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Patterns of Molecular Population Genetic and Phenotypic Variation Associated with
Urbanization in the Western Black Widow Spider

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of
Philosophy at Virginia Commonwealth University.

by

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Abstract

PATTERNS OF MOLECULAR POPULATION GENETIC AND PHENOTYPIC VARIATION ASSOCIATED WITH URBANIZATION IN THE WESTERN BLACK WIDOW SPIDER

By Lindsay S. Miles, Ph.D.

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

Virginia Commonwealth University, 2018.

Major Director: Dr. Brian Verrelli, Graduate program director, Integrative Life Sciences Doctoral Program

In urban population genetic studies, the "urban fragmentation model" predicts that urbanization acts as a barrier that isolates native populations, and can lead to reduced gene flow and increased genetic drift between populations. The "urban facilitation model" predicts urban areas act as corridors to increase dispersal among urban areas, and can lead to higher genetic diversity within and lower differentiation between urban areas.

In a review of the current literature, we found that there is no consistent signature of reduced within-population genetic diversity or increased between-population genetic differentiation. Analyses that investigate the urban barriers to gene flow also found no consistent results. Thus, the response to urbanization may be species and city specific.

We used social network genetic analyses, which can identify connections that both fragment and facilitate gene flow, to investigate the impact of anthropogenic disturbance on

connectivity in a model urban pest of significant medical-relevance, the Western black widow spider, *Latrodectus hesperus*. In comparison to non-urban locales, urban locales have higher within-population genetic diversity, lower between-population genetic differentiation, and higher overall estimates of genetic connectivity. We found that not all cities are highly connected, with specific urban hubs driving gene flow among historically isolated non-urban locales.

We compared and contrasted our previous broad-scale patterns of urban gene flow with a new fine-scale locale sampling from within three Southwestern U.S. cities. Urban areas have significantly different patterns of connectivity to the overall network that generate contrasting patterns of within- and between-city genetic diversity. There is significant heterogeneity among the fine-scale city samples, such that certain urban hubs are impacting the network of urban and non-urban locales on the whole.

We examined differences in gene expression between three paired urban and non-urban populations from the cephalothorax (metabolism), ovary (fertility), and silk glands (web architecture). There is significant differential expression in each tissue type observed between urban and non-urban locales, among both urban and among non-urban locales, and specific to geographic locations independent of urban or non-urban habitat. These results imply that not all cities are created equal with respect to demographic and gene flow patterns, but also with phenotypic patterns.

Chapter 1: URBANIZATION EFFECTS ON GENE FLOW ACROSS TAXA, A REVIEW OF PRIOR WORK

Introduction

Currently, over half the human population lives in urban areas (United Nations, 2015). With an ever-growing urban population at the global level, these urban areas fragment and eliminate natural habitat, which results in the loss of biodiversity (Seto et al. 2011, 2012). The loss of biodiversity can have negative impacts on conservation and invasion biology, as well as on ecosystems services that provide resources to humans (McKinney, 2002, 2006; Keyghobadi, 2007; McDonald et al., 2008; Alberti, 2015; Donihue and Lambert, 2015; McDonnell and Hahs, 2015). Recently, there have been an abundance of empirical studies that seek to address the eco-evolutionary dynamics that arise from organisms living in the novel urban environment (Alberti 2015; Donihue and Lambert, 2015; McDonnell and Hahs, 2015). Studies have addressed the phenotypic changes resulting from urbanization and, more recently, there is a surge in studies that seek to identify whether these changes are phenotypically plastic or have an underlying genetic explanation consistent with adaptation (Alberti 2015; Donihue and Lambert, 2015; McDonnell and Hahs, 2015; Johnson & Munshi-South, 2017; Schell, 2018). In fact, there has been a steady increase in molecular population and evolutionary genetic studies conducted in urban areas with the advent of affordable next-generation sequencing technologies for non-

model organisms (Ekblom and Galindo, 2011; Andrews et al., 2016). However, despite this emergence, it is still unclear what impact urbanization has had on gene flow, which is a key force behind both urban adaptive and non-adaptive evolution, and is the focus of this review.

The impact that urbanization has on organisms has recently been studied through the lens of population genetics, which tends to come at this problem from multiple directions. For example, conservation genetics looks at the level of genetic diversity within populations, with the intent that it can be used to interpret inbreeding or management units (Frankham & Ralls, 1998; Høglund, 2009). Landscape genetics tends to focus on measures of genetic diversity within and between populations with the intent to examine correlations with biotic and abiotic features in identifying landscape barriers to gene flow (Manel et al., 2003; 2013). Evolutionary genetics use measures of genetic diversity to focus on making conclusions about the relative contributions that evolutionary forces of gene flow, drift, mutation, and selection make in explaining patterns of diversity on both temporal and spatial scales (e.g., Wright, 1982; Oyler-McCance et al., 2016; Lowry et al., 2017). These fields can have different questions and analyses, which will ultimately alter our perception of the impact of urbanization on evolution. For example, conservation and landscape genetic studies are usually "individual-based" to learn something about how individuals are related (conservation) or impacted by barriers (landscape), whereas, evolutionary genetics studies focus on analyses of populations, as these are the evolutionary units of selection, gene flow, drift and mutation.

While these disciplines have different questions that they address, they incorporate similar data and analyses (Dyer, 2015). Specifically, for urban population genetic studies, there is an interest in how genetic diversity may be decreased within urban areas as a result of fragmentation of the landscape, an ideology long adopted in urban ecology as the "urban

fragmentation model". This model predicts that urbanization acts as a barrier that isolates native populations, and can lead to ecological divergence through reduced gene flow, reduced effective population (N_e) sizes, and increased genetic drift among populations (Keyghobadi, 2007; Holderegger & Di Giulio, 2010; Munshi-South & Kharchenko, 2010; Parks et al., 2015; Xuereb et al., 2015; Fairman et al., 2016). This model is typically associated with detrimental fitness consequences, in part due to the fragmentation and isolation of populations that leads to increased drift and inbreeding (Cheptou et al., 2008; Brady, 2012; Mueller et al., 2013).

A contrasting view to this urban fragmentation model is that organisms have adapted to these urban environments as “urban adapters” (Blair, 1996; Shochat, 2004). These urban adapters possess traits that enable them to successfully thrive in urban ecosystems, in part due to human movement among urban areas that facilitates gene flow for them (Blair 1996; McKinney & Lockwood, 1999; Holderegger & DiGiulio, 2010, Crispo et al., 2011). This proposed model of “urban facilitation” rivals the traditional model of urban fragmentation in that it predicts that urban areas can act as corridors to increase dispersal within and between urban areas, resulting in higher genetic diversity within and lower differentiation between urban areas (Crispo et al., 2011).

In testing the hypotheses set by each of these competing models of urban gene flow, multiple disciplines have also approached landscape barriers using a similar population genetic framework. For example, a standard population genetic model is isolation-by-distance (IBD, Wright, 1943; Slatkin 1993), such that as geographic distance between populations increases, so does genetic differentiation. On the other hand, isolation-by-resistance (IBR, McRae, 2006) models predict that in addition to geographic distance, other factors, such as urbanization, can act as barriers under “urban fragmentation”, or even as conduits to gene flow under “urban

facilitation” (Holderegger & DiGiulio 2010; Crispo et al., 2011; LaPoint et al., 2015; deGroot et al., 2016; Tang et al., 2016). In the latter case, it is expected that urbanization increases genetic connectivity among urban adapter populations, and this increased gene flow may lead to increased connections among even previously isolated non-urban populations. Given the polarizing outcomes for conservation priorities predicted by models of urban fragmentation vs. facilitation, population genetic studies are necessary to distinguish among these models in the face of continued urban growth (McDonnell & Hahs, 2015).

The effects of urbanization on evolutionary processes have become the focus of several recent reviews (e.g., Johnson and Munshi-South, 2017). However, with the growing use of population genetic studies in urban areas, we review evidence mounting for the competing models of fragmentation and facilitation, with an overall goal to determine if cities have predictable effects on non-adaptive evolutionary processes across taxa. With these two models in mind, in this review, we focused on three specific questions:

- 1) What effects does urbanization have on within-population genetic diversity?
- 2) What effects does urbanization have on between-population genetic differentiation?
- 3) How have studies explored barriers, both biotic and abiotic, to gene flow in urban environments?

Trends in urban gene flow studies

We used Google Scholar and ISI Web of Science to search for studies which included the following terms: “genetic drift”, “genetic diversity”, “landscape genetics”, “population genetics”,

or “gene flow” together with the terms “urban” or “city”. We define “urban” as human-modified landscape with human dwellings which can include towns, cities, and metropolitan areas, whereas a “city” is a distinct unit within an urban area with human defined borders. We identified 160 empirical research articles that met these criteria. For each study, we identified variables that we hypothesized could influence population genetic structure between urban and nonurban populations, as related to our aforementioned questions. These included: the study organism, the number of urban and nonurban populations sampled, the number of cities sampled, and the type of genetic marker (e.g., microsatellite, SNPs) used to measure population structure.

Taxon sampling

The biology and life history of an organism are necessary to consider when making conclusions about the overall impact of urbanization. These considerations include the organisms range (does it occur in an urban area or in multiple urban areas?) and the ability for movement (see review Medina et al., 2018). Urban studies have covered a variety of taxa, including mammals ($N = 62$), arthropods ($N = 48$), amphibians ($N = 21$), plants ($N = 20$), birds ($N = 15$), reptiles ($N = 15$), and viruses ($N = 3$), with several studies sampling multiple taxa. The most common type of organism studied is mammals, dominating the current literature at 38%. The lack of taxonomic diversity in urban evolution studies has been discussed in several recent reviews (Holderreger & DiGiulio, 2010; Johnson & Munshi-South, 2017; Schell 2018). This dominance of mammals in the urban gene flow literature is likely due to the contribution of conservation genetic studies as large mammals are considered “flagship species” in conservation (Schipper et al., 2008; Francis et al., 2010; Zachos & Hacklander, 2011). Additionally, rodents are commonly studied in urban areas ($N = 19$) due to their prevalence within urban areas and

their pest concerns (Johnson & Munshi-South, 2017). Arthropod studies are also quite prevalent in urban literature, accounting for 30% of the taxa represented. Similar to mammals, arthropods such as bees are of conservation concern because of their role in pollination in both wild plants and agricultural crops (Estoup et al., 1996; Cameron et al., 2011). Additionally, arthropods such as mosquitoes, bed bugs, and cockroaches are also studied because of their prevalence in urban areas as human commensals, but more specifically because of the medical concerns as pest species (reviewed in Johnson & Munshi-South 2017). While both species of conservation and pest concerns are important to study in urban environments, interestingly, these reflect the extreme outcomes of anthropogenic effects on species, with species of conservation concern becoming extirpated in urban areas and species of pest concern thriving in urban areas.

There are different expectations of gene flow for different types of organisms, regardless of conservation or pest delimitation. For example, in vertebrate species, those that fly (e.g., birds) have fewer geographic barriers to gene flow than those that move on the ground (e.g., pumas) and thus have different patterns of gene flow between them (Medina et al., 2018). Furthermore, plants, which are vastly underrepresented in urban gene flow studies, are expected to be sensitive to urban fragmentation because of their sessile habit, but may also overcome fragmentation because of life history traits such as wind-dispersed pollen (Young et al., 1996; Cresswell, 2005). Given these differences, when we are evaluating different models of urban gene flow, we must consider this organismal diversity so as not to bias our conclusions.

Genetic marker sampling

Studies have used a several types of molecular markers including microsatellites ($N = 126$), mitochondrial DNA ($N = 26$), allozymes ($N = 14$), genome-wide SNPs ($N = 10$), AFLPs ($N = 6$), and ISSRs ($N = 4$), with only a total of 13 studies using multiple markers (e.g., microsatellites and mitochondrial DNA). Not surprisingly, most studies used microsatellite markers as traditionally these markers have been easier to develop and less expensive to apply to large samples, especially when most organisms in these studies are non-model ones (Ekblom and Galindo, 2011; Andrews et al., 2016). This approach is largely reflective of the conservation genetics discipline interest in urban ecological research. Microsatellite markers, while they are rapidly evolving and can reflect recent or contemporary gene flow, have caveats in that they can violate various population genetic assumptions of identity-by-descent and with widely varying mutation rates (Hartl & Clark, 1997). Some of these conclusions have little bearing on simple estimates of genetic diversity contrasted within and between populations, but do have implications for evolutionary genetic modeling of demographic and adaptive scenarios.

Geographic sampling

We identified two urban-specific variables which we hypothesized could be related to how urbanization alters genetic diversity within and between populations: city area (km^2) of the city(ies) sampled and human population size. Because these two variables were often not included in the study details we estimated these variables from population censuses (US Census 2014; United Nations, 2015). We found that 32 studies sampled non-urban habitats that occur near urban areas, and because the sampling was not specific to within an urban area, we did not identify the nearby urban area size or human population for these studies. The remaining 128 studies that sampled within urban areas, had urban areas that range from 0.37 km^2 to $809,000$

km². These urban areas have human population sizes as small as 222 to as large as 21 million. Most of the studies reviewed here were conducted in North America and Europe ($N = 103$), with temperate zones within these areas overrepresented. Although from a broad perspective, cities are often considered replicates of each other (Pickett et al., 2016; Alberti et al., 2017), they have clear differences in ecological, climatic, anthropogenic, temporal, and spatial characteristics (Grimm et al., 2008; Alberti, 2015). For example, cities in the tropics and deserts have different climatic conditions than those in temperate regions, therefore, in this review, we address how the response to these vastly different urban areas may be quite different.

Geographic extent and human population size can both be used as proxies to the level of urbanization (see Munshi-South et al., 2016) and therefore can differ in the extent that urbanization has fragmented the habitat and reduces gene flow. For example, Leidner & Haddad (2010) sampled individuals from a small urban area (17 km² and 701 human population size), and identified that urbanization was not a barrier to gene flow, but instead identified that ocean inlets were driving patterns of genetic structure. On the other hand, Wang et al (2010) sampled individuals from a large urban area (16,000 km² and 2.15 million human population size) and identified that urbanization was a barrier to gene flow. However, Desender et al. (2005) sampled two different urban areas with similar geographic and population size, Brussels and Birmingham, and found that genetic diversity in dung beetles was significantly higher in Brussels and differentiation was greater in Birmingham. Additionally, when multiple cities are sampled across a broad geographic scale to look at connectivity among them, some cities can act as hubs of genetic connectivity regardless of their relative size, driving gene flow across the landscape (Miles et al., 2018). Therefore, it is likely that the size and level of urbanization of a city does not

always impact genetic connectivity in the same way, and in fact, as discussed in this review, can have different implications for fragmentation or facilitation of gene flow.

Genetic diversity in urban environments

The primary measures of genetic diversity in these urban population genetic studies were observed (H_O) and expected heterozygosity (H_E), allelic richness (Ar), and the inbreeding coefficient (F_{IS}) from microsatellite studies, with a few using π or the average pairwise nucleotide differences among SNPs. Given the few number of studies using SNPs, we found that 18 studies measured π , with only three using NGS nuclear data, and the remaining 15 using mtDNA data. Only two studies that measured π compared urban to non-urban populations, reporting the differences in π between them. For both studies, π was lower in urban compared to non-urban, although not statistically significant (Hirota et al., 2004; Asgharian et al., 2015). With the majority of the studies using microsatellite markers ($N = 126$) the standard measures of diversity were broken down as: $H_O = 111$, $H_E = 98$, $Ar = 91$, $F_{IS} = 50$. Of these studies, there were 47 that sampled at least one urban and one non-urban locale to perform a paired contrast (only 29% of the overall studies).

While identifying the level of genetic diversity within urban areas is a valuable benchmark in assessing the current standing genetic variation of urban populations, without a background estimate from a "non-urban" sample as a paired contrast, it is unclear whether or not a reduction of diversity in these samples is associated with urbanization. To address the overall differences in genetic diversity between urban and non-urban populations, we used a multi-model averaging approach to identify the best fitting model implemented using the *dredge*

function in the R package *MuMIn* (Barton, 2015), then performed an ANOVA on each response variable, using the corresponding best-fitting model from the *dredge* output (see Supplemental Methods for model fitting). We found that A_r was 12.69% lower in urban populations compared to non-urban populations ($F_{1, 143.22} = 7.37$, $p = 0.007$). Although there was a trend towards decreased H_O (1.57%), H_E (9.38%), and F_{IS} (22.14%), in urban populations, this trend was not statistically significant ($p > 0.05$). Measures of genetic diversity for microsatellite markers are strongly influenced by the number of microsatellites used, the number of alleles per locus, and the sample size (Bashalkhanov et al., 2009; Hale et al., 2012; Landguth et al., 2012). While these factors may play a role in estimates of diversity using microsatellite markers, given the large number of studies sampled here, it is not clear that this explanation can account for the lack of a pattern. In this respect, after accounting for diversity in several factors including taxon sampling, the data do not support a drop in genetic diversity in urban areas, which would be predicted by the urban fragmentation model.

Genetic differentiation in urban environments

To address the overall differences in genetic differentiation between urban and non-urban populations, we used the same multi-model averaging approach identified above. The most common measure of genetic differentiation among urban population genetic studies was F_{ST} ($N = 118$), with the majority ($N = 77$) having calculated F_{ST} between populations in different cities and 9 studies calculating F_{ST} between populations within the same city. Although high F_{ST} values might reflect low gene flow, they do not necessarily reflect a reduction in gene flow due to urbanization. For example, many urban studies have shown high estimates of F_{ST} in both plants and animals (e.g., Hitchings & Beebe, 1997; Saenz et al., 2012; Ascunce et al., 2013; Munshi-

South et al., 2013; Bartlewicz et al., 2015; Booth et al., 2015; Johnson & Munshi-South, 2017). However, without background estimates of population structure and population differentiation outside of urban areas, it is unclear whether these values truly represent gene flow reduction or just simply high population differentiation for the species. For example, Miles et al. (Chapter 2) found that the average F_{ST} between urban populations of the Western black widow spider was as high as 0.42, however, this was statistically significantly lower than the average F_{ST} between non-urban populations ($F_{ST} = 0.56$). In our review of the literature, 33 studies that calculated F_{ST} sampled both urban and non-urban locales. Although F_{ST} was higher in pairwise analyses of urban populations ($F_{ST} = 0.12 \pm 0.16$) compared to pairwise analyses of non-urban populations ($F_{ST} = 0.07 \pm 0.06$), this trend was non-significant ($\chi^2_1 = 0.403$, $p = 0.525$). Therefore, both sampling design and urban influences appear to play a large role in our ability to detect significant differences in genetic differentiation between urban and non-urban populations. Indeed, the lack of significance in comparing urban to non-urban population genetic differentiation suggests that while urbanization may sometimes lead to increased differentiation between populations as predicted by the urban fragmentation model of gene flow, this is not always the case, and may be taxon-specific.

Although the most common measure used in urban studies was F_{ST} , there are several different measures of genetic differentiation, including G_{ST} (Nei and Chesser, 1983), G'_{st} (Hedrick, 2005) and D (Jost, 2008), one of the caveats to F_{ST} is that the pairwise distance between two populations does not account for the genetic variation present among populations on the whole. Other measures such as conditional genetic distance (cGD, Dyer & Nason, 2004) take into account shared variation across all sampled populations at once and can outperform F_{ST} when describing the spatial distribution of genetic diversity (Dyer et al., 2010). Additionally,

there is no consensus on what is considered a significantly high value of F_{ST} (see value bins in Hartl & Clark, 1997; Frankham et al., 2002, 2010; Lowe & Allendorf, 2010). For example, when $F_{ST} = 0.18$, this is evidence for low differentiation for a mosquito (*Culex pipens*, Asgharian et al., 2015), however it is evidence for significantly high differentiation for an Arroyo chub (Benjamin, et al., 2016) and an ocelot (Janecka et al., 2011). Therefore, these differences in significant high or low differentiation are complex and related to the life history of an organisms, and thus conclusions about these values alone may not reflect the impact of urban fragmentation, but rather a specific species' estimated genetic differentiation.

Barriers to gene flow in urban environments

Both the urban fragmentation and facilitation models of gene flow explore barriers and conduits for dispersal in urban environments. We recorded the number of studies which explored isolation-by-distance (IBD) and/or isolation-by-resistance (IBR) in urban environments, and found that 108 studies examined the role that environmental factors can play in shaping gene flow. In order to test for the relationship between genetic and geographic distance (IBD approach), researchers calculate the genetic distance as pairwise F_{ST} and the geographic distance is measured as Euclidean distance (km) between sampled locales. Of the 108 studies that tested IBD, 88 studies conducted a Mantel test, and 57 of those studies reported a measure of associated (i.e., the Mantel r^2). Of the 57 studies that reported Mantel r^2 , 22 sampled only urban locales and the remaining 35 studies combined both urban and non-urban measures of genetic and geographic distance in their IBD analyses. For the studies that sampled urban locales only, Mantel r^2 ranged from 0.0001 to as high as 0.79 (average = 0.12 ± 0.19), and was not statistically significant ($\chi^2_2 = 4.96$, $p = 0.08$), which could be due to the relatively small sample size.

Nonetheless, the pattern is still in the opposite direction (i.e. low IBD) than expected under an IBD model. Interestingly, signatures of IBD may be weak under both the urban fragmentation model and the urban facilitation model. The former predicts higher genetic differentiation among urban populations even at small geographic scales, whereas the latter predicts lower genetic differentiation among urban locales at all spatial scales. Therefore the lack of significance of IBD within studies may be due to either model of gene flow, and thus it is necessary to identify potential barriers or conduits that may be reducing the strength of IBD.

In testing IBR, resistance variables for both urban and natural/non-urban are calculated to tease apart the relative contributions of these landscapes to patterns of gene flow. Resistance variables that are associated with urbanization include percent impervious surface (higher % is more urbanization), canopy cover (values inversely related to urbanization), road density, and human population density (Grimm et al., 2008; Nowak and Greenfield, 2012; Alberti, 2015, Alberti et al., 2017). Resistance variables that are associated with the natural landscape that can also impact gene flow included land-use types (e.g., forest), canopy cover (higher canopy cover for natural landscape), and rivers (Manel et al., 2003; Manel and Holderegger, 2013). There were 47 studies that specifically tested IBR in an urban context that employed linear regression analyses. There were no consistent trends that identified urbanization as a barrier to gene flow both within and between studies. For example, Emaresi et al. (2011) identified forest, urban, and orchard landscape classifications as barriers to gene flow for the alpine newt (*Mesotriton alpestris*). In fact, several studies found both urban and natural barriers to gene flow (Unfried et al., 2013; Parks et al., 2015; Nagamatsu et al., 2016; Oritz et al., 2017). Other studies found no significant urban or natural barriers to gene flow (Gortat et al., 2015, 2017). Urban land-use was identified as a statistically significant barrier for many of the remaining IBR studies, but had

varying degrees of correlation. For example, Delaney et al., (2010) sampled three lizard species and one bird species from the same urban locales and identified roads as barriers to gene flow, but that the strength of the barrier varied from 0.09-0.16. Therefore, given the lack of consistent trends both within and between studies, the urban fragmentation model of gene flow which identifies urban land use as a barrier to gene flow is unlikely to explain these differences in patterns of gene flow. There appears to be support for both the urban fragmentation and the urban facilitation models of gene flow, which are study specific, and no one model explains all patterns of gene flow in urban environments.

Although genetic distances measured as pairwise F_{ST} and geographic distances measured between samples have been the standard for these IBD/IBR analyses, both the statistical method to assess significance and the landscape variables used in these analyses are not consistent across studies. Traditionally, Mantel and partial Mantel tests were used to identify a significant correlation between these measures. However, recently Legendre et al (2015) criticized the use of Mantel tests for use in spatial analyses because the null of the Mantel is the absence of a relationship between two dissimilarity matrices and thus the Mantel R^2 should not be considered the same as an R^2 of correlation. Thus while many researchers have used general linear models (GLMs) prior to the Legendre et al. (2015) criticism, the use of GLMs has since increased. However, there is a lack of consistency on what results (e.g., AIC, r^2) of these GLMs are reported. For example, similar to the results in Mantel tests, when a GLM is not significant, neither the AIC nor the r^2 values are reported. Additionally, landscape variables that are modeled into these GLMs are not always consistent across studies. For example, when modeling historic land-use vs contemporary urban land-use researchers have used "time since urban/park establishment" (Munshi-South & Nagy, 2014; Lourenco et al., 2017) and others have used

historic GIS layers that run multiple models based on urban resistance via Circuitscape (Jha & Kremen, 2013). These differences in statistical modeling and land use as urbanization proxies make the identification of consistent urban patterns difficult to compare. Currently, the paucity of studies that used a GLM approach (N = 47) have not all reported the same statistics. However, the increased popularity of using GLMs to analyze spatial data, if reporting results becomes consistent, may allow future analyses to have the statistical power to detect potential differences in the signature IBD/IBR among urban population genetic studies.

Overall Synthesis

Although the conventional wisdom has been that urbanization acts as a barrier to reduce gene flow, decrease within population diversity and increase between population diversity, here, in a review of 160 urban population genetic studies, we found that there is no consistent signature of reduced within-population genetic diversity or increased between-population genetic differentiation. In addition, a further review of analyses that investigate the urban barriers to gene flow also found no consistent results. Urban gene flow studies need more organism and environment diversity to understand the diverse impacts that urban evolution can have. For example, German cockroaches have been found in many major urban areas and have experienced human-mediated dispersal across the globe (Booth et al 2011). However, there are species such as pumas that are only in remnant patches near urban habitats and have experienced extreme isolation in part due to road mortality (Lee et al., 2012). These organisms are vastly different in their biology and life history, and thus we may expect to find differences in the signature of gene flow between them. However, even when organisms have similar life history traits, there are still very different responses to urbanization. For example, although different bee species were

sampled in several studies, some bee species are unable to disperse through urban areas and have lower genetic diversity and higher genetic differentiation for urban populations, consistent with the urban fragmentation model of gene flow (Davis et al 2010; Jha & Kremen 2013). However, other bee species were able to disperse through urban areas, making use of local urban gardens, and have higher genetic diversity and lower genetic differentiation in urban populations, consistent with the urban facilitation model of gene flow (Chapman et al., 2003; Soro et al 2017). We may expect that certain groups, such as mammals, may be expected to follow the urban fragmentation model. However, we note that urban pests do follow the urban facilitation model, but some pests are mammals (e.g., mice, rats). Additionally, some animals of conservation concern (e.g., tarantulas, Machkour-M'Rabet et al., 2012) follow urban facilitation model of gene flow. Therefore, whether an organism experiences urban fragmentation or urban facilitation of gene flow, our current understanding is that these are highly taxon specific, where certain taxa are no more susceptible to urban fragmentation than others.

While the response to urbanization is likely taxa-specific, it may also be city- and environment-specific. With the current literature dominated by North American and European temperate zones, our understanding of the interaction between urbanization and environmental conditions is lacking. However, even though the literature reviewed here is in similar environmental regions, we still find that there are differences in response to urbanization that may be city-specific. For example, studies of salamanders show reduced gene flow in Baltimore, MD (Gardner-Santana et al., 2009; Garcia-Gonzalez et al., 2012); however, salamanders in Montreal did not experience reduced gene flow (Noel & Laponte, 2010). Given that the amphibians in these studies have similar life history traits and the cities are both in temperate zones, it is likely that the response to gene flow is due to differences in these cities. Future

studies are needed that incorporate cities in more diverse ecological, climatic, anthropogenic, temporal, and spatial characteristics (Grimm et al., 2008; Alberti, 2015). For example, the Western United States has recently become highly urbanized (US Census 2014) and has a diversity of ecoregions including deserts, plains, Mediterranean-like chaparrals, forested mountains, and coastal forests (Western Ecology Division EPA, 2018). These areas need to be investigated if we are to learn not only how these unique ecological regions are impacted by urbanization, but also if we are to learn how contemporary and recent urban growth evolves with respect to local biodiversity.

We found that 78% of the studies we identified have used microsatellite markers, which as noted above, have been historically easier to generate and less expensive and have utility in measuring more recent changes in genetic diversity. However, recent advances in next generation sequencing (NGS), have recently made it possible to collect genome-wide SNPs. In fact, NGS has recently become more affordable and available for non-model organisms, which allows researchers to estimate genome-wide nucleotide diversity (Ekblom and Galindo, 2011; Andrews et al., 2016). The number of single nucleotide polymorphisms (SNPs) that can be examined using next generation sequencing (NGS) are magnitudes higher than the number of microsatellite markers traditionally used (Allendorf et al., 2010; Andrews et al., 2016). The genome-wide putatively neutral SNPs that are genotyped by NGS data provide ample genetic variation in which researchers can detect differences between populations even at small spatiotemporal scales (Ekblom and Galindo, 2011; Richardson et al., 2014). Therefore, in the next 10 years, we will have enough studies that have used these NGS markers in urban studies that we will be able to identify urban impacts on genetic diversity at multiple spatiotemporal scales.

In the review of the studies' "Introductions", $N = 118$ studies (73%) hypothesized that urban fragmentation was the primary mechanism which altered patterns in gene flow and drift in their study system while $N = 11$ (7%) hypothesized human-mediated facilitation was the mechanism which altered patterns non-adaptive evolution. Interestingly, the 11 studies that hypothesized that urbanization facilitates gene flow are all studies investigating human-health pests. The remaining 20% of the studies were descriptive-driven studies investigating general patterns of gene flow/structure of organisms in urban environments. These differences in hypotheses have influenced how researchers set up their study design, what analyses they will use, and thus the conclusions they will make. For example, when a study hypothesizes that urbanization creates a barrier to gene flow, individuals can be sampled from only one urban area and do not require a non-urban comparison. When genetic diversity estimates are “low” and genetic differentiation estimates (F_{ST}) are “high”, one may conclude that urbanization is acting as a barrier to gene flow. However, as noted previously, without a non-urban comparison, these values may be indicative of a species-specific signature of gene flow. For example, bed bugs, which are globally distributed, human-commensal pests, have high genetic diversity within urban populations but also high genetic differentiation ($F_{ST}=0.68$) between populations due to their infestation patterns (Saenz et al., 2012). While pest species typically experience human-mediated gene flow, some species of conservation concern have signatures of urban facilitated gene flow, even though researchers hypothesized that they experience urban fragmentation. These patterns are not taxon-specific; studies have indicated human-mediated dispersal in conserved species such as tarantulas (Machkour-M'Rabet et al., 2012), pine martens (deGroot et al., 2016), and even a brushland plant (Roberts et al., 2007). Thus, as mentioned previously, the response to urbanization, either fragmentation or facilitation, is likely taxon-specific.

The urban fragmentation and facilitation models of gene flow explore barriers and conduits for dispersal in urban environments, which are reliant on resistance models. Resistance models are really in their infancy given that the field of landscape genetics has only emerged since 2003 (Manel et al., 2003), resistance models such as Circuitscape were introduced in 2006 (McRae, 2006), and the widespread use of GLMs to detect significant resistances has blossomed since 2015 (Legendre et al., 2015). Additionally, we have only recently started getting at different organisms that allow us to look at different resistors. For example, if we have been focused on large mammals, then maybe we do not have the power and geographic distances to have the resolution to test IBR, whereas, other studies of global organisms such as birds, bees, spiders, and roaches have enabled such ideas to develop. Nonetheless, we are also just starting to build consistent ways to test IBR. For example, while many have looked at single cities and within these cities for IBR, we learn very different things than when we have multiple cities, different geographic scales, and vastly different resistors. Indeed, this has been the case for the rat, the black widow spider, and trees (Aplin et al., 2011; Miles et al., in review; Noreen et al., 2016). Therefore, the sampling of a diversity of organisms, different locales, populations, and markers, now technically changes the questions we can ask with regards to urban fragmentation or facilitation of gene flow.

Although there is a trend towards decreased genetic diversity and increased genetic differentiation, the response to urbanization may not always be negative. Indeed many have proposed the urban fragmentation model of gene flow as the primary response to urbanization. However, the urban facilitation model of gene flow is equally as common a response to urbanization. For the studies that have identified barriers to gene flow, the loss of genetic variation in cities could hinder the ability of urban populations to adapt to the new urban

environment (Barrett & Schluter, 2007). However, if gene flow is reduced between urban and non-urban populations, but sufficient genetic diversity remains, urbanization may be facilitating local adaptation through the isolation of these urban populations (e.g., Wright, 1982).

Additionally, if gene flow is reduced between urban and non-urban populations, but is facilitated between urban populations, an urban “ecotype” may emerge (Krtinic et al., 2012; Schapira & Boutsika, 2012). Therefore, populations may be able to adapt and persist in these human-dominated landscapes.

Supplemental Methods

Database construction

We used Google Scholar and ISI Web of Science to search for studies which included terms such as “genetic drift”, “genetic diversity”, “landscape genetics”, “population genetics”, or “gene flow” together with the terms “urban” or “city”. From these studies, we extracted estimates of genetic diversity, including observed (H_o) and expected heterozygosity (H_E), allelic richness (Ar), and the inbreeding coefficient F_{IS} . We extracted estimates of F_{ST} as a measure of population genetic differentiation. We also recorded the number of studies which observed evidence of isolation-by-distance (IBD) and/or isolation-by-resistance (IBR) in urban environments. We also extracted other measures of diversity and differentiation (e.g., π , $N = 21$), but give the low sample sizes we excluded them from our statistical analyses.

