

Population Genetics of Red Foxes (*Vulpes vulpes*) in the Greater Sydney Region

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This thesis is written in the form of a manuscript for submission to Journal of Wildlife Management, with the following exceptions: 1.5 space text, continuous line numbers and running head are absent, figures are integrated into the text, a table of contents has been included, headers are present in the introduction and the introduction, methods, results, and discussion are extended. An aims section has also been added.

Declaration

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All other research detailed in this report is my own work.

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ABSTRACT

The Eurasian red fox (*Vulpes vulpes*) is an invasive predator that causes significant damage to native species and agriculture. In Australian cities, red fox density reaches up to 16 foxes per km² and is much higher than in surrounding rural and natural bush areas. Limited information on population distributions, genetic mixing and dispersal in urban compared to rural habitats hampers the ability of management authorities to safely and effectively control red foxes in these areas. This study examined whether inferred levels of red fox dispersal differ across three habitats; urban, peri-urban and rural, across the Sydney region. We collected fox carcasses from each of these habitats and genotyped thousands of SNPs from DNA extracted from 94 individuals. We found that geneflow was sufficient prevent localized differences in allele frequency and the estimate of effective population size of red foxes in the Greater Sydney region was 212. Analysis of relatedness showed that dispersal was not sex-biased, and levels of dispersal were significantly less in urban compared with peri-urban and rural habitats. Urban landcover, road, railways, and waterways limited dispersal of red foxes within the Sydney region. These results provide insight into the effect of urbanization on red foxes in Sydney and can be used to tailor management to urban and rural populations.

KEYWORDS: Red Fox, Population Genetics, Urban, Distribution, Management

INTRODUCTION

Urban regions are some of the fastest growing environments on the planet (Grimm, Faeth et al. 2008) with 55% of the global human population residing in cities and metropolitan areas. This percentage is predicted to rise to 68% by 2050 (Nations 2018). Despite the concrete canopies and vehicular rivers found in urban environments, nature persists and can even thrive in these rapidly expanding novel habitats.

While the destruction of native habitat required for urban expansion is generally incompatible with the persistence of native wildlife, there are some ‘urban specialists’ like pigeons (*Columba livia domestica*) (Sol and Senar 1995, Rose, Nagel et al. 2006), black rats (*Rattus rattus*) (Himsworth, Parsons et al. 2013, Feng and Himsworth 2014), and some plant species (Godefroid and Koedam 2007) that can be found in abundance in the highly modified environments of cities across the globe (Rebele 1994). In Australian cities, some native species have adapted to urbanised environments in a relatively short period of time. For example, native brushtail possums (*Trichosurus vulpecula*) (Eymann, Herbert et al. 2006), rainbow lorikeets (*Trichoglossus moluccanus*) (Daoud-Opit and Jones 2016), Australian white ibis (*Threskiornis molucca*) (Smith 2009), sulfur-crested cockatoos (*Cacatua galerita*) (Temby 2004), noisy miners (*Manorina melanocephala*) (Taylor, Taylor et al. 2013) and Australian brush turkeys (*Alectura lathamii*) (Jones and Everding 1991) can all be found in relative abundance in Australian suburbia (Temby 2004).

Urban ecologists recognise three broad categories of wildlife responses to urbanisation: 1) urban avoiders, 2) urban adapters, and 3) urban exploiters (Blair 2001, McKinney 2002, Shochat, Warren et al. 2006, Kark, Iwaniuk et al. 2007, Roetman and Daniels 2011). Native wildlife that are rarely found in urban landscapes are categorised as urban avoiders. For these species, urban expansion is a direct threat. Urban adapters, by comparison, are able to persist in urban areas. They are often habitat generalists such as small to medium aerial or arboreal species like brushtail possums and Australian magpies (*Gymnorhina tibicen*) (Roetman and Daniels 2011). Urban exploiters, often non-native species such as black rats (Kark, Iwaniuk et al. 2007), thrive in cities and rely on urban resources such as human food waste and artificial shelters. Such resources allow urban exploiters to become more abundant in urban areas compared to non-urban areas (Blair 2001, Roetman and Daniels 2011).

Urban Predators

While cities provide a challenging environment for carnivores with limited canopy cover and high habitat fragmentation, many predators are able to survive and thrive in and around large metropolitan areas (Bateman and Fleming 2012). For example, predators such as coyotes (*Canis latrans*) (Gehrt, Brown et al. 2011) and American black bears (*Ursus americanus*) (Beckmann and Lackey 2008) live in many North American cities, red foxes (*Vulpes vulpes*) in European and Australian cities (Robinson and Marks 2001, Wandeler, Funk et al. 2003, Bateman and Fleming 2012, DeCandia, Brzeski et al. 2019), and spotted hyenas (*Crocuta crocuta*) in many African cities (Abay, Bauer et al. 2011, Soulsbury and White 2016, Yirga, Leirs et al. 2016).

The incursion of predators into urban areas creates potential for negative implications including increased probability for human-wildlife conflict and spread of zoonotic diseases. Examples of human-wildlife conflict include attacks or fatalities of humans and domestic animals including livestock and pets, and damage to property as seen for coyotes (Gehrt, Brown et al. 2011, Poessel, Mock et al. 2017, Poessel, Gese et al. 2017), cougars (*Puma concolor*) (Gehrt, Riley et al. 2010), and black bears (Don Carlos, Bright et al. 2009) in North American cities. Increased human exposure to parasites and pathogens vectored by urban predators poses significant health risks. Zoonotic disease such as mange, rabies, and parasitic diseases can be spread in urban habitats by coyotes (Bradley and Altizer 2007, Dunbar, Sterner et al. 2007), red foxes (Marks and Bloomfield 1998, Soulsbury and White 2016) and feral dogs (Sparkes, McLeod et al. 2016, Harriott, Gentle et al. 2019).

