soneja, A., Drews, M., & Malinski, T. (2005). Role of nitric oxide, nitroxidative and oxidative stress in wound healing. *Pharmacological reports*, 57, 108.
- Sorci, G., & Faivre, B. (2009). Inflammation and oxidative stress in vertebrate host.
- Soria-Carrasco, V., Gompert, Z., Comeault, A. A., Farkas, T. E., Parchman, T. L., Johnston, J. S., Buerkle, C. A., Feder, J. L., Bast, J., & Schwander, T. (2014). Stick insect genomes reveal natural selection's role in parallel speciation. *Science*, 344(6185), 738-742.
- Stebbins, R. C., & McGinnis, S. M. (2018). *Peterson field guide to western reptiles & amphibians*. Houghton Mifflin.

- Stow, A., & Sunnucks, P. (2004). Inbreeding avoidance in Cunningham's skinks (*Egernia cunninghami*) in natural and fragmented habitat. *Molecular Ecology*, *13*(2), 443-447.
- Su, Y. S., & Yajima, M. (2015). Package 'r2jags'. *R package version 0.03-08*.
- Sun, W., & Hu, Y. (2013). eQTL mapping using RNA-seq data. *Statistics in biosciences*, *5*, 198-219.
- Team, R. C. (2016). R: A language and environment for statistical computing.
- Teixeira, J. C., & Huber, C. D. (2021). The inflated significance of neutral genetic diversity in conservation genetics. *Proceedings of the National Academy of Sciences*, *118*(10), e2015096118.
- Theodorou, P., Radzevičiūtė, R., Kahnt, B., Soro, A., Grosse, I., & Paxton, R. J. (2018). Genome-wide single nucleotide polymorphism scan suggests adaptation to urbanization in an important pollinator, the red-tailed bumblebee (*Bombus lapidarius* L.). *Proceedings of the Royal Society B: Biological Sciences*, *285*(1877), 20172806.
- Thomassen, H. A., Harrigan, R. J., Semple Delaney, K., Riley, S. P., Serieys, L. E., Pease, K., Wayne, R. K., & Smith, T. B. (2018). Determining the drivers of population structure in a highly urbanized landscape to inform conservation planning. *Conservation Biology*, *32*(1), 148-158.
- Thompson, M., Capilla-Lasheras, P., Dominoni, D., Réale, D., & Charmantier, A. (2021). Phenotypic variation in urban environments: mechanisms and implications. *Trends in Ecology & Evolution*.
- Tinkle, D. W. (1969). The concept of reproductive effort and its relation to the evolution of life histories of lizards. *The American Naturalist*, *103*(933), 501-516.
- Tom, J. A., Reeder, J., Forrest, W. F., Graham, R. R., Hunkapiller, J., Behrens, T. W., & Bhangale, T. R. (2017). Identifying and mitigating batch effects in whole genome sequencing data. *BMC bioinformatics*, *18*, 1-12.

- Treidel, L., Carter, A., & Bowden, R. (2016). Temperature experienced during incubation affects antioxidant capacity but not oxidative damage in hatchling red-eared slider turtles (*Trachemys scripta elegans*). *Journal of Experimental Biology*, 219(4), 561-570.
- Turner, F. B., Hoddenbach, G. A., Medica, P. A., & Lannom, J. R. (1970). The demography of the lizard, *Uta stansburiana* Baird and Girard, in southern Nevada. *The Journal of Animal Ecology*, 505-519.
- Tylan, C., Camacho, K., French, S., Graham, S. P., Herr, M. W., Jones, J., McCormick, G. L., O'Brien, M. A., Tennessen, J. B., & Thawley, C. J. (2020). Obtaining plasma to measure baseline corticosterone concentrations in reptiles: How quick is quick enough? *General and Comparative Endocrinology*, 287, 113324.
- Urban, M. C., Richardson, J. L., & Freidenfelds, N. A. (2014). Plasticity and genetic adaptation mediate amphibian and reptile responses to climate change. *Evolutionary applications*, 7(1), 88-103.
- Van Buskirk, J. (2017). Spatially heterogeneous selection in nature favors phenotypic plasticity in anuran larvae. *Evolution*, 71(6), 1670-1685.
- Virens, E., Krauss, S. L., Davis, R. A., & Spencer, P. B. (2015). Weak genetic structuring suggests historically high genetic connectivity among recently fragmented urban populations of the scincid lizard, *Ctenotus fallens*. *Australian Journal of Zoology*, 63(4), 279-286.
- Vitousek, M. N., Johnson, M. A., Downs, C. J., Miller, E. T., Martin, L. B., Francis, C. D., Donald, J. W., Fuxjager, M. J., Goymann, W., & Hau, M. (2019). Macroevolutionary patterning in glucocorticoids suggests different selective pressures shape baseline and stress-induced levels. *The American Naturalist*, 193(6), 866-880.
- Walsh, B., & Lynch, M. (2018). *Evolution and selection of quantitative traits*. Oxford University Press.

- Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews Genetics*, *10*(1), 57-63.
- Waples, R. S. (1989). A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics*, *121*(2), 379-391.
- Warner, D. A., Du, W.-G., & Georges, A. (2018). Introduction to the special issue-Developmental plasticity in reptiles: Physiological mechanisms and ecological consequences. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *329*(4), 153-161.
- Watson, H., Videvall, E., Andersson, M. N., & Isaksson, C. (2017). Transcriptome analysis of a wild bird reveals physiological responses to the urban environment. *Scientific Reports*, *7*(1), 1-10.
- Willi, Y., Van Buskirk, J., & Hoffmann, A. A. (2006). Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics*, 433-458.
- Winchell, K. M., Campbell-Staton, S. C., Losos, J. B., Revell, L. J., Verrelli, B. C., & Geneva, A. J. (2023). Genome-wide parallelism underlies contemporary adaptation in urban lizards. *Proceedings of the National Academy of Sciences*, *120*(3), e2216789120.
- Winchell, K. M., Reynolds, R. G., Prado-Irwin, S. R., Puente-Rolón, A. R., & Revell, L. J. (2016). Phenotypic shifts in urban areas in the tropical lizard *Anolis cristatellus*. *Evolution*, *70*(5), 1009-1022.
- Wingfield, J. C., Maney, D. L., Breuner, C. W., Jacobs, J. D., Lynn, S., Ramenofsky, M., & Richardson, R. D. (1998). Ecological bases of hormone—behavior interactions: the “emergency life history stage”. *American Zoologist*, *38*(1), 191-206.
- Wobeser, G. A. (2013). *Essentials of disease in wild animals*. John Wiley & Sons.

- Yewers, M. S. C., Stuart-Fox, D., & McLean, C. A. (2019). Space use and genetic structure do not maintain color polymorphism in a species with alternative behavioral strategies. *Ecology and evolution*, 9(1), 295-306.
- Zera, A. J., Harshman, L. G., & Williams, T. D. (2007). Evolutionary endocrinology: the developing synthesis between endocrinology and evolutionary genetics. *Annu. Rev. Ecol. Evol. Syst.*, 38, 793-817.
- Zhou, X., Carbonetto, P., & Stephens, M. (2013). Polygenic modeling with Bayesian sparse linear mixed models. *PLoS genetics*, 9(2), e1003264.



## CHAPTER 5

## SUMMARY &amp; CONCLUSIONS

The goal of this research was to explore population-level responses to urbanization in an indicator reptile species, the side-blotched lizard (*U. stansburiana*). Monitoring the responses of such species is crucial for conservation management because of the uncertainties in how populations decline or persist in human-modified landscapes. By identifying environmental factors or individual traits that are key to a species' sensitivity or tolerance, conservationist can better understand how to prevent local extirpations. In addition, assessing the evolutionary impact of urbanization on functionally relevant traits and genetic variation within urban populations can reveal the potential for adaptation. Here, comparisons of urban and natural *U. stansburiana* population vital rates and genetics were tested, while considering the ecological and evolutionary implications for physiological parameters that contribute to self-maintenance.

To address how *U. stansburiana* population vital rates can vary across natural and urban environments, models were developed for a six-year demographic data set (Chapter 2). Accounting for variation in physiological traits (*e.g.*, metabolic hormone levels, oxidative stress, and immunocompetence) when building such models allowed not only for better comparisons of annual survival rates, but also for identification of physiological traits predictive of survival. Here, survival estimates were lower for urban lizard populations overall, though there was high variability in survival across both habitat types over time. There was also strong support for a direct relationship between immune function and survival, with survival rates predicted to increase for lizards with greater bactericidal ability.