For each study, we identified variables which we hypothesized could influence population genetic structure between urban and nonurban populations. These included data on basic features of the studies, such as the kingdom of the study organism, and the year the study was published. We included data on the experimental design of the study, such as the number of urban and nonurban populations sampled, the number of cities sampled and the type of marker used to measure population structure (e.g., microsatellite, SNPs, etc). We also identified two variables which we hypothesized could be related to how urbanization alters genetic structure: city human population size and city area (km^2) of the city sampled. Because these final two variables were often not included in the study details we obtained these data from population censuses (US Census 2014; United Nations, 2015).

Statistical Analyses

All data were analyzed in R v. 3.4.1 (R Development Core Team, 2016). To address questions 1 and 2, we tested if urbanization explained variation in the five response variables: H_O , H_E , F_{IS} , and A_r (question 1), and F_{ST} (question 2), using linear mixed model regression analysis. To address questions 3, we used linear mixed model regressions to compare the Mantel r^2 values of studies, which found evidence of IBD. For each response variable, we built two models, a simple model which only contained urbanization as a predictor variable (i), and a more complex model which contained urbanization and covariates as predictor variables (ii). For H_O , H_E , A_r , and IBD (Mantel r^2 (Mantel, 1967) we ran each response using a normal distribution, and we ran F_{IS} and F_{ST} using a generalized mixed model on a binomial distribution. We used the following linear equation models:

$$\text{Response} = \text{intercept} + \text{urbanization} + \text{study ID} + \text{error} \quad (i)$$

and

$$\text{Response} = \text{intercept} + \text{urbanization} + \text{year} + \text{kingdom} + \text{marker} + \text{number of (ii) populations} + \text{city area} + \text{city population} + \text{native} + \text{study ID} + \text{error}$$

Year, number of populations, city area, and city population were treated as continuous predictor variables and were transformed to normalize the distribution of the residuals. Urbanization was treated as a categorical fixed effect variable with two levels (urban or nonurban), marker was treated as a categorical fixed effect with 8 levels (ALFPs, allozymes ISSRs, microsatellites, mitochondrial genes, RAPD, or SNPs), and native was treated as a categorical variable with two levels (native or non-native). Study ID was included as a random factor to prevent pseudoreplication for studies that included multiple marker types or organisms.

To determine which variables best predicted the species response to urbanization, we used a multi-model averaging approach to identify the best fitting model implemented using the *dredge* function in the R package *MuMIn* (Barton, 2015). The *dredge* function tests all possible combinations of models and ranks best-fitting models based on their AICc weights. We used a model selection approach to determine whether any additional variables were important to include in the final model. We took weighted-averages from all models $<2 \Delta AICc$ scores of the best model, with better fitting models weighted more heavily, to determine final model-averaged parameters. We interpreted parameter outputs from the more conservative ‘full’ model output of the analysis rather than the ‘conditional’ output, because the conditional output tends to be biased away from zero, which can inflate type I error rates (Barton, 2015).

In addition to using model averaging, we also performed an ANOVA on each response variable, using the corresponding best-fitting model from the *dredge* output. For H_0 , H_E , A_r , and IBD (Mantel r^2) the significance of fixed effects were estimated using the *LmerTest* package (Kuznetsova et al., 2017), which uses sums-of-squares III to calculate partial F-tests. The denominator degrees of freedom were estimated using the Satterthwaite correction for finite sample sizes (Kenward and Roger, 1997). For F_{IS} and F_{ST} , we assessed significance of fixed effects using type III sums-of-squares run using a Wald’s Chi-square distribution to account for non-normal residuals. We present the ANOVA results described here in tables included in the main text, and the results from multimodel averaging approach in the supplementary materials.

Table 1.1 Urban Gene Flow Papers Used for Analyses:

1	Adams AL, van Heezik Y, Dickinson KJM, Robertson BC. 2014. Identifying eradication units in an invasive mammalian pest species. <i>Biological Invasions</i> , 16(7), 1481-1496. doi:10.1007/s10530-013-0586-9
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6	Balkenhol N, Holbrook JD, Onorato D, Zager P, White C, Waits LP. 2014. A multi-method approach for analyzing hierarchical genetic structures: A case study with cougars, puma concolor. <i>Ecography</i> , 37(6), 552-563. doi:10.1111/j.1600-0587.2013.00462.x
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9	Beninde J, Feldmeier S, Werner M, Peroverde D, Schulte U, Hochkirch A, Veith M. 2016. Cityscape genetics: structural vs. functional connectivity of an urban lizard population. <i>Molecular Ecology</i> 25:4984-5000
10	Benjamin A, May B, O'Brien J, Finger AJ. 2016. Conservation Genetics of an Urban Desert Fish, the Arroyo Chub, <i>Transactions of the American Fisheries Society</i> , 145:2, 277-286, DOI: 10.1080/00028487.2015.1121925
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12	Blanchong JA, Sorin AB, Scribner KT. 2013. Genetic diversity and population structure in urban white-tailed deer. <i>Journal of Wildlife Management</i> , 77(4), 855-862. doi:10.1002/jwmg.521
13	Booth W, Balvín O, Vargo EL, Vilímová J, Schal C. 2015. Host association drives genetic divergence in the bed bug, <i>Cimex lectularius</i> . <i>Mol. Ecol.</i> 24, 980-992.

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15	Booth W, Santangelo RG, Vargo EL, Mukha DV, Schal C. 2011. Population genetic structure in German Cockroaches (<i>Blattella germanica</i>): Differentiated islands in an agricultural landscape. <i>Journal of Heredity</i> 102, 175-183.
16	Bozarth CA, Gardner B, Rockwood LL, Maldonado JE. 2015. Using fecal DNA and spatial capture-recapture to characterize a recent coyote colonization. <i>Northeastern Naturalist</i> 22(1):144-162.
17	Braaker S, Kormann U, Bontadina F, Obrist MK. 2017. Prediction of genetic connectivity in urban ecosystems by combining detailed movement data, genetic data and multi-path modelling. <i>Landscape and Urban Planning</i> 160: 107-114.
18	Brashear WA, Ammerman LK, Dowler RC. 2015. Short-distance dispersal and lack of genetic structure in an urban striped skunk population. <i>Journal of Mammalogy</i> 96(1): 72-80.
19	Breinholt JW, Van Buren R, Kopp OR, Stephen CL. 2009. Population genetic structure of an endangered utah endemic, <i>astragalus ampullarioides</i> (fabaceae). <i>American Journal of Botany</i> , 96(3), 661-667. doi:10.3732/ajb.0800035
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Chapter 2: URBANIZATION AS A FACILITATOR TO GENE FLOW IN A HUMAN HEALTH PEST

Introduction

By 2050, two-thirds of the human population are predicted to live in urban areas (United Nations, 2014). In the United States the most rapid urban growth occurs in the West, where urban centers expand outward into pristine natural habitat (US Census, 2010; Seto et al., 2011, 2012). The loss of these natural habitats reduces local and global biodiversity, which has implications for conservation and human health (McKinney, 2002, 2006; Keyghobadi, 2007; McDonald et al., 2008). Although negative eco-evolutionary consequences of urbanization have been documented (McKinney, 2006; McDonald et al., 2008; Shochat et al., 2010; Faeth et al., 2011; McDonnell & Hahs, 2015; Johnson & Munshi-South, 2017), we know very little about species that thrive in urban ecosystems, known as urban adapters (Blair, 1996; Shochat, 2004). Urban adapters thrive in urban areas because they are able to take advantage of new or more abundant resources, and also have increased opportunities for higher population connectivity, or gene flow on the landscape, due to human-mediated transport (Crispo et al., 2011). Although urban adapters can have demonstrated benefits for humans, many are pests that cause structural damage, mental anguish, and human health concerns (Crissman et al., 2010; Booth et al., 2012; Puckett et al., 2016). As urban areas and human population size continue to expand, an understanding of what factors influence how urban pests disperse across and adapt to the urban landscape is necessary for the control and management of pest species (Hauser & McCarthy,

2009; Crissman et al., 2010; Menke et al., 2010; Booth et al., 2012; Saenz et al., 2012; Puckett et al., 2016).

From an eco-evolutionary perspective, the urban fragmentation model of gene flow predicts populations become isolated because of urbanization fragmenting the landscape (Debinski & Holt, 2000; Trizio et al., 2005; Allendorf & Luikart, 2007; Keyghobadi, 2007; Vandergast et al., 2007, 2009; Holderegger & Di Giulio, 2010; Storfer et al., 2010). These isolated populations have reduced dispersal between patches that leads to reduced gene flow, which is expected to lead to increased drift, reducing genetic diversity within patches and increasing genetic differentiation among them (Keyghobadi, 2007). For example, white-footed mice (*Peromyscus leucopus*) in urban parks experience high genetic differentiation between parks, and as canopy cover decreases with increasing impervious surfaces, genetic diversity is reduced (Munshi-South & Karchenko, 2010; Munshi-South, 2012). This trend is consistent across a variety of taxa, including both vertebrates and invertebrates (Booth et al., 2007; Davis et al., 2010; Beninde et al., 2016; Lourenco et al., 2016). However, as urban adapters may take advantage of these new urban habitats as corridors to dispersal, studies have invoked the alternative urban facilitation model that predicts increased dispersal within and between urban areas (Crispo et al., 2011). Under this model, not only would higher gene flow among urban areas be expected compared to that among non-urban areas (Crispo et al., 2011), but evidence of isolation-by-distance (IBD) is expected to be weak as urban-mediated human transportation can result in adapter populations that are distantly geographically separated being more genetically similar. For example, human commensals such as bed bugs and German cockroaches show low population structure across global geographic areas due to their high association with human expansion and colonization (Booth et al., 2012, 2015; Vargo et al., 2014). Thus, while urban

fragmentation studies have dominated the literature, our understanding of how urbanization facilitates gene flow and increases genetic diversity is particularly poor despite the utility this model has for urban adapter pests and applications to conservation and human health.

In characterizing gene flow patterns on variable spatial and temporal scales, interdisciplinary approaches in landscape, population, and evolutionary genetics have emerged (Dyer 2015). For example, traditional pairwise measures such as F_{ST} are typically used to impart information on genetic differentiation between sampled populations. However, measures such as conditional genetic distance (cGD), which is derived from population networks that are generated under principles of network theory, inform about connections among all sampled populations, and in fact, outperform other genetic distance metrics (Dyer & Nason, 2004; Dyer et al., 2010). Social network theory has gained significant exposure in disciplines such as sociology (Easley & Kleinberg, 2010), economics (Seiler et al., 2014), ecology (Greenbaum et al., 2015), and evolutionary biology (Pickrell & Pritchard, 2012; Greening & Fefferman, 2014); however, this approach has been unexplored in the context of the human impact of urbanization, despite urban areas being models of social networks that reflect human interactions. Instead of simply identifying evidence of population structure overall, these analyses would be invaluable to our understanding of how urban areas act as a biological network with specific connections identified that both fragment and facilitate gene flow among urban pest populations.

The Western black widow spider, *Latrodectus hesperus*, is a perfect urban eco-evolutionary model because it (i) inhabits an area of rapid Western U.S. urbanization, (ii) is recognized as an urban adapter due to its recent expansion and success in urban areas from its native desert habitat, (iii) maintains a large geographic distribution among multiple urban and non-urban areas (Fig. 1), and (iv) is an urban pest with significant medical-relevance, which has

implications for the evolving social and physical interactions between humans and our natural environment. We have previously documented urban ecological differences in fertility, behavior, web-building, and diet (Johnson et al., 2012; Trubl et al., 2012), as well as more dense urban aggregations, which are of health concern given its highly toxic venom (Vetter & Ibister, 2008). There is sex-biased dispersal in this species where adult female widow spiders are the sedentary sex, build a web as a juvenile, and only migrate when resources are depleted; whereas, males and spiderlings have the potential for aerial “ballooning” dispersal (Chamberlin & Ivie, 1935). Although there are well-documented ecological differences between urban and non-urban Western black widow spiders, we know little about the evolutionary potential of these ecological differences and how they may impact humans, especially as secondary contact among these habitats continues to be inevitable. In this respect, evolutionary population genetic analyses are needed to address how urbanization fragments or facilitates gene flow for this pest species.

Here, we used social network genetic analyses to investigate the impact of anthropogenic disturbance on connectivity in a model urban pest with human health concerns. Specifically, we conduct population structure, phylogeographic, genetic connectivity, and network analyses of the Western black widow spider across multiple urban and non-urban locales using both mitochondrial and genomewide nuclear ddRAD-seq markers. Although a null model of urban fragmentation is typically invoked, we also consider whether urban areas have acted to facilitate gene flow (e.g., via human-mediated transport). If the urban facilitation model drives evolution for urban adapter pests like the Western black widow spider, then we hypothesize that urban locales have higher genetic diversity, experience less population structure, more recent admixture, higher phylogeographic similarity, higher genetic connectivity and overall lower isolation-by-distance in contrast to non-urban locales.

Materials and methods

Sampling

The Western black widow spider (*L. hesperus*) is a nocturnal web-building predator that is both asocial and highly cannibalistic in all life stages. In urban areas, populations can be densely aggregated with abundant food resources of crickets and cockroaches in open xeric-landscaping; whereas, their non-urban distribution is very patchy, isolated, and associated with arid, rocky-outcrops and dry river-bed banks that are highly-sheltered. In considering these distributions, the difficulty in access to Western black widow spider habitat, and our objective in making contrasts between multiple urban and non-urban locales, we assembled a sample of 210 individuals from 11 urban and 10 non-urban locales (10 individuals from each locale) spanning the Western U.S. (Fig. 1, Table S1). Each locale constituted an area of ~0.5 km sq. As males are significantly smaller and rarely found, our sample is almost completely of females (<10 males). For comparative phylogenetic analyses, we also sampled three Southern black widow spiders (*L. mactans*) from Richmond, VA to be used as an outgroup as this species is the sister taxon to *L. hesperus* (Garb et al., 2004). After collection, samples were immediately stored in 90% EtOH, and then placed at -20°C.

DNA sequence collection

Both mitochondrial DNA (mtDNA) and nuclear DNA (nuDNA) were collected to tease apart temporal and spatial characteristics of gene flow from demographic contributions, i.e., maternally-inherited and more-rapidly evolving mtDNA provides the opportunity to test for patterns of potentially further reduced gene flow independent of urbanization. Using tissue

dissected from individuals' legs, DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen). For both *L. hesperus* and *L. mactans*, the mtDNA ND1 region was collected from PCR-amplified fragments of 480 bp using previously published primers (Hedin, 1997). PCR products were purified by treatment with Exonuclease I and Shrimp Alkaline Phosphatase (US Biochemicals), and then sequenced using an Applied Biosystems 3730 capillary sequencer. Sequences were aligned and edited manually using SEQUENCHER v3.1.1 (GeneCodes).

For both *L. hesperus* and *L. mactans*, genome-wide nuDNA fragments were generated from double digest RAD sequencing (ddRADseq) according to the protocol outlined in Peterson et al. (2012). Each pool of 20 individuals was sequenced on a single-end, 100bp Illumina HiSeq 2500 lane. The Illumina reads were processed to identify and genotype loci across all individuals using the STACKS v1.44 pipeline (Catchen et al., 2011, 2013) with program parameters set to default unless otherwise noted. The raw fastq files were demultiplexed, filtered for quality (Phred quality score >10) and the presence of barcodes, trimmed to 90 bp in length, and filtered for reads that did not contain the EcoRI recognition site using *process_radtags*. Because a well-annotated genome is not yet available for *L. hesperus* or a closely related species, a *de novo* assembly of raw reads into RAD tags was generated using *ustacks*, with the minimum number of reads set at $m=5$. Next, a catalog of consensus loci was generated using *cstacks* with the number of mismatches allowed between tags set to $n\leq 5$. After alleles were identified for each individual against the catalog using *sstacks*, the data were further filtered using *populations* with a minimum coverage of 5x per allele for each individual. One SNP per fragment was randomly chosen to reduce effects of locus-specific natural selection and clustering due to LD. Genotype data were exported from STACKS in each of the formats needed for subsequent analyses.

Data analyses

Estimates of nucleotide diversity as the average number of pairwise differences (π), the number of polymorphic sites (S), and distributions reflecting contrasts between these two (Tajima's D, 1989) were estimated within all locales and for our groupings of urban and non-urban samples. For estimates of genetic differentiation, standard pairwise F_{ST} estimates and tests for statistical significance were calculated between all locales, as well as for comparisons of urban and non-urban samples. Principal component analysis (PCA) was used to make inferences about clustering and structure of individuals, and urban and non-urban locales, as this analysis is model-free (Jombart 2008; Novembre & Stephens 2008; Novembre et al. 2008; Jombart et al. 2009, 2010). The PCA was generated in the *gstudio* package (Dyer et al., 2010) in R.

The program BEAST v2.4.5 (Bouckaert et al., 2014) was used to generate *L. hesperus* haplotype tree topologies, with our *L. mactans* samples as an outgroup, to estimate phylogeographic relationships among urban and non-urban individuals and locales. All analyses were performed using a TrN+G model of substitution, identified here as the appropriate model using JModelTest (Posada, 2008), on the basis of the Akaike Information Criterion (AIC). To calibrate the mtDNA tree, BEAST v2.4.5 was first run using published CO1 mtDNA sequence data and mutation rate estimates (Garb et al., 2004), and the fossil calibration divergence of the *Latrodectus* clade estimated at ~65 MYA (as in Dimitrov et al., 2012). Using a similar iterative approach employed by others using fossil calibrations (Dornburg et al., 2012; Heled & Drummond, 2012; Valente et al., 2012; Boykin et al., 2013; Qu et al., 2014), our initial BEAST run used the relaxed uncorrelated lognormal clock estimate and speciation yule process model, with five chains for 5000000 generations (with the first 5000 discarded as burn-in), and logging every subsequent 5000. Once the estimate for divergence between the *L. hesperus* and *L.*

mactans clade was generated, and because mtDNA regions are inherently linked, we set the T_{MRCA} at the node estimated from the published CO1 data for our ND1 sequence data here. This run used the lognormal relaxed clock (uncorrelated) estimate and coalescent constant size model, with five chains for 50000000 generations (with the first 5000 discarded as burn-in) and logging every subsequent 5000. All runs were checked for convergence of the chains in the program TRACER 1.5 (Drummon & Rambaut, 2007). Log files for each run were combined using LogCombiner and a consensus tree was summarized using TreeAnnotator v1.6.1 (Drummon & Rambaut, 2007). For our nuDNA tree, we again used BEAST with our *L. mactans* samples as an outgroup. We used the same run parameters as above (except for mutation rate, Masta, 2000) and visualized both the mtDNA and nuDNA datasets using FigTree (Rambaut, 2012).

To further place our phylogenetic results in a demographic perspective (e.g., temporal changes in population size), the program *ms* (Hudson, 2002) was used to generate simulations under a coalescent model (i.e., given sample sizes and estimates of θ in overall sample). We simulated multiple models of migration between and among urban and non-urban locales, by varying migration rates and population sample sizes. Within these simulations, we also varied the coalescent time for urban populations. Since black widow spiders have approximately one generation per year (Herms et al., 1935), and urbanization in the Western U.S. emerged ~200-500 years ago (US Census, 2010), we set the number of generations since divergence to between 200 and 500. After each set of simulations, we calculated all pairwise F_{ST} values as well as other summary statistics of nucleotide diversity, and determined statistically significant differences by comparing observed and simulated data using an in-house R script.

Genetic connectivity among sampled locales was estimated from the conditional genetic distance statistic cGD (Dyer & Nason, 2004), which is estimated from the genetic covariance

among locales. This measure of genetic covariance among all locales can be visualized as a popgraph using the *popgraph* R package (Dyer et al., 2010), where nodes represent sampled locales and edges represent genetic connections among locales. Popgraph topology is not only a visualization of cGD as genetic covariance but also of social network parameters that define the popgraph. Our social network analyses evaluate genetic relationships among locales and relative contributions of key “actors” using mathematical graph theory (Wasserman & Faust, 1994), which here, visually represent gene flow among all sampled locales to identify hubs of higher connectivity on the landscape. Social network node-specific parameters including closeness, degree, betweenness, and eigenvector centrality were estimated from the popgraphs using the *popgraph* R package. “Closeness” measures the degree to which a node is genetically similar to all other nodes in the network, where higher closeness values indicate further genetic distance to the next node. “Degree” is the number of edges a node has connecting it to other nodes. “Betweenness” is the sum of the shortest paths (i.e., the combination of edges among multiple nodes), where higher betweenness values indicate more paths that pass through a node. Eigenvector “centrality” computes the extent to which each node is centrally located among all other nodes within the popgraph topology. To identify statistical differences and congruence between mtDNA and nuDNA popgraph topologies, as well as differences within these topologies with respect to urban and non-urban contrasts, we performed t-tests for all of these network parameters, with Bonferroni corrections for multiple comparisons.

Finally, to test for signatures of isolation-by-distance (IBD), Euclidian geographic distances were estimated from latitude and longitude coordinates using the *fields* package (Nychka et al., 2015) in R and genetic distances were calculated as cGD (see above). Mantel tests were performed on the geographic and genetic distances in R.

Results

We generated >100K single nucleotide polymorphisms (SNPs), from which a filtered high-quality dataset of 40,533 SNPs from nuDNA and 124 SNPs from mtDNA sequences were analyzed. Estimates of nucleotide diversity for both marker types (Table S2) are high and consistent with arthropod studies (Garb et al., 2004; Burns et al., 2017). Genetic diversity for the overall urban sample was lower than for the non-urban sample, and this was true for both mtDNA and nuDNA datasets. However, we find a significant excess of rare alleles for the urban sample in both the mtDNA (Taj D = -1.44, $p < 0.01$) and nuDNA (Taj D = -1.57, $p < 0.01$) datasets. In fact, when we examined how variation is distributed within locales, we found that although average locale mtDNA diversity was similar for urban and non-urban samples ($t_{16} = 0.83$, $p = 0.42$), the average nuDNA diversity was significantly higher within urban locales compared to within non-urban locales ($t_{14} = -1.66$, $p = 0.02$).

The average F_{ST} of all pairwise comparisons for mtDNA and nuDNA was 0.53 ± 0.02 and 0.23 ± 0.01 , respectively, and these two estimates were significantly different ($t_{391} = -9.85$, $p < 0.001$; Fig. S1). Although F_{ST} estimates are significantly high for both the urban and non-urban datasets, urban locales are significantly less-genetically differentiated from each other for mtDNA variation, in contrast to that observed between non-urban locales ($F_{ST} = 0.42 \pm 0.04$ vs. 0.56 ± 0.05 ; $t_{96} = -1.33$, $p < 0.01$). Although the overall level of differentiation is lower than that seen with mtDNA variation, the nuDNA variation shows the same significant pattern in the contrast of urban vs. non-urban locales ($F_{ST} = 0.15 \pm 0.03$ vs. 0.03 ± 0.04 ; $t_{90} = -0.99$, $p < 0.05$).

The PCA of SNP genotypes for each marker dataset identified different patterns of genetic structure among individuals. For mtDNA, urban locales are predominantly clustered

(Fig. 2a), while non-urban locales are individually more isolated, shown by PC1 and PC2 explaining 55% of the genetic variance (Fig. 2b). Further PCs explain significantly less variance with no additional separation of urban or non-urban locales. The nuDNA variation exhibits far less genetic clustering for both urban (Fig. 2a) and non-urban (Fig. 2b) locales compared to mtDNA variation, with PC1 and PC2 explaining only 27% of the genetic variance. Further inspection of PCs resulted in far less variance explained, and even less separation compared to that seen with the mtDNA dataset.

Our phylogenetic analysis (Fig. 3) of mtDNA haplotype variation shows that an ancestral clade is dominated by monophyletic groups of non-urban locales with deeper evolutionary divergence. In contrast, urban locales form a predominant phylogenetic clade of mixed lineages more recently derived from non-urban ones. In fact, our ms simulations under different demographic scenarios indicate that observed urban genetic diversity is significantly more comprised of rare rather than common haplotypes (Taj D = -2.05, $p < 0.001$), compared to non-urban locales (Taj D = -0.92, ns). On the other hand, the phylogenetic analysis of the nuDNA dataset resulted in no statistical support for any clade structure, whether they correspond to urban or non-urban locales.

Our popgraph topologies for mtDNA and nuDNA datasets show contrasting patterns of genetic connectivity (Fig. 4). Although our cGD estimate was significantly higher in overall mtDNA than nuDNA dataset ($t_{68} = 9.50$, $p < 0.0001$), this estimate among urban locales is higher than among non-urban locales for both marker types (mtDNA $t_{55} = 3.30$, $p < 0.001$; nuDNA $t_{97} = 1.70$, $p < 0.05$). The Mantel tests found weak associations for IBD analyses for both the mtDNA ($r^2 = 0.030$, $p = 0.02$) and nuDNA ($r^2 = 0.008$, $p = 0.15$) datasets. When Mantel tests were applied individually to the urban and non-urban datasets, no significant patterns of IBD emerged.

The social network parameters underlying the popgraph also have contrasting patterns between mtDNA and nuDNA datasets (Table S3). “Closeness” is significantly higher in the mtDNA popgraph for non-urban than urban locales ($t_{15} = -2.50$, $p < 0.001$) and is also higher (which again, means greater distance) overall in the mtDNA popgraph than the nuDNA popgraph ($t_{20} = -18.18$, $p < 0.00001$). Overall there are a fewer number of connections, and even more visible disconnections, in the mtDNA popgraph compared to the nuDNA popgraph. This result is evidenced by the parameter “degree” being significantly higher in the nuDNA than the mtDNA popgraph ($t_{20} = -3.50$, $p < 0.0001$); however, we note “degree” is not significantly different between urban and non-urban locales for mtDNA or nuDNA popgraphs. We found no significant results for the “betweenness” parameter analyses overall; however, non-urban locales are among the highest ranked values in the mtDNA popgraph, whereas, multiple urban locales are among the highest values in the nuDNA popgraph. The results of “degree” and “betweenness” indicate that while urban locales have higher network connections overall, urban locales do not drive genetic connectivity similarly. In fact, our analyses of “centrality” or hub determinism, which is a function of the aforementioned social network parameters combined, identifies Phoenix (PHX), Reno (RNO), and Las Vegas (LVN) as major urban hubs of connectivity, whereas, urban locales Albuquerque (ABQ), Davis (DAV), and Denver (DEN) show little to no influence on popgraph network structure.

Discussion

With the Western black widow spider as an urban pest model, this study set out to specifically test two competing hypotheses, that urbanization primarily acts as a barrier to gene flow, or instead, that urbanization facilitates gene flow for this urban adapter likely due to human-

mediated transport. From multiple perspectives of population, phylogenetic, and network analyses, the primary observations here are that in comparison to non-urban locales, urban locales have higher within-population genetic diversity, lower between-population genetic differentiation, and higher overall estimates of genetic connectivity. We discuss these results in light of their support for an urban facilitation model, and how these evolutionary approaches help our perception of conservation and human health in an ever-growing urban environment.

The urban fragmentation model predicts that urbanization acts as a barrier to dispersal (e.g., Holderegger & DiGiulio, 2010; Storfer et al., 2010), which inherently predicts that measures of Western black widow spider genetic differentiation among urban areas would be elevated. Our F_{ST} values at first glance are significantly high for both the mtDNA and nuDNA datasets. In fact, these measures rival that of multiple studies in both plants and animals (e.g., Hitchings & Beebee, 1997; Saenz et al., 2012; Ascunce et al., 2013; Munshi-South et al., 2013; Bartlewicz et al., 2015; Booth et al., 2015; Johnson & Munshi-South, 2017). However, while high dissimilarity indices such as F_{ST} might reflect low gene flow, it is not necessarily a reduction in gene flow due to urbanization. In fact, our measures of genetic differentiation among multiple geographically dispersed non-urban locales, are significantly greater than that of measures among urban locales for both mtDNA and nuDNA datasets. Thus, the contrasts of these patterns actually prove to be more consistent with gene flow being relatively facilitated, and not reduced among urban locales.

Consistent with the pattern of lower between-locale genetic differentiation observed among urban locales, estimates of genetic diversity and phylogeographic history of Western black widow spiders also support the urbanization facilitation model. Although the overall non-urban population has greater genetic diversity than the overall urban population for both mtDNA

and nuDNA datasets, on average, urban within-locale genetic diversity is significantly higher than that seen within non-urban locales. These results are surprising given that urban population genetic studies more often identify reduced genetic diversity within urban locales. As gene flow reduces, classic theoretical and empirical studies in population genetics predict that while individual non-urban locales may suffer from reduced effective population sizes, increased inbreeding, and higher probability of fixation of alleles by drift alone, this process happens randomly across locales. This process results in possibly even higher levels of allelic diversity maintained in the overall non-urban population (e.g., Dobzhansky, 1937; Wright, 1982). On the other hand, the urban population maintains higher within-locale diversity expected from relatively higher gene flow, but a lower overall level of genetic diversity as expected if it was more recently derived from non-urban areas. Our phylogenetic analysis and demographic simulations support this exact hypothesis. While the nuDNA dataset provides little phylogenetic information owing to the likely higher level of admixture and diversity of biparental variation (more below), the maternally-inherited mtDNA preserves the phylogenetic history showing older and ancestral clades of seemingly more evolutionarily isolated non-urban locales, in contrast to the urban locales that form a larger, more homogenous and recently derived clade. Thus, while we expected a signature of colonization of urban areas from non-urban areas, what is more interesting and potentially concerning is that our demographic simulations identify a signature of a recent population expansion (i.e., a significant excess of rare alleles) associated with the movement of Western black widow spiders into urban locales. These observations demonstrate not only that these organisms are successfully invading urban environments and maintaining high genetic diversity, but that they do so in rapidly spreading across large geographic areas;

these patterns should be noted as a unique molecular signature of an urban adapter and pest (Booth et al., 2012, 2015; Vargo et al., 2014).

Our final set of analyses that examine population clustering and network characteristics show the most demonstrable evidence of urban facilitation with higher genetic connectivity overall driven by urban locales. The PCA results show very little clustering for nuclear variation (as noted above, and discussed more below), whereas, for the mtDNA haplotypes, patterns are again consistent with the historical isolation of non-urban locales compared to the large clustering of urban locales, consistent with our phylogenetic analysis. However, not seen previously are non-random patterns of urban and non-urban haplotypes clustering, which hints at how certain locales influence introgression and gene flow more than others. Our popgraph topologies test these hypotheses, as unlike dissimilarity measures based on pairwise contrasts such as F_{ST} , they capture the genetic covariance among all populations sampled at once to demonstrate where and how gene flow moves through the network. For example, while our mtDNA and nuDNA popgraphs indicate fewer and greater connections among locales, respectively (as expected by their demographic histories, more below), overall, urban locales have significantly more connections and have higher genetic proximity to other locales than non-urban locales do. Furthermore, this degree of high urban connectivity results in the surprising observation that several non-urban locales are connected to each other only via gene flow through certain urban locales on the landscape. This surprising pattern has potentially negative evolutionary impacts for the phenotypes within these historically isolated non-urban locales. In fact, our IBD analysis shows a consequence of urban facilitation is that Western black widow spiders, even from non-urban locales, are more genetically similar than expected given their geographically distant separation. Altogether, this popgraph evidence clearly shows that urban

facilitation increases gene flow overall, even hinting at certain urban areas as drivers of this increased genetic connectivity through both urban and non-urban locales.

With a pattern of urban facilitation having emerged, a final step is to test hypotheses of what specific locales and potential factors may influence urban genetic connectivity. Our social network analyses that take a unique look at topologies identified Phoenix, Reno, and Las Vegas as major urban hubs, whereas, other urban locales such as Denver have little influence on genetic connectivity. Thus, while we find urbanization overall may facilitate gene flow, not all urban locales behave the same in this role. Multiple urban features have been implicated as factors driving urban gene flow such as percent impervious surface, canopy cover, or human population size (Alberti, 2015; McDonnell & Hahs, 2015; Johnson & Munshi-South, 2017). However, these studies are often focused on fine-scale gene flow within a single urban area (Emaresi et al., 2011; Munshi-South et al., 2012; Van Buskirk, 2012; Sacks et al., 2016), whereas, our study uniquely focused on characterizing broad-scale genetic connectivity among multiple urban locales. With this in mind, we expect human population size to be a mitigating factor, as human-mediated transport among these areas may be tied to the simple volume of humans in each urban area. That said, there is no such association here; specifically, while Phoenix and Denver have high population sizes, they have contrasting high and low influence on genetic connectivity, respectively; yet Reno and Albuquerque have low population sizes, but have high and low influence on genetic connectivity, respectively (U.S. Census, 2014; Table S3). Interestingly, these four urban areas also differ with respect to age of colonization and population expansion, as well as their proximity along urban corridors. These social network analyses help identify not only drivers of gene flow, but help test hypotheses of how features interact in complex ways in

helping us understand how urban development and movement among these areas impacts biodiversity on large spatial scales.

The overall population genetic pattern shown here is consistent with an urban facilitation model, nonetheless, we still observe clear signatures of sex-biased dispersal patterns. Compared to nuDNA, the mtDNA dataset for non-urban locales reveals patterns of reduced gene flow, including lower within-locale genetic diversity, higher between-locale differentiation, and higher locale clustering. The phylogenetic analyses show strong evidence of population and temporal structure consistent with ancestral non-urban isolation and more derived urban expansion, but only for the mtDNA dataset, which validates life history observations of females being the sedentary sex (Chamberlin & Ivie, 1935). One possible problem in urban landscape studies is that sex-specific spatial autocorrelation, when not accounted for, may result in erroneously concluding that urbanization reduces dispersal distances (as shown by Brashear et al., 2015). In fact, high site-fidelity is common for the majority of web-building spiders (Foelix, 2014), so without different genetic markers to contrast, it is possible that sex-biased dispersal does dampen signatures of urban facilitation overall. As our sample is almost completely derived from females, our comparative datasets of mtDNA- and nuDNA-specific patterns enable us to identify strong signatures of urban facilitation despite high-site fidelity for females. In addition, our network analyses found that higher gene flow among urban areas has had significantly less influence on mtDNA than nuDNA variation. Under an urban facilitation model for sedentary females, this pattern makes sense as maternally-inherited genetic material is less impacted by higher rates of gene flow in contrast to bi-parentally inherited genetic material. Thus, although sex-biased dispersal may be looked at as a caveat in gene flow studies, altogether, our results make clear that comparative marker and network approaches can indeed reveal the impact that

sex-dispersal and urban facilitation each independently have on genotypic and phenotypic variation.