Australia, the European red fox has moved into cities and is commonly found at a much greater density within urban habitats than rural surrounds (Marks and Bloomfield 1999, Robinson and Marks 2001, Short, Kinnear et al. 2002, Temby 2004, PestSmart 2011, Newsome, Crowther et al. 2014, Stepkovitch, Martin et al. 2019). The cryptic nature of these highly adaptable predators means that many Australians are unaware of their presence throughout even the most highly urbanised areas. For example, in a 2018 study using non-toxic baited camera traps in highly urbanised areas of Sydney, foxes were sighted at 90% of sampling sites across 16 locations (5 sites at each location) over an average sampling period of 19 nights at each site (Gil Fernández 2018). This suggests high levels of activity, even in the most densely populated areas.

Red Foxes in Australia

While Australia's historical isolation over evolutionary timescales has resulted in a large degree of endemism (Major 1988, Cracraft 1991), since European settlement in 1788 more than 3000 non-native species have invaded the island continent (McLeod and Norris 2004, Hoffmann and Broadhurst 2016). Introduced species have significant environmental impact upon native ecosystems. Notable examples in Australia include cane toads (*Rhinella marina*), feral cats (*Felis catus*), red foxes (*Vulpes vulpes*), and West Indian lantana (*Lantana camara*), (Kloot 1986, McLeod and Norris 2004, Shine 2010, Hoffmann and Broadhurst 2016). The European red fox was initially released in Australia in the 1850s for recreational hunting (Saunders, Coman et al. 1995, Contesse, Hegglin et al. 2004). By the 1890s, red fox populations had expanded so rapidly that they were declared pests in Victoria and had become a common sight in Armidale, New South Wales (over 1000km away). This initial rapid spread is thought to have been assisted by human translocations (Abbott 2011). Despite attempted control measures, the red fox now inhabits most of mainland Australia (Figure 1) and is one of the key species linked to the extinction and/or decline of many native species, such as little terns (*Sterna albifrons*) and eastern barred bandicoots (*Perameles gunnii*) (McLeod and Norris 2004, Thomsen and Willerslev 2015, Hoffmann and Broadhurst 2016).

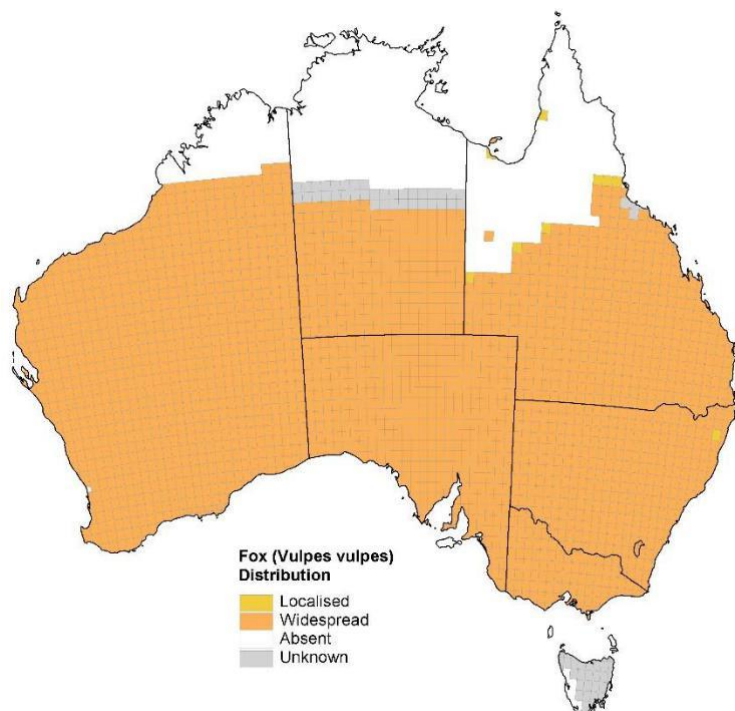


Figure 1: Current distribution of foxes within Australia (West 2008, PestSmart 2011).

The red fox has a significant impact on agricultural and native species across Australia. Red foxes cost the Australian economy more than \$200 million in damages annually, with an additional \$16 million spent on management measures (Saunders, Coman et al. 1995, McLeod and Norris 2004, Towerton, Kavanagh et al. 2016). Red foxes are opportunistic omnivores and surplus killers, meaning they will hunt and kill in excess of their nutritional requirements (Short, Kinnear et al. 2002, Contesse, Hegglin et al. 2004). This behaviour negatively influences Australian native species and livestock animals. Ground nesting birds, small-medium sized mammals, chickens and lambs are most greatly affected by red fox predation (Short, Kinnear et al. 2002, West 2008, PestSmart 2011, Hoffmann and Broadhurst 2016).

Red foxes breed once a year in the winter months, resulting in an average of four cubs born in spring (Saunders, Coman et al. 1995). As red foxes are highly territorial, once the offspring reach a mature age (approximately 6 months) they are forced to disperse to obtain their own territory. Red fox dispersal within a landscape is, on average, double the home range size, resulting in resource-rich environments being able to support a greater number of foxes with smaller territories (Trewhella, Harris et al. 1988, Šálek, Kreisinger et al. 2010, Hradsky, Kelly et al. 2019).

Control methods for red foxes in Australia focus on the spring birthing months and the autumn dispersal period in an attempt to reduce fox population growth (Saunders, Coman et al. 1995, Towerton, Kavanagh et al. 2016). Baiting, trapping, shooting, and den fumigation (Saunders, Coman et al. 1995, Towerton, Kavanagh et al. 2016) are all effective at reducing red fox numbers in treated areas. However, they are often not applied widely enough or with sufficient frequency to cause substantial, large-scale population reduction. Control methods are particularly difficult to implement within an urban setting due to risk to humans or pets from baiting or shooting (Thompson and Fleming 1994, Saunders, Coman et al. 1995).

Urban Red Foxes

In their native extent, red foxes were first observed within cities across the United Kingdom in the 1930s (Bateman and Fleming 2012). However, introduced red foxes in Australia have been observed in and around the metropolitan region of Sydney since about 1910 (Abbott, Peacock et al. 2014). The high availability of anthropogenic food resources in urban areas has been thought to have strongly contributed to this urban expansion of red foxes (Gortázar, Travaini et al. 2000, Yom-Tov and Geffen 2011, Oro, Genovart et al. 2013).