To determine the genetic basis of these physiological traits, a genome-wide association study was conducted on the same side-blotched lizards (Chapter 3). By fitting polygenic Bayesian mapping models, heritability was estimated for each of the traits, and their associated genetic loci were identified. Here, variation in each trait exhibited a polygenic basis, with low to moderate heritability and few measurable effect loci. To infer evolutionary impacts of urbanization, genomic time-series data from the same populations were used to test for environmental differences (urban versus natural) in genetic variation and selection on the physiological traits and their associated genetic loci (Chapter 4). In doing so, population-level comparisons of genetic structure and differentiation, variance effective population size, and molecular diversity were tested across environments. Here, lizards inhabiting urban patches were highly differentiated from those residing in natural areas, but moderate admixture and high levels of molecular diversity were present across populations. In addition, selection was not detected for any of the physiological traits or genetic loci, whereas indicators of drift were evident across the landscape, especially for urban populations.

Altogether, investigating the intersection of population physiology, demography, and genetics helped to resolve ongoing uncertainties regarding how variation in physiological traits across an urban-natural landscape (i) contribute to survival, (ii) are under genetic control, and (iii) are subject to evolutionary processes. The results herein have multifaceted implications for conservation and illustrate the effectiveness of integrated approaches to studying urban impacts on wildlife, but also the utility of organismal indicators for understanding both individual and population level processes. Incorporating these approaches into future work, while considering the results herein, will be crucial to parsing the processes responsible for population persistence or decline among wildlife occupying city environments.

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**Education**


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**Doctor of Philosophy** (Ecology), Spring 2023. Utah State University, Logan, Utah. GPA 3.78 / 4.00. Advisor - Dr. Susannah French

**Bachelor of Science** (Biology/Chemistry), Spring 2015. Millikin University, Decatur, Illinois. GPA 3.87 / 4.00. Advisor - Dr. Travis Wilcoxon

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**Teaching and Mentoring Experience**


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**Utah State University – Logan, Utah**

2021 Teaching Assistant: Herpetology  
 2020 Teaching Assistant: Human Physiology Lab  
 2017 - 2020 Guest Instructor: Comparative Animal Physiology Lecture  
 2016 - 2021 Research Mentor: 7 Undergraduate Students  
 2016 Instructor: Comparative Animal Physiology Lab  
 2016 Guest Instructor: Human Physiology Lecture  
 2016 Teaching Assistant: Human Physiology Lab  
 2015 Teaching Assistant: Principles of Biology Lab

**Millikin University – Decatur, Illinois**

2014 Tutor: Organic Chemistry II  
 2013 Tutor: Organic Chemistry I  
 2013 Tutor: Essentials of Organic & Biochemistry  
 2015 Teaching Assistant: Animal Behavior Lab  
 2013 Teaching Assistant: Essentials of Organic & Biochemistry Lab  
 2013 Teaching Assistant: Diversity of Life Lab  
 2013 Teaching Assistant: Ecology & Evolution Lab

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**Honors and Awards**


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2021 Doctoral Researcher of the Year, Utah State University  
 2020 Ivan J. Palmblad Award, Utah State University: \$2000  
 2018 Canyonlands Research Center Grant, Nature Conservancy: \$3000  
 2018 Joseph E. Greaves Award, Utah State University: \$5000  
 2017 - 2018 Ecology Center Award, Utah State University: \$9500  
 2017 Love of Learning Award, Phi Kappa Phi: \$500  
 2016 - 2017 RGS Travel Award, Utah State University: \$600  
 2016 Fort Carson Wildlife Grant, U.S. Department of Defense: \$2500  
 2016 John C. Johnson Travel Award, Beta Beta Beta: \$750  
 2015 - 2018 Charlotte Mangum Award, Integrative & Comparative Biology: \$900

2015	Summa Cum Laude Honors, Millikin University: \$500
2014	Scientific Meeting Travel Award, Millikin University: \$500
2012 - 2013	Barry M. Goldwater Scholarship Nominee, Millikin University