The impacts this model of urban facilitation have on the evolutionary ecology of urban adapters can be speculated on given our choice of the Western black widow spider here. In fact, the majority of spider species are able to aeri ally disperse through ballooning as spiderlings (Bell et al., 2005), and this dispersal mechanism that enables them to successfully colonize new areas makes spiders ideal models for studying gene flow into novel urban habitats. Although this is the case, there is a paucity of studies that have investigated urbanization impacts in arachnids. For one species of wolf spider, urbanization appears to act as a barrier to gene flow (Reed et al., 2011), while for another, urbanization appears to facilitate gene flow (Colgan et al., 2002). Additionally, another has shown that an endangered species of tarantula experiences human-mediated gene flow (Machkour-M'Rabet et al., 2012). Given the polarizing outcomes for conservation priorities predicted by models of urban fragmentation vs. facilitation, population genetic studies need to target urban adapters on broad geographic scales to document the impact of continued urban growth. To this point, our study is the first that has used this approach, and despite the inherent limited dispersal in females, we show a significant association between urbanization and higher gene flow and genetic diversity in the Western black widow spider.

This specific urban facilitation scenario can abruptly alter the fitness landscape, especially since we show that non-urban locales appear to have been historically isolated, with some showing very little within-locale diversity. That is, it is unknown to what extent the varying degrees of population divergence identified here among non-urban locales has led to local adaptation (e.g., Wright, 1982), and how the now recent secondary contact among these locales, ushered in by rapid urban networking at geographically distant scales, has altered these

phenotypes. In fact, if selective pressures differ sufficiently among urban and non-urban, and even among non-urban environments, “urban ecotypes” may evolve and easily sweep across geographically distant urban areas as a result of human-mediated transport (Krtinic et al., 2012; Schapira & Boutsika, 2012). As one example previously noted, our group has already documented Western black widow spider phenotypic differences in behavior between urban and non-urban habitats, where urban spiders are more densely aggregated, larger, and are more aggressive (Johnson et al., 2012, 2017; Trubl et al., 2012). Our research here shows that these behavioral changes together with the potentially lethal venom these spiders wield present one such urban ecotype. These ecotypes may be the result of coadapted gene and phenotype complexes that are emerging in urban environments due to increased gene flow among urban areas.

Conclusions

Our integrated approach examining urban and non-urban locales across a broad landscape scale raises several larger questions with respect to both population genetics and functional evolutionary ecology in urban settings. One thought is with respect to how cities are true “replicates” of each other. They do share some characteristics, but they can vary in how they impact gene flow, including effects of human population size. Without the use of popgraphs and social network analyses that identify specific hubs that increase and decrease gene flow, we argue that we cannot even begin to understand what features act as barriers and conduits. Differences in urbanization effects among different cities have been documented previously (e.g., Thompson et al., 2016); however, without multiple contrasts of urban and non-urban areas, we would erroneously conclude patterns are consistent with an urban fragmentation model. This

point begs the question of how common urban facilitation is, especially with respect to urban adapters that give us the best chance at understanding urban evolution. Currently, urban population genetic studies tend to focus on fine-scale within city boundaries or on broad-scale between cities, and our work argues that studies combining genomewide sampling at both scales is necessary. Specifically, while previous population genetic studies have studied pest invasion, we find that with added social network analyses that we can pinpoint hubs that have an influence on pests' spread on the natural landscape. Together with future phenotypic studies across multiple urban environments, this network approach would enable us to identify those traits most associated with urban adaptation, as we seek to reduce spread of pests with negative medical impacts, or on the other hand, increase dispersal of organisms currently under conservation management.

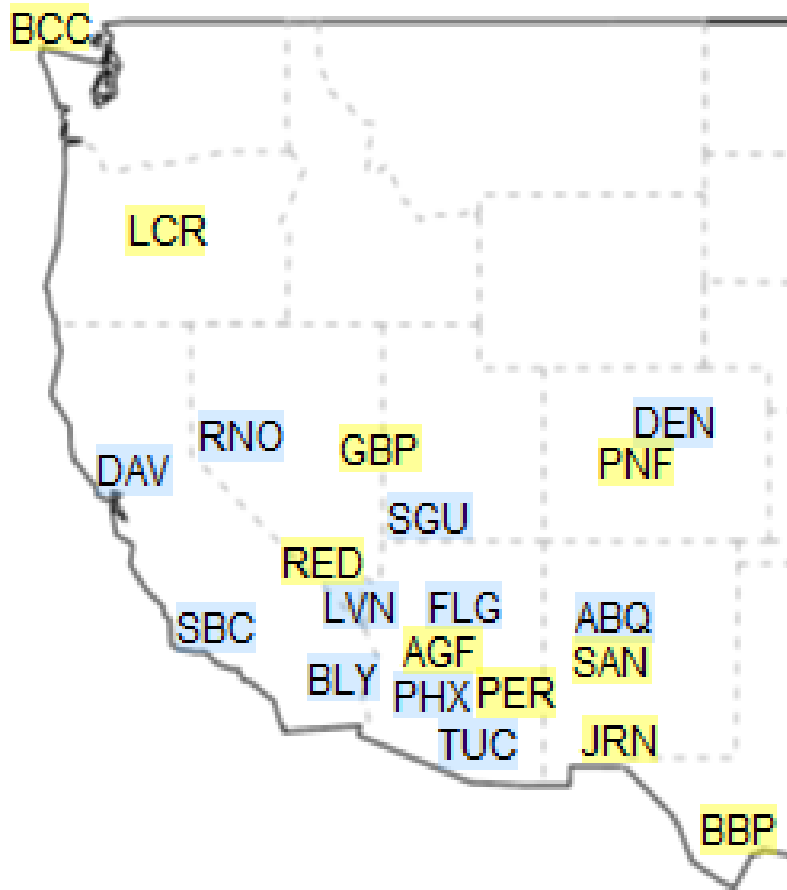


Figure 2.1. Western black widow spider geographic distribution. Distribution of the sampling locales, available from the geographic range of the Western black widow spider across the Western U.S. Abbreviations highlighted in blue and yellow reflect urban and non-urban locale sampling, respectively (Table S1).

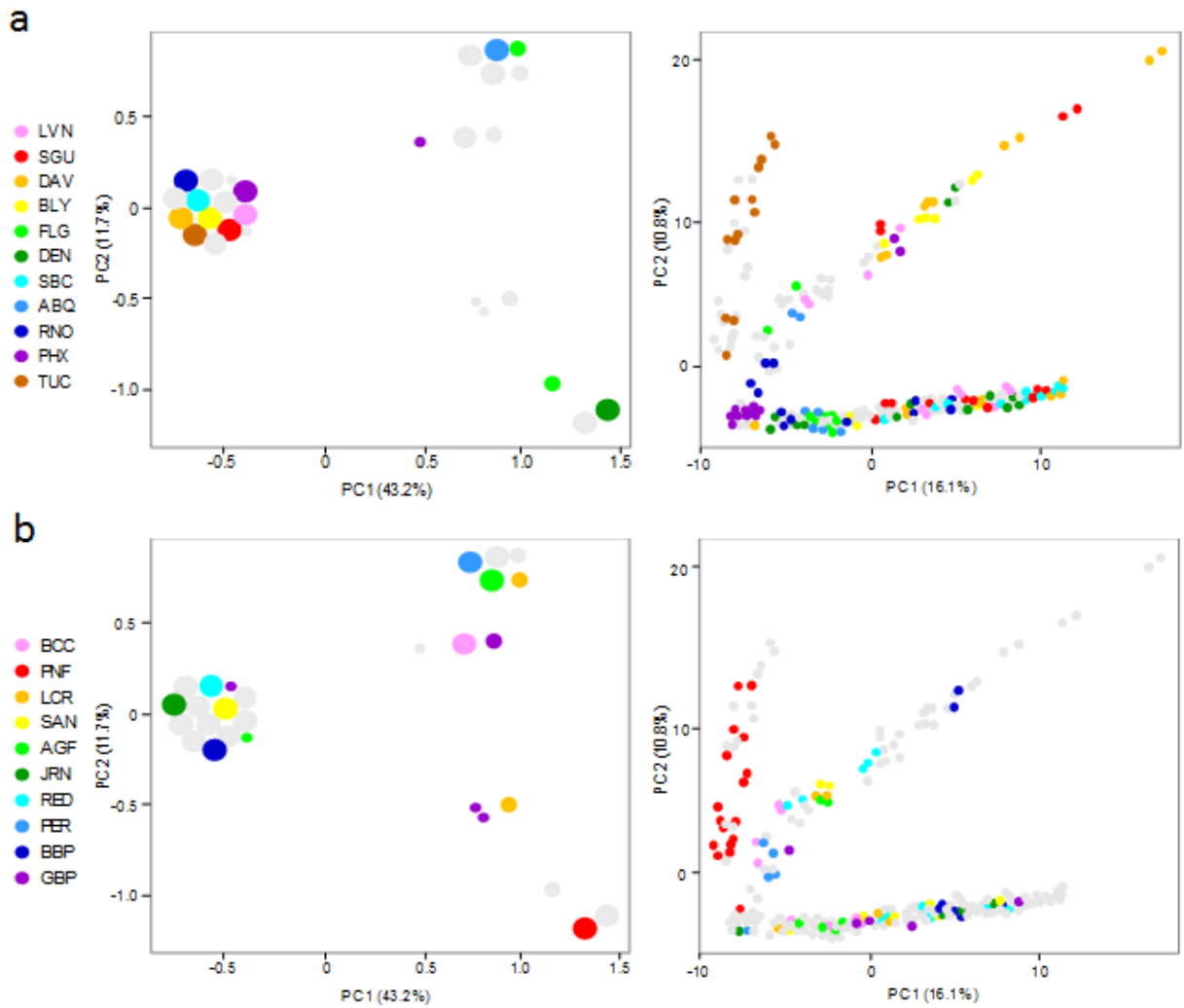


Figure 2.2. PCA biplot. a, PCA of mtDNA (left) and nuDNA (right), with urban samples highlighted, and **b**, PCA of mtDNA (left) and nuDNA (right), with non-urban locales highlighted. Each circle size reflects relative number of individual samples at that point (see Table S1 for locale abbreviations).

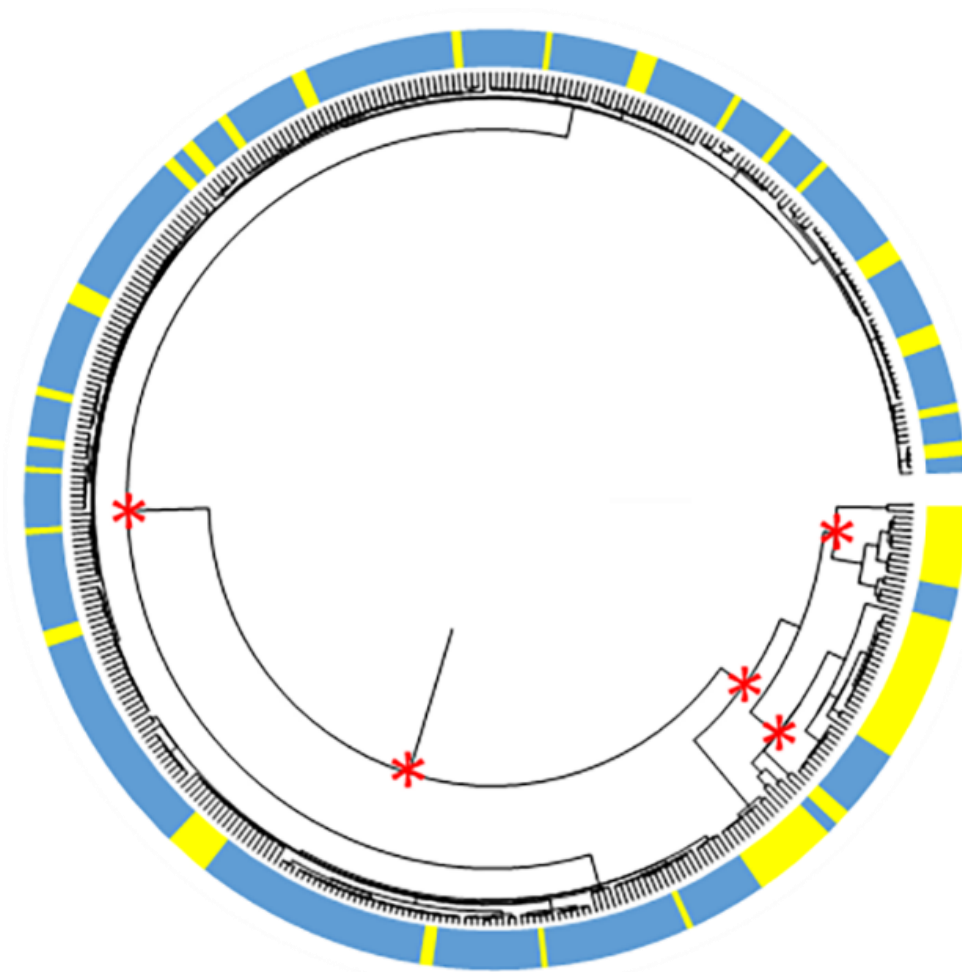


Figure 2.3. Phylogenetic tree. BEAST analysis of 210 mtDNA haplotypes with highlights in blue and yellow reflecting urban and non-urban locale sampling, respectively. Major node support as posterior probabilities above 70% are noted as red asterisks.

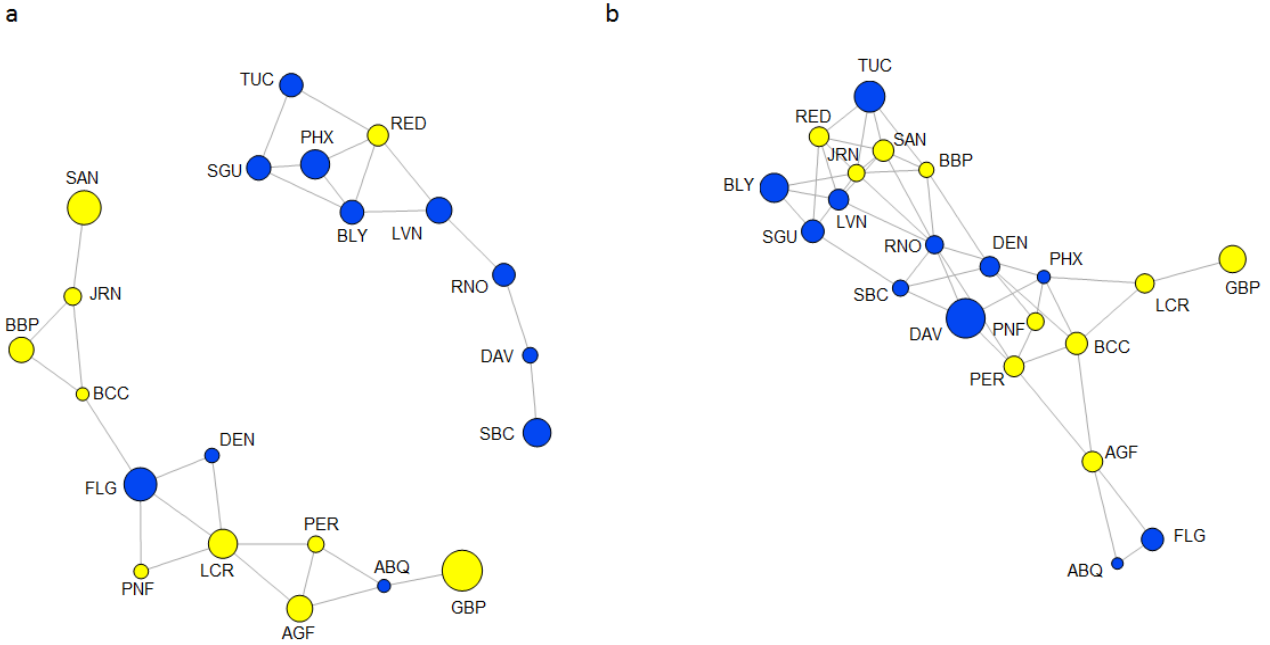


Figure 2.4. Social network popgraph analysis. a, mtDNA popgraph and **b**, nuDNA popgraph among urban (yellow) and non-urban (blue) locales (Table S1). The size of each locale node reflects the amount of genetic variance within the locale, and length of connections between nodes is proportional to cGD (conditional genetic distance) as a measure of genetic covariance reflecting gene flow among locales.

Table S2.1. Sampling locales of Western black widow spiders

Locale Name	Locale Abbreviation	Latitude	Longitude	Habitat
Agua Fria, AZ	AGF	34.192	-112.101	non-urban
Albuquerque, NM	ABQ	35.084	-106.621	urban
Big Bend, TX	BBP	29.329	-103.208	non-urban
British Columbia, Canada	BCC	48.581	-123.374	non-urban
Blythe, CA	BLY	33.616	-114.598	urban
Pine National Forest, CO	PNF	39.543	-105.163	non-urban
Flagstaff, AZ	FLG	35.192	-111.645	urban
Great Basin, NV	GBP	39.010	-114.123	non-urban
Jornada Basin, NM	JRN	32.366	-106.525	non-urban
Lower Creek River, OR	LCR	44.135	-120.813	non-urban
Las Vegas, NV	LVN	36.003	-115.289	urban
Peralta, AZ	PER	33.403	-111.348	non-urban
Phoenix, AZ	PHX	33.454	-112.065	urban
Red Rock, NV	RED	36.144	-115.406	non-urban
Reno, NV	RNO	39.530	-119.814	urban
San Acacia, NM	SAN	34.206	-107.027	non-urban
Santa Barbara, CA	SBC	34.736	-120.134	urban
Tucson, AZ	TUC	32.180	-111.014	urban
Davis, CA	DAV	38.537	-121.746	urban
Saint George, UT	SGU	37.209	-112.980	urban
Denver, CO	DEN	39.722	-104.969	urban

Table S2.2. Population diversity summary statistics for mtDNA and nuDNA

Marker	Habitat	Locale Abbreviation	$\theta\pi$ (%)	θ_s (%)	TajD	
mtDNA		All	2.20	3.48	-1.14	
	Urban	All	1.60	1.47	-1.44	
		ABQ	0.00	0.00	0.00	
		BLY	0.48	0.64	-1.01	
		DEN	0.45	0.83	1.95	
		FLG	2.40	4.79	1.44	
		LVN	0.64	1.87	-0.64	
		RNO	0.22	0.42	1.64	
		SBC	0.25	1.04	-1.39	
		TUC	0.61	1.87	-0.82	
		UCD	0.04	0.21	-1.11	
		VBP	1.65	7.08	-1.90	
		SGU	0.47	1.67	-0.92	
		Non-urban	All	2.63	2.69	-0.07
			AGF	0.95	1.27	-1.40
	BBP		0.32	1.46	-1.57	
	BCC		0.00	0.00	0.00	
	GBP		2.70	5.83	0.62	
	JRN		0.30	0.36	-0.78	
	LCR		2.30	2.50	-0.62	
	PER		0.09	0.41	-1.36	
	PNF		0.45	0.83	1.95	
	RED		0.34	0.46	-1.08	
	SAN		2.46	2.70	-1.45	
	nuDNA			All	0.30	0.14
		Urban	All	0.13	0.25	-1.57
ABQ			0.06	0.28	-2.60	
BLY			0.39	0.27	1.77	

	DAV	0.44	0.36	-2.55
	DEN	0.66	0.30	5.33
	FLG	0.11	0.27	-2.46
	LVN	0.33	0.27	1.03
	PHX	0.43	0.33	1.33
	RNO	0.03	0.27	-1.62
	SBC	0.05	0.26	0.18
	SGU	0.24	0.27	1.64
	TUC	0.05	0.30	-0.35
Non-urban	All	0.24	0.12	3.17
	AGF	0.11	0.27	-3.33
	BBP	0.28	0.28	0.01
	BCC	0.17	0.27	-1.70
	GBP	0.22	0.29	-1.08
	JRN	0.28	0.26	0.18
	LCR	0.11	0.30	-2.86
	PER	0.05	0.34	-3.64
	PNF	0.04	0.27	-0.75
	RED	0.06	0.26	1.07
	SAN	0.04	0.27	-0.76
	SAN	0.04	0.27	-0.76

Table S2.3. Social Network Node Parameters

Marker	Locale	closeness	betweenness	degree	eigenCent	Type
mtDNA	AGF	0.0025	16	3	0.11	non-urban
	BBC	0.0022	24	3	1.00	non-urban
	BBP	0.0019	0	2	0.44	non-urban
	GBP	0.0020	0	1	0.01	non-urban
	JRN	0.0019	10	3	0.48	non-urban
	LCR	0.0026	29	5	0.46	non-urban
	PER	0.0025	0	3	0.11	non-urban
	PNF	0.0025	0	2	0.35	non-urban
	RED	0.0029	4	4	0.00	non-urban
	SAN	0.0018	0	1	0.07	non-urban
	ABQ	0.0023	10	3	0.04	urban
	BLY	0.0029	9	4	0.00	urban
	DEN	0.0025	0	2	0.35	urban
	FLG	0.0026	28	4	0.99	urban
	LVN	0.0029	15	3	0.00	urban
	PHX	0.0028	0	3	0.00	urban
	RNO	0.0027	12	2	0.00	urban
	SBC	0.0023	0	1	0.00	urban
	TUC	0.0027	0	2	0.00	urban
	UCD	0.0024	7	2	0.00	urban
SGU	0.0028	2	3	0.00	urban	
nuDNA	AGF	0.0117	36	4	0.45	non-urban
	BBP	0.0153	9	5	0.34	non-urban
	BCC	0.0120	16	5	1.00	non-urban
	GBP	0.0090	0	1	0.06	non-urban
	JRN	0.0168	7	7	0.15	non-urban

LCR	0.0104	19	3	0.57	non-urban
PER	0.0155	36	5	0.84	non-urban
PNF	0.0121	2	3	0.53	non-urban
RED	0.0167	18	5	0.03	non-urban
SAN	0.0173	11	6	0.14	non-urban
ABQ	0.0095	0	2	0.06	urban
BLY	0.0154	0	3	0.02	urban
DEN	0.0131	10	4	0.63	urban
FLG	0.0098	0	2	0.05	urban
LVN	0.0174	28	4	0.08	urban
PHX	0.0157	37	5	0.86	urban
RNO	0.0193	76	8	0.62	urban
SBC	0.0163	11	4	0.33	urban
TUC	0.0156	0	4	0.06	urban
UCD	0.0158	0	4	0.58	urban
SGU	0.0158	14	4	0.05	urban

	ABQ	BLY	DEN	FLG	LVN	RNO	SBC	TUC	DAV	PHX	SGU	AGF	BBP	BCC	GBP	JRN	LCR	PER	PNF	RED	SAN
ABQ	-	0.17	0.73	0.08	0.14	0.20	0.18	0.16	0.21	0.53	0.09	0.11	0.14	0.21	0.16	0.17	0.17	0.55	0.73	0.13	0.15
BLY	0.92	-	0.64	0.12	0.07	0.13	0.17	0.10	0.07	0.17	0.05	0.13	0.12	0.11	0.14	0.16	0.15	0.17	0.64	0.08	0.11
DEN	0.16	0.39	-	0.68	0.63	0.74	0.74	0.67	0.65	0.85	0.51	0.69	0.66	0.74	0.72	0.74	0.74	0.88	0.00	0.62	0.68
FLG	0.49	0.64	0.45	-	0.10	0.16	0.16	0.12	0.16	0.39	0.07	0.09	0.12	0.15	0.13	0.15	0.13	0.37	0.45	0.10	0.12
LVN	0.91	0.03	0.90	0.63	-	0.09	0.11	0.07	0.10	0.24	0.04	0.10	0.08	0.11	0.10	0.10	0.12	0.25	0.90	0.05	0.08
RNO	0.96	0.45	0.93	0.66	0.43	-	0.10	0.13	0.15	0.53	0.06	0.16	0.12	0.20	0.15	0.13	0.20	0.57	0.74	0.09	0.12
SBC	0.96	0.34	0.93	0.65	0.36	0.21	-	0.13	0.19	0.53	0.07	0.15	0.11	0.20	0.14	0.10	0.19	0.56	0.74	0.10	0.11
TUC	0.91	0.00	0.90	0.64	0.02	0.42	0.33	-	0.13	0.36	0.04	0.12	0.08	0.15	0.12	0.12	0.16	0.39	0.67	0.07	0.09
DAV	0.99	0.47	0.95	0.67	0.46	0.30	0.03	0.43	-	0.16	0.08	0.17	0.16	0.14	0.18	0.20	0.19	0.15	0.65	0.12	0.15
PHX	0.77	0.01	0.81	0.54	0.05	0.20	0.15	0.00	0.19	-	0.15	0.44	0.37	0.46	0.49	0.54	0.52	0.48	0.85	0.27	0.41
SGU	0.93	0.00	0.91	0.64	0.07	0.37	0.22	0.01	0.36	0.01	-	0.06	0.05	0.07	0.06	0.07	0.08	0.15	0.51	0.04	0.05
AGF	0.33	0.72	0.30	0.31	0.70	0.76	0.76	0.71	0.79	0.56	0.73	-	0.11	0.16	0.13	0.14	0.15	0.42	0.69	0.10	0.11
BBP	0.96	0.50	0.25	0.70	0.49	0.70	0.65	0.40	0.76	0.23	0.50	0.79	-	0.15	0.10	0.08	0.14	0.39	0.66	0.07	0.07
BCC	1.00	0.93	0.46	0.64	0.91	0.97	0.96	0.91	0.99	0.79	0.93	0.79	0.95	-	0.18	0.19	0.21	0.50	0.74	0.11	0.15
GBP	0.42	0.28	0.63	0.22	0.27	0.33	0.30	0.27	0.33	0.16	0.27	0.13	0.35	0.57	-	0.13	0.14	0.53	0.63	0.09	0.11
JRN	0.96	0.38	0.93	0.68	0.39	0.65	0.58	0.29	0.72	0.14	0.38	0.78	0.05	0.96	0.33	-	0.18	0.57	0.93	0.09	0.08
LCR	0.24	0.64	0.65	0.22	0.62	0.65	0.65	0.63	0.67	0.52	0.64	0.05	0.70	0.62	0.16	0.68	-	0.58	0.65	0.12	0.15
PNF	0.16	0.39	0.00	0.68	0.63	0.95	0.94	0.89	0.98	0.74	0.91	0.30	0.25	0.46	0.72	0.74	0.74	-	0.93	0.29	0.44
PER	0.83	0.91	0.93	0.43	0.89	0.93	0.93	0.90	0.95	0.81	0.91	0.05	0.94	0.98	0.35	0.94	0.11	0.88	-	0.62	0.68
RED	0.94	0.03	0.92	0.65	0.13	0.49	0.38	0.01	0.53	0.00	0.05	0.74	0.47	0.95	0.28	0.34	0.65	0.93	0.92	-	0.07
SAN	0.90	0.04	0.89	0.63	0.10	0.34	0.23	0.01	0.34	0.00	0.02	0.70	0.26	0.90	0.26	0.11	0.63	0.88	0.89	0.02	-

Figure S2.1. FST matrix of mtDNA and nuDNA for 11 urban and 10 non-urban locales. The top half of the matrix consists of nuDNA pairwise FST, the bottom half of the matrix consists of mtDNA pairwise FST. Highlighted in light grey are urban by urban pairwise FST, highlighted in dark grey are non-urban by non-urban pairwise FST

Chapter 3: URBAN HUBS OF CONNECTIVITY: CONTRASTING PATTERNS OF GENE FLOW WITHIN AND AMONG CITIES IN THE WESTERN BLACK WIDOW SPIDER

Introduction

While the global human population continues to grow, the regions most impacted by this growth are urban areas, where half of the human population already resides (United Nations, 2014). This urban growth fragments and eliminates the surrounding natural habitat, and can have negative effects on local flora and fauna (McKinney, 2006; Keyghobadi, 2007). From an evolutionary perspective, the urban fragmentation model of gene flow predicts populations become isolated because natural corridors are fragmented by urbanization, which reduces dispersal and gene flow (Debinski & Holt 2000; Trizio et al., 2005; Allendorf & Luikart, 2007; Vandergast et al., 2007, 2009; Holderegger & Di Giulio 2010; Storfer et al., 2010). These isolated patches are vulnerable to increased genetic drift, reduced genetic diversity within patches, and increased genetic differentiation among them (Keyghobadi, 2007). However, urban adapters, which thrive in the urban habitats and can be pests of human health concern (Blair, 1996; Shochat, 2004), may use these novel urban habitats and human transportation as corridors to increase dispersal (Crispo et al., 2011). In this respect, the alternative urban facilitation model of gene flow predicts populations become more connected because of these artificial corridors (Crispo et al., 2011). These urban adapters are necessary models for understanding the evolutionary success of urban adaptation juxtaposed with the many negative accounts of

urbanization; however, there is a paucity of population genetic studies that have focused on these models. Additionally, urban population genetic studies have traditionally focused either on fine-scale, within single-city patterns of gene flow (Munshi-South 2012, Munshi-South & Karchenko 2010; Munshi-South et al., 2013) or on broad-scale global, between-city patterns (Booth et al., 2012, 2015; Vargo et al 2014). A perspective that bridges fine- and broad-scale patterns of urban connectivity is necessary given that the eco-evolutionary changes that emerge within and between urban areas will differ depending on the organism and the growing realization that cities are not replicates of the processes that lead to urban adaptation (Grimm et al., 2008; Johnson & Munshi-South 2017).

Across broad-scales, cities have some similarities such as buildings and impervious surfaces since they have been designed specifically to meet the needs of humans (McKinney, 2006); however, it is unclear the extent to which heterogeneity both within and between cities impacts genetic connectivity (Grimm et al., 2008; Holderegger & DiGiulio 2010; Storfer et al., 2010). Urban population genetic studies have been dominated by analyses within temperate ecosystems and in older, developed cities (Johnson & Munshi-South, 2017), where urban expansion is typically upon the backbone of landscapes that are human-modified, and thus, much of the eco-evolutionary dynamic has already been established (Alberti, 2015; McDonnell & Hahs, 2015). More than any other region of the U.S., urban expansion has been significantly rapid in the West (US Census, 2014). This urban growth is unique as much of the increased urban areas are built upon natural landscapes, have the highest increase in human population size, and represent a new urbanization model. In fact, the Southwestern U.S. is further unique as an urban model in that growth is in arid regions where supplemental water use and increased artificial urban heat island temperatures greatly impact local biodiversity (Chow et al., 2014;

Bateman et al., 2015; Wang et al., 2016). These new urban models are perfect habitats for invasive urban adapters, and thus, our need to determine the similarities and differences in how these unique urban areas impact genetic connectivity is even more imperative given their recent and rapid growth.

In characterizing evolutionary changes on multiple spatial scales, interdisciplinary approaches in landscape, population, and evolutionary genetics have emerged that provide measures of how gene flow moves across the network as a whole (Dyer et al., 2010). Additional social network approaches that compare fine- and broad-scale connectivity increase our understanding of how cities act as a biological network with connections that not only fragment but also facilitate gene flow among them (Wasserman & Faust, 1994). From a classical population genetic perspective, when gene flow is sufficiently high between locales, genetic diversity within individual locales can be maintained at high levels (Dobzhansky, 1937; Wright, 1982). While previous studies have characterized dispersal patterns of pest species and their associated patterns of genetic diversity (Crissman et al., 2010; Booth et al., 2012, 2015; Vargo et al., 2014), the use of population genetic and social network analyses that specifically identify urban hubs of connectivity, which maintain genetic diversity and stable population structure, are critical for management of both endangered and pest species (Paupy et al., 2008; Piccinali et al., 2009).

The Western black widow spider, *Latrodectus hesperus*, is an ideal eco-evolutionary model for examining urban gene flow across both fine- and broad-scales. Our previous population genetic work (Chapter 2) focused on this organism as it maintains a large geographic distribution across the arid Western U.S., inhabiting multiple urban and non-urban areas, and most importantly, is an urban adapter and pest with medical-relevance. Specifically, we and our

colleagues have previously documented ecological differences between urban and non-urban *L hesperus* for changes in fertility, behavior, web-building, and diet (Johnson et al 2012, 2014; Trubl et al., 2012), with dense aggregations in urban areas (Trubl et al., 2012), all of which have health concerns given its highly toxic, vertebrate-specific venom (Vetter & Isbister, 2008). Our previous sampling of thousands of genomewide mitochondrial and nuclear SNPs from 11 urban and 10 non-urban locales found urban-specific patterns of higher within-locale genetic diversity, lower between-locale genetic differentiation, and higher genetic connectivity, all of which are predicted by the urban facilitation model of gene flow (Chapter 2). Additionally, we found that not all cities are highly connected, with specific urban hubs driving gene flow among historically isolated non-urban locales. While this study provides needed support for our understanding of urban facilitation models and urban pest adaptation, as previously noted, how this higher gene flow on the urban landscape impacts genetic diversity and gene flow within different cities as replicates in the urban network is still unknown.