Red foxes are generalist opportunist omnivores meaning that their diet varies widely, including everything from seasonal fruits and berries to small mammals, lizards and birds (Contesse, Hegglin et al. 2004). In an urban setting this behaviour can result in a significant proportion of an urban fox's diet being derived from human resources such as garbage and food waste (Stepkovitch 2017). The high availability of anthropogenic food sources found within urban areas can support a higher red fox population than in surrounding areas (Marks and Bloomfield 1998, Saunders and McLeod 2007) and also likely influences animal size with red foxes in urban environments in Sydney found to have significantly larger body size and mass in both sexes than their rural counterparts (Stepkovitch, Martin et al. 2019).

In the Greater Sydney region, urban red fox densities are estimated at around 12 foxes per square kilometre (Saunders and McLeod 2007). This is significantly more than the average 2-3 foxes per square kilometre in surrounding rural areas. Management practices are limited within urban areas due to the proximity to humans and pets, meaning that baiting, trapping and shooting programs are heavily restricted. As a result, any reduction in fox numbers within a certain area is usually short lived as foxes will readily disperse in from surrounding areas (Towerton, Kavanagh et al. 2016). This may be particularly true in areas with high density fox populations, such as those seen in Australian cities.

Effects of Urbanisation on Genetic Connectivity

Urban areas create unique environments that vary greatly from natural habitats (Grimm, Faeth et al. 2008, Johnson and Munshi-South 2017). Matrices of roads and buildings separate and degrade natural habitats within urban landscapes, limiting functional dispersal among habitat types (Forman, Sperling et al. 2003, Beninde, Feldmeier et al. 2016). For smaller ground-dispersing species, these anthropogenic developments can create impermeable barriers, causing habitat fragmentation (Riley, Sauvajot et al. 2003, Keyghobadi 2007, Delaney, Riley et al. 2010). Population genetics using neutral genetic markers (from loci not affected by selection) can be used to identify and explore the effects that these human-made structures have on genetic connectivity and gene flow.

Landscape barriers are one of the most common restrictions to gene flow (Balkenhol and Waits 2009). In urban environments these barriers can come in the form of roads and train lines, buildings, and rivers or landscape features such as elevated ridges, that limit species dispersal (Johnson and Munshi-South 2017). When part of the population is separated from the rest for a prolonged period of time, it can result in a fragmented population (Forman, Sperling et al. 2003, Balkenhol, Dudaniec et al. 2017). A recent study of urban-dwelling common wall lizards (*Podarcis muralis*) (Beninde,

Feldmeier et al. 2016) using species distribution models identified that while rivers provided the most significant dispersal barrier to this species, urban architecture and roads also significantly limited population gene flow. Similar studies on koalas (*Phascolarctos cinereus*) found that highways and freeways provided more significant gene flow barriers than other road types (Dudaniec, Rhodes et al. 2013). This was predicted to be due to higher levels of traffic causing koala mortality and/or physical barriers surrounding the highways and freeways preventing the koalas from crossing. Reduced canopy cover was also found to limit dispersal in this species (Dudaniec, Rhodes et al. 2013). These examples highlight the compounding effect of urban development and the multiple ways that urbanisation can manifest gene flow barriers for different species.

Urbanisation can also negatively impact the gene flow of aerially dispersing species. Despite being able to fly over some dispersal barriers, such as roads, buildings and rivers, species dispersal of some birds and bees is limited by urban development. Recent studies on song sparrows (*Melospiza melodia*) (Unfried, Hauser et al. 2013) and yellow-faced bumble bees (*Bombus vosnesenskii*) (Jha and Kremen 2013) found that urban areas with more than 50% impervious surface (such as concreted areas or roads) significantly reduced gene flow between populations. In the case of song sparrows, the observed genetic variation between populations was also correlated with the age of the urban developments, suggesting that individuals could move across the landscape but at reduced levels to pre-urban development (Unfried, Hauser et al. 2013).

Landscape genetics can also be used to explore dispersal patterns in invasive species to target eradication or management efforts. A recent study in Singapore on the invasive Javan myna (*Acridotheres javanicus*) detected a gene flow barrier correlated with areas with lower density human populations, low-rise housing estates, reduced open-air eateries (this species' primary food source is scavenged from human eateries), and parkland (Low, Chattopadhyay et al. 2018). Through the identification of this dispersal barrier the authors were able to advise that management of the invasive species should focus on reducing public waste and incorporating low-rise housing and parklands into planning policy to reduce both resource availability (food and nesting) and gene flow potential.

Landscape Genetics of Urban Red Foxes in Sydney, Australia

In the greater Sydney area, little is known about the genetic population structure, connectivity, or dispersal patterns of red foxes in urban, peri-urban, and rural areas. Genetic studies of red fox populations in Melbourne found that genetic relatedness between individuals within urban areas

was significantly less than rural individuals (Marks and Bloomfield 1999). This suggests reduced dispersal within Australian urban areas, which agrees with previous findings of reduced red fox dispersal in European (Wandeler, Funk et al. 2003, Atterby, Allnutt et al. 2015, Šálek, Drahníková et al. 2015) and Japanese (Kato, Amaike et al. 2017) cities. The study also identified dense urban residential housing in Melbourne as a dispersal barrier for foxes, however, increases in urban development density may affect dispersal differently in the 20 years post publication. No studies have been conducted on the genetic structure or landscape effects of urbanisation on red foxes in the Greater Sydney region.

Information about the landscape scale genetic structure of red fox populations in urban areas would significantly benefit control efforts and enable management professionals to focus eradication events in strategically important areas thus utilising limited management funding to achieve the greatest impact. Genetic population information, such as an effective population size estimate for the Sydney red fox population(s), could be used as an important reference for assessing the impact of future red fox control projects.

Project Aims

Through this study I aimed to answer four key questions:

- 1) How is genetic variation structured in red foxes across the Greater Sydney region?
- 2) Are levels of gene flow in red foxes influenced by habitat type across the Greater Sydney region?
- 3) Is there sex-biased dispersal of red foxes within the Greater Sydney region?
- 4) What is the effective population size of the red fox population(s) across the Greater Sydney region?