## Publications

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1. Virgin, E. E., Kepas, M. E., Lewis, E. L., Lidgard, A. D., Marchetti, J., **Hudson, S.B.**, Smith, G. D., & French, S. S. (2023). Egg viability and egg mass underly immune tradeoffs and differences in urban and rural lizard egg yolk physiology. *General and Comparative Endocrinology*, *1*, 1.
2. Kepas, M., Sermersheim, L. O., **Hudson, S.B.**, Lehmicke, A. J. J., French, S. S., & Aubry, L. M. (2023). Behavior, Stress, and Metabolism of a Parthenogenic Lizard in response to Flyover Noise. *Frontiers in Amphibian and Reptile Science*, *1*, 1.
3. Virgin, E. E., **Hudson, S. B.**, Webb, A. C., & French, S. S. (2022). The Immunological and Oxidative Costs of Reproduction and Associations between Maternal and Egg Yolk Physiology in a Reptile. *Ichthyology & Herpetology*.
4. French, S. S., **Hudson, S. B.**, Webb, A. C., Knapp, C. R., Virgin, E. E., Smith, G. D., Lewis, E. L., Iverson, J. B., & DeNardo, D. F. (2022). Glucose tolerance of iguanas is affected by high-sugar diets in the lab and supplemental feeding by ecotourists in the wild. *Journal of Experimental Biology*.
5. Caracalas, H. E., French, S. S., **Hudson, S. B.**, Kluever, B. M., Webb, A. C., Eifler, D., Lehmicke, A. J., & Aubry, L. M. (2021). Reproductive trade-offs in the colorado checkered whiptail lizard (*Aspidoscelis neotesselatus*): an examination of the relationship between clutch and follicle size. *Evolutionary Ecology*.
6. **Hudson, S. B.**, Virgin, E. E., Kepas, M. E., & French, S. S. (2021). Energy expenditure across immune challenge severities in a lizard: consequences for innate immunity, locomotor performance and oxidative status. *Journal of Experimental Biology*.
7. Denlinger, D. S., **Hudson, S. B.**, Keweshan, N. S., Gompert, Z., & Bernhardt, S. A. (2021). Standing genetic variation in laboratory populations of insecticide-susceptible *Phlebotomus papatasi* and *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae) for the evolution of resistance. *Evolutionary Applications*, *14*(5), 1248-1262.
8. **Hudson, S.B.**, E.E. Virgin, E.D. Brodie Jr, and S.S. French. (2021) Recovery from discrete wound severities in side-blotched lizards (*Uta stansburiana*): implications for energy budget, locomotor performance, and oxidative stress. *Journal of Comparative Physiology B*.
9. Spence, A. R., French, S. S., Hopkins, G. R., Durso, A. M., **Hudson, S. B.**, Smith, G. D., & Neuman-Lee, L. A. (2020). Long-term monitoring of two snake species reveals immune–endocrine interactions and the importance of ecological context. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *333*(10), 744-755..