Here, we combine fine-scale and broad-scale population genetic and social network analyses to test the hypothesis that urban areas show similar levels of genetic diversity. Alternatively, because we have previously documented patterns of higher population structure associated with non-urban locales, and that urban locales differentially contribute to genetic connectivity on broad-scales, we predict that cities more connected to the urban network will tend to have higher levels of within-city genetic diversity. We compare and contrast our previous broad-scale patterns of urban gene flow (Chapter 2) with a new fine-scale locale sampling from within three Southwestern U.S. cities. Our novel social network approach enables us to determine how patterns of genetic connectivity within multiple cities are consistent, as well as to determine whether these patterns are predicted by their connectivity to the overall urban network.

These population genetic networks have implications for applied urban development, management of both endangered and pest species diversity within and across cities, and human health management across different local and global urban areas.

Methods

Sampling

We used our previously collected samples and published data (Chapter 2) from the Western black widow spider distributed across its geographic distribution of 11 urban and 10 non-urban locales (Fig. 1). From this previous study, we chose three Southwestern U.S. cities (Albuquerque, NM; Las Vegas, NV; Phoenix, AZ) that had non-urban locale counterparts as samples. These three cities are each located within an arid landscape, with recent human population and geographic size expansion. However, these three cities also have varying urbanization histories in the Southwest with respect to colonization time, geographic size, and human population size (US Census 2014), with which to contrast the impact of urbanization on genetic connectivity within urban areas (Fig. 2). Albuquerque is the smallest and oldest of the three cities founded in 1706, and covers 490 km² with a current human population of 560,000; Las Vegas is the most recently founded in 1905, covers 1600 km², and is one of the fastest growing metropolitan areas with a population of 1.9 million; Phoenix is the largest of the three, having been founded in 1881, covers 235000 km² and has a population of 4.5 million as the 12th largest metropolitan area in the US. Although the size of Albuquerque has remained relatively small, likely due to it being bounded by the Sandia Mountains on the East and Native American land on the West, Phoenix and Las Vegas have been two of the fastest growing metropolitan areas, expanding over 45% in the last 30 years (US Census 2014).

In urban areas, populations of Western black widow spiders are typically densely aggregated in open xeric-landscaping (Trubl et al., 2012); whereas, their non-urban distribution is very patchy and isolated associated with arid, rocky-outcrops and dry river-bed banks that are highly-sheltered (Chamberlin & Ivie, 1935), making their discovery and sampling very difficult. We sampled 330 Western black widow spider individuals from each of the three urban areas (Albuquerque, Las Vegas, and Phoenix), with each having samples of 10 urban locales to address our main focus here of within-city diversity, and then 1 non-urban locale as a contrast (Fig. 2, Table S1). Herein, the 11 single urban and 10 single non-urban locales are referred to as the "broad-scale" sample, whereas, the 10 urban and 1 non-urban locales from each of the 3 urban areas are referred to overall as the "fine-scale" sample. After additional collection of the fine-scale samples for this study, individuals were placed in 90% EtOH and stored at -20° C.

Data collection

Genomic DNA was extracted from tissue dissected from one front and one hind leg of each spider using the DNeasy Blood and Tissue Kit (Qiagen). We collected genomewide nuclear DNA (nuDNA) sequence fragments by generating reduced representation, double-digest RAD sequencing (ddRADseq) libraries according to previous protocol (Peterson et al., 2012). Extracted DNA was digested with MseI and EcoRI (New England Biolabs), ligated with adapters containing Illumina amplification and sequencing primers and unique barcodes (Petersen et al., 2012), and then PCR amplified. Barcoded individuals were pooled (20 per library), then size selected using gel electrophoresis for fragments ranging from 300-500 bp. Fragments were excised and purified using QIAquick Gel Extraction Kits (Qiagen). Each library was sequenced

in one lane of an Illumina HiSeq 4000 (150bp, single end) at the VCU Nucleic Acids Research Facility.

The STACKS v1.44 *de novo* pipeline (Catchen et al., 2011, 2013) was used to demultiplex, quality filter, and call genotypes with the following programs' parameters set to default unless otherwise noted: *process_radtags*, *ustacks*, *cstacks*, *sstacks*, and *populations*. *Process_radtags* demultiplexed reads and filtered for both quality and the presence of barcodes, then trimmed reads to 90 bp in length. The *ustacks* minimum number of reads was set at $m=5$. The *cstacks* number of mismatches allowed between tags was set to $n \leq 5$. The *populations* minimum coverage was set to 5x per allele for each individual. For analyses not dependent upon estimates of nucleotide site diversity (e.g., population structure), only one SNP per fragment was randomly sampled as a standard way to reduce impact of linkage disequilibrium and selection. Genotype data were exported from STACKS in each of the formats needed for analyses.

Data analysis

Our analyses include 48 locales, which include 10 non-urban locales from the broad-scale sample, and 38 urban locales, 30 of which are from the 3 cities for our fine-scale sample. Estimates of genetic diversity within and between cities, within and between non-urban locales, and between urban and non-urban locales within urban areas were performed. Estimates of genetic diversity were calculated as the average number of pairwise differences (π), the number of polymorphic sites (S), and the distributions reflecting contrasts between these two (Tajima's D, 1989). We used standard pairwise F_{ST} measures of overall genetic differentiation between all locales, as well as for measures of genetic differentiation among urban locales within each city to contrast across cities. To examine hierarchical partitioning of genetic variance within and

between cities, we performed an AMOVA in R using the *adonis* function in the *vegan* package (Oksanen et al., 2017). To identify potential population clustering, principal component analyses (PCA) were performed in the *gstudio* R package (Dyer, 2016). These PCAs were performed for each of the three urban areas individually including their non-urban locale, as well as for the combined broad-scale and fine-scale samples of a total of 48 locales.

Genetic connectivity among sampled locales was determined from the conditional genetic distance statistic cGD (Dyer & Nason, 2004), which is estimated from the genetic covariance among locales. This measure of genetic covariance derived from all locales can be visualized as a popgraph using the *popgraph* R package (Dyer 2017), where nodes represent sampled locales and edges represent genetic connections among locales. Popgraph topology is not only a visualization of cGD as genetic covariance but also of social network parameters that define the popgraph. Our social network model evaluates genetic relationships among locales and relative contributions of key “actors” using mathematical graph theory (Wasserman & Faust, 1994), which here, visually represent gene flow among all sampled locales to identify hubs of higher connectivity on the landscape. Social network node-specific parameters including closeness, degree, betweenness, and eigenvector centrality were estimated from the popgraphs using the *popgraph* R package. “Closeness” measures the degree to which a node is genetically similar to all other nodes in the network, where higher closeness values indicate further “distance” to the next node. “Degree” is the number of edges a node has connecting it to other nodes. “Betweenness” is the sum of the shortest paths (i.e., the combination of edges among multiple nodes), where higher betweenness values indicate more paths that pass through a node. Eigenvector “centrality” computes the extent to which each node is centrally located among all other nodes within the popgraph topology. To test competing hypotheses, a popgraph was

generated for each of the three urban areas of the fine-scale sample, independently, as well as for the combined overall 48 locale dataset. To identify statistical differences and congruence between popgraph topologies, we performed t-tests for network parameters noted above, with Bonferroni corrections for multiple comparisons.

From a spatial perspective, patterns of gene flow even on a fine-spatial scale within cities may be due to geographic distance when dispersal distance is low, and this is typically expected in web-building spiders (Foelix, 2014). Therefore, we test a simple isolation-by-distance (IBD) model for each of the three cities. As this analysis is specifically contrasting patterns of gene flow within and across cities, we excluded the non-urban sample from each of the three analyses. Euclidian geographic distances were estimated from latitude and longitude coordinates using the *fields* package in R (Nychka et al., 2015), and genetic distances were calculated as cGD (see above). Mantel tests were performed on the geographic and genetic distances in R. To compare with this analysis of geographic distance, we also used percent impervious surface (PIS) as a standard resistance distance proxy (Storfer et al., 2010; Johnson & Munshi-South, 2017; Alberti, 2015) for the degree of urbanization (national land cover database: <https://www.mrlc.gov/finddata.php>). As in Dyer et al. (2012), we performed a permutation analysis using the *gstudio* package in R to test for significant relationships between PIS and genetic connectivity. In the permutation analysis, each sample locale, or node, was fixed on the landscape of the popgraph. The connections among the nodes, or edges of the popgraph, were overlaid on the raster maps of PIS to generate the observed mean and variance resistance distances. We simulated 1,000 popgraphs with the observed number of nodes fixed on the landscape and the edges randomized among these popgraphs to generate a null distribution for

both the mean and variance of PIS. Statistical significance was assessed by determining the probability of the observed popgraph values compared to our simulated distributions.

Results

After quality filtering, the final dataset included 1.9 million SNPs for the 48 locales, which includes the broad-scale and fine-scale samples. Estimates of Western black widow spider genetic diversity in the entire sample (Table S3) are consistent with estimates of arthropod nuclear genetic diversity in general (Burns et al., 2017). For the fine-scale analysis, within-locale estimates of genetic diversity are on average significantly lower for Albuquerque locales ($\pi_{ave} = 0.07$) than Las Vegas (t-test $p < 0.001$) and Phoenix (t-test, $p < 0.001$) locales, with Las Vegas and Phoenix having similar estimates ($\pi_{ave} = 0.20$ and 0.20 , respectively). Although estimates of within-city locale genetic differentiation (i.e., among locales within a city) are moderately high, Albuquerque has the statistically highest average pairwise F_{ST} ($F_{ST} = 0.29$, Fig. S1a) compared to each of Las Vegas ($F_{ST} = 0.19$, t-test $p < 0.01$, Fig. S1b) and Phoenix ($F_{ST} = 0.22$, t-test $p < 0.01$, Fig S1c). In the combined dataset of 48 locales, urban locales are statistically significantly less genetically differentiated from each other than non-urban locales ($F_{ST} = 0.15$ vs. 0.30 ; t-test, $p < 0.01$, Fig S2). When combining the 10 locales within each city as a sampled unit, there is statistically significantly less genetic differentiation (t-test, $p < 0.01$) between Las Vegas and Phoenix ($F_{ST} = 0.06$) when compared to Albuquerque and each of these two cities (vs. Las Vegas, $F_{ST} = 0.12$; vs. Phoenix $F_{ST} = 0.18$). Each of the three cities has a statistically significant negative Tajima's D value, although these values are not significantly different from each other (Table S3). Finally, the AMOVA resulted in 8.2% variance explained by city and 20.4% variance explained by locale, with the remaining 71.4% among individuals. That is, the majority

of the genetic variance is found among individuals, and overall, more genetic variance is found among locales within cities than is found between cities.

The first 10 PCs for each urban area are statistically significant and account for 52% (Albuquerque), 44% (Las Vegas), and 47% (Phoenix) of the genetic variance among individuals (Fig. 3). The previous PCA of the broad-scale sample had shown significant independent non-urban clusters, with the majority of urban individuals forming a single cluster (Chapter 2). With the independent PCAs of the fine-scale samples, we see that each of the three urban areas show a pattern of no clustering of specific urban locales, and apparent clustering for the non-urban individuals, with Phoenix showing the strongest cluster. In the combined PCA of 48 locales, the locales for each of the three cities show some weak clustering in PC1-2 (29% variance explained), with most of this variance among the three cities here, and in other PCs, explained by Albuquerque locales (Fig. S3).

For our popgraph analyses, although the number of edges or connections do not significantly differ among the three cities' networks (Fig. S4), the measures of cGD and "closeness" are statistically significantly higher in Albuquerque and Phoenix than in Las Vegas (Table S4). Each of the three cities' popgraphs have contrasting patterns of "betweenness" such that this parameter is statistically significantly different between all three cities (Table S4). Specifically, the Albuquerque popgraph has one locale (BEL) with the highest betweenness value, whereas Las Vegas has nearly each node equally weighted, and Phoenix has two equally weighted locales (BRO & GCC) that have the highest value (Table S5). When combining all 48 locales into one popgraph (Fig. 4), Albuquerque locales have the highest connection distances (least "central") from all other locales in the network, whereas, Phoenix and Las Vegas locales are centrally connected with all other urban locales in the broad-scale sample. Except for the

Albuquerque non-urban locale, all non-urban locales are peripherally-linked outside of the network, which at its core are urban locales, and many non-urban locales are only connected via urban locales. The analysis of “centrality” identified ERN (Las Vegas), CHU (Albuquerque), and BUC (Phoenix) as the top three major hubs of connectivity in the entire network. On the other hand, non-urban locales have the least influence on connectivity in the overall network; in fact, 7 of the 10 non-urban locales sampled have the lowest “centrality” of all 48 locales in the network (Table S6).

The Mantel tests found both Albuquerque and Phoenix have statistically significant patterns of IBD ($r^2=0.18$ and $r^2=0.17$, respectively, both $p<0.01$), whereas, Las Vegas shows no such pattern ($r^2=0.01$, $p=0.49$; Fig. S5). For our PIS resistance distance analyses, the mean and variance for PIS in each of the three cities showed no statistical significant association with genetic connectivity (Fig. S6).

Discussion

Our previous work on the Western black widow spider as an urban pest model documented population genetic signatures consistent with the urban facilitation model of gene flow on a broad geographic scale, yet it raised questions about how this model explains patterns on fine-scales within cities. While many studies have focused on fine-sampling of a single city and its surrounding areas to document genetic diversity and gene flow patterns in testing hypotheses about impacts of urbanization (Munshi-South & Karchenko, 2010; Munshi-South 2012; Booth et al., 2012, 2015; Munshi-South et al., 2013; Vargo et al., 2014), here, we used a unique analysis of fine-scale sampling of Western black widow spider genetic variation from three Southwestern cities in combination with our previous broad-scale urban and non-urban

sampling. The primary observation is that urban areas have significantly different patterns of connectivity to the overall network that generate contrasting patterns of within- and between-city genetic diversity. We discuss these results as they challenge the use of cities as replicates of urban eco-evolution, and have implications for conservation and human health in a rapidly growing urban habitat.

The observations of significantly higher within-locale genetic diversity, lower between-locale genetic diversity, and most interestingly, higher connectivity among 11 urban locales compared to 10 non-urban locales were all patterns consistent with the urban facilitation model (Holderegger & DiGiulio, 2012; Crispo et al., 2011) for our previous analysis of Western black widow spiders (Chapter 2). These patterns are overall consistent with the fine-scaled analyses of 30 locales from three Southwestern U.S. cities, whether independently analysed or in combination with the previous broad-scale sample, indicating at the outset that broad-scale and fine-scale analyses were not reflecting different general urban evolutionary forces. In fact, the hierarchical variance analysis of these broad-scale and fine-scale samples shows that overall, urban facilitated gene flow both within and among cities results in more genetic diversity being distributed among locales within cities than is found between cities. As we have previously noted (Chapter 2), these patterns should be expected in emerging studies as signatures of urban facilitated gene flow for urban adapter and pest species (Booth et al., 2012, 2015; Vargo et al., 2014).

Underlying this urban facilitation model, the most interesting find is the significant heterogeneity among the fine-scale city samples. Specifically, the locales sampled from within each of Las Vegas and Phoenix show similar levels of within- and between-locale genetic diversity, similar population clustering, and significantly higher connections to the urban

network; however, Albuquerque has significantly lower within-locale and higher between-locale diversity compared to the other two cities. In fact, Albuquerque locales share more in common with the 11 geographically distributed non-urban locales, which appear to have been relatively isolated with lower diversity and higher population structure (Chapter 2), and from our popgraph here of all 48 locales, show significantly reduced connectivity to the urban network at large. Thus, while urban and non-urban areas are different with respect to genetic diversity, even urban areas cannot be classified as a single group with respect to effects of urbanization.

Our previous broad-scale analysis first revealed that certain urban areas act as "drivers" of the overall higher genetic connectivity of the Western black widow spider population network, with surprisingly, even non-urban locales becoming more connected via urban areas (Chapter 2). With the popgraph analysis of the overall 48 locales here, our fine-scale samples are consistent with this initial observation, yet now reveal how urban areas specifically drive connectivity. For example, our social network analysis finds that while Las Vegas and Phoenix locales overall are still highly connected to the network, Phoenix has multiple locales identified as "hubs" of connectivity, whereas, Las Vegas locales each equally drive gene flow. Alternatively, Albuquerque locales, which overall are significantly disconnected from the network, have one identified hub, but note that this hub simply connects the other nine Albuquerque locales to the network. Therefore, while certain urban hubs are impacting the network of urban and non-urban locales on the whole, other urban hubs only connect peripheral populations, albeit loosely, to the network. These results reveal one of the powerful characteristics of using conditional genetic distances (cGD) in that the addition or removal of populations alters the covariance across the network, as seen from contrasts of individual city popgraphs to the overall popgraph (Dyer et al., 2010; Koen et al., 2013; Naujokaitis-Lewis et al., 2013). Thus, social network analyses are

ideally suited for investigating evolutionary changes across multiple urban environments, in modeling how the applied management of specific urban hubs may alter and especially create corridors on multiple spatial scales.

Given the underlying urban facilitation model here, we may predict that broad- and fine-scale patterns of urban genetic connectivity predict patterns of within-urban area genetic diversity, which can be a long-term measure of sustainability (Debinski & Holt 2000; Trizio et al., 2005; McKinney, 2006; Allendorf & Luikart, 2007; Keyghobadi, 2007; Vandergast et al., 2007, 2009). In testing this hypothesis in the 38 urban locales, we initially find a negative correlation between connectivity (as the parameter betweenness) and genetic diversity. However, this analysis revealed multiple statistical outliers with high genetic diversity that all coincidentally have the lowest measures of connectivity of all 38 locales. In fact, when these three outliers were removed, the correlation became significantly positive ($r^2 = 0.20$, $p < 0.01$). The outlier locales are all from the broad-scale sample (BLY, DAV, DEN; Table S3), and reflect different human population and geographic sizes. Thus, this observation reveals that while a proportion of Western black widow spider genetic diversity within urban locales can be predicted by how well-connected these locales are to the urban network, underlying this correlation is significant heterogeneity among urban areas that reveals multiple "urban signatures". More to the point, several designated "urban" areas (e.g., Albuquerque) mimic even non-urban areas in that they have similarly low levels of genetic diversity and connectivity due to their isolation on the landscape. Thus, while urbanization appears to facilitate gene flow among even geographically distant populations (as evidenced by the IBD results), some urban locales do show the effects of reduced connectivity further rejecting urban areas as simple replicates of the same urbanization process.

Under an urban facilitation model of gene flow, it has often been proposed that the similarities among cities, such as human population size, canopy cover, and human transportation networks, can be dispersal corridors (Hoderegger & DiGiulio, 2010; Crispo et al., 2011; Alberti, 2015; McDonnell & Hahs, 2015; Johnson & Munshi-South, 2017). Our previous investigation of human population size (Chapter 2) and this study's investigation of PIS as potential drivers of genetic connectivity for broad- and fine-scale samples, respectively, were not statistically significant for the Western black widow spider. However, the contrast in patterns of connectivity across scales shown here further emphasizes the importance of identifying corridors and barriers that evolve differently, especially for cities that vary in size, timing and magnitude of human habitation. For example, while PIS was not a significant predictor of within-city connectivity, we note that not only are the PIS distributions different among cities, but they do not show a predictable pattern (i.e., cities with high PIS do not have the lowest genetic connectivity). Thus, as we characterize patterns of genetic connectivity within and among multiple urban areas for multiple organisms, only then will we be able to successfully model how landscape features that are typically implicated as factors driving urban gene flow (Hoderegger & DiGiulio, 2010; Crispo et al., 2011; Alberti, 2015; McDonnell & Hahs, 2015; Johnson & Munshi-South, 2017), interact in complex ways both within and across cities.

One of the predictions of this urban facilitation model is that an "urban ecotype" sweeps across not only urban areas, but invades non-urban areas as well given the patterns of overall connectivity we have observed (Krtinic et al., 2012; Shapira & Boutsika, 2012). While our previous broad-scale analyses hinted at this speculation, our popgraph network analyses here find that only specific urban locales may have the opportunity to drive and spread phenotypes into specific urban and non-urban locales (i.e., a standard source-sink dynamic). This model

would predict that not only would we see divergent phenotypes each locally-adapted between urban and non-urban environments, but that even multiple urban and non-urban phenotypes emerge (Thompson et al., 2016), possibly due to local adaptation, and as predicted by the urban network of gene flow. For example, our group has already documented Western black widow spider behavioral differences between urban and non-urban habitats, where urban spiders are significantly more densely aggregated and are more aggressive towards prey and conspecifics (Johnson et al., 2014; Trubl et al., 2012), as well as gene expression differences among even urban habitats related to metabolism and fertility (our data unpublished). Thus, as the field of urban eco-evolution is focused on characterizing the adaptive traits that define invasion into human habitats, it must consider not only how these traits differ from ancestral habitats, but also how multiple urban ecotypes emerge in response to the heterogeneity of urbanization selective pressures on different spatial scales.

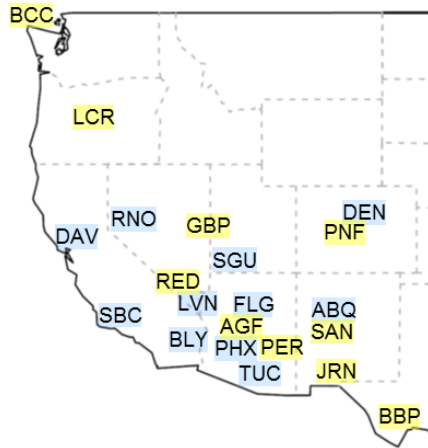


Figure 3.1. Geographic distribution of the broad-scale sampled locales of the Western black widow spider across the Western U.S. (see Table S2). Highlighted locales in blue and yellow reflect urban and non-urban samples, respectively.

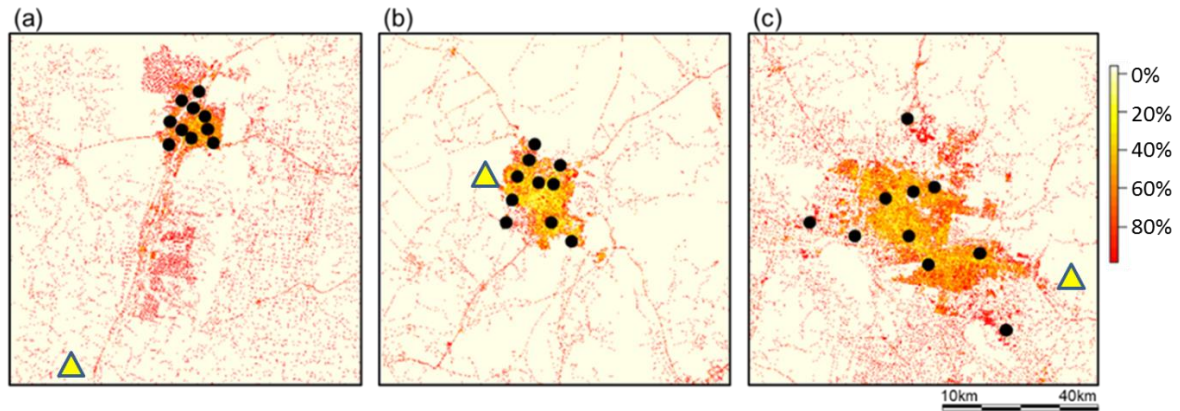


Figure 3.2. Geographic distribution of the fine-scale sampled locales of the Western black widow spider from three urban areas. Color-scale represents the percent of impervious surface for the cities and surrounding non-urban areas of (a) Albuquerque, (b) Las Vegas, and (c) Phoenix. Triangles represent non-urban locales (see Table S1 for sampling locales).

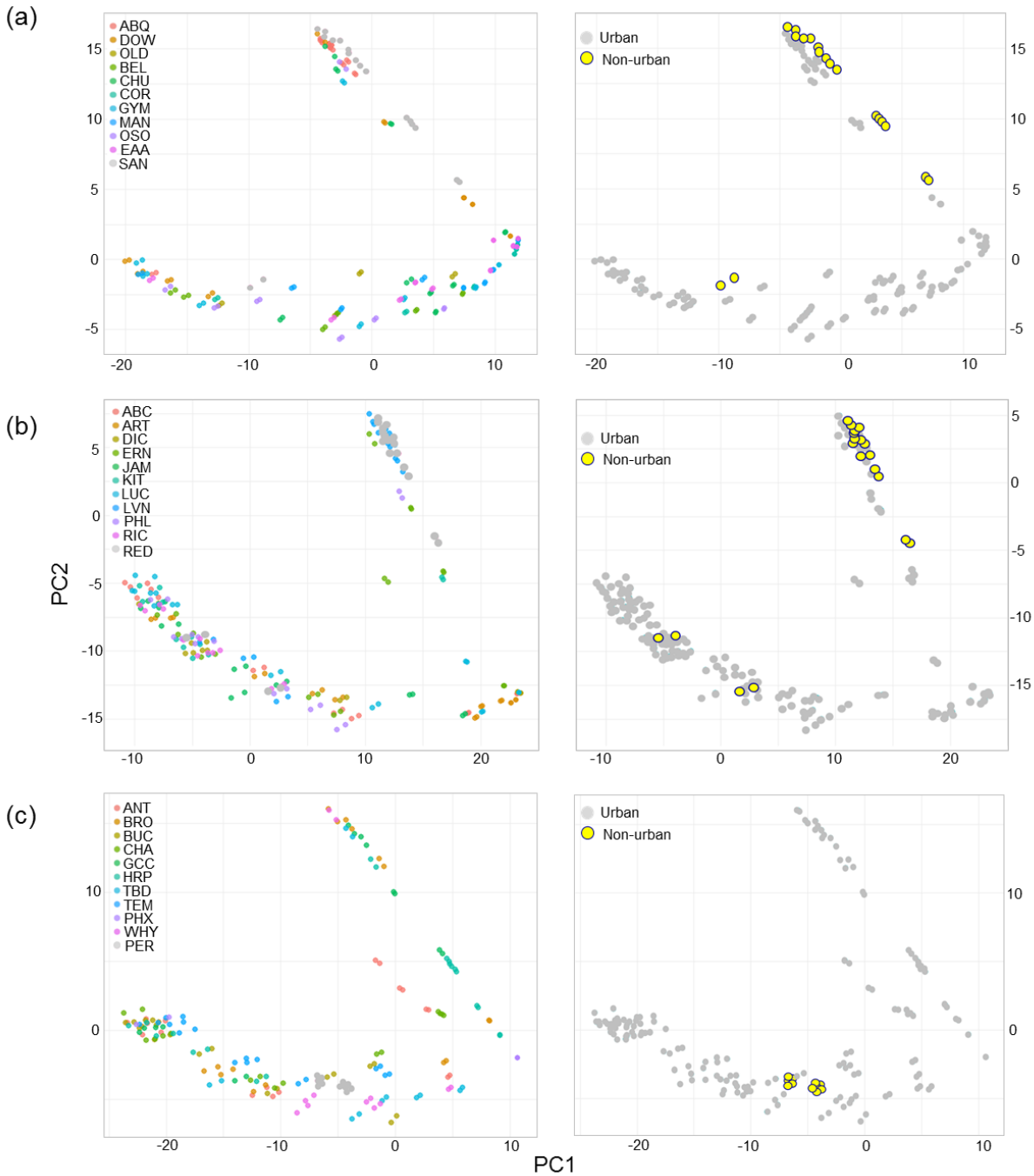


Figure 3.3. PC1 and PC2 biplots of individual genotypes are shown for fine-scale sampled locales within (a) Albuquerque, (b) Las Vegas, and (c) Phoenix urban areas. The left and right panels reflect urban samples highlighted (color-scheme) and non-urban samples highlighted (yellow), respectively (see Table S1).

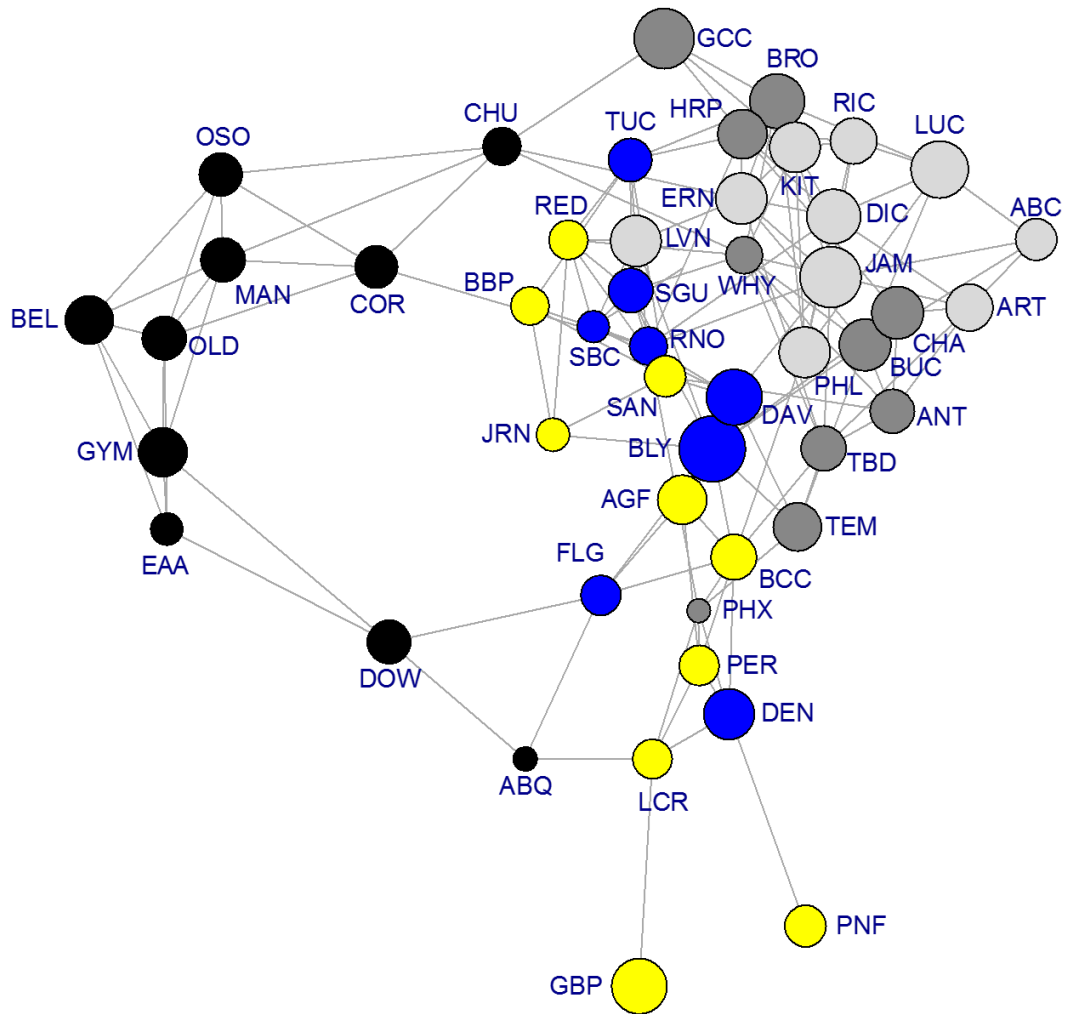


Figure 3.4. Social network popgraph analysis among urban (blue) and non-urban (yellow) locales for the broad-scale sample (see Figure 1), as well as the fine-scale sample from Albuquerque (black), Las Vegas (light grey), and Phoenix (dark grey) cities (see Table S1, S2). The relative size of each node reflects the locale-specific genetic variance, and the length of the edges is proportional to the conditional genetic distance (cGD, see Methods) between locales.

(a)

	ABQ	DOW	OLD	BEL	CHU	COR	GYM	MAN	EAA	OSO	SAN
ABQ		0.44	0.56	0.58	0.38	0.53	0.53	0.57	0.51	0.55	0.32
DOW			0.48	0.33	0.43	0.29	0.34	0.44	0.40	0.31	0.29
OLD				0.32	0.56	0.31	0.30	0.33	0.51	0.31	0.54
BEL					0.57	0.25	0.27	0.27	0.39	0.28	0.60
CHU						0.49	0.55	0.62	0.45	0.55	0.25
COR							0.24	0.29	0.30	0.30	0.57
GYM								0.28	0.38	0.26	0.54
MAN									0.50	0.26	0.55
EAA										0.35	0.30
OSO											0.57
SAN											

(b)

	ABC	ART	DIC	ERN	JAM	KIT	LUC	LVN	PHL	RIC	RED
ABC		0.21	0.20	0.23	0.20	0.19	0.16	0.34	0.19	0.19	0.36
ART			0.24	0.24	0.23	0.20	0.17	0.34	0.20	0.21	0.38
DIC				0.24	0.24	0.20	0.17	0.34	0.20	0.22	0.38
ERN					0.24	0.22	0.22	0.24	0.21	0.24	0.26
JAM						0.21	0.17	0.34	0.21	0.21	0.38
KIT							0.17	0.31	0.19	0.20	0.33
LUC								0.33	0.17	0.16	0.36
LVN									0.29	0.34	0.10
PHL										0.20	0.31
RIC											0.37
RED											

(c)

	ANT	BRO	BUC	CHA	GCC	HRP	TBD	TEM	PHX	WHY	PER
ANT		0.27	0.16	0.19	0.26	0.24	0.37	0.19	0.33	0.43	0.34
BRO			0.26	0.27	0.19	0.22	0.22	0.26	0.30	0.23	0.33
BUC				0.17	0.25	0.22	0.34	0.17	0.30	0.41	0.25
CHA					0.26	0.24	0.37	0.19	0.33	0.44	0.35
GCC						0.21	0.27	0.26	0.32	0.29	0.37
HRP							0.30	0.24	0.32	0.35	0.37
TBD								0.36	0.45	0.34	0.49
TEM									0.32	0.41	0.31
PHX										0.47	0.52
WHY											0.55
PER											

Figure S3.1. Pairwise F_{ST} values of urban and non-urban locales for the fine-scale samples of (a) Albuquerque, (b) Las Vegas, and (c) Phoenix city areas. Locale abbreviations found in Table S1.