To answer these questions, I used a population genetic based analysis conducted on 94 foxes collected from across urban, peri-urban, and rural environments around Greater Sydney. I assessed population structure, connectivity, and dispersal patterns within urban, peri-urban, and rural habitats, as well as between sexes. I conducted landscape genetic analyses to assess the effect of urbanisation, roads and railways, and water ways on red fox dispersal. Lastly, I estimated the effective population size of red foxes of the Greater Sydney region.

STUDY AREA

I collected a total of 94 red fox (*Vulpes vulpes*) tissue samples across the three habitat types (figure 2). The habitats were defined based on human density per local government area, with urban classified as > 2000 people/km², peri-urban within 10km of 500 – 2000 people/km², and rural < 500 people/km². However, as baiting is widely used to control red foxes in rural areas, and baited fox carcasses are not as easily located as shot fox carcasses, it was much more difficult to obtain rural than urban or peri-urban samples. Based on information from the Catchment Scale Land Use of Australia (CLUM) 2018 dataset (Australian Bureau of Agricultural and Resource Economics and Sciences 2018) the primary types of land cover found in rural areas around Sydney are forest and low impact agriculture such as grazing land or cropping, with low levels of urbanisation in the form of roads, rural residential housing, and farm infrastructure. Peri-urban habitats are made up of a combination of forests, grazing land, cropping, irrigated farming, intensive animal production (dairy sheds, horse studs etc.) and rural manufacturing. Peri-urban habitats have a medium level of urbanisation with more roads, railways, rural residential housing, and urban residential housing. Urban habitats largely consist of urban industrial, public/commercial services, urban defence facilities, research facilities and low levels of forest and farming. Urban habitats have dense road and railway matrices as well as high levels of urban residential housing and industrial land cover.

METHODS

Sample Collection

I used samples from red foxes killed between May 2016 and May 2019. Some samples ($n = 81$) were provided from a previous study (Stepkovitch 2017), and I collected 29 new samples from red fox carcasses provided by authorised wildlife management shooters and trappers. A total of 94 samples were chosen from a much larger pool ($n = 190$) of available samples, including 40 from urban habitats, 38 from peri-urban habitats, and 16 from rural habitats (Figure 1). While efforts were made to obtain an even spread of foxes from each habitat, far fewer fox carcasses were collected from rural areas due to factors outlined above. The selected samples comprised of 40 females and 54 males, due to a male bias in collection. A bias towards males in red fox sampling in New South Wales has previously been observed (Kay, Gifford et al. 2000). The sex, size (femur length and nose to tailbone length in cm), condition of the fox, and geographic location was recorded. I removed 1cm x 1cm tissue samples from each fox's ear. These tissue samples were placed in 1.5 ml plastic sample tubes, immersed in 70% ethanol and refrigerated. Samples were then

sent to Diversity Arrays Technology Pty. Ltd. (Canberra, Australia – <http://www.diverityarrays.com>) for DNA extraction, Sequencing and SNP development.

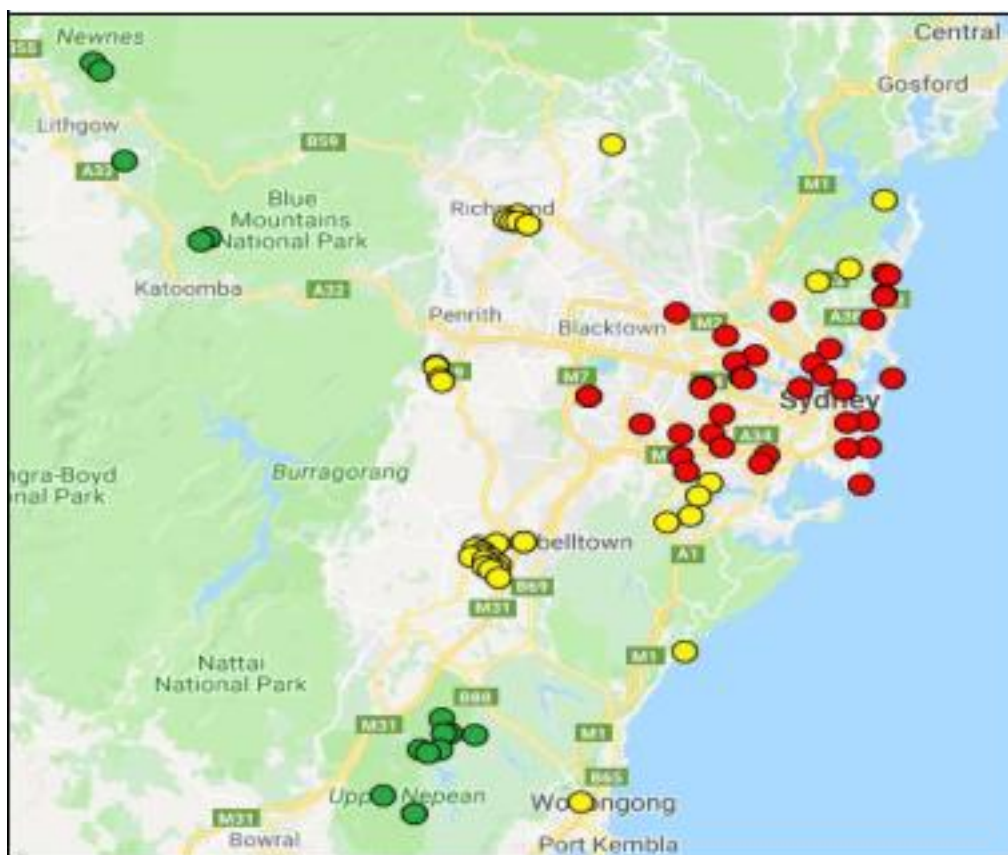


Figure 1: Locations of the 94 red fox samples used in this study. Points are coloured to represent the three different habitats sampled. Urban = red, peri-urban = yellow, rural = green.