10. Walker, R. H., Smith, G. D., **Hudson, S. B.**, French, S. S., & Walters, A. W. (2020). Warmer temperatures interact with salinity to weaken physiological facilitation to stress in freshwater fishes. *Conservation Physiology*, 8(1), coaa107.
11. Aubry, L. M., **Hudson, S. B.**, Kluever, B. M., Webb, A. C., & French, S. S. (2020). Competing reproductive and physiological investments in an all-female lizard, the Colorado checkered whiptail. *Evolutionary Ecology*, 34, 999-1016.
12. **Hudson, S.B.**, A.D. Lidgard, and S.S. French. (2020) Glucocorticoids, energy metabolites, and immunity vary across allostatic states for plateau side-blotched lizards (*Uta stansburiana uniformis*) residing in a heterogeneous thermal environment. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*.
13. **Hudson, S.B.** A.D. Lidgard, and S.S. French. (2020) Acute stress-reactivity for male plateau side-blotched lizards *Uta stansburiana uniformis* in a heterogeneous environment: implications for hormone-mediated energetics and innate immunity. *Journal of Experimental Zoology A*.
14. Durso, A. M., Smith, G. D., **Hudson, S. B.**, & French, S. S. (2020). Stoichiometric and stable isotope ratios of wild lizards in an urban landscape vary with reproduction, physiology, space and time. *Conservation Physiology*, 8(1), coaa001.
15. **Hudson, S.B.**, B.M. Kluever, A.C. Webb, and S.S. French. (2020) Steroid hormones, energetic state, and immunocompetence vary across reproductive contexts in a parthenogenetic lizard. *General and Comparative Endocrinology*.
16. Neuman-Lee, L. A., **Hudson, S. B.**, Webb, A. C., & French, S. S. (2020). Investigating the relationship between corticosterone and glucose in a reptile. *Journal of Experimental Biology*, 223(2), jeb203885.
17. **Hudson, S.B.**, and S.S. French. (2020) Color Polymorphism in *Uta stansburiana uniformis*. *Herpetological Review: Natural History Notes*.
18. **Hudson, S.B.**, M. Robertson, and T.E. Wilcoxon. (2019) Fecal glucocorticoid response to periodic social stress in male green anoles, *Anolis carolinensis*. *Copeia*.
19. French, S. S., Webb, A. C., **Hudson, S. B.**, & Virgin, E. E. (2018). Town and country reptiles: a review of reptilian responses to urbanization. *Integrative and Comparative Biology*, 58(5), 948-966.
20. **Hudson, S.B.** and T.E. Wilcoxon. (2018) Condition-dependence of structural plumage coverage in indigo buntings *Passerina cyanea*. *Avian Biology Research*.

### Manuscripts in Review

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21. Clifton, I.T., M.R. Duffy, **S.B. Hudson**, C.D. Robinson, E.E. Virgin, S.S. French, K.E. McCluney, and J. M. Refsnider. Compensation for exposure to increased environmental temperatures is costly in a montane, desert lizard. *Journal of Arid Environments*.

## Professional Presentations

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- **Hudson, S.B.**, E. Virgin, G. Smith, E. Brodie Jr, and S.S. French. Energetic strategy, locomotor performance, and oxidative cost vary according to magnitude of an integrated immune challenge.
  - Annual meeting of the Society for Integrative and Comparative Biology. Austin, TX. 2020.
- **Hudson, S.B.**, A. Durso, G. Smith, L. Lucas, and S.S. French. Physiology, demography, and genetics of side-blotched lizards (*Uta stansburiana*) across an urban-rural landscape.
  - Biology Department Seminar. Millikin University, Decatur, IL. 2018
- **Hudson, S.B.**, A. Durso, G. Smith, Z. Gompert, and S.S. French. Selection across and urban-rural landscape in side-blotched lizards, *Uta stansburiana*.
  - Annual meeting of the Society for Integrative and Comparative Biology. San Francisco, CA. 2018.
- **Hudson, S.B.**, A. Durso, G. Smith, and S.S. French. Reproductive coloration and physiology in female side-blotched lizards, *Uta stansburiana*.
  - Annual meeting of the Society for Integrative and Comparative Biology. New Orleans, LA. 2017.
- **Hudson, S.B.** and T.E. Wilcoxon. Structurally based plumage coloration as an honest signal for individual quality in indigo buntings, *Passerina cyanea*.
  - Tri-Beta National Meeting. St. Paul, MN. 2016
  - Tri-Beta North Central Regional Meeting. Monmouth College, Monmouth, IL. 2015. (First Place – Best Zoology Student Oral Presentation)
  - Annual meeting of the Society for Integrative and Comparative Biology. West Palm Beach, FL. 2015.
  - Illinois State Academy of Science Annual Meeting. Governors State University, University Park, IL. 2014. (First Place – Best Zoology Student Oral Presentation)
  - Tri-Beta North Central Regional Meeting. St. Xavier University, Chicago, IL. 2014. (Honorable Mention – Best Organismal Poster Presentation)
- **Hudson, S.B.** and T.E. Wilcoxon. Effects of social habituation on coloration and stress in male green anoles, *Anolis carolinensis*.
  - Illinois State Academy of Science Annual Meeting. Western Illinois University, Macomb, IL. 2015. (First Place – Best Student Poster Presentation)
  - Society for Integrative and Comparative Biology Annual Meeting. West Palm Beach, FL. 2015.
  - Celebration of Scholarship Research Poster Symposium. Decatur, IL. 2014. (Second Place – Best Student Poster Presentation)
- **Hudson, S.B.**, M. Robertson, and T.E. Wilcoxon. Thermally dependent color change and stress physiology in green anoles, *Anolis carolinensis*.
  - Celebration of Scholarship Research Poster Symposium. Millikin University, Decatur, IL. 2015. (Third Place – Best Student Poster Presentation)