	AGP	BBP	BCC	BLY	DEN	FLG	GBP	JRN	LCR	INF	RNO	SBC	TUC	UCD	SCU	ABQ	DOW	OLD	BEL	CHU	COR	DYM	MAN	BAA	OSO	SHN	ART	DIC	ERN	JAM	KIT	LUC	LYN	ABC	PHL	RIC	RED	ANT	BRO	BUC	CHA	TBD	TEM	PHX	WHY	QCC	HSP	PER				
AGP		0.16	0.39	0.23	100	0.31	0.30	0.20	0.38	100	0.33	0.22	0.22	0.22	0.09	0.31	0.41	0.65	0.59	0.39	0.56	0.54	0.62	0.46	0.56	0.22	0.65	0.63	0.40	0.61	0.63	0.54	0.17	0.58	0.49	0.60	0.16	0.59	0.29	0.56	0.60	0.48	0.57	0.63	0.43	0.38	0.46	0.45				
BBP			0.22	0.17	100	0.18	0.16	0.10	0.24	100	0.20	0.17	0.16	0.20	0.05	0.26	0.23	0.46	0.58	0.21	0.48	0.45	0.46	0.26	0.48	0.10	0.42	0.42	0.29	0.41	0.37	0.40	0.14	0.40	0.35	0.41	0.13	0.40	0.23	0.38	0.40	0.29	0.39	0.42	0.24	0.27	0.32	0.49				
BCC				0.16	100	0.38	0.32	0.23	0.41	100	0.34	0.24	0.24	0.26	0.23	0.10	0.44	0.43	0.66	0.69	0.43	0.66	0.64	0.63	0.47	0.67	0.25	0.61	0.60	0.40	0.58	0.51	0.51	0.17	0.54	0.46	0.56	0.17	0.55	0.38	0.52	0.56	0.47	0.53	0.61	0.41	0.36	0.43	0.69			
BLY					100	0.29	0.21	0.20	0.23	100	0.20	0.21	0.16	0.10	0.09	0.27	0.21	0.29	0.34	0.28	0.33	0.32	0.30	0.22	0.32	0.16	0.23	0.24	0.17	0.24	0.22	0.24	0.10	0.24	0.21	0.24	0.15	0.24	0.16	0.24	0.18	0.24	0.18	0.23	0.28	0.17	0.17	0.20	0.27			
DEN						100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100		
FLG							100	0.37	0.28	0.39	0.27	0.10	0.36	0.46	0.66	0.64	0.41	0.63	0.61	0.63	0.49	0.64	0.29	0.61	0.60	0.44	0.59	0.53	0.52	0.22	0.55	0.49	0.57	0.21	0.56	0.35	0.51	0.57	0.50	0.55	0.62	0.43	0.41	0.47	0.70							
GBP								100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100		
JRN									100	0.20	0.16	0.17	0.23	0.05	0.29	0.29	0.63	0.61	0.25	0.57	0.51	0.58	0.30	0.57	0.10	0.54	0.63	0.37	0.51	0.45	0.48	0.14	0.50	0.44	0.51	0.10	0.50	0.27	0.49	0.50	0.39	0.48	0.51	0.31	0.34	0.46	0.52					
LCR										100	0.37	0.28	0.39	0.27	0.10	0.36	0.46	0.66	0.64	0.41	0.63	0.61	0.63	0.49	0.64	0.29	0.61	0.60	0.44	0.59	0.53	0.52	0.22	0.55	0.49	0.57	0.21	0.56	0.35	0.51	0.57	0.50	0.55	0.62	0.43	0.41	0.47	0.70				
INF											100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100		
RNO												100	0.24	0.10	0.09	0.43	0.41	0.61	0.67	0.36	0.61	0.63	0.65	0.40	0.64	0.21	0.60	0.59	0.41	0.58	0.51	0.53	0.14	0.56	0.48	0.57	0.14	0.57	0.30	0.55	0.57	0.46	0.54	0.51	0.39	0.39	0.46	0.68				
SBC													100	0.20	0.20	0.11	0.32	0.29	0.63	0.61	0.29	0.88	0.55	0.88	0.30	0.57	0.17	0.54	0.64	0.38	0.62	0.47	0.49	0.16	0.50	0.45	0.52	0.19	0.51	0.29	0.56	0.51	0.39	0.49	0.51	0.32	0.36	0.42	0.57			
TUC														100	0.68	0.34	0.27	0.48	0.51	0.26	0.50	0.47	0.48	0.28	0.49	0.16	0.43	0.42	0.29	0.41	0.37	0.38	0.16	0.40	0.34	0.41	0.15	0.40	0.22	0.39	0.41	0.29	0.39	0.43	0.26	0.27	0.32	0.48				
UCD															100	0.31	0.24	0.25	0.32	0.21	0.30	0.32	0.28	0.22	0.30	0.22	0.22	0.22	0.23	0.18	0.24	0.22	0.24	0.16	0.23	0.21	0.23	0.18	0.24	0.16	0.24	0.18	0.24	0.27	0.18	0.18	0.21	0.27				
SCU																100	0.11	0.22	0.25	0.09	0.25	0.21	0.21	0.11	0.23	0.09	0.16	0.16	0.15	0.16	0.15	0.16	0.15	0.16	0.15	0.16	0.15	0.16	0.15	0.16	0.15	0.16	0.15	0.16	0.15	0.16	0.15	0.16	0.15	0.16		
ABQ																	100	0.58	0.38	0.53	0.53	0.57	0.51	0.55	0.32	0.63	0.62	0.45	0.59	0.56	0.56	0.27	0.56	0.54	0.50	0.29	0.58	0.39	0.54	0.57	0.54	0.54	0.61	0.43	0.43	0.53	0.69					
DOW																		100	0.48	0.33	0.43	0.29	0.34	0.44	0.43	0.31	0.29	0.64	0.62	0.40	0.59	0.51	0.52	0.20	0.56	0.46	0.50	0.21	0.57	0.28	0.54	0.58	0.48	0.56	0.63	0.43	0.36	0.43	0.60			
OLD																			100	0.57	0.25	0.27	0.39	0.29	0.66	0.42	0.38	0.36	0.36	0.34	0.29	0.45	0.33	0.32	0.32	0.47	0.34	0.36	0.29	0.36	0.50	0.34	0.51	0.60	0.40	0.39	0.53					
BEL																				100	0.57	0.25	0.27	0.39	0.29	0.66	0.42	0.38	0.36	0.36	0.34	0.29	0.45	0.33	0.32	0.32	0.47	0.34	0.36	0.29	0.36	0.50	0.34	0.51	0.60	0.40	0.39	0.53				
CHU																					100	0.55	0.62	0.45	0.55	0.25	0.64	0.63	0.30	0.60	0.51	0.55	0.16	0.58	0.48	0.60	0.18	0.59	0.28	0.56	0.60	0.46	0.57	0.63	0.39	0.37	0.44	0.70				
COR																					100	0.29	0.30	0.30	0.57	0.39	0.36	0.33	0.33	0.32	0.26	0.41	0.33	0.31	0.34	0.42	0.33	0.34	0.26	0.32	0.50	0.32	0.49	0.57	0.39	0.39	0.46					
DYM																						100	0.38	0.26	0.54	0.43	0.40	0.37	0.39	0.37	0.33	0.42	0.37	0.35	0.37	0.43	0.38	0.35	0.34	0.39	0.50	0.36	0.51	0.64	0.38	0.46	0.43					
MAN																						100	0.29	0.35	0.36	0.36	0.32	0.34	0.31	0.26	0.39	0.31	0.28	0.30	0.41	0.31	0.32	0.27	0.31	0.48	0.31	0.50	0.51	0.35	0.35	0.36						
BAA																							100	0.39	0.20	0.64	0.62	0.38	0.59	0.49	0.52	0.20	0.58	0.44	0.58	0.29	0.58	0.27	0.54	0.50	0.48	0.57	0.66	0.45	0.34	0.40	0.66					
OSO																								100	0.67	0.39	0.36	0.34	0.35	0.34	0.28	0.42	0.37	0.33	0.34	0.44	0.35	0.36	0.29	0.34	0.49	0.33	0.49	0.55	0.39	0.38	0.44					
SHN																									100	0.50	0.35	0.49	0.44	0.45	0.10	0.47	0.41	0.49	0.21	0.48	0.38	0.46	0.40	0.36	0.46	0.48	0.49	0.29	0.33	0.39	0.56					
ART																										100	0.24	0.24	0.23	0.21	0.17	0.34	0.21	0.20	0.21	0.38	0.21	0.27	0.18	0.22	0.39	0.21	0.38	0.45	0.27	0.26	0.40					
DIC																											100	0.26	0.24	0.21	0.17	0.34	0.20	0.20	0.22	0.38	0.21	0.27	0.18	0.22	0.39	0.22	0.38	0.46	0.28							

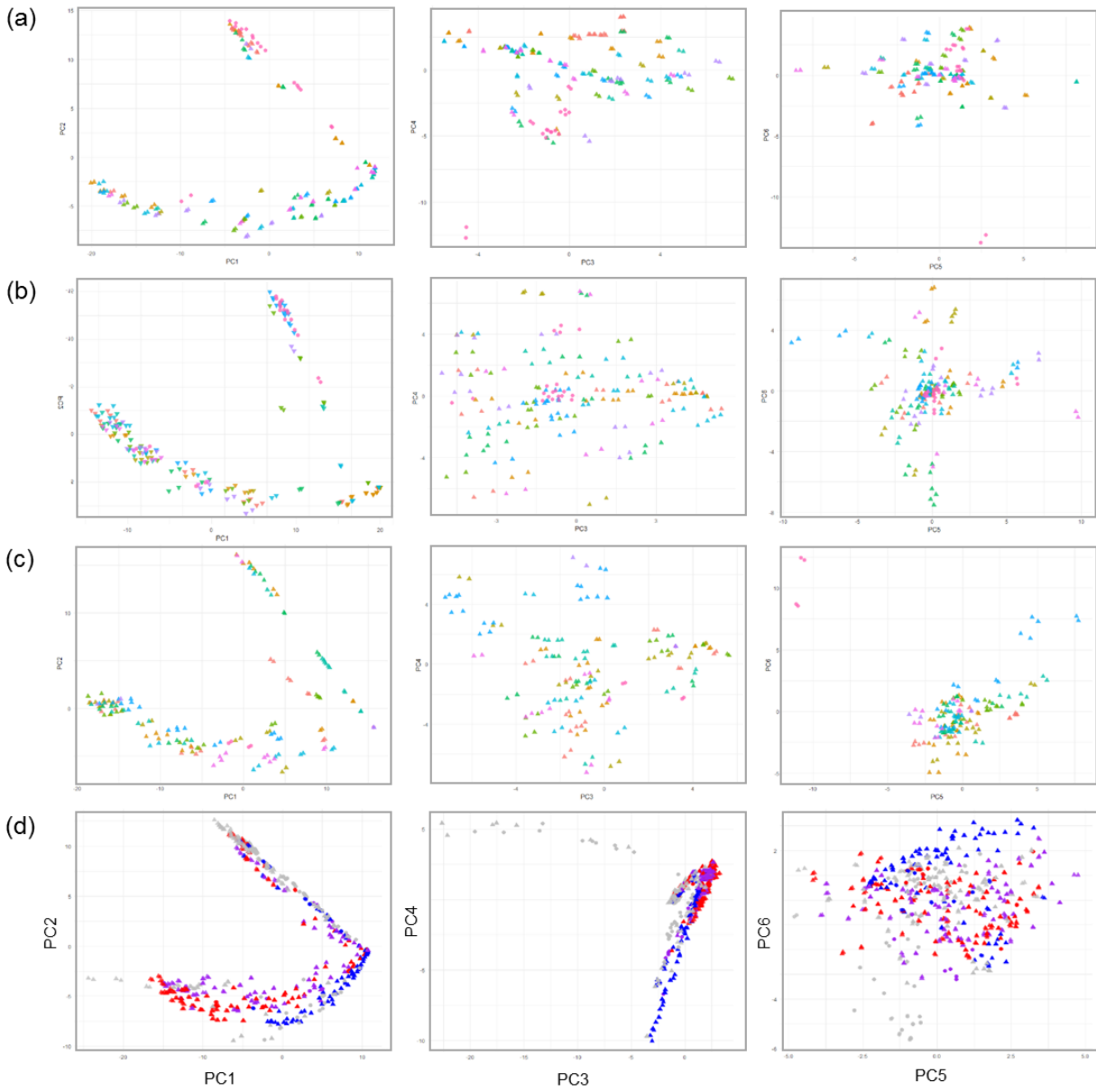


Figure S3.3. PCA biplots for PC1-6 of urban (circles) and non-urban (triangles) locales within (a) Albuquerque, (b) Las Vegas, (c) Phoenix, and (d) combined broad- and fine-scale samples of 48 locales.

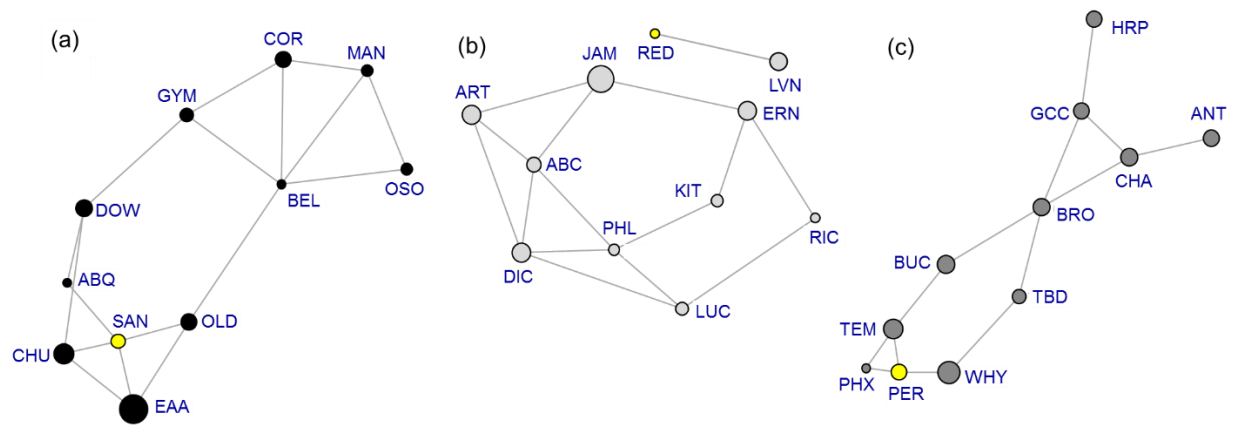


Figure S3.4. Social network popgraph analyses for each of the fine-scale sampled (a) Albuquerque, (b) Las Vegas and (c) Phoenix city areas, with urban (grey-scale) and non-urban (yellow) locales highlighted (locale abbreviations found in Table S1). For each network, the relative size of each node reflects the locale-specific genetic variance and the length of the edges is proportional to the conditional genetic distance (cGD, see Methods) between locales.

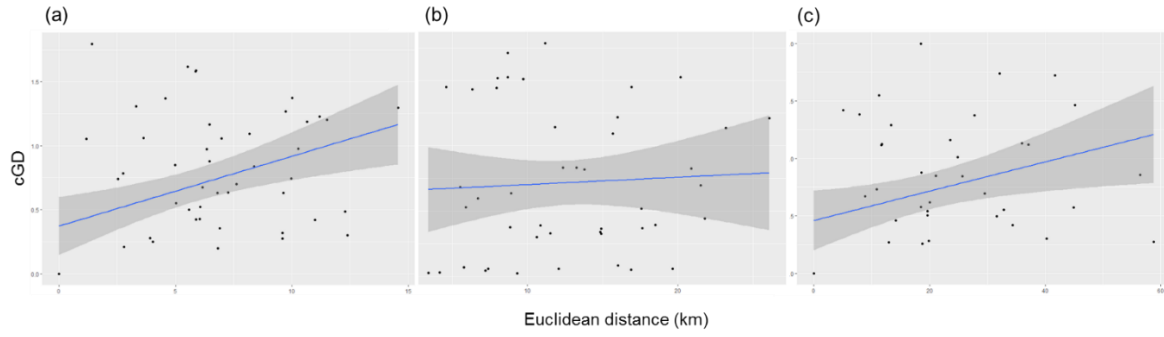


Figure S3.5. Isolation-by-distance analysis for locales within (a) Albuquerque, (b) Las Vegas, and (c) Phoenix cities.

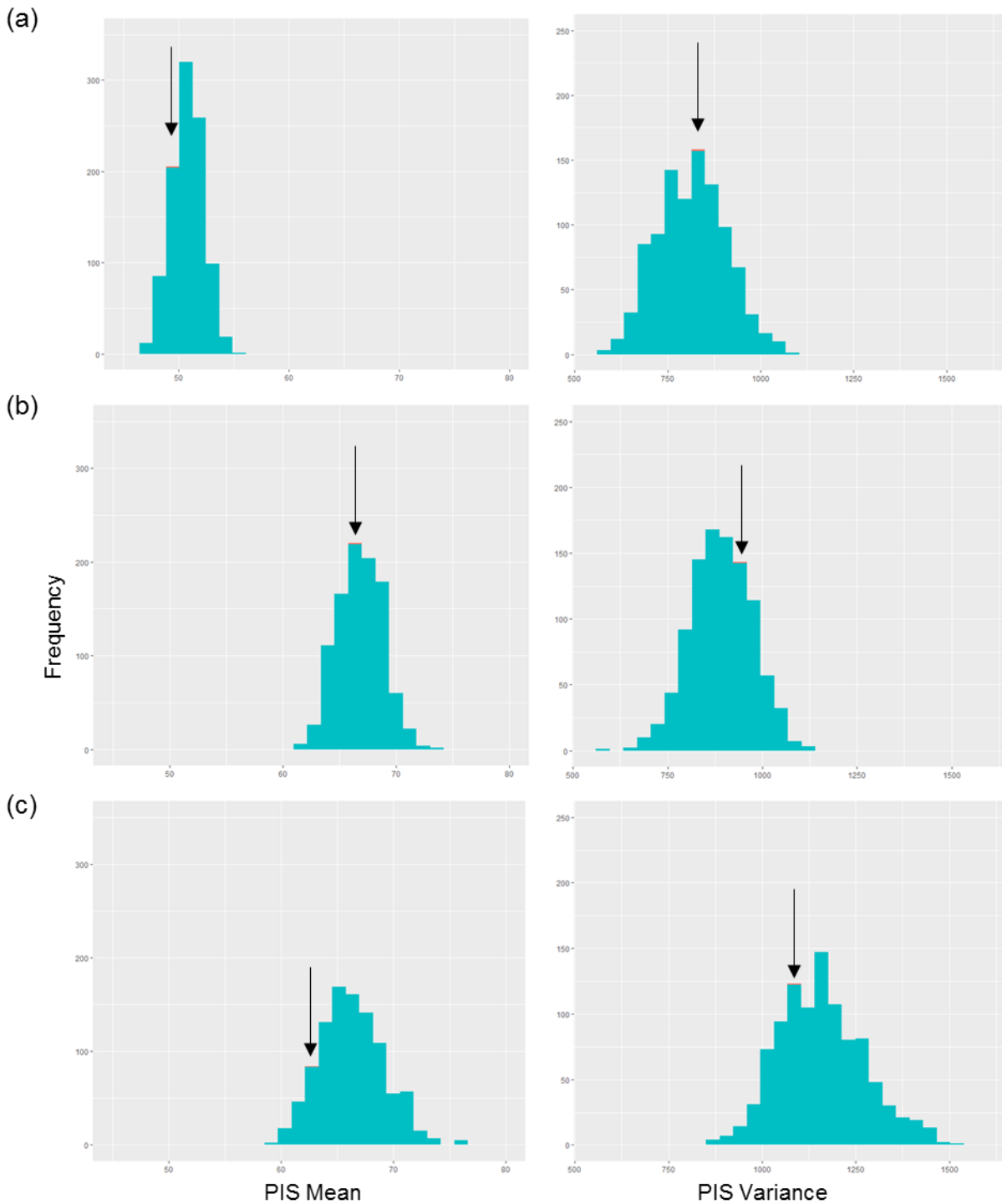


Figure S6. Permutation distribution of percent impervious surface (PIS) between locales presented as mean (left) and variance (right) for (a) Albuquerque, (b) Las Vegas, and (c) Phoenix cities. The arrows point to the observed PIS value within each simulated distribution.

Table S3.1. Fine-scale sample locales of Western black widow spiders.

City	Locale	Abbreviation	Latitude	Longitude	Habitat
Albuquerque, NM	San Acacia	SAN	34.205844	-107.027108	non-urban
	Albuquerque	ABQ	35.083183	-106.625317	urban
	Bellehaven	BEL	35.098728	-106.546769	urban
	Church	CHU	35.176675	-106.625486	urban
	Cortez	COR	35.227547	-106.610217	urban
	Downtown	DOW	35.096331	-106.667097	urban
	Elementary	EAA	35.033394	-106.709769	urban
	Gymnasium	GYM	35.196158	-106.667831	urban
	Manzano	MAN	35.062433	-106.524894	urban
	Oldtown	OLD	35.086997	-106.649681	urban
Oso	OSO	35.151983	-106.575333	urban	
Las Vegas, NV	Red Rock Park	RED	36.144175	-115.405719	non-urban
	Bonanza	ABC	36.178333	-115.171700	urban
	Arthur	ART	36.178067	-115.110550	urban
	Dickens	DIC	36.260317	-115.092067	urban
	Ernest	ERN	36.254433	-115.234217	urban
	James	JAM	36.046367	-115.074950	urban
	Kitty	KIT	36.313517	-115.219783	urban
	Lucille	LUC	36.088367	-115.284433	urban
	Las Vegas	LVN	36.003439	-115.289411	urban
	Paradise	PHL	35.992033	-114.975983	urban
Richard	RIC	36.194267	-115.268633	urban	
Phoenix, AZ	Peralta Park	PER	33.402600	-111.348410	non-urban
	Anthem	ANT	33.874583	-112.155622	urban
	Brown	BRO	33.437014	-111.736914	urban
	Buckeye	BUC	33.437958	-112.495933	urban
	Chandler	CHA	33.179380	-111.570640	urban
	Glendale	GCC	33.571044	-112.190178	urban
	Horse Ranch	HRP	33.647860	-111.984920	urban
	Thunderbird	TBD	33.617722	-112.065208	urban
	Tempe	TEM	33.365439	-111.954681	urban
	Phoenix	PHX	33.454456	-112.064992	urban
Whyman	WHY	33.424422	-112.294017	urban	

Table S3.2. Broad-scale sample locales of Western black widow spiders.

Locale	Abbreviation	Latitude	Longitude	Habitat
Agua Fria, AZ	AGF	34.192	-112.101	non-urban
Albuquerque, NM	ABQ	35.084	-106.621	urban
Big Bend, TX	BBP	29.329	-103.208	non-urban
British Columbia, Canada	BCC	48.581	-123.374	non-urban
Blythe, CA	BLY	33.616	-114.598	urban
Pine National Forest, CO	PNF	39.543	-105.163	non-urban
Flagstaff, AZ	FLG	35.192	-111.645	urban
Great Basin, NV	GBP	39.010	-114.123	non-urban
Jornada Basin, NM	JRN	32.366	-106.525	non-urban
Lower Creek River, OR	LCR	44.135	-120.813	non-urban
Las Vegas, NV	LVN	36.003	-115.289	urban
Peralta, AZ	PER	33.403	-111.348	non-urban
Phoenix, AZ	PHX	33.454	-112.065	urban
Red Rock, NV	RED	36.144	-115.406	non-urban
Reno, NV	RNO	39.530	-119.814	urban
San Acacia, NM	SAN	34.206	-107.027	non-urban
Santa Barbara, CA	SBC	34.736	-120.134	urban
Tucson, AZ	TUC	32.180	-111.014	urban
Davis, CA	DAV	38.537	-121.746	urban
Saint George, UT	SGU	37.209	-112.980	urban
Denver, CO	DEN	39.722	-104.969	urban

Table S3.3. Population diversity summary statistics for 48 sampled locales.

Scale	City	Habitat	Locale		$\theta\pi$ (%)	θs (%)	TajD
			Abbreviation				
both	Albuquerque, NM	non-urban	SAN		0.04	0.27	-0.76
both	Albuquerque, NM	urban	ABQ		0.06	0.28	-2.60
fine-scale	Albuquerque, NM	urban	BEL		0.06	0.33	-3.65
fine-scale	Albuquerque, NM	urban	CHU		0.11	0.32	-2.78
fine-scale	Albuquerque, NM	urban	COR		0.06	0.33	-3.66
fine-scale	Albuquerque, NM	urban	DOW		0.11	0.32	-2.79
fine-scale	Albuquerque, NM	urban	EAA		0.06	0.34	-3.80
fine-scale	Albuquerque, NM	urban	GYM		0.06	0.31	-3.44
fine-scale	Albuquerque, NM	urban	MAN		0.06	0.31	-3.44
fine-scale	Albuquerque, NM	urban	OLD		0.06	0.42	-4.78
fine-scale	Albuquerque, NM	urban	OSO		0.06	0.31	-3.44
both	Las Vegas, NV	non-urban	RED		0.06	0.26	1.07
fine-scale	Las Vegas, NV	urban	ABC		0.22	0.32	-1.30
fine-scale	Las Vegas, NV	urban	ART		0.17	0.31	-1.90
fine-scale	Las Vegas, NV	urban	DIC		0.17	0.33	-2.18
fine-scale	Las Vegas, NV	urban	ERN		0.22	0.30	-1.09
fine-scale	Las Vegas, NV	urban	JAM		0.17	0.33	-2.18
fine-scale	Las Vegas, NV	urban	KIT		0.22	0.36	-1.84
fine-scale	Las Vegas, NV	urban	LUC		0.22	0.31	-1.17
both	Las Vegas, NV	urban	LVN		0.33	0.27	1.03
fine-scale	Las Vegas, NV	urban	PHL		0.22	0.33	-1.44
fine-scale	Las Vegas, NV	urban	RIC		0.04	0.36	-1.84
both	Phoenix, AZ	non-urban	PER		0.05	0.34	-3.64
fine-scale	Phoenix, AZ	urban	ANT		0.17	0.31	-1.96
fine-scale	Phoenix, AZ	urban	BRO		0.28	0.32	-3.90
fine-scale	Phoenix, AZ	urban	BUC		0.22	0.32	-1.30
fine-scale	Phoenix, AZ	urban	CHA		0.17	0.32	-2.14
fine-scale	Phoenix, AZ	urban	GCC		0.28	0.34	-0.88
fine-scale	Phoenix, AZ	urban	HRP		0.22	0.31	-1.18
both	Phoenix, AZ	urban	PHX		0.43	0.33	1.33
fine-scale	Phoenix, AZ	urban	TBD		0.03	0.36	-2.55
fine-scale	Phoenix, AZ	urban	TEM		0.03	0.32	-2.04
fine-scale	Phoenix, AZ	urban	WHY		0.17	0.27	2.55
broad-scale	-	non-urban	AGF		0.11	0.27	-3.33
broad-scale	-	non-urban	BBP		0.28	0.28	0.01
broad-scale	-	non-urban	BCC		0.17	0.27	-1.70
broad-scale	-	non-urban	GBP		0.22	0.29	-1.08
broad-scale	-	non-urban	JRN		0.28	0.26	0.18
broad-scale	-	non-urban	LCR		0.11	0.30	-2.86
broad-scale	-	non-urban	PNF		0.04	0.27	-0.75
broad-scale	-	urban	BLY		0.39	0.27	1.77
broad-scale	-	urban	DAV		0.44	0.36	-2.55
broad-scale	-	urban	DEN		0.66	0.30	5.33
broad-scale	-	urban	FLG		0.11	0.27	-2.46
broad-scale	-	urban	RNO		0.03	0.27	-1.62
broad-scale	-	urban	SBC		0.05	0.26	0.18
broad-scale	-	urban	SGU		0.24	0.27	16.40
broad-scale	-	urban	TUC		0.05	0.30	-0.35

Table S3.4. Fine-scale sample social network analysis parameter t-tests.

Comparison	cGD	closeness	betweenness	degree	centrality
Albuquerque x Phoenix	-1.68	1.79	2.61	0.89	-0.52
Albuquerque x Las Vegas	6.47	10.78	2.64	0.88	-1.64
Phoenix x Las Vegas	6.74	5.75	3.19	0.08	-0.94

Values represent student's t, bold italics are significant after Bonferroni correction.

Table S3.5. Fine-scale sample social network node-specific parameters.

City	Locale	closeness	betweenness	degree	centrality	Habitat
Albuquerque, NM	ABQ	0.07371	0	2	0.57941	urban
	BEL	0.14066	19	5	0.11921	urban
	CHU	0.10743	6	3	0.48827	urban
	COR	0.11900	0	3	0.02292	urban
	DOW	0.13028	16	3	0.26432	urban
	EAA	0.09713	1	3	0.62193	urban
	GYM	0.13659	15	3	0.05351	urban
	MAN	0.11748	1	3	0.01389	urban
	OLD	0.11461	7	3	0.62998	urban
	OSO	0.11087	0	2	0.01303	urban
Las Vegas, NV	SAN	0.07002	0	4	1	non-urban
	ABC	0.03903	3	4	1	urban
	ART	0.03833	2	3	0.75970	urban
	DIC	0.03914	5	4	0.98724	urban
	ERN	0.03831	3	3	0.53032	urban
	JAM	0.03828	2	3	0.74011	urban
	KIT	0.03764	1	2	0.52250	urban
	LUC	0.03896	5	3	0.59082	urban
	LVN	0.01007	0	1	0.00001	urban
	PHL	0.03943	4	4	0.97933	urban
Phoenix, AZ	RED	0.01007	0	1	0.00001	non-urban
	RIC	0.03824	2	2	0.30785	urban
	ANT	0.08933	0	1	0.00015	urban
	BRO	0.13690	30	4	0.01670	urban
	BUC	0.11620	14	2	0.13750	urban
	CHA	0.11538	9	3	0.00163	urban
	GCC	0.11444	9	3	0.00168	urban
	HRP	0.08988	0	1	0.00014	urban
	PER	0.05616	0	3	1	non-urban
	TBD	0.11116	13	2	0.03151	urban
Phoenix, AZ	TEM	0.08760	9	3	0.74286	urban
	PHX	0.04924	0	2	0.87681	urban
	WHY	0.09329	6	2	0.37036	urban

Table S3.6. Social network node-specific parameters for 48 locales.

Locale	closeness	betweenness	degree	centrality	Habitat
ABC	0.010	33	8	0.015	urban
ABQ	0.005	2	3	0.132	urban
AGF	0.004	2	4	0.570	non-urban
ANT	0.010	77	7	0.030	urban
ART	0.010	76	9	0.098	urban
BBP	0.009	94	9	0.047	non-urban
BCC	0.005	0	6	1.000	non-urban
BEL	0.007	5	4	0.001	urban
BLY	0.009	33	10	0.195	urban
BRO	0.010	36	8	0.029	urban
BUC	0.010	122	10	0.058	urban
CHA	0.009	24	6	0.054	urban
CHU	0.009	124	8	0.020	urban
COR	0.006	4	2	0.001	urban
DAV	0.008	3	7	0.028	urban
DEN	0.003	2	4	0.990	urban
DIC	0.009	11	9	0.100	urban
DOW	0.008	68	8	0.048	urban
EAA	0.007	28	6	0.010	urban
ERN	0.010	127	11	0.025	urban
FLG	0.006	13	4	0.123	urban
GBP	0.004	0	2	0.135	non-urban
GCC	0.010	61	10	0.193	urban
GYM	0.008	18	6	0.004	urban
HRP	0.009	35	9	0.067	urban
JAM	0.009	18	8	0.013	urban
JRN	0.009	16	9	0.026	non-urban
KIT	0.009	7	8	0.029	urban
LCR	0.004	2	5	0.923	non-urban
LUC	0.010	86	10	0.016	urban
LVN	0.010	53	11	0.036	urban
MAN	0.007	21	5	0.001	urban
OLD	0.008	38	6	0.010	urban
PER	0.005	2	4	0.524	non-urban
PHL	0.009	24	9	0.034	urban
PHX	0.006	2	7	0.673	urban
PNF	0.004	40	2	0.139	non-urban
RED	0.009	35	7	0.017	non-urban
RIC	0.009	10	9	0.015	urban
RNO	0.009	31	11	0.014	urban
SAN	0.009	46	9	0.021	non-urban
SBC	0.008	7	8	0.016	urban
SGU	0.009	12	9	0.021	urban
TBD	0.010	50	10	0.040	urban
TEM	0.007	109	7	0.411	urban
TUC	0.009	17	7	0.011	urban
WHY	0.009	67	9	0.102	urban

Chapter 4: DIFFERENTIAL EXPRESSION BETWEEN URBAN AND NON-URBAN WESTERN BLACK WIDOW SPIDERS

Introduction

By 2050, two-thirds of the human population are predicted to live in urban areas (United Nations, 2014). In the United States, the most rapid urban growth in the last 30 years has taken place in the Western U.S. (US Census, 2010). This urban expansion eliminates natural pristine habitats, the fragmentation of which reduces genetic connectivity among most populations, and reduces local and global biodiversity (McKinney, 2002; Keyghobadi, 2007). Conservation of species diversity is seen as a cost to land and resource development profit; however, the loss of endemic biodiversity also has direct negative impact on ecosystem services that provide for human survival (Wu, 2008). Conservation efforts need to use an evolutionary perspective to determine how, not whether, species locally adapt to these novel landscapes that we have generated.