DNA Sequencing and SNP Development

Library Preparation

Prior to library preparation, DNA was extracted at Diversity Arrays Technology Pty. Ltd. using the chloroform:isoamyl alcohol procedure (Sambrook and Russell 2006). DNA was incubated at 37 °C for 2 hours in a 1X solution of Multi-CoreTM restriction enzyme buffer to check for quality.

Restriction enzymes *Pst*I and *Sph*I were used to digest each sample before amplification using barcode and adaptor PCR primers. The PCR amplification used the following conditions: Initial denaturation for 1 minute at 94 °C, then 30 cycles of; 20 second denaturation at 94 °C, 30 seconds annealing at 58 °C, and 45 seconds extension at 72 °C, the amplification finished with a 7 min extension at 72 °C. Using NaOH 10 µL of each sample was pooled, diluted and denatured in preparation for SN hybridization to the flow cell. Sequencing of the library occurred using an

Illumina HiSeq®2500 platform (single read) for 77 cycles, resulting in 77 base pair long fragments. More than 40% of the of the samples underwent a second run of the whole preparation protocol to create a set of technical replicates to test reproducibility of SNP sequences.

Initial SNP Calling, Quality Control, and Data Filtering

Sequences were converted to fastq format using the Illumina HiSeq®2500 software and samples were de-multiplexed based on their ligated barcode using DArT developed customized scripts. Using Phred (Ewing, Hillier et al. 1998) quality scores (Q-scores), reads with Q-scores <25 were removed and were check for contamination using at the DArT database using GenBank viral and bacterial sequences. Single nucleotide polymorphism (SNPs) were identified and called using in DArT proprietary pipeline DArTSoft14TM (Diversity Arrays Technology) standard procedures. Monomorphic clusters were removed retaining only homozygous and heterozygous forms. Filtering for SNPs with average ratio of read depths between alleles of 0.75, reproducibility average of >90%, and a minimum read depth of 5

The initial dataset I received from DArT, contained 25528 single nucleotide polymorphisms (SNPs) from 90 individuals. Following quality control four individuals were removed due to poor sequencing. I used a script I created in the program Rstudio (Allaire 2012) to further filter the dataset received from DArT to retain loci with; a 100% reproducibility average, <5% missing data, a maximum average read depth of 20.95 (average read depth + 4 x $\sqrt{\text{average read depth}}$), an average minimum read depth of >5, a minor allele frequency of >0.2, and no duplicate clone IDs. I also filtered for individuals with <10% missing data.

To complete the population and landscape genetic analysis, I first removed loci potentially associated with selection. I used a F_{ST} outlier approach with the programs BayeScan v2.0 (Foll and Gaggiotti 2008) and Arlequin v3.5.2.2 (Schneider, Roessli et al. 2000) and the package “OutFLANK” (Whitlock and Lotterhos 2015) and removed any loci putatively under selection.

I then removed loci significantly deviating from Hardy-Weinberg equilibrium (HWE) that may be attributable to genotyping errors. HWE tests were conducted using Arlequin. To correct for multiple tests p-values were modified to q-values using the package “qvalue” (Dabney, Storey et al. 2010) in R. False discovery rates of less than 0.05 (q-value < 0.05) were removed.

Population Structure

The package “StrataG” (Archer, Adams et al. 2017) was used to output summary statistics for the 33 habitat types and the whole sample.

I used the package “Adegenet” (Jombart 2008) within R to conduct a principle component analysis (PCA) to identify clustering within the samples. Using Admixture with the package “LEA” (Frichot and François 2015) I then inferred the likely number of populations (K). The program “fineRADstructure” and the R package “RADpainter” (Malinsky, Trucchi et al. 2018) was used to estimate K based on nearest neighbour haplotype coancestry. I also used the program “STRUCTURE v2.3.4” (Pritchard, Stephens et al. 2000) to assign individuals to populations. I ran STRUCTURE from 1k to 10k with a burnin period of 1000 and 10,000 iterations. The online program “Structure Harvester” (Earl 2012) was used to analyse the results from the STRUCTURE analysis to ascertain the best estimate of K.

Spatial Structure

Effect of Distance, Habitat, and Sex on Spatial Structure

To examine the spatial genetic structure within the total population, pairwise genetic and geographic distance matrices were created using GenAlEx v 6.5 (Smouse and Peakall 2012). A spatial autocorrelation analysis in GenAlEx v 6.5 was then used to examine the effect of distance on genetic relatedness between individuals. Spatial autocorrelation analysis uses a multivariate method to concurrently test the spatial signal generated by multiple loci (Smouse and Peakall 1999, Peakall, Ruibal et al. 2003, Smouse, Peakall et al. 2008). This approach was used to generate a genetic correlation (r) between individuals who were located within specified geographic distance classes. Isolation by distance is indicated by r decreasing as geographic distance increases.

I selected eight distance classes (5, 10, 20, 30, 50, 75, 100, and 130 km) for my spatial autocorrelation. These were chosen to provide adequate sampling density while covering a broad range of distances within the maximum extent between individuals of 130 km. I also ran spatial autocorrelation analysis with stacked r values at even distance classes in increments of 10 km up to the maximum of 130 km (from 0km to $x + 10$ km). Significance was evaluated by generating 95% confidence intervals around mean r for each distance class and the null hypothesis of no spatial structure ($r = 0$) using 9999 bootstraps. The presence of isolation-by-distance was examined using heterogeneity tests (Banks and Peakall 2012).

To test habitat type effects on spatial structure, I conducted three separate spatial autocorrelation analyses on the samples within the respective habitats, with the following distance classes were chosen for each habitat: urban = (5, 10, 15, 20, 30, and 45 km), peri-urban = (5, 20, 40, 70, and 100 km), and rural = (10, 50, and 120 km). I also ran spatial auto correlation analysis for the three different habitat treatments with stacked r values calculated at increasing distance increments (urban = increments of 4 km, peri-urban = increments of 5 km, and rural = increments of 10 km).