## Research in Media

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1. “USU College of Science Recognizes Top Scholars of 2021” Utah State University Press Release, 2019. <https://www.usu.edu/today/story/usu-college-of-science-recognizes-top-scholars-of-2021>
2. “The pandemic stilled human activity. What did this ‘anthropause’ mean for wildlife?” Science Magazine, 2020. <https://www.sciencemag.org/news/2020/08/pandemic-stilled-human-activity-what-did-anthropause-mean-wildlife>
3. “Leapin’ Lizards: USU Undergrad Biologist Discovers Passion for Research” Utah State University Press Release, 2019.  
[http://www.usu.edu/today/index.cfm?id=58556&nl=628&utm\\_source=todaynewsletter&utm\\_medium=email&utm\\_campaign=nl628&utm\\_content=58556](http://www.usu.edu/today/index.cfm?id=58556&nl=628&utm_source=todaynewsletter&utm_medium=email&utm_campaign=nl628&utm_content=58556)
4. Canyonlands Research Center: Sundial Newsletter 2019-2020
5. “MU biology undergrads take top honor at Tri Beta Convention” Millikin University Press Release, 2015.
  - <http://www.millikin.edu/press-release/mu-biology-undergrads-take-top-honors-tri-beta-convention>
6. “Millikin Bird Feeding Study” *Illinois Stories* with Mark McDonald, WSEC-TV (PBS)
  - <https://www.youtube.com/watch?v=x3owxYPaINo>
7. “Biology students awarded research grants from National Biological Honor Society” Millikin University Press Release, 2013.
  - <https://www.millikin.edu/media/releases/Lists/Posts/Post.aspx?ID=942>
8. “SICB 2015: Color and Stress in Green Anoles” *Anole Annals*, 2015.
  - <http://www.anoleannals.org/2015/01/09/sicb-2015-color-and-stress-in-green-anoles/>

## Professional Organizations

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2017 – 2018	Herpetologists League
2016 – present	American Society of Ichthyologists and Herpetologists
2014 – present	Society for Integrative and Comparative Biology
2013 – present	Beta Beta Beta National Biological Honor Society
2013 – 2015	American Chemical Society
2013 – present	Phi Kappa Phi Honor Society
2013 – 2015	Illinois State Academy of Science

## Student Involvement

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2017 – 2020	Aggie Nerd Herd, Utah State University
2017 – 2018	BGSA President, Utah State University
2016 – 2017	BGSA Outreach Chair, Utah State University
2016 – 2017	Ecology Seminar Committee Officer, Utah State University
2012 – 2015	Environmental Affairs Council, Millikin University
2012 – 2014	International Student Organization, Millikin University
2012 – 2014	Asian Student Organization, Millikin University
2012 – 2013	Decaturian Newspaper Photographer, Millikin University
2013 – 2015	Biology Lab Van Driver, Millikin University

**Community Service and Leadership**

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2018 – 2020	Youth Teaching Youth – Canyonlands Research Center, Field Outreach
2018 - 2019	Mountain Crest Middle School – Hyrum Utah, Guest Expert
2017 - 2020	Natural History Museum – Salt Lake City Utah, Animal Outreach
2017	March for Science – Logan Utah, Event Coordinator
2017 - 2018	Edith Bowen Elementary School, Animal Outreach
2015 - 2017	Science Unwrapped Outreach Program, Coordinator & Demonstrator
2017	Venture Academy Elementary School, Animal Outreach
2016	Intech Collegiate High School, Science Fair Judge
2015	Dennis Elementary School Plant Study, Guest Instructor
2012 - 2013	Fido Scurry: Animal Shelter Charity Race, Volunteer
2012	Sangamon River Clean-up, Volunteer
2006 - 2008	LeaderCorps, Volunteer
2005 - 2009	Highway Clean-up, Volunteer