One overlooked perspective in this urban eco-evolutionary model is that of “urban adapters” (Blair 1996), a term given to species that have increased population densities and show phenotypic modifications in urban compared to their natural, or non-urban habitats. Some have proposed the urban facilitation model of gene flow, which suggests that this adaptation is facilitated by gene flow among previously isolated populations via human-mediated transport or

the provision of alternate habitat patches (Hoderegger & Di Giulio, 2010; Crispo et al., 2011). While most research in urban areas has focused on the urban fragmentation model of gene flow that describes landscape fragmentation and declining species diversity, the urban adapter model and its potential to facilitate population persistence has largely been ignored.

The Western black widow spider, *Latrodectus hesperus*, is considered an urban adapter (Johnson et al., 2012; Trubl et al., 2012), and is an excellent test case for understanding how evolutionary change occurs in urbanized environments. This species is found across the Western U.S., primarily in the desert landscape, within and outside of urban areas. In natural habitats, *L. hesperus* feed on diverse prey, including insects, crustaceans, and small lizards that become trapped in their webs (Salomon, 20017), but they experience reduced prey diversity in urban areas (predominantly crickets and cockroaches, Trubl et al., 2012). In comparison to non-urban spiders, urban spiders also make smaller webs, and have higher population densities, but females have significantly lower body mass and fewer eggs per egg sac (Johnson et al., 2012). Prey capture by spiders involves two protein-based secretions, venom and silk. *L. hesperus* venoms are composed of a wide variety of toxic proteins used to immobilize prey, including multiple latrotoxins with variable phyletic specificity (Haney et al., 2014). Black widows also use multiple protein-based silk fiber types and glues to capture prey in webs and physically wrap them (Foelix, 2011). The abundance or identity of venom and silk proteins can vary during an individual's lifetime, or over evolutionary timescales, in response to dietary changes (Tso et al, 2005; Gibbs et al., 2011; Morgenstern & King, 2013), where interspecific associations between diet and both silk and venom composition have been linked to niche adaptation (Daltry et al., 1996; Binford, 2001; Sanz et al., 2006; Remigio & Duda, 2008; Zevenbergen et al., 2008; Boutry & Blackledge, 2008; Blamires et al., 2010, 2012).

These life history and behavioral observations predict that a suite of traits involved in diet and metabolism, venom and silk production, and fertility have been recently altered by urban selective pressures. For example, the reduced diversity, but relatively higher abundance of prey in urban environments suggests that urban spiders may not require such complex venoms or web architecture. In addition, relaxed predation, increased population densities, and more abundant resources in urban environments may also select for altered egg development both in size and number. If this is the case, we may expect to see signatures of phenotypic variation associated with these specific traits, specifically in differential gene expression in contrasts of urban and non-urban populations. As there have recently been *L. hesperus* transcriptome analyses documenting hundreds of transcripts that exhibit tissue-specific expression (Clarke et al., 2014, 2015; Haney et al., 2014), there is a valuable resources already available with which to test these hypotheses. Characterizing this urban adapter model requires bringing together genetic connectivity results with phenotypic trait analyses to shed light on the potential signatures of urban adaptation.

Given the polarizing outcomes for conservation priorities predicted by models of urban fragmentation vs. facilitation, population genetic studies targeting urban adapters on broad geographic scales are necessary to document the impact of continued urban growth (McDonnell & Hahs 2015). In addressing urban patterns of gene flow, we have conducted the only study of Western black widow population genetic connectivity. We sampled thousands of genomewide mitochondrial and nuclear SNPs from 11 urban and 10 non-urban locales and found urban-specific patterns of higher within-locale genetic diversity, lower between-locale genetic differentiation, and higher genetic connectivity, all of which are predicted by the urban facilitation model of gene flow. One interesting find was that although urbanization appears to

facilitate gene flow, even among non-urban areas, that not all cities are highly connected in the population network, with specific urban hubs driving gene flow among both urban and historically isolated non-urban locales. To further investigate how this higher gene flow on the urban landscape impacts genetic diversity and gene flow in the urban network, we analysed 1.9 million genomewide SNPs, with an additional 30 urban locales from three Southwestern cities. As urban population genetic studies focus on single urban vs non-urban contrasts or within-urban locale diversity, this second study served as the first to sample multiple pairs of urban and non-urban locales, with fine-scale sampling within urban locales, to test hypotheses of how urbanization uniquely impacts population diversity across multiple spatial scales. The primary observation is that urban areas have significantly different patterns of genetic connectivity to the overall urban network, and this result also generates contrasting patterns of within- and between-city genetic diversity. The most interesting implication here is that not all cities can be assumed to be “replicates” of the urbanization process and its effects on the eco-evolutionary changes within them. Therefore, given the patterns of heterogeneity in gene flow found within and among urban and non-urban populations, it is likely that there is heterogeneity in how phenotypes have evolved within and among urban and non-urban locales in response to urbanization.

Here, we will characterize differential gene expression in tissues associated with urban phenotypes of the Western black widow spider. Specifically, we will examine differences in gene expression between urban and non-urban populations from the cephalothorax (metabolic processes), ovary (fertilization and egg development), and silk glands (web architecture, prey capture, egg protection) to test for the presence of an “urban ecotype” (Kritinic et al., 2012; Schapira & Boutsika, 2012). We use our unique sampling of multiple pairs of urban and non-urban populations to test the model of an urban ecotype, which we predict would be the result of

an overall phenotype that shows consistent differences in gene expression patterns between these multiple pairs of locales. This model is in contrast to one that predicts urban locales are sufficiently different from each other in their gene expression responses as a result of demographic history and connectivity in the urban network, as well as the different selective pressures that exist among urban areas on the landscape.

Methods

Sampling

In September of 2016, we collected 10 live adult female spiders from each of 3 urban and 3 non-urban paired locales: Phoenix (AZ), Las Vegas (NV), and Denver (CO) (Fig. 1), for a total of 60 individuals. Spiders were transferred to -80 C within 48 hours of collection.

These three cities have each experienced recent human population and geographic size expansion. However, these three cities also have varying urbanization histories in the Southwest with respect to colonization time, geographic size, and human population size (US Census 2014), with which to contrast the impact of urbanization on genetic connectivity within urban areas (Fig. 2). Denver was founded in 1858 oldest of the three cities founded in 1858, and covers 402 km² with a current metropolitan area human population of 2.8 million. Las Vegas is the most recently founded in 1905, covers 1600 km², and is one of the fastest growing metropolitan areas with a population of 1.9 million. Phoenix is the largest of the three, having been founded in 1881, covers 235000 km² and has a population of 4.5 million as the 12th largest metropolitan area in the US. Phoenix and Las Vegas have been two of the fastest growing metropolitan areas, expanding over 45% in the last 30 years (US Census 2014). Phoenix and Las Vegas are also in

arid, desert climates (Sonoran and Mojave deserts, respectively) with similarly high summer temperatures reaching 43°C. In contrast, Denver is located at the highest elevation of the three cities, as well as being the highest major city in the United States, at 1609 meters above sea level with a semi-arid climate, but still experiences significant precipitation and much cooler temperatures.

RNA-seq Data collection

The cephalothorax, ovaries, and silk glands were dissected from each of the 60 individuals, after which total RNA was isolated from the tissue samples in TRIzol (Invitrogen), purified using the RNeasy kit (Qiagen), and any contaminating DNA was removed with Turbo DNase (Ambion). RNA yield and purity were analyzed using an Agilent 2100 Bioanalyzer (Santa Clara, USA). The cDNA library for each individual tissue sample (n=180) was generated with the TruSeq RNA Sample Preparation Kit (Illumina), followed by paired-end, 150 bp sequencing in single lanes of HiSeq 4000 (Illumina) by Novogene. The reads were cleaned using *Trim Galore!* (version 0.3.7) with FastQC (version 0.11.2) that removed Illumina adaptors and low quality reads.

Differential Expression Analyses:

We used Bowtie2 (Version 2.2.6) to align the sequence reads to a previously published *Latrodectus hesperus* transcriptome that covered 28 individual-based libraries (Haney et al 2014), followed by estimation of expected read counts per transcript with RSEM (version 1.2.19, Li & Dewey, 2011), which accounts for the possibility of a single read mapping to multiple transcripts. Read counts for each individual in each tissue type were used to determine

differential expression (DE) using a general linear model (GLM) in edgeR (Robinson et al., 2010; Ritchie et al., 2015). This DE analysis was used to contrast (1) the pair of urban and non-urban locales for each of the three geographic locations of Phoenix, Las Vegas, and Denver, (2) the three urban locales from the three geographic locations, and (3) the three non-urban locales from the three geographic locations. In addition, to compare differences in the pattern of differential expression among these comparisons, we used a Mann-Whitney U statistic as a non-parametric test with the assumption that the differences in gene expression log-fold change do not follow a normal distribution and have unequal variance.

Results

Sequencing and de novo assembly

Transcriptomes were successfully generated from 59 cephalothorax, 58 ovary, and 52 silk gland cDNA libraries. For each of the 169 libraries, 35M-63M raw sequence reads were collected, and 98% of clean reads were retained after pre-processing (e.g., adaptor removal, quality trimming, “N” removal).

Differentially expressed transcripts

To identify differentially expressed transcripts between urban and non-urban, and among 3 geographic regions, cephalothorax, ovary, and silk tissues were compared. For each tissue type individuals’ overall gene expression profiles cluster by geographic location, regardless of habitat

origin (urban vs non-urban). While this geographic clustering was the case, Denver samples clustered independently of a cluster of both Phoenix and Las Vegas samples together (Figure 2).

Tissue-specific DE between urban and non-urban pairs

For the cephalothorax, there were 99 significant up- and 225 significant down-regulated gene isoforms in Phoenix, 33 significant up- and 35 significant down-regulated gene isoforms in Las Vegas, and 166 significant up- and 174 significant down-regulated gene isoforms in Denver (Figure 3). For the ovary, there were 87 significant up- and 49 significant down-regulated gene isoforms in Phoenix, 197 significant up- and 129 significant down-regulated gene isoforms in Las Vegas, and 230 significant up- and 246 significant down-regulated gene isoforms in Denver (Figure 3). For the silk glands, there were 15 significant up- and 29 significant down-regulated gene isoforms in Phoenix, 4 significant up- and 4 significant down-regulated gene isoforms in Las Vegas, and 14 significant up- and 3 significant down-regulated gene isoforms in Denver (Figure 3). Table 1 presents the top 20 significantly up- and down-regulated gene isoforms for each of the cephalothorax, ovary, and silk gland tissues across the three geographic locations.

Tissue-specific DE among urban and non-urban locales

For the cephalothorax tissue, for Phoenix compared to Las Vegas, regardless of habitat type, there are less differentially expressed genes than when Denver is compared to Phoenix or Las Vegas (Figure 4). Similarly for the ovary tissue, for Phoenix compared to Las Vegas, regardless of habitat type, there are less differentially expressed genes than when Denver is compared to Phoenix or Las Vegas (Figure 5). Finally, for silk glands, again, for Phoenix compared to Las

Vegas, regardless of habitat type, there are less differentially expressed genes than Denver compared to Phoenix or Las Vegas (Figure 6). The least differential expression in silk occurs between Phoenix and Las Vegas urban samples. For all three tissue types, the top 20 most significantly differentially expressed genes have higher fold changes (both for up- and down-regulated genes) when comparing Denver to either Phoenix or Las Vegas, regardless of habitat type (Table 2). In fact, when comparing the patterns of log fold changes in statistically significant differentially expressed gene isoforms in our comparisons of urban to urban and non-urban to non-urban, we find statistically significant differences across geographic locations, such that Denver samples, regardless of habitat type, are significantly differentiated from both Phoenix and Las Vegas samples across all tissue types (Table 3, Mann-Whitney U tests).

Discussion

We utilized RNA-seq to investigate the phenotypic variation associated with differential gene expression in genes associated with urban phenotypes of the Western black widow spider, *Latrodectus hesperus*. Specifically, we tested the hypothesis that there may be differences in differential expression between urban and non-urban areas for different tissue types and that these genes are related to phenotypes we have previously identified among urban and non-urban locales. Our main finding is that there is significant differential expression in each tissue type of cephalothorax (metabolic processes), ovary (fertilization and egg development), and silk glands (web architecture, prey capture, egg protection) that is observed between urban and non-urban locales, among both urban and among non-urban locales, as well as specific to geographic locations independent of urban or non-urban habitat. We discuss these results in light of the

hypothesis that urban locales may consistently select for an urban ecotype, and the assumption that cities are replicates in urban eco-evolutionary research.

Our previous work on the Western black widow spider as an urban pest model documented population genetic signatures consistent with an urban facilitation model of gene flow on both a broad- and fine-scale (Chapters 2 & 3). Previously, our group has documented several urban phenotypes in the Western black widow spider, including dense aggregations and increased egg sac production compared to non-urban population (Johnson et al., 2014; Trubl et al., 2012). One of the predictions of the urban facilitation model that is supported by our previous work is that an "urban ecotype" could potentially sweep across urban areas (Krtinic et al., 2012; Shapira & Boutsika, 2012) dispersing these urban phenotypes to all locales; however, this would only be the case if urban areas had consistent and similar local selective pressures. Although it is the case that we find some gene expression patterns that are shared among urban areas, the majority of our results are consistent with patterns specific to individual urban and non-urban areas, which may be explained by multiple demographic and selective pressures.

One consistent pattern that we observed is that transcription factors are up-regulated in the cephalothorax in all three urban areas, which is consistent with our initial hypotheses related to metabolism, fertility, and web architecture and prey availability. This upregulation in transcription factors within the cephalothorax tissue may be increasing a suite of genes involved in metabolism given the increased food availability and consumption in urban compared to non-urban habitats. In the ovaries, there are significantly up-regulated gene isoforms involved in cellular transport that may be indicative of cellular proliferation of eggs. For example, in Las Vegas "zinc transporter ZIP9" is three times more expressed in urban compared to non-urban locales and is involved in cell growth and proliferation (Taniguchi et al., 2003), which is

consistent with proliferation of new eggs in the ovary that increases overall fertility. There is an up-regulation trend in silk proteins across each of our urban to non-urban comparisons. For example, “egg case silk protein-1” is 8.5 times higher expressed in Denver urban than non-urban samples. This upregulation of genes that are involved in cell proliferation and growth in the ovaries, and the egg case silk protein in the silk glands may be related to previous observations of increased egg production in urban locales, and was predicted by us, as the potential for increased fertility would be high with increased population densities of black widow spiders in urban locales

While we found similar functional groups of genes up-regulated in urban compared to non-urban samples, there is no significant overlap in the specific gene isoforms that are differentially expressed. For example, “succinate dehydrogenase [ubiquinone] flavoprotein subunit”, which is involved in the citric acid cycle and the electron transport chain, is 6 times more expressed in Denver urban compared to Denver non-urban individuals. This specific gene isoform is not found to be significantly differentially expressed in contrasts between urban and non-urban populations in Las Vegas and Phoenix. Therefore, while certain genes are shared across urban areas, and even across geographic regions, there are genes that are differentially expressed specific to urban locales. This phenotypic variation mirrors that of genetic variation and connectivity that our previous work has found. For example, there is significant variation in genetic connectivity across urban and non-urban locales that reflects urban facilitated gene flow across urban locales, where a few urban locales act as hubs of genetic connectivity, and into a few non-urban locales (Chapter 2; Miles et al. 2018). Additionally, we have found that genetic variation on a fine-scale varies between cities and that some of the locales within cities drive overall connectivity on the landscape, such that there is both shared variation among urban

locales and certain urban locales are less connected to the genetic connectivity network (Chapter 3). These patterns of genetic connectivity and phenotypic variation support the conclusion that there can be both shared and locale specific variation in spider populations.

Given the differences that we see between our urban and non-urban comparisons, the genes found differentially expressed in each of the three cities are simply a reflection of the individual contrasts with their non-urban counterparts. Previously, we found that non-urban populations reflect ancestral genetic diversity in that they are significantly more differentiated from each other on the landscape due to the sedentary nature of female black widow spiders (Chapter 2; Miles et al. 2018). Thus, it is likely that we would find differences among our non-urban populations here not only because of this historical demography, but also due to the differences in environments between the sampled areas. Here, we found that there are consistently different patterns of expression among each of our three cities, but some of the non-urban environments are more similar to each other than to non-urban environments. For example, in the cephalothorax, one of the most significantly up-regulated genes in Denver individuals was associated with multiple variants of "nose resistant to fluoxetine protein 6", which is responsible for the uptake of lipids and transporting lipids to the reproductive tract (Choy et al., 2006; Dzitoyeva et al., 2003). Additionally, we identified an up-regulation in Denver individuals for "Long-chain-fatty-acid--CoA ligase 6" which is used in fatty acid metabolism (Dai et al., 2015). Both of these genes are related to fat metabolism, and their upregulation is common for high-altitude populations (Kennedy et al., 2001; Simonson et al., 2010; Palmer & Clegg, 2014). Denver is not only at a significantly high elevation in general, it is also higher elevation than both Phoenix and Las Vegas (USGS, 2018). In ovary tissue, we identified down-regulation in Denver for "THO complex subunit", which is involved in cell

proliferation and required for proper export of heat-shock mRNAs under heat stress (El Bounkari et al., 2009; Yu et al., 2012). Given the higher elevation of Denver compared to Las Vegas and Phoenix, temperatures and their variance over the year are significantly higher and lower, respectively, which could explain the reduced expression in genes related to heat shock. In silk glands, we find “ATPase family AAA domain-containing protein 3” is significantly up-regulated in Denver compared to Phoenix and Las Vegas. This gene acts as a molecular chaperone in many cellular activities, such as membrane fusion, cell-cycle regulation, and stress response (Bolbaatar et al., 2002). This up-regulation is likely linked to increasing productivity of silk strands required to capture prey in the non-urban, colder and more variably climatic habitat where fewer prey types and numbers are available. Overall, when comparing differential expression patterns between non-urban locales, much of the differences are related to environmental features associated with their geographic locations.

Interestingly, we find that there are also significant differences in the pattern of gene expression across urban locales. There are more differentially expressed genes, and the most differentially expressed genes are shared when comparing Denver to either Phoenix or Las Vegas, regardless of habitat type. For example, in the cephalothorax, there are 2129 significantly differentially expressed gene isoforms between Phoenix and Las Vegas urban locales but there are 6183 and 5455 significantly differentially expressed gene isoforms between Denver and Phoenix and between Denver and Las Vegas urban locales, respectively. Additionally, each of the genes noted previously that are shared across non-urban samples, are also shared across urban samples. Currently, much of the urban-eco-evolutionary literature assesses that urban areas are likely replicates of each other (Chapter 1; Alberti, 2015). However, given that the urban locales here have significant differential expression between them that is largely due to local

environmental differences, and our previous work has shown significant differences in genetic connectivity between urban locales (Chapter 2; Miles et al., 2018; Chapter 3), cities may not be the replicates that were previously assumed. Therefore, while we find that several species are able to thrive in multiple urban areas, they may be responding to these multiple urban environments in different ways.

Additionally, these results indicate that there is hierarchical variation in gene expression, such that geography plays the strongest role in patterns of differential expression, followed by habitat type, and finally by comparisons of urban and non-urban pairs. For example, “aqueous glue droplet peptide” is significantly down-regulated in Phoenix, compared to Las Vegas in both urban and non-urban comparisons. This gene is involved in both web-building to adhere silk to a substrate and is used as part of a defense mechanism (Foelix, 2014). Therefore, there are significant differences in web building and defense among Phoenix and Las Vegas. Interestingly, the aqueous glue droplet protein is also significantly up-regulated in urban compared to non-urban spiders in Las Vegas. These differential patterns at both the geographic and local scale imply that Western black widow spiders not only have to respond to different environments, but they also have to respond to different environments associated with different cities.

Conclusion

The existence of consistencies and differences in gene expression profiles between urban and non-urban Western black widow spiders, suggests they are shared by both adaptive and non-adaptive processes. While urban eco-evolutionary studies have been limited by considering only a single pair of urban and non-urban populations, the current study has explored differences at

both the geographic environmental level and local conditions that vary between urban and non-urban pairs. Several of the up-regulated gene isoforms were in accordance with previous ecological and behavioral studies in black widow spiders. For example, many genes linked to fertility were expressed at higher levels in urban compared to non-urban populations, suggesting that there is an overall increase in egg production overall, even though black widow spiders produce less eggs per egg sac, they produce significantly more egg sacs than non-urban spiders (Johnson et al., 2014). However, many of the differentially expressed gene isoforms between urban and non-urban pairs are locale specific. Thus, while we find evidence to support the phenotypic differences identified in previous studies, these phenotypes may not be consistent across all urban areas. Indeed, we have found that several urban locales can act as hubs of genetic connectivity across a broad-scale (Chapter 2; Miles et al., 2018) and that even on a fine-scale, genetic connectivity varies within cities. Therefore, these results imply that not all cities are created equal with respect to demographic and gene flow patterns, but also with phenotypic patterns. Future studies should aim to address the fitness consequences related to this variation in expression to determine the role that these varying urban and non-urban environments have in shaping adaptation.

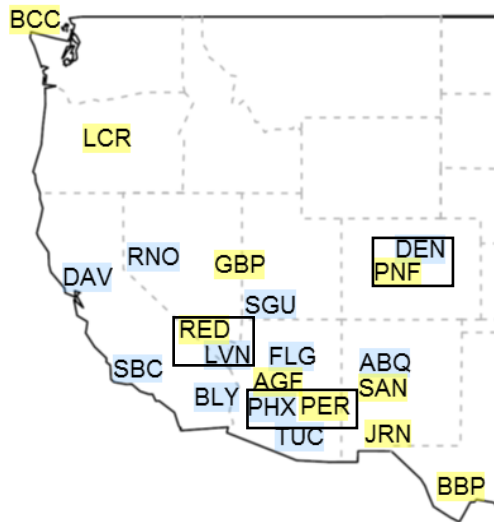


Figure 4.1. Geographic distribution of the broad-scale sampled locales of the Western black widow spider across the Western U.S. Highlighted locales in blue and yellow reflect urban and non-urban samples, respectively. Boxed locales are the paired urban and non-urban samples for Denver, CO, Las Vegas, NV, and Phoenix, AZ.

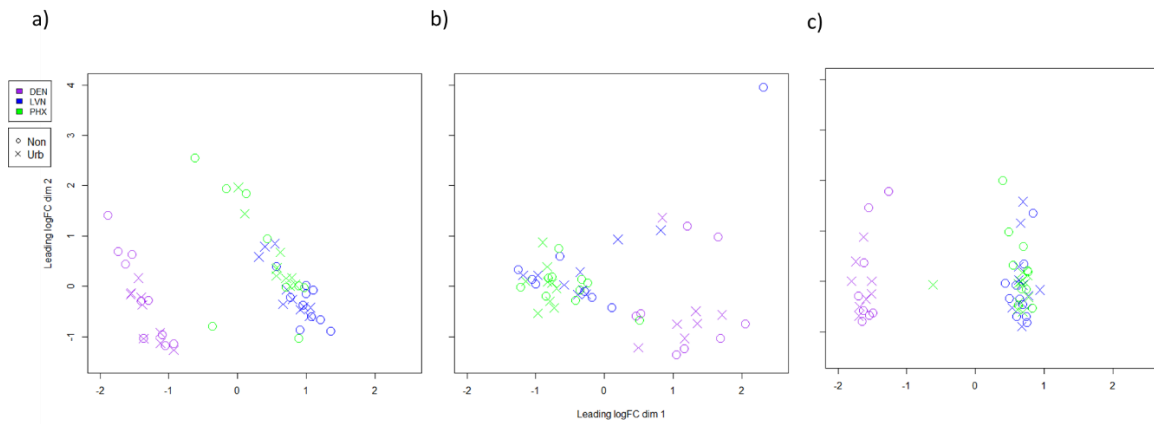


Figure 4.2. Multidimensional scaling plot of the overall differential expression for each individual for a) cephalothorax, b) ovary, and c) silk tissue transcripts. Highlighted individuals reflect Denver (“purple”), Las Vegas (“blue”), and Phoenix (“green”), respectively. Shapes represent urban (“x”) and non-urban (“o”) samples.

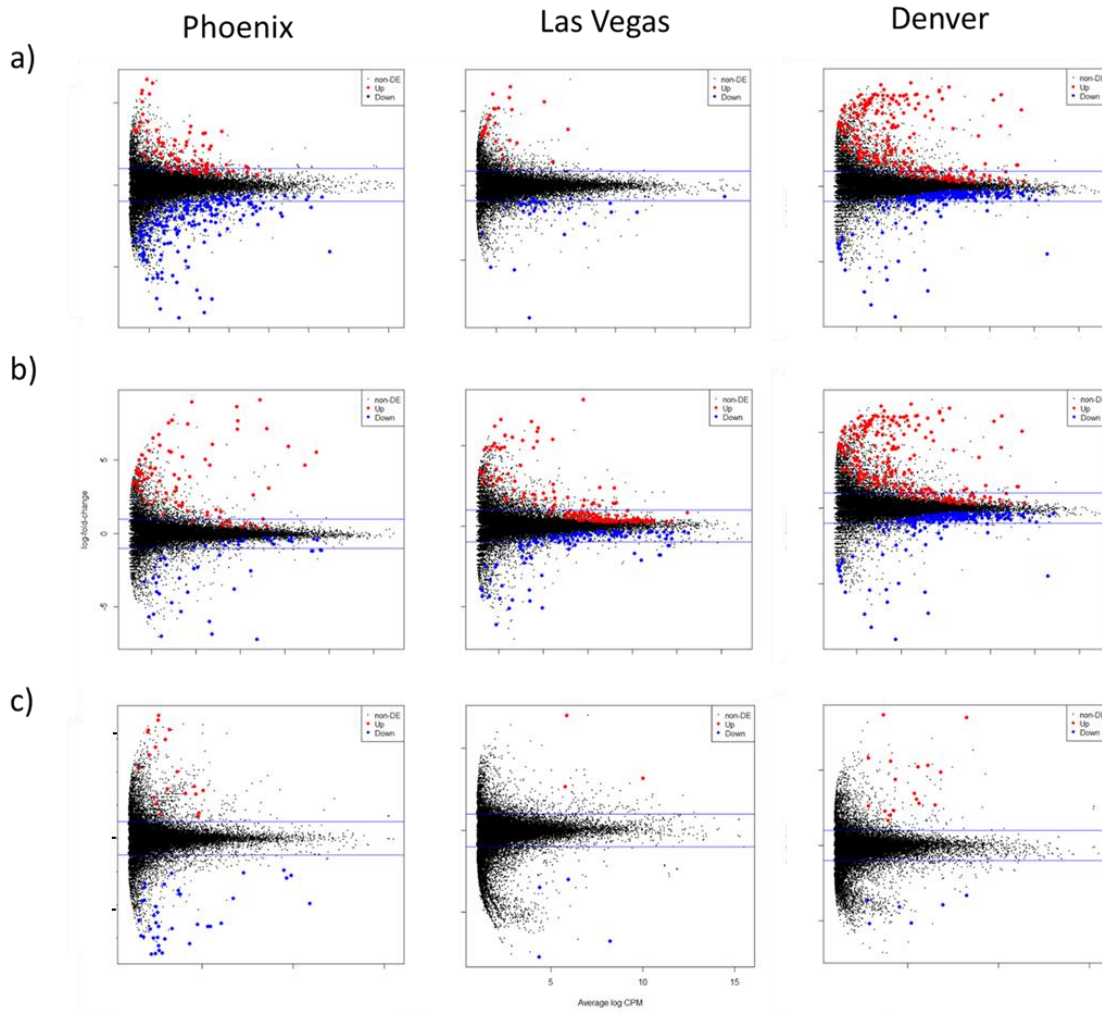


Figure 4.3. Volcano plot of the differential expression between urban and non-urban locales for each of a) cephalothorax, b) ovary, and c) silk tissue transcripts. Highlighted within each plot are gene isoforms that are significantly up-regulated (“red”) and significantly down-regulated (“blue”), with bars reflecting one log fold change in expression. Each row reflects the comparison between urban and non-urban pairs in Phoenix, Las Vegas, and Denver, respectively.

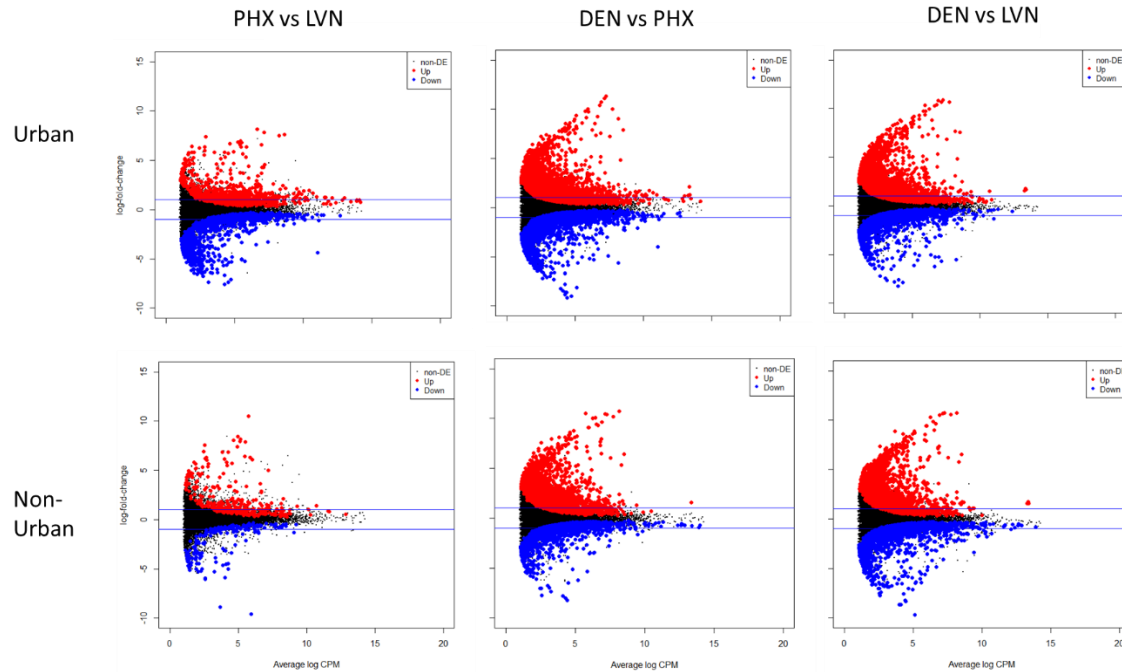


Figure 4.4. Volcano plot of the differential expression in the cephalothorax. Highlighted within each plot are gene isoforms that are significantly up-regulated (“red”) and significantly down-regulated (“blue”), with bars reflecting one log fold change in expression. Each row reflects comparisons between urban and non-urban locales, respectively. Each column reflects geographic area comparisons of Phoenix vs. Las Vegas, Denver vs Phoenix, and Denver vs Las Vegas, respectively.

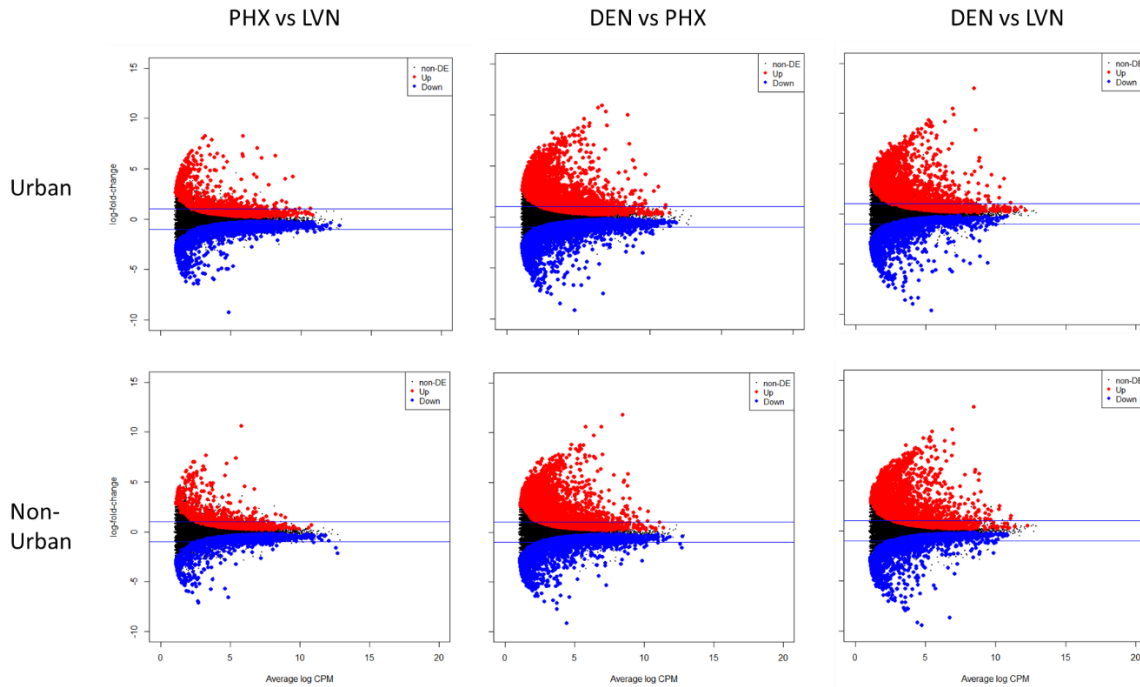


Figure 4.5. Volcano plot of the differential expression in the ovary. Highlighted within each plot are gene isoforms that are significantly up-regulated (“red”) and significantly down-regulated (“blue”), with bars reflecting one log fold change in expression. Each row reflects comparisons between urban and non-urban locales, respectively. Each column reflects geographic area comparisons of Phoenix vs. Las Vegas, Denver vs Phoenix, and Denver vs Las Vegas, respectively.