To test the effect of sex on spatial structure I conducted separate spatial autocorrelation analyses for males and females within the total region and within the urban habitat. The following distance classes were chosen for each habitat: total males = (5, 20, 40, 80, and 130 km), total females = (10, 20, 40, 80, and 105 km), urban males = (9, 20, and 43 km), and urban females = (11, 20, and 42 km). I also ran spatial auto correlation analysis for the four different treatments with stacked r values calculated at increasing distance increments (total males = increments of 10 km, total females = increments of 10 km, urban males = increments of 4 km, and urban females = increments of 4 km).

Evaluating the Influence of Landscape Variables on Genetic Structure

To assess the impact of landscape features on genetic structure I first needed to create raster maps of the variables I wanted to test: urbanisation, roads and railways, and waterways. I chose to test for these landscape features because previous studies have suggested that they create dispersal barriers for foxes (Robinson and Marks 2001, Kato, Amaike et al. 2017).

Creating Land Cover Resistance Raster Maps

A base raster map of landcover variables was obtained from the Catchment Scale Land Use of Australia (CLUM) 2018 dataset (Australian Bureau of Agricultural and Resource Economics and Sciences 2018). As the initial raster map contained 110 different landcover variables, I used the program “QGIS v 3.8” (QGIS Developer Team 2009) to condense the raster to five variables reflecting low to high urbanisation (Figure 2).

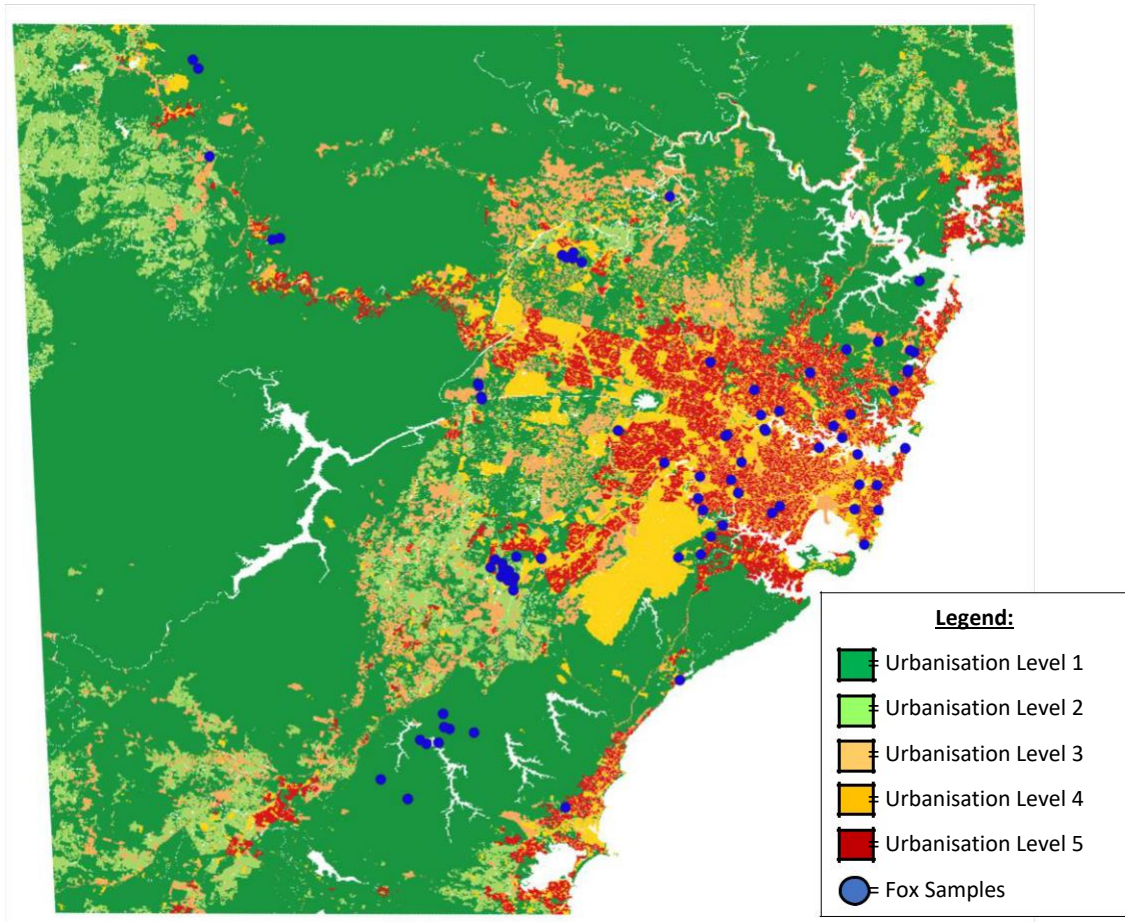


Figure 2: Map showing the five different urbanisation scores with red fox sample locations.

Using QGIS v 3.8 five separate raster maps were created from the modified urbanisation landcover map. The five maps each showed only one level of urbanisation (map 1 = urbanisation level 1, map 2 = urbanisation level 2, map 3 = urbanisation level 3, map 4 = urbanisation level 4, and map 5 = urbanisation level 5). A further two maps were created from the original CLUM raster file; one showing land covered by roads and railways, and another showing land covered by inland waterways (not the ocean). As I wanted to test each landcover variable as a potential barrier to dispersal, notepad++ was used to edit each raster map so that the landcover variable interest was assigned a resistance value of 10,000 (very high resistance) and all other landcover types were assigned a resistance value of 1 (very low resistance). Notepad++ was used to create a final resistance map with all land cover variable (excluding the ocean) set at a resistance value of 1. This map would be used to create a least cost geographic distance pairwise matrix.

Creating Pairwise Matrices of Landcover Resistance

I used the program “Circuitscape” (McRae, Shah et al. 2016) to create pairwise resistance matrices between each sample for each land cover variable. Circuitscape uses a circuit theory-based approach to establish least cost pathways between each individual and create a pairwise matrix showing the average resistance between each pair of individuals. Eight pairwise matrices were created, one for each of our resistance maps. Circuitscape was also used to create dispersal current maps for each resistance map, showing the combined least cost pathway between each pair of individuals.