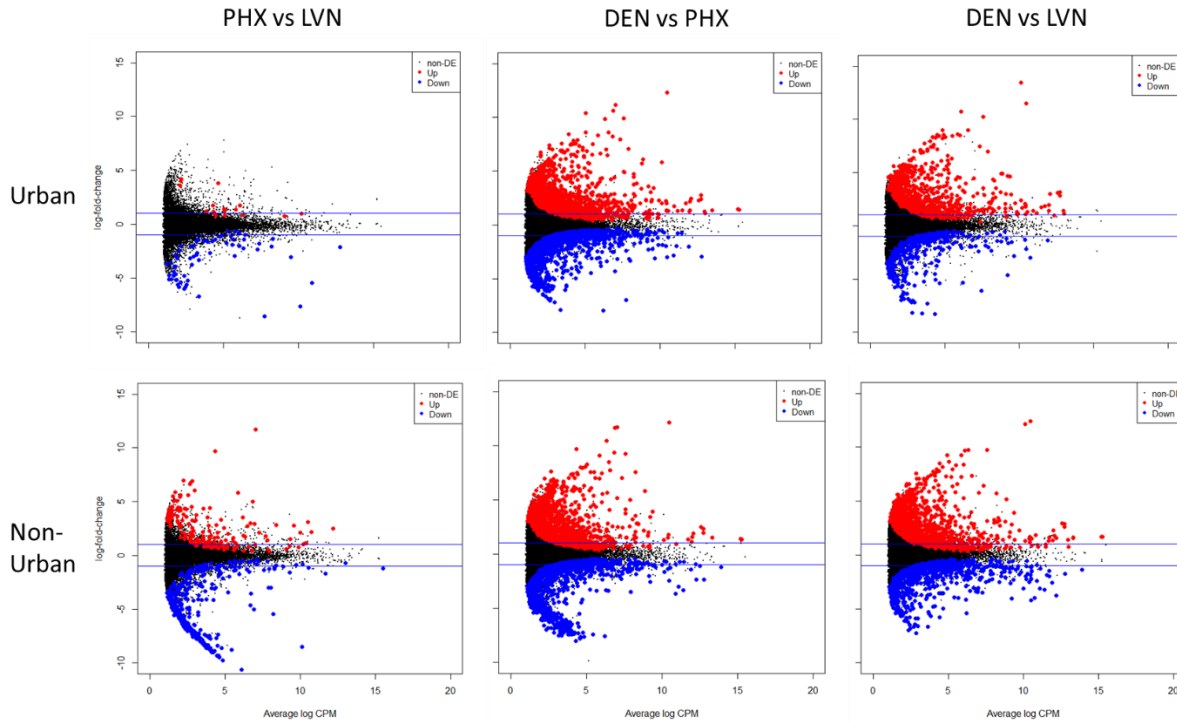


Figure 4.6. Volcano plot of the differential expression in the silk. Highlighted within each plot are gene isoforms that are significantly up-regulated (“red”) and significantly down-regulated (“blue”), with bars reflecting one log fold change in expression. Each row reflects comparisons between urban and non-urban locales, respectively. Each column reflects geographic area comparisons of Phoenix vs. Las Vegas, Denver vs Phoenix, and Denver vs Las Vegas, respectively.

Table 4.1 Top 20 differentially expressed gene isoforms for non-urban compared to urban locales in Phoenix, Las Vegas, and Denver.

Tissue	Comparison	Accession	BLAST identity	Log		P-Value	FDR
				FC	CPM		
Cephalothorax	PHX_UvN	XP_015905503.1	PREDICTED: protein transport protein Sec24C-like	2.40	3.84	4.16E-10	4.18E-06
		KFM68364.1	Transposable element Tcb1 transposase	2.39	4.69	8.53E-08	2.14E-04
		KFM62172.1	Histone-arginine methyltransferase CARMER	1.50	4.14	8.05E-10	5.77E-06
		XP_003396019.1	PREDICTED: septin-2	1.34	4.91	3.89E-08	1.22E-04
		XP_015928295.1	PREDICTED: 60S ribosomal protein L13a-like	-1.17	4.86	6.02E-08	1.68E-04
		XM_016067859	PREDICTED: gamma-soluble NSF attachment protein-like	-1.25	4.79	1.32E-08	6.16E-05
		ADV40094.1	ribosomal protein L32 isoform B	-1.32	4.96	1.58E-08	6.16E-05
		XP_015930827.1	PREDICTED: glutathione peroxidase-like isoform	-1.51	5.34	5.09E-10	4.25E-06
		OBS80197.1	hypothetical protein A6R68_21600	-1.51	4.37	7.29E-09	4.06E-05
		HQ005863	clone CV93 putative 60S ribosomal protein L5	-1.79	5.13	8.57E-11	1.08E-06
		XP_013775155.1	PREDICTED: 60S ribosomal protein L11	-1.79	7.18	4.99E-09	3.13E-05
		XM_016050316	PREDICTED: ras-related protein rab7	-1.84	5.15	7.84E-08	2.07E-04
		AII97591.1	BLTX194	-1.87	6.72	7.44E-12	1.24E-07
		ADV40088.1	nucleoside diphosphate kinase	-1.89	5.76	3.79E-08	1.22E-04
		NA		-2.30	5.32	5.69E-08	1.68E-04
		XM_024094244	PREDICTED: keratin, type I cytoskeletal 9	-2.69	3.31	1.60E-08	6.16E-05
		ADV40094.1	ribosomal protein L32 isoform B	-2.80	3.48	1.48E-08	6.16E-05
		KFM61120.1	Transitional endoplasmic reticulum ATPase TER94	-4.66	3.81	2.60E-08	9.32E-05
		NA		-6.94	2.36	3.38E-13	1.50E-08
		KFM62591.1	hypothetical protein X975_04353	-8.11	3.48	5.98E-13	1.50E-08

LVN_UvN	KFM61963.1	Lysosome-associated membrane glycoprotein 5	5.92	2.65	3.89E-07	3.13E-03
	XM_003900080	PREDICTED: CDC42 small effector 2	5.18	1.86	1.19E-09	5.99E-05
		PREDICTED: general transcription factor II-I repeat				
	XP_017758189.1	domain-containing protein 2-like	5.13	1.94	8.85E-08	8.88E-04
	XM_015753358	PREDICTED: DNA topoisomerase 1	4.71	1.68	9.52E-09	2.39E-04
	XP_015921979.1	PREDICTED: uncharacterized protein	4.48	1.56	4.28E-08	7.16E-04
	NA		4.00	1.45	5.03E-07	3.15E-03
	XP_015931144.1	PREDICTED: uncharacterized protein	3.93	1.44	1.19E-06	4.27E-03
	XP_015925596.1	PREDICTED: uncharacterized protein	3.93	1.43	8.78E-07	4.20E-03
	KFM71996.1	Speckle-type POZ protein B	3.84	1.45	8.66E-07	4.20E-03
		PREDICTED: mitochondrial 2-oxoglutarate/malate				
	XP_015905468.1	carrier protein-like	3.78	5.61	3.96E-06	1.05E-02
	KFM62227.1	hypothetical protein X975_10841	3.06	2.68	1.84E-06	5.44E-03
	NA		2.46	2.25	9.48E-07	4.20E-03
	XP_015928112.1	PREDICTED: ceramide synthase 1-like isoform X1	-1.10	4.14	1.09E-06	4.20E-03
	BAD91058.2	Pt1-cadherin	-1.19	4.46	4.37E-07	3.13E-03
		putative sodium-coupled neutral amino acid transporter				
	KFM80688.1	7	-1.50	2.50	4.47E-06	1.12E-02
	XM_016062946	PREDICTED: sal-like protein 1	-1.70	4.45	2.03E-06	5.65E-03
	XP_015919728.1	PREDICTED: laminin subunit alpha-like	-1.72	3.34	1.59E-06	4.98E-03
	JX978171	clone 28K13 aciniform spidroin 1 (AcSp1) gene	-1.77	9.08	1.04E-06	4.20E-03
	JX978171	clone 28K13 aciniform spidroin 1 (AcSp1) gene	-1.77	7.79	1.35E-06	4.53E-03
	XP_015919728.1	PREDICTED: laminin subunit alpha-like	-1.87	3.69	8.34E-08	8.88E-04
DEN_UvN	XM_004493118	PREDICTED: DNA ligase 1	6.96	4.13	4.02E-11	1.01E-06
	AAZ15706.1	egg case fibroin	6.92	3.21	6.97E-08	2.06E-04

			PREDICTED: succinate dehydrogenase [ubiquinone]				
		XP_015785335.1	flavoprotein subunit, mitochondrial	6.50	6.80	2.02E-16	1.02E-11
		WP_051068643.1	hypothetical protein	6.46	2.28	3.15E-08	1.49E-04
		FJ973621	pyriform spidroin 1 mRNA	6.42	2.32	1.24E-09	1.56E-05
		AAZ15706.1	egg case fibroin	6.38	3.26	1.33E-08	7.39E-05
		EF595245	clone 46B18 major ampullate spidroin 2 (MaSp2)	6.32	4.25	6.38E-08	2.00E-04
		XM_021866677	PREDICTED: basic proline-rich protein-like	6.12	2.21	6.98E-10	1.17E-05
		AMK48676.1	aggregate spidroin 1	6.01	2.22	1.31E-08	7.39E-05
		AFP57565.1	aggregate gland silk factor 1	5.87	1.97	4.81E-08	1.86E-04
		ADV40352.1	hypothetical protein	5.63	1.98	5.82E-08	1.95E-04
		ADV40263.1	hypothetical protein	5.49	1.77	3.26E-08	1.49E-04
		ADV40380.1	putative nidogen 1	5.31	1.90	9.00E-08	2.51E-04
		ADV40263.1	hypothetical protein	4.99	1.58	1.28E-07	2.91E-04
		ADV40308.1	putative fibropellin	3.38	4.49	3.80E-08	1.59E-04
			PREDICTED: vascular non-inflammatory molecule 3- like				
		XP_015929207.1	like	1.94	6.05	1.15E-07	2.91E-04
		ADV40088.1	nucleoside diphosphate kinase	1.87	5.76	5.21E-08	1.87E-04
		XM_017153543	PREDICTED: suppressor protein SRP40-like	-2.74	3.24	7.48E-09	5.36E-05
		HQ006005	clone CV174 putative secreted salivary gland peptide	-4.48	2.81	4.13E-09	4.14E-05
			PREDICTED: coiled-coil domain-containing protein				
		XM_016056140	149	-5.61	4.95	6.97E-09	5.36E-05
Ovary	PHX_UvN	EF595245	clone 46B18 major ampullate spidroin 2 (MaSp2)	8.97	3.83	3.67E-08	3.68E-04
		EF595245	clone 46B18 major ampullate spidroin 2 (MaSp2)	8.64	5.85	3.42E-07	1.43E-03
		ABR68858.1	major ampullate spidroin 2	7.73	2.90	3.54E-09	9.42E-05
		ABY67425.1	major ampullate spidroin 1 locus	7.72	2.94	3.75E-09	9.42E-05

EF595245	clone 46B18 major ampullate spidroin 2 (MaSp2)	7.55	2.78	7.64E-07	2.94E-03
NA		7.15	7.19	1.61E-07	8.29E-04
EF595245	clone 46B18 major ampullate spidroin 2 (MaSp2)	7.11	5.86	1.65E-07	8.29E-04
ABR68855.1	major ampullate spidroin 2	6.01	2.38	1.40E-07	8.29E-04
ADV40223.1	hypothetical protein	4.46	2.16	9.19E-07	2.94E-03
JAT05635.1	hypothetical protein g.5631	4.08	2.02	7.77E-09	1.30E-04
	PREDICTED: intraflagellar transport protein 80				
XP_015920280.1	homolog	2.11	3.41	1.28E-06	3.39E-03
XM_021146117	PREDICTED: translocation protein SEC63 homolog	1.89	3.35	8.29E-07	2.94E-03
XP_015905503.1	PREDICTED: protein transport protein Sec24C-like	1.53	5.13	9.38E-07	2.94E-03
XM_021145653	PREDICTED: vigilin	0.52	7.19	2.71E-07	1.23E-03
ADV40298.1	putative tumor differentially expressed protein	-1.11	9.61	7.67E-08	6.42E-04
ADV40298.1	putative tumor differentially expressed protein	-1.14	9.65	3.62E-08	3.68E-04
XP_015919260.1	PREDICTED: nicotinamide N-methyltransferase-like	-2.29	4.07	1.10E-06	3.06E-03
NA		-3.79	5.73	1.39E-06	3.47E-03
XP_015929235.1	PREDICTED: uncharacterized protein isoform X1	-3.99	3.63	1.57E-07	8.29E-04
	PREDICTED: betaine--homocysteine -				
XM_517686	methyltransferase	-6.84	4.72	1.06E-06	3.06E-03
	PREDICTED: nuclear pore complex protein Nup88-				
LVN_UvN	XP_015930929.1 like	4.86	3.57	2.49E-10	2.08E-06
	XM_016454989 PREDICTED: zinc transporter ZIP9-like	2.87	2.13	5.38E-11	9.00E-07
	ABX75436.1 protein disulfide isomerase	2.35	7.19	2.62E-08	6.58E-05
	KFM69666.1 T-complex protein 1 subunit zeta	1.77	5.09	2.15E-08	5.69E-05
	XP_014230480.1 PREDICTED: 60S ribosomal protein L10 isoform X1	1.75	4.51	1.52E-10	1.52E-06
	XP_013780714.1 PREDICTED: calreticulin-like	0.86	5.97	3.06E-11	7.67E-07

ABX75466.1	ribosomal protein l24	0.85	5.14	3.56E-10	2.55E-06	
	PREDICTED: ribosomal L1 domain-containing protein					
XP_015920263.1	1	0.82	5.62	8.72E-09	2.57E-05	
XP_015918668.1	PREDICTED: rab11 family-interacting protein 3	0.48	6.54	1.22E-08	3.40E-05	
ADV40072.1	60S ribosomal protein l27a	0.36	8.64	5.20E-09	2.01E-05	
XP_015909199.1	PREDICTED: T-complex protein 1 subunit zeta-like	0.32	7.94	6.28E-09	2.22E-05	
KFM69053.1	Speckle-type POZ protein B	-0.70	8.30	7.82E-11	9.80E-07	
KFM69053.1	Speckle-type POZ protein B	-0.78	9.50	4.82E-12	2.42E-07	
XP_015903132.1	PREDICTED: protein bicaudal C homolog 1-like	-0.79	9.76	2.10E-09	1.17E-05	
ADV40298.1	putative tumor differentially expressed protein	-1.20	9.61	7.67E-09	2.41E-05	
ADV40298.1	putative tumor differentially expressed protein	-1.22	9.65	4.20E-09	1.86E-05	
ADV40332.1	hypothetical protein	-1.79	9.22	2.96E-09	1.49E-05	
ADV40332.1	hypothetical protein	-1.80	9.25	1.88E-09	1.17E-05	
KRZ48411.1	Uncharacterized protein T02_11458	-2.11	8.40	6.65E-09	2.22E-05	
KRZ48411.1	Uncharacterized protein T02_11458	-2.12	8.39	4.45E-09	1.86E-05	
DEN_UvN	KFM56629.1	Tubulin beta-1 chain	6.06	8.52	3.02E-22	1.52E-17
	PREDICTED: succinate dehydrogenase [ubiquinone]					
XP_015785335.1	flavoprotein subunit	5.00	5.35	1.44E-10	1.03E-06	
XM_021146242	PREDICTED: organic cation transporter protein-like	4.43	5.44	1.15E-09	4.50E-06	
JAT96147.1	putative beta tubulin	3.98	8.50	5.46E-21	1.37E-16	
XM_021146099	PREDICTED: uncharacterized	1.46	7.79	2.36E-11	2.96E-07	
	PREDICTED: zinc finger CCCH-type and G-patch					
XM_020534947	domain containing (zgpap)	1.27	6.94	5.24E-11	4.38E-07	
	PREDICTED: probable serine/threonine-protein kinase					
XP_013789052.1	nek3	1.18	6.24	2.37E-11	2.96E-07	

XP_015911780.1	PREDICTED: polyadenylate-binding protein 4-like	0.50	7.65	3.06E-10	1.71E-06		
XP_015929571.1	PREDICTED: protein transport protein Sec23A-like	-0.49	7.34	6.30E-09	1.58E-05		
XP_011405146.1	PREDICTED: transcriptional activator GLI3-like	-0.50	8.30	2.94E-10	1.71E-06		
XP_015916635.1	PREDICTED: clathrin heavy chain 1-like	-0.55	6.97	2.70E-09	8.46E-06		
KFM81532.1	Ubiquitin-conjugating enzyme E2 Z, partial	-0.60	6.57	9.34E-10	4.26E-06		
XP_015931157.1	PREDICTED: beta,beta-carotene 9',10'-oxygenase-like	-0.63	7.92	6.17E-09	1.58E-05		
XP_015921824.1	PREDICTED: fatty acyl-CoA reductase 1-like	-0.73	8.35	1.25E-09	4.50E-06		
XP_015922626.1	PREDICTED: uncharacterized protein LOC107451137	-0.82	6.46	3.85E-09	1.14E-05		
KFM56803.1	E3 ubiquitin-protein ligase UBR4	-0.93	5.41	4.16E-10	2.09E-06		
XP_015910390.1	PREDICTED: oxidation resistance protein 1-like	-1.04	7.87	1.86E-09	6.24E-06		
KFM79038.1	hypothetical protein X975_16632	-1.32	4.64	1.24E-09	4.50E-06		
XM_016073420	PREDICTED: uncharacterized	-1.60	7.88	5.04E-09	1.40E-05		
XP_015929083.1	PREDICTED: uncharacterized protein	-6.92	5.20	2.95E-11	2.96E-07		
Silk	PHX_UvN	AFP57561.1	putative integral membrane protein	7.11	2.61	1.21E-06	4.67E-03
		NA		6.56	3.23	4.90E-12	2.46E-07
		HQ006027	clone GW19 hypothetical protein mRNA	4.22	1.51	5.14E-06	1.36E-02
		XP_015926383.1	PREDICTED: bicaudal D-related protein homolog	2.87	5.08	4.09E-06	1.14E-02
		XP_013776068.1	PREDICTED: protein Mpv17-like isoform X1	2.71	4.10	6.03E-06	1.51E-02
		XM_021144651	PREDICTED: sorting nexin-5-like	2.49	2.38	3.63E-07	3.03E-03
		KFM60593.1	DnaJ-like protein subfamily C member 21	2.06	2.49	3.81E-06	1.13E-02
			PREDICTED: glycine-rich cell wall structural protein-				
		XM_018984297	like	-2.05	7.28	9.70E-07	4.57E-03
		XP_003396019.1	PREDICTED: septin-2	-2.09	3.33	4.99E-07	3.58E-03
			PREDICTED: glycine-rich cell wall structural protein-				
		XM_018984297	like	-2.23	9.89	3.58E-06	1.12E-02

	XM_021145987	PREDICTED: gastrula zinc finger protein XICGF71.1	-2.75	2.95	1.07E-06	4.57E-03
	JX262195	clone 549 aggregate gland silk factor 1 mRNA	-3.92	10.91	2.88E-07	2.89E-03
	NA		-5.11	6.06	1.72E-06	6.18E-03
	NA		-5.17	4.75	2.51E-07	2.89E-03
	NA		-5.29	5.34	8.93E-07	4.57E-03
		PREDICTED: brefeldin A-inhibited guanine				
	XP_015926421.1	nucleotide-exchange protein 2-like	-5.29	2.63	1.09E-06	4.57E-03
	NA		-5.92	1.80	3.04E-06	1.02E-02
		PREDICTED: betaine--homocysteine S-				
	XM_517686	methyltransferase	-6.05	2.96	6.10E-07	3.83E-03
	KP241087	MADS17 (MADS17) gene	-6.73	2.65	2.21E-10	5.54E-06
	KFM62591.1	hypothetical protein X975_04353	-6.94	2.45	7.34E-09	1.23E-04
		PREDICTED: uncharacterized PE-PGRS family				
LVN_UvN	XM_021145598	protein	7.00	5.87	2.88E-06	2.06E-02
	NA		3.84	3.66	3.49E-05	1.03E-01
	ADV40348.1	hypothetical protein	3.19	10.00	7.83E-11	3.93E-06
	NA		2.94	1.68	3.16E-05	1.03E-01
	EF153412	aqueous glue droplet peptide (SCP-2)	2.66	5.77	1.39E-06	1.16E-02
	NA		2.49	3.98	3.01E-05	1.03E-01
	ABO09798.1	aqueous glue droplet peptide	2.34	6.16	3.50E-05	1.03E-01
	AC215348	BAC clone CH251-482M21	1.99	6.33	2.77E-05	1.03E-01
	XP_015911003.1	PREDICTED: cytochrome P450 3A21-like	1.82	4.73	4.54E-05	1.16E-01
	JX262192	clone 2525 aggregate gland silk factor 2 mRNA	1.65	8.75	8.09E-06	5.07E-02
	ADV40308.1	putative fibropellin	1.35	3.94	4.08E-05	1.14E-01
	XP_015908617.1	PREDICTED: transmembrane protein 214-like	-1.15	4.52	4.60E-05	1.16E-01

	KRZ48411.1	Uncharacterized protein T02_11458	-2.65	11.43	2.80E-05	1.03E-01
	KRZ48411.1	Uncharacterized protein T02_11458	-2.81	11.47	1.25E-05	6.99E-02
	XP_015910547.1	PREDICTED: ribosome-binding protein 1-like	-3.02	5.94	4.77E-07	4.78E-03
		PREDICTED: putative polypeptide N-				
	XP_015912971.1	acetylgalactosaminyltransferase 9 isoform X1	-3.48	4.40	1.76E-08	2.94E-04
	KFM79735.1	hypothetical protein X975_26264	-4.28	3.21	3.20E-05	1.03E-01
		PREDICTED: ADP-ribosylation factor GTPase				
	XM_005924728	activating protein 1 (arfgap1)	-5.14	2.14	2.98E-05	1.03E-01
	KFM78240.1	hypothetical protein X975_22095	-6.78	8.21	1.58E-08	2.94E-04
	KFM59877.1	Pre-mRNA-processing factor 17	-7.75	4.35	2.18E-07	2.73E-03
DEN_UvN	XP_013915757.1	PREDICTED: long-chain-fatty-acid--CoA ligase 5	8.69	3.67	1.70E-05	4.59E-02
	AAX92677.1	egg case silk protein-1	8.50	8.24	1.04E-05	3.25E-02
	XP_015926487.1	PREDICTED: FK506-binding protein 2-like	5.80	2.85	3.23E-06	1.62E-02
	KFM82954.1	Acetoacetyl-CoA synthetase	5.62	4.06	2.82E-06	1.62E-02
		PREDICTED: succinate dehydrogenase [ubiquinone]				
	XP_015785335.1	flavoprotein subunit	5.23	5.59	4.44E-11	2.23E-06
	XP_015919953.1	PREDICTED: peroxidase-like isoform X3	4.89	6.83	5.12E-06	2.34E-02
	XP_015907595.1	PREDICTED: long-chain-fatty-acid--CoA ligase 5-like	4.40	4.34	3.17E-06	1.62E-02
	NA		3.45	5.38	8.10E-06	2.93E-02
	XP_015926824.1	PREDICTED: lipase member I-like	3.10	5.49	3.27E-09	8.20E-05
	XP_015926824.1	PREDICTED: lipase member I-like	2.80	5.63	5.83E-09	9.75E-05
	ADV40128.1	putative lipase precursor	2.69	6.52	1.07E-08	1.35E-04
	KFM62623.1	Retrovirus-related Pol polyprotein from transposon 412	2.65	2.85	1.29E-05	3.80E-02
	XP_015927416.1	PREDICTED: uncharacterized protein	2.32	4.25	1.83E-05	4.59E-02

	clone 119_P5 alpha-latrotoxin and latrotoxin-like				
KM382064	protein genes	2.28	3.76	2.33E-06	1.62E-02
ADV40308.1	putative fibropellin	1.66	3.94	1.41E-06	1.18E-02
	PREDICTED: glycine-rich cell wall structural protein-				
XM_016061392	like	-3.61	2.80	8.18E-06	2.93E-02
XM_016049162	PREDICTED: translation initiation factor IF-2	-3.92	6.96	6.27E-06	2.62E-02
EF595245	clone 46B18 major ampullate spidroin 2 (MaSp2)	-4.04	4.55	9.96E-06	3.25E-02
KFM73227.1	60S ribosomal protein L10	-5.14	5.22	2.44E-08	2.45E-04
XM_016050243	PREDICTED: E3 ubiquitin-protein ligase SMURF2	-5.16	2.93	1.82E-05	4.59E-02

*D = Denver, L = Las Vegas, P = Phoenix, U = urban, and N = non-urban

Table 4.2 Top 20 differentially expressed gene isoforms for comparisons of locales in Phoenix, Las Vegas, and Denver.

Tissue	Comparison	Accession	BLAST identity	Log FC	Log CPM	P-Value	FDR
Cephalothorax	LUxPU	XP_015923309.1	PREDICTED: hemocyanin A chain-like	6.95	5.43	7.03E-13	2.07E-09
		XP_015928477.1	PREDICTED: b(0,+)-type amino acid transporter 1-like	2.66	5.42	3.68E-13	1.23E-09
		KFM70302.1	Neuronal acetylcholine receptor subunit alpha-10	2.58	7.84	7.55E-19	7.58E-15
		XP_015926011.1	PREDICTED: twitchin-like	2.21	6.49	3.30E-14	1.51E-10
		XP_015922437.1	PREDICTED: hexokinase-2-like isoform X1	2.18	4.83	2.44E-17	2.04E-13
		KFM62172.1	Histone-arginine methyltransferase CARMER	1.87	4.14	1.07E-14	5.38E-11
		XP_015926210.1	PREDICTED: uncharacterized protein	1.71	4.16	6.04E-17	4.33E-13
		KFM65083.1	Ryanodine receptor 44F	1.69	7.23	4.19E-19	6.09E-15
		NA		1.61	6.96	6.14E-23	3.08E-18
		XP_013776029.1	PREDICTED: twitchin-like	1.59	5.85	5.65E-16	3.54E-12
		KFM76651.1	4-hydroxybutyrate coenzyme A transferase	1.51	7.01	4.86E-19	6.09E-15
		KFM76553.1	Junctophilin-1	1.46	7.84	1.85E-12	4.63E-09
		XP_015911478.1	PREDICTED: uncharacterized protein	1.43	4.32	3.08E-13	1.10E-09
		KFM65083.1	Ryanodine receptor 44F	1.43	5.63	1.70E-12	4.49E-09
		NA		1.35	8.28	4.87E-13	1.53E-09
		XP_015920771.1	PREDICTED: serine/threonine-protein kinase fray2-like	1.33	4.57	8.69E-15	4.85E-11
		KFM65083.1	Ryanodine receptor 44F	1.27	7.29	1.22E-12	3.40E-09
		XP_011262166.1	PREDICTED: furin-like protease 1	1.23	5.55	3.01E-22	7.55E-18
		XP_015924135.1	PREDICTED: LOW QUALITY PROTEIN: furin-like protease 1	1.03	5.48	1.24E-13	5.17E-10
		XP_015905336.1	PREDICTED: histone-lysine N-methyltransferase SETMAR-like	0.92	5.99	2.32E-13	8.94E-10
DUxPU		XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	11.33	7.29	4.96E-49	2.26E-45
		XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	11.12	7.16	1.83E-51	1.31E-47
		XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	10.70	6.92	3.03E-49	1.52E-45
		XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	10.04	5.96	8.66E-46	2.90E-42
		XP_015920146.1	PREDICTED: 60S ribosomal protein L36-like	10.04	7.74	5.13E-88	1.29E-83
		XP_015913973.1	PREDICTED: nose resistant to fluoxetine protein 6-like	9.14	5.83	1.58E-40	3.95E-37
		XP_015927728.1	PREDICTED: fatty acid synthase-like	8.11	4.21	4.00E-45	1.25E-41
		XP_015921317.1		8.01	4.45	8.89E-47	3.42E-43
		KFM62484.1	Long-chain-fatty-acid--CoA ligase 6	7.87	8.51	5.26E-54	5.28E-50
		NA		7.71	4.05	4.85E-50	2.70E-46

	KFM62484.1	Long-chain-fatty-acid--CoA ligase 6	7.57	6.90	9.53E-47	3.42E-43
	NA		7.32	3.49	7.07E-45	1.97E-41
	KFM62484.1	Long-chain-fatty-acid--CoA ligase 6	7.25	7.55	2.32E-47	9.72E-44
	ADV40374.1	putative transcription factor XBP-1	7.15	3.71	3.30E-51	2.07E-47
	XM_017059456	PREDICTED: glycerophosphodiester phosphodiesterase 1	7.08	3.48	1.71E-43	4.53E-40
	XM_016067092	PREDICTED: stress response protein NST1	5.28	4.31	5.71E-45	1.69E-41
	KFM76317.1	hypothetical protein X975_15322	4.77	5.79	3.76E-66	6.29E-62
	XM_005831914	CCMP2712 hypothetical protein	4.62	6.86	6.49E-60	8.14E-56
	NA		-8.47	4.12	7.36E-53	6.16E-49
	KFM76317.1	hypothetical protein X975_15322	-8.63	4.29	1.10E-104	5.53E-100
DUxLU	XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	10.88	7.29	3.45E-46	1.44E-42
	XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	10.84	6.92	9.51E-51	6.82E-47
	XP_015920146.1	PREDICTED: 60S ribosomal protein L36-like	10.68	7.74	1.98E-100	4.96E-96
	XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	10.66	7.16	3.01E-48	1.68E-44
	XP_015913973.1	PREDICTED: nose resistant to fluoxetine protein 6-like	9.76	5.83	1.38E-46	6.31E-43
	XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	9.36	5.96	1.95E-40	5.15E-37
	XP_015921317.1	PREDICTED: uncharacterized protein	7.72	4.45	4.59E-43	1.28E-39
	KFM62484.1	Long-chain-fatty-acid--CoA ligase 6	7.57	8.51	1.51E-51	1.26E-47
	NA		7.44	4.05	5.38E-46	2.08E-42
	JAS58553.1	hypothetical protein g.793	7.17	4.45	2.04E-44	7.32E-41
	KFM62484.1	Long-chain-fatty-acid--CoA ligase 6	7.16	6.90	2.93E-43	8.64E-40
	ADV40374.1	putative transcription factor XBP-1	7.08	3.71	1.43E-51	1.26E-47
	KFM62484.1	Long-chain-fatty-acid--CoA ligase 6	6.88	7.55	3.45E-44	1.15E-40
	XM_016067092	PREDICTED: stress response protein NST1	5.67	4.31	3.62E-58	4.54E-54
	KFM76317.1	hypothetical protein X975_15322	5.08	5.79	1.26E-78	2.11E-74
	XM_005831914	CCMP2712 hypothetical protein	4.01	6.86	8.43E-48	4.23E-44
	XM_020289277	PREDICTED: pumilio RNA binding family member 3 (PUM3)	3.09	8.17	1.43E-43	4.48E-40
	XP_015909736.1	PREDICTED: small nuclear ribonucleoprotein Sm D1	2.75	4.61	2.28E-39	5.73E-36
	NA		-5.51	4.12	8.33E-49	5.22E-45
	KFM76317.1	hypothetical protein X975_15322	-7.83	4.29	1.25E-119	6.28E-115
LNxPN	NA		4.67	2.36	4.43E-10	2.47E-06
	XP_015923755.1	PREDICTED: uncharacterized protein	1.65	5.83	3.39E-18	8.50E-14

	XP_015920332.1	PREDICTED: uncharacterized protein	1.56	3.85	6.79E-09	2.27E-05
	AII97591.1	BLTX194	1.42	6.72	1.52E-08	4.76E-05
	XP_015923784.1	PREDICTED: Down syndrome cell adhesion molecule-like protein 1 homolog	1.35	4.74	7.18E-20	3.60E-15
	XP_015906117.1	PREDICTED: 32 kDa beta-galactoside-binding lectin-like	1.24	4.88	2.88E-08	7.24E-05
	XP_015928295.1	PREDICTED: 60S ribosomal protein L13a-like	1.22	4.86	3.41E-10	2.14E-06
	XP_015923501.1	PREDICTED: uncharacterized protein	1.16	4.33	1.33E-11	1.66E-07
	NA		1.15	6.11	1.73E-10	1.45E-06
	KFM65083.1	Ryanodine receptor 44F	1.11	7.23	7.84E-10	3.58E-06
	NA		1.04	6.96	1.05E-10	1.05E-06
	XP_015920771.1	PREDICTED: serine/threonine-protein kinase fray2-like	0.94	4.57	3.11E-09	1.11E-05
	XP_015926208.1	PREDICTED: uncharacterized protein	0.83	5.35	2.97E-09	1.11E-05
	XP_015930288.1	PREDICTED: F-box/WD repeat-containing protein 7-like	0.72	6.12	6.75E-12	1.13E-07
	XP_015922994.1	PREDICTED: uncharacterized protein	0.72	5.10	1.96E-08	5.19E-05
	XP_015927756.1	PREDICTED: bcl-2-like protein 1	0.71	5.61	1.77E-08	5.10E-05
	NA		-0.72	6.38	2.40E-10	1.72E-06
	ADV40204.1	40S ribosomal protein S9	-0.82	8.16	1.83E-08	5.10E-05
	NA		-1.04	5.77	1.15E-09	4.83E-06
	NA		-1.27	4.66	5.55E-10	2.78E-06
DNxPN	XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	10.43	7.16	2.86E-40	2.39E-36
	XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	10.39	7.29	3.92E-37	1.97E-33
	XP_015920146.1	PREDICTED: 60S ribosomal protein L36-like	10.18	7.74	6.94E-82	1.74E-77
	XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	9.62	6.92	2.34E-35	9.79E-32
	XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	9.08	5.96	9.91E-33	2.92E-29
	XP_015921317.1	PREDICTED: uncharacterized protein	7.93	4.45	1.19E-38	7.47E-35
	ADV40374.1	putative transcription factor XBP-1, partial	7.06	3.71	2.37E-40	2.38E-36
	NA		7.03	4.05	8.76E-31	2.20E-27
	XM_017059456	PREDICTED: glycerophosphodiester phosphodiesterase 1	6.78	3.48	6.23E-31	1.65E-27
	JAS58553.1	hypothetical protein g.793	6.58	4.45	2.22E-31	6.20E-28
	KFM62484.1	Long-chain-fatty-acid--CoA ligase 6	6.47	6.90	2.54E-34	7.95E-31
	KFM62484.1	Long-chain-fatty-acid--CoA ligase 6	6.43	8.51	1.14E-38	7.47E-35
	KFM62484.1	Long-chain-fatty-acid--CoA ligase 6	6.05	7.55	1.14E-34	4.10E-31
	XM_016067092	PREDICTED: stress response protein NST1	4.97	4.31	6.96E-38	3.88E-34
	KDR18366.1	Reactive oxygen species modulator 1	4.45	4.32	1.06E-34	4.09E-31
	XP_015928426.1	PREDICTED: high mobility group protein B2-like	3.88	6.02	1.98E-36	9.05E-33

		KFM76317.1	hypothetical protein X975_15322	3.52	5.79	3.61E-46	4.53E-42
		XP_002433495.1	protein-tyrosine phosphatase	3.38	5.93	1.70E-34	5.68E-31
		NA		-7.81	4.12	1.91E-58	3.19E-54
		KFM76317.1	hypothetical protein	-8.04	4.29	1.54E-122	7.71E-118
DNxLN		XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	10.74	7.29	2.65E-39	1.11E-35
		XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	10.66	7.16	6.51E-42	5.44E-38
		XP_015920146.1	PREDICTED: 60S ribosomal protein L36-like	10.59	7.74	2.09E-88	5.25E-84
		XP_015928808.1	PREDICTED: nose resistant to fluoxetine protein 6-like	10.52	6.92	8.13E-42	5.82E-38
		XP_015921317.1	PREDICTED: uncharacterized protein	7.94	4.45	8.22E-39	2.95E-35
		NA		7.59	4.05	2.64E-40	1.33E-36
		NA		7.55	3.80	4.94E-36	1.24E-32
		JAS58553.1	hypothetical protein g.793	7.21	4.45	2.66E-40	1.33E-36
		ADV40374.1	putative transcription factor XBP-1	6.95	3.71	3.46E-38	1.16E-34
		KFM62484.1	Long-chain-fatty-acid--CoA ligase 6	6.69	6.90	2.99E-36	8.83E-33
		KFM62484.1	Long-chain-fatty-acid--CoA ligase 6	6.52	8.51	1.77E-39	8.07E-36
		KFM62484.1	Long-chain-fatty-acid--CoA ligase 6	6.21	7.55	4.70E-36	1.24E-32
		XM_016067092	PREDICTED: stress response protein NST1	5.27	4.31	4.10E-45	4.11E-41
		KFM76317.1	hypothetical protein	3.60	5.79	1.37E-48	1.71E-44
		XM_005831914	CCMP2712 hypothetical protein	3.56	6.86	1.55E-36	4.85E-33
		XP_002433495.1	protein-tyrosine phosphatase	3.46	5.93	4.26E-36	1.19E-32
		XP_015909736.1	PREDICTED: small nuclear ribonucleoprotein Sm D1	3.17	4.61	2.39E-40	1.33E-36
		XM_016070581	PREDICTED: silent chromatin protein ESC1	-2.53	4.63	6.24E-39	2.41E-35
		KFM76317.1	hypothetical protein X975_15322	-7.56	4.29	4.97E-126	2.50E-121
		NA		-8.68	4.12	8.83E-63	1.48E-58
Ovary	PUxLU	XP_015921819.1	PREDICTED: glutamine--fructose-6-phosphate aminotransferase [isomerizing]	1.26	6.45	6.55E-33	1.83E-29
		JAN31158.1	Small ubiquitin-related modifier	-0.75	8.69	3.08E-42	3.86E-38
		XP_015918389.1	PREDICTED: 28 kDa heat- and acid-stable phosphoprotein	-0.78	7.30	5.20E-36	2.61E-32
		XP_015911142.1	PREDICTED: transcription factor BTF3 homolog 4-like	-0.82	7.19	2.70E-32	6.77E-29
		ABX75436.1	protein disulfide isomerase	-0.88	9.54	1.43E-52	3.58E-48
		XP_015928339.1	PREDICTED: 60S ribosomal protein L6-like	-0.93	8.17	6.55E-51	1.09E-46
		KFM77976.1	Proteasome subunit alpha type-5	-0.93	6.95	3.22E-33	9.49E-30
		XP_015925153.1	PREDICTED: 60S ribosomal protein L23a-like	-0.95	7.78	2.75E-36	1.53E-32

	XP_015926887.1	PREDICTED: eukaryotic translation initiation factor 3 subunit H-like	-0.97	6.78	5.04E-37	3.61E-33
	KFM82963.1	60S ribosomal protein L12	-0.98	7.33	5.98E-34	2.45E-30
	HQ006005	clone CV174 putative secreted salivary gland peptide	-1.10	6.79	2.48E-36	1.53E-32
	ADV40369.1	putative ribosomal protein S24	-1.13	9.26	2.86E-39	2.39E-35
	KFM72359.1	60S ribosomal protein L22	-1.24	7.36	3.16E-41	3.17E-37
	XP_015928755.1	PREDICTED: nose resistant to fluoxetine protein 6-like	-1.26	7.89	1.18E-32	3.13E-29
	XP_015907976.1	PREDICTED: 40S ribosomal protein S12-like	-1.27	8.31	1.35E-52	3.58E-48
	ACH48193.1	40S ribosomal protein S15	-1.29	8.05	2.06E-34	9.38E-31
	XP_015909052.1	PREDICTED: uncharacterized protein	-1.29	8.57	6.36E-34	2.45E-30
	XP_015913298.1	PREDICTED: 60S acidic ribosomal protein P1-like	-1.32	9.72	2.31E-33	7.25E-30
	KFM68269.1	Zinc finger protein 330-like protein	-1.50	5.59	8.29E-34	2.97E-30
	KFM81238.1	DET1- and DDB1-associated protein 1	-2.19	4.40	9.66E-34	3.23E-30
DUXPU	XP_015920146.1	PREDICTED: 60S ribosomal protein L36-like	8.98	6.99	9.45E-68	1.58E-63
	NA		7.54	4.00	3.24E-48	1.81E-44
	XM_017059456	PREDICTED: glycerophosphodiester phosphodiesterase 1	7.36	4.74	9.41E-57	9.44E-53
	KFM78087.1	Integrator complex subunit 8	7.12	5.23	6.14E-57	7.70E-53
	NA		6.45	5.09	5.45E-47	2.28E-43
	NA		6.32	5.88	4.72E-48	2.15E-44
	XP_015906006.1	PREDICTED: DNA-directed RNA polymerase I subunit RPA2-like	4.62	6.48	1.17E-45	3.93E-42
	OEH77433.1	hypothetical protein cyc_01245	4.53	5.40	1.10E-46	4.23E-43
	XM_010199706	PREDICTED: ADP-ribosylation factor-like 14 effector protein-like	3.62	6.91	4.50E-46	1.61E-42
	KFM71066.1	Serine/threonine-protein kinase TBK1	3.08	5.22	5.24E-42	1.31E-38
	NA		2.33	5.54	2.93E-45	9.19E-42
	ABX75436.1	protein disulfide isomerase	-0.96	9.54	1.68E-56	1.40E-52
	AHH29554.1	dynein light chain type 1	-1.43	8.70	9.77E-52	7.01E-48
	AHH29554.1	dynein light chain type 1	-1.43	7.28	4.32E-48	2.15E-44
	XP_015909052.1	PREDICTED: uncharacterized protein	-1.53	8.57	2.56E-43	7.14E-40
	XP_015928561.1	PREDICTED: ATP-dependent Clp protease ATP-binding subunit clpX-like	-1.60	6.21	1.08E-49	6.76E-46
	NA		-1.61	8.00	3.65E-43	9.64E-40
	XP_015924158.1	PREDICTED: coiled-coil domain-containing protein 47	-2.62	4.47	1.86E-43	5.49E-40
	XP_002414852.1	THO complex subunit	-2.80	8.40	4.48E-70	1.12E-65
	KFM76317.1	hypothetical protein X975_15322	-5.91	3.99	8.59E-71	4.31E-66

DUxLU	XP_015920146.1	PREDICTED: 60S ribosomal protein L36-like	9.90	6.99	1.48E-83	7.41E-79
	NA		8.75	5.24	1.85E-42	5.45E-39
	NA		7.46	4.00	1.16E-45	4.17E-42
	XM_017059456	PREDICTED: glycerophosphodiester phosphodiesterase 1	6.92	4.74	1.66E-46	7.58E-43
	KFM78087.1	Integrator complex subunit 8, partial	6.52	5.23	1.28E-44	4.29E-41
	NA		6.44	5.88	8.13E-50	5.82E-46
	NA		6.07	5.09	1.16E-40	2.91E-37
	XM_010199706	PREDICTED: ADP-ribosylation factor-like 14 effector protein-like	4.11	6.91	1.33E-57	1.33E-53
	XP_015926048.1	PREDICTED: cytosolic purine 5'-nucleotidase-like	3.77	6.17	3.90E-48	1.95E-44
	KFM71066.1	Serine/threonine-protein kinase TBK1	3.06	5.22	6.22E-41	1.64E-37
	NA		3.02	8.75	5.20E-46	2.17E-42
	XP_015906006.1	PREDICTED: DNA-directed RNA polymerase I subunit RPA2-like	2.75	6.57	4.65E-43	1.46E-39
	NA		2.44	5.54	2.68E-49	1.68E-45
	XP_015905420.1	PREDICTED: coiled-coil-helix-coiled-coil-helix domain-containing protein 5	2.17	8.06	1.03E-45	3.99E-42
	XP_015909199.1	PREDICTED: T-complex protein 1 subunit zeta-like	2.11	6.35	2.84E-48	1.58E-44
	KFM60678.1	CDGSH iron-sulfur domain-containing protein 1	1.97	6.86	6.62E-60	8.30E-56
	KFM76317.1	hypothetical protein X975_15322	1.90	6.22	1.10E-62	1.84E-58
	XP_002414852.1	THO complex subunit	-2.41	8.40	1.36E-53	1.14E-49
	KFM76317.1	hypothetical protein	-5.61	3.99	5.22E-65	1.31E-60
	PNxLN	KFM62172.1	Histone-arginine methyltransferase CARMER	1.93	4.95	3.71E-23
KFM83567.1		Protein trapped in endoderm-1	1.24	6.77	7.78E-22	2.44E-18
JAN31158.1		Small ubiquitin-related modifier	-0.62	8.69	2.12E-28	1.33E-24
ADV40369.1		putative ribosomal protein S24	-0.84	9.26	1.41E-22	5.88E-19
XP_009702107.1		PREDICTED: calmodulin-like	-0.84	8.77	8.60E-20	2.16E-16
HQ006005		clone CV174 putative secreted salivary gland peptide	-0.87	6.79	2.52E-22	9.02E-19
KFM82963.1		60S ribosomal protein L12	-0.90	7.33	1.47E-28	1.05E-24
NA			-0.93	7.25	5.33E-23	2.43E-19
XP_015907976.1		PREDICTED: 40S ribosomal protein S12-like	-0.93	8.31	1.16E-28	9.70E-25
KFM72359.1		60S ribosomal protein L22	-1.04	7.36	8.14E-29	9.70E-25
NA			-1.07	10.97	4.98E-23	2.43E-19
XP_015928703.1		PREDICTED: immediate early response 3-interacting protein 1	-1.10	6.12	2.42E-29	4.05E-25
KFM77012.1		hypothetical protein X975_14454	-1.10	5.50	3.95E-20	1.04E-16

	KFM71106.1	GTP-binding protein 128up	-1.11	5.67	1.54E-20	4.29E-17
	KFM72542.1	U6 snRNA-associated Sm-like protein LSm3	-1.12	5.52	1.73E-22	6.66E-19
	ACH48193.1	40S ribosomal protein S15	-1.17	8.05	9.71E-29	9.70E-25
	ADV40204.1	40S ribosomal protein S9	-1.31	7.88	2.12E-33	1.06E-28
	NA		-1.45	5.44	4.18E-21	1.23E-17
	KFM68269.1	Zinc finger protein 330-like protein	-1.46	5.59	1.50E-32	3.76E-28
	XP_015914041.1	PREDICTED: flavin reductase (NADPH)-like	-1.46	6.24	3.41E-22	1.14E-18
DNxPN	NA		8.38	5.09	4.13E-46	1.38E-42
	XP_015920146.1	PREDICTED: 60S ribosomal protein L36-like	7.86	6.99	1.47E-62	1.85E-58
	KFM78087.1	Integrator complex subunit 8	7.42	5.23	2.49E-63	4.16E-59
	NA		7.12	5.88	6.32E-49	2.88E-45
	XM_017059456	PREDICTED: glycerophosphodiester phosphodiesterase 1	6.57	4.74	8.13E-50	4.08E-46
	OEH77433.1	hypothetical protein	6.26	5.40	3.50E-55	2.51E-51
	NA		5.79	7.81	3.36E-41	8.88E-38
	XP_015926048.1	PREDICTED: cytosolic purine 5'-nucleotidase-like	3.99	6.17	4.87E-46	1.53E-42
	KFM71066.1	Serine/threonine-protein kinase TBK1	3.89	5.22	4.17E-57	3.49E-53
	XP_015928426.1	PREDICTED: high mobility group protein B2-like	3.62	6.76	4.51E-55	2.83E-51
	XM_010199706	PREDICTED: ADP-ribosylation factor-like 14 effector protein-like	3.41	6.91	8.80E-44	2.60E-40
	XP_013789052.1	PREDICTED: probable serine/threonine-protein kinase nek3	3.22	6.11	4.27E-48	1.79E-44
	NA		2.25	5.54	2.98E-43	8.30E-40
	KFM60292.1	hypothetical protein X975_13925	-1.46	6.45	3.56E-41	8.92E-38
	M5B4R7.1	RecName: Full=Translationally-controlled tumor protein homolog; Short=GTx-TCTP1	-1.46	8.03	5.13E-48	1.98E-44
	NA		-1.52	6.58	5.66E-54	3.15E-50
	AHH29554.1	dynein light chain type 1	-1.56	7.28	3.93E-57	3.49E-53
	NA		-1.70	8.00	3.34E-47	1.20E-43
	XP_002414852.1	THO complex subunit	-2.72	8.40	1.66E-66	4.16E-62
	KFM76317.1	hypothetical protein	-6.32	3.99	1.70E-73	8.52E-69
DNxLN	NA		9.14	5.24	1.53E-46	5.47E-43
	XP_015920146.1	PREDICTED: 60S ribosomal protein L36-like	8.73	6.99	2.28E-77	1.14E-72
	NA		8.37	5.09	8.01E-46	2.68E-42
	NA		7.75	4.74	2.13E-64	3.56E-60
	NA		7.29	5.88	1.41E-51	7.88E-48

		KFM78087.1	Integrator complex subunit 8	6.91	5.23	5.13E-52	3.22E-48
		XM_017059456	PREDICTED: glycerophosphodiester phosphodiesterase 1	6.32	4.74	3.30E-44	8.72E-41
		OE77433.1	hypothetical protein	6.01	5.40	9.51E-50	4.77E-46
		XP_015926048.1	PREDICTED: cytosolic purine 5'-nucleotidase-like	4.43	6.17	1.95E-56	1.63E-52
		CAC44751.1	hemocyanin subunit 3	4.27	6.94	2.26E-43	5.68E-40
		XP_015928426.1	PREDICTED: high mobility group protein B2-like	3.70	6.76	3.63E-57	3.64E-53
		KFM71066.1	Serine/threonine-protein kinase TBK1	3.66	5.22	1.59E-48	7.27E-45
		XM_010199706	PREDICTED: ADP-ribosylation factor-like 14 effector protein-like	3.44	6.91	1.49E-44	4.39E-41
		NA		3.06	8.75	3.80E-47	1.59E-43
		NA		2.75	7.10	6.36E-45	1.99E-41
		XP_015903573.1	PREDICTED: insulin-degrading enzyme-like	2.53	5.39	2.70E-44	7.52E-41
		KFM76317.1	hypothetical protein X975_15322	1.81	6.22	2.07E-52	1.48E-48
		KFM60678.1	CDGSH iron-sulfur domain-containing protein 1	1.76	6.86	1.09E-46	4.20E-43
		XP_002414852.1	THO complex subunit	-2.61	8.40	1.17E-61	1.47E-57
		KFM76317.1	hypothetical protein X975_15322	-6.45	3.99	2.48E-70	6.23E-66
Silk	PUxLU	KFM71801.1	hypothetical protein X975_19628	3.80	4.67	6.63E-07	1.89E-03
		KFM62172.1	Histone-arginine methyltransferase CARMER	1.76	4.30	1.92E-08	2.41E-04
		XP_015916472.1	PREDICTED: actin-related protein 2	1.25	5.07	1.17E-07	6.03E-04
		ADV40152.1	G protein beta subunit-like protein	0.77	9.09	7.33E-08	5.25E-04
		NA		-0.99	6.47	2.02E-07	7.92E-04
		XP_015909052.1	PREDICTED: uncharacterized protein	-1.46	6.37	9.91E-07	2.62E-03
		AFP57562.1	aggregate gland silk factor 2	-1.60	4.28	1.20E-07	6.03E-04
		ADV40308.1	putative fibropellin	-1.67	3.94	2.85E-07	9.52E-04
		JX262195	clone 549 aggregate gland silk factor 1 mRNA	-1.83	7.38	1.29E-06	3.24E-03
				-2.15	12.79	1.37E-08	2.29E-04
		XM_018984297	PREDICTED: glycine-rich cell wall structural protein-like	-2.35	7.28	1.02E-07	6.03E-04
		ADV40223.1	hypothetical protein	-2.68	4.12	2.54E-08	2.54E-04
		NA		-2.68	3.26	3.79E-07	1.19E-03
		EF153412	aqueous glue droplet peptide (SCP-2)	-2.96	5.77	2.19E-07	7.92E-04
		WP_051068643.1	hypothetical protein	-3.04	9.50	3.28E-11	1.64E-06
		NA		-3.34	3.37	1.48E-07	6.74E-04
		NA		-4.67	1.82	4.36E-08	3.64E-04
				-5.12	1.55	2.21E-07	7.92E-04
		WP_051068643.1	hypothetical protein	-5.47	10.91	1.70E-10	4.26E-06

	ABO09798.1	aqueous glue droplet peptide	-7.65	10.13	6.78E-07	1.89E-03
DUxPU	XM_023423376	PREDICTED: growth/differentiation factor 11-like	12.31	10.49	1.92E-22	8.77E-19
	XP_015920146.1	PREDICTED: 60S ribosomal protein L36-like	9.92	7.61	1.17E-46	5.87E-42
	NA		8.57	5.04	1.05E-23	6.61E-20
	KFM63144.1	Lysosomal acid lipase/cholesteryl ester hydrolase	8.35	5.88	4.86E-45	1.22E-40
	XM_023155205	PREDICTED: CDK5 regulatory subunit associated protein 2 (cdk5rap2)	7.49	4.06	3.15E-21	1.13E-17
	XP_014251251.1	PREDICTED: lipase 3-like	6.94	3.49	3.32E-27	3.33E-23
	XM_016067092	PREDICTED: stress response protein NST1	6.73	3.55	2.40E-25	1.72E-21
	XM_017059456	PREDICTED: glycerophosphodiester phosphodiesterase 1	6.14	3.72	1.18E-27	1.48E-23
	XM_023155205	PREDICTED: CDK5 regulatory subunit associated protein 2 (cdk5rap2)	5.71	5.28	1.09E-25	9.14E-22
	ADV40374.1	putative transcription factor XBP-1	5.41	4.10	1.64E-42	2.74E-38
	XP_015929191.1	PREDICTED: serine/arginine repetitive matrix protein 2	5.20	3.62	1.98E-23	1.11E-19
	XP_015905468.1	PREDICTED: mitochondrial 2-oxoglutarate/malate carrier protein-like	4.47	3.53	8.83E-22	3.41E-18
	XM_005831914	CCMP2712 hypothetical protein	4.43	5.18	7.17E-23	3.60E-19
	KFM67949.1	SPRY domain-containing protein 3	3.60	4.62	6.35E-22	2.65E-18
	XP_015904620.1	PREDICTED: protein-tyrosine sulfotransferase 1-like	2.37	4.77	7.69E-19	2.27E-15
	NA		1.78	6.31	1.60E-17	4.02E-14
	KFM75168.1	hypothetical protein X975_11824	-3.19	7.94	3.91E-18	1.03E-14
	NA		-5.33	2.16	3.65E-20	1.14E-16
	KFM61703.1	Zinc finger protein 36, C3H1 type-like 1	-6.46	2.21	4.00E-21	1.34E-17
	XP_015916914.1	PREDICTED: inositol-3-phosphate synthase-like	-7.97	6.23	1.54E-18	4.31E-15
DUxLU	XM_023423376	PREDICTED: growth/differentiation factor 11-like	11.46	10.49	2.42E-20	1.21E-16
	XP_015920146.1	PREDICTED: 60S ribosomal protein L36-like	10.23	7.61	4.45E-48	2.23E-43
	KFM63144.1	Lysosomal acid lipase/cholesteryl ester hydrolase	8.61	5.88	1.73E-46	4.34E-42
	NA		8.48	6.01	8.09E-18	2.39E-14
	NA		8.31	5.04	1.37E-21	8.61E-18
	XM_023155205	PREDICTED: CDK5 regulatory subunit associated protein 2 (cdk5rap2)	7.41	4.06	7.53E-20	2.99E-16
	XP_014251251.1	PREDICTED: lipase 3-like	6.95	3.49	7.04E-26	7.07E-22
	XM_016067092	PREDICTED: stress response protein NST1	6.93	3.55	1.94E-26	2.44E-22
	XM_023155205	PREDICTED: CDK5 regulatory subunit associated protein 2 (cdk5rap2)	5.83	5.28	1.47E-25	1.23E-21
	XM_017059456	PREDICTED: glycerophosphodiester phosphodiesterase 1	5.80	3.72	5.71E-22	4.10E-18

	ADV40374.1	putative transcription factor XBP-1	5.49	4.10	1.34E-41	2.24E-37
	XP_015929191.1	PREDICTED: serine/arginine repetitive matrix protein 2	5.03	3.62	6.88E-20	2.99E-16
	KFM83083.1	Solute carrier family 41 member 2	4.46	3.70	2.50E-17	6.26E-14
	XM_005831914	CCMP2712 hypothetical protein	4.08	5.18	9.19E-19	2.88E-15
	KFM64043.1	Dimethylaniline monooxygenase [N-oxide-forming] 5	3.63	4.70	1.06E-20	5.91E-17
	KFM67949.1	SPRY domain-containing protein 3	3.52	4.62	1.43E-19	5.13E-16
	ADV40093.1	dehydrogenase/reductase SDR family member 11	3.33	6.23	7.75E-20	2.99E-16
	XP_015930588.1	PREDICTED: elongation of very long chain fatty acids protein 7	2.80	4.86	1.25E-17	3.29E-14
	NA		2.51	4.62	5.94E-19	1.99E-15
	XM_015334535	PREDICTED: targeting protein for Xklp2-like	-7.05	2.87	9.96E-18	2.78E-14
LNxPN	NA		9.68	4.34	2.12E-07	6.52E-04
	KP241087	MADS17 (MADS17) gene	6.66	2.65	2.45E-09	3.07E-05
	NA		2.17	3.51	1.46E-07	4.90E-04
	NA		1.75	6.36	8.07E-09	7.50E-05
	XP_015922437.1	PREDICTED: hexokinase-2-like isoform X1	1.63	4.30	9.72E-13	4.88E-08
	NA		1.52	3.57	6.58E-08	3.00E-04
	XP_015911304.1	PREDICTED: transcriptional coactivator YAP1-like	1.05	5.21	4.04E-08	2.25E-04
	XP_971914.1	PREDICTED: transmembrane emp24 domain-containing protein 2	0.99	4.51	2.67E-07	6.52E-04
	ADV40376.1	putative galactosyltransferase	0.85	9.32	1.05E-08	7.50E-05
	XP_015911478.1	PREDICTED: uncharacterized protein	0.84	5.10	2.60E-07	6.52E-04
	XP_015908446.1	PREDICTED: elongation factor 1-beta-like	-0.92	8.41	2.46E-07	6.52E-04
	XM_021145653	PREDICTED: vigilin	-1.16	10.57	3.52E-08	2.21E-04
	NA		-1.30	6.47	3.02E-12	6.64E-08
	XP_015928339.1	PREDICTED: 60S ribosomal protein L6-like	-2.39	3.66	3.97E-12	6.64E-08
	XP_015912971.1	PREDICTED: putative polypeptide N-acetylgalactosaminyltransferase 9	-3.00	4.40	6.38E-08	3.00E-04
	NA		-4.56	1.52	9.92E-08	3.83E-04
	XP_015919260.1	PREDICTED: nicotinamide N-methyltransferase-like	-4.63	6.70	8.16E-08	3.41E-04
	KFM78240.1	hypothetical protein	-5.47	8.21	1.37E-07	4.90E-04
	NA		-5.58	3.23	9.71E-09	7.50E-05
	ABO09798.1	aqueous glue droplet peptide	-8.54	10.13	2.39E-07	6.52E-04
DNxPN	XM_023423376	PREDICTED: growth/differentiation factor 11-like	12.12	10.49	3.12E-24	1.42E-20
	XP_015920146.1	PREDICTED: 60S ribosomal protein L36-like	9.25	7.61	2.08E-49	1.04E-44

	KFM63144.1	Lysosomal acid lipase/cholesteryl ester hydrolase	9.08	5.88	4.31E-48	7.21E-44
	NA		8.30	5.04	8.11E-25	4.52E-21
	XM_023155205	PREDICTED: CDK5 regulatory subunit associated protein 2 (cdk5rap2)	7.55	5.28	5.09E-33	6.38E-29
	ADV40374.1	putative transcription factor XBP-1	7.38	4.10	1.67E-48	4.18E-44
	XM_023155205	PREDICTED: CDK5 regulatory subunit associated protein 2 (cdk5rap2)	7.26	4.06	5.50E-22	1.84E-18
	XP_014251251.1	PREDICTED: lipase 3-like	6.59	3.49	2.58E-25	1.62E-21
	XM_017059456	PREDICTED: glycerophosphodiester phosphodiesterase 1	6.13	3.72	6.08E-29	6.10E-25
	NA		5.82	4.57	1.44E-24	7.22E-21
	XM_016067092	PREDICTED: stress response protein NST1	4.76	3.55	3.67E-23	1.42E-19
	XP_015926824.1	PREDICTED: lipase member I-like	4.35	5.63	1.03E-20	2.88E-17
	XP_015929191.1	PREDICTED: serine/arginine repetitive matrix protein 2	4.35	3.62	1.17E-22	4.21E-19
	XP_015905468.1	PREDICTED: mitochondrial 2-oxoglutarate/malate carrier protein	3.95	3.53	2.35E-21	6.93E-18
	XP_015931247.1	PREDICTED: uncharacterized protein	3.83	4.06	3.24E-26	2.33E-22
	XP_015922025.1	PREDICTED: ATPase family AAA domain-containing protein 3-like	3.59	5.10	1.00E-21	3.15E-18
	KFM67949.1	SPRY domain-containing protein 3	3.32	4.62	3.29E-20	8.25E-17
	KFM80597.1	Arginine--tRNA ligase, cytoplasmic	2.12	5.95	1.81E-26	1.51E-22
	KFM75168.1	hypothetical protein	-3.49	7.94	2.73E-20	7.21E-17
	XM_015334535	PREDICTED: targeting protein for Xklp2-like	-7.28	2.87	8.89E-24	3.72E-20
DNxLN	XM_023423376	PREDICTED: growth/differentiation factor 11-like	12.41	10.49	4.64E-25	1.94E-21
	XP_015920146.1	PREDICTED: 60S ribosomal protein L36-like	9.73	7.61	3.00E-54	1.51E-49
	KFM63144.1	Lysosomal acid lipase/cholesteryl ester hydrolase	9.26	5.88	5.26E-51	1.32E-46
	NA		8.68	5.04	2.27E-27	1.90E-23
	NA		8.40	6.01	5.73E-20	1.44E-16
	XM_023155205	PREDICTED: CDK5 regulatory subunit associated protein 2 (cdk5rap2)	7.96	5.28	2.36E-37	2.96E-33
	XM_023155205	PREDICTED: CDK5 regulatory subunit associated protein 2 (cdk5rap2)	7.47	4.06	1.04E-23	3.74E-20
	ADV40374.1	putative transcription factor XBP-1	7.32	4.10	1.05E-47	1.76E-43
	XP_014251251.1	PREDICTED: lipase 3-like	6.55	3.49	4.03E-25	1.84E-21
	XM_017059456	PREDICTED: glycerophosphodiester phosphodiesterase 1	5.85	3.72	1.53E-25	8.52E-22
	NA		5.46	4.57	4.81E-22	1.34E-18
	XM_016067092	PREDICTED: stress response protein NST1	5.00	3.55	4.47E-27	3.20E-23
	XP_015929191.1	PREDICTED: serine/arginine repetitive matrix protein 2	4.46	3.62	1.41E-24	5.45E-21

KFM83083.1	Solute carrier family 41 member 2	4.39	3.70	1.41E-23	4.72E-20
XP_015912092.1	PREDICTED: thioredoxin domain-containing protein 9	4.04	3.62	2.62E-21	6.92E-18
KFM67949.1	SPRY domain-containing protein 3	3.74	4.62	2.40E-26	1.51E-22
XP_015922025.1	PREDICTED: ATPase family AAA domain-containing protein 3	3.60	5.10	3.79E-22	1.19E-18
XP_015931247.1	PREDICTED: uncharacterized protein	3.55	4.06	4.78E-22	1.34E-18
KFM80597.1	Arginine--tRNA ligase, cytoplasmic, partial	2.16	5.95	3.74E-28	3.75E-24
XM_015334535	PREDICTED: targeting protein for Xklp2-like	-7.28	2.87	2.96E-25	1.49E-21

*D = Denver, L = Las Vegas, P = Phoenix, U = urban, and N = non-urban

Table 4.3 Mann Whitney U tests for comparisons among urban and non-urban locales down- and up-regulated gene isoforms

Tissue	Comparison	Down	Up
cephalothorax	LUxLN vs PUxPN	2882	1687
	LUxLN vs DUxDN	561	0
	PUxPN vs DUxDN	2145	0
	LUxDU vs PUxLU	472430	1547300
	LUxDU vs PUxDU	911630	5402500
	PUxLU vs PUxDU	414660	66480
	LNxDN vs PNxLN	70492	325400
	LNxDN vs PNxDN	42650	2932300
	PNxLN vs PNxDN	35225	19260
	ovary	LUxLN vs PUxPN	767
LUxLN vs DUxDN		5222	1114
PUxPN vs DUxDN		7652	609
LUxDU vs PUxLU		46470	1367300
LUxDU vs PUxDU		1151700	4097600
PUxLU vs PUxDU		836550	1110500
LNxDN vs PNxLN		240980	760650
LNxDN vs PNxDN		780160	3378700
PNxLN vs PNxDN		366560	509660
silk		LUxLN vs PUxPN	767
	LUxLN vs DUxDN	25	6
	PUxPN vs DUxDN	99	40

LUxDU vs PUxLU	12611	7362
LUxDU vs PUxDU	149720	661990
PUxLU vs PUxDU	21542	5577
LNxDN vs PNxLN	897030	325210
LNxDN vs PNxDN	434420	680360
PNxLN vs PNxDN	677610	266030

*values are Mann-Whitney W, bold values denote significance after Bonferonni correction, D =

Denver, L = Las Vegas, P = Phoenix, U = urban, and N = non-urban

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