Comparing the Matrices

To test which level of urbanisation best explained the observed genetic variation, I used the package “coreMLPE” (<https://github.com/nspope/corMLPE> Supporting Information) (Pinheiro, Bates et al. 2017) in R to conduct linear based maximum likelihood population effects (MLPE). Each pairwise resistance matrix for urbanisation levels was compared to the genetic distance matrix (GD) created in GenAlEx v 6.5. GD was also compared to each resistance matrix + the geographic distance (GGD) matrix created in GenAlEx v 6.5, and each resistance matrix + the least cost geographic distance matrix (LC-GGD) created in Circuitscape. Lastly, genetic distance was compared to all resistance models combined + GGD, and each resistance model combined + LC-GGD. In separate MLPE analyses, GD was compared to waterways (WW) (GD was compared to WW, WW + GD, and WW + LC-GGD) and roads/railways (RR) (GD was compared to RR, RR + GD, and RR + LC-GGD). Akaike information criteria (AIC) and Bayesian information criteria (BIC) was used to select the best model (Row, Knick et al. 2017).

Effective Population Size

The effective population size (N_e) is a hypothetical ‘ideal’ population that would lose genetic variation at the same rate that is observed within the known population (Frankham 1995). A skewed sex ratio, low founder population, or a genetic bottleneck can result in a N_e lower than the actual population. To estimate the effective population size, I used the program “NeEstimator 2.1” (Do, Waples et al. 2014). The N_e estimation via linkage disequilibrium models’ output was corrected for physically linked loci, as described by Waples, Antao et al. (2014), using the number of chromosomes of the red fox (34) (Buckton and Cunningham 1971). The following equation was used:

$$N_e (Adj) = 0.098 + 0.219 * \ln \left(\frac{1}{h^2} \right)$$

RESULTS

Filtering

A total of 25,528 single nucleotide polymorphisms (SNPs) were received from 90 individual foxes. Due to an error in the DNA extraction and sequencing process DNA could not be obtained from four foxes. Due to more than 10% missing data I excluded a further four individuals from the data set. F_{st} outlier test found no loci under selection and 259 loci were removed because they significantly deviated from HWE. Following the filtering process, I retained 2078 SNPs from 86 individuals (see Appendix A for further details on the filtering process). The proportion of each sex in the three habitat types was as follows: urban = 36 foxes (males = 20, females = 16), peri-urban = 35 foxes (males = 24, females = 11), rural = 15 foxes (males = 8, females = 7).

Genetic Structure in the Greater Sydney Region

Summary statistics can be found in table 1.

Table 1: Summary statistics from the total region, as well as urban, peri-urban, and rural habitats.

Location	Allelic Richness	Proportion Unique Alleles	Expected Heterozygosity (H_e)	Observed Heterozygosity (H_o)	Inbreeding Coefficient (F_{is})
Urban	0.0559	0.0156	0.3080	0.2742	0.1083
Peri-Urban	0.0577	0.0142	0.3052	0.2682	0.1150
Rural	0.1299	0.0397	0.3048	0.2930	0.0326
Total	0.0812	0.0232	0.3060	0.2784	0.0853

The PCA analysis provided some evidence for genetic structure (Figure 3A). Most of the individuals belonged to one large cluster (grey), while three smaller clusters were located in the urban north shore of Sydney (green), around peri-urban Camden (blue), and in rural Upper Nepean (red) (Figure 3B). The presence of these clusters was supported by coancestry inferred clusters found in the fineRADstructure analysis (Figure 4). Despite the distribution of genetic distances apparent in the PCA, the best model for the data resolved using Admixture and STRUCTURE was $K = 1$ (Figure 5).

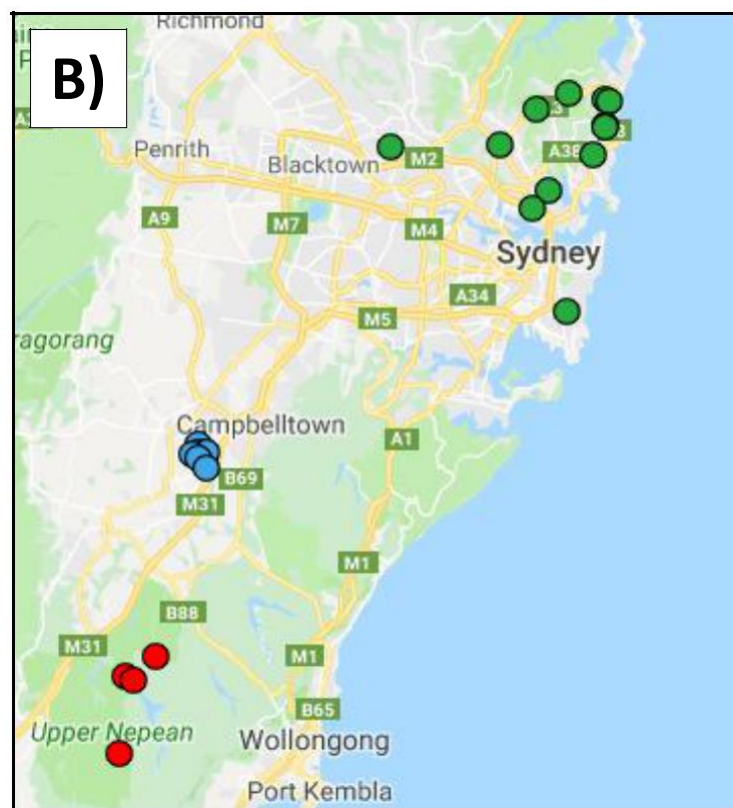
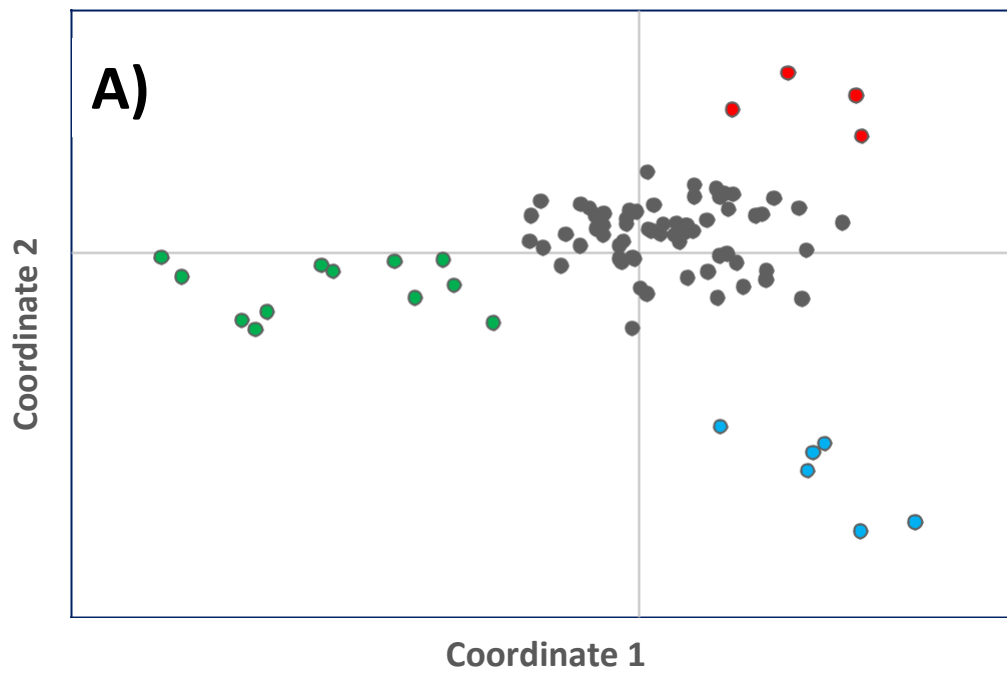


Figure 3: A) PCA of genetic variation within the region indicating possible presence of four genetic clusters. One large cluster of x individuals (grey) and three smaller cluster containing four (red), six (blue), and twelve (green) individuals. B) Map showing the location of individuals within the three smaller clusters (red, blue, and green).

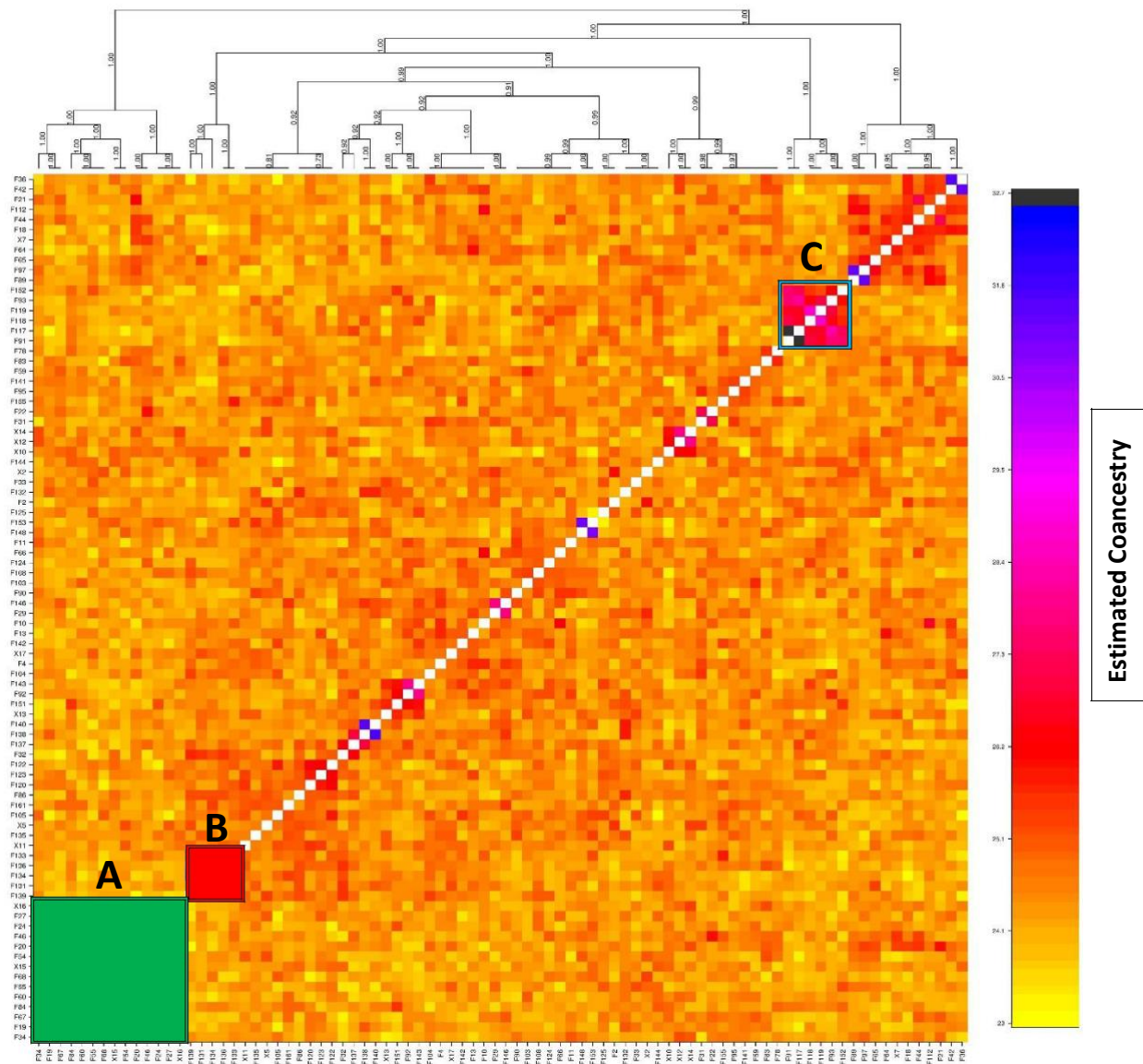


Figure 4: Pairwise estimated coancestry matrix with the three smaller clusters highlighted as following: A = north shore (green), B = Upper Nepean (red), and C = Camden (blue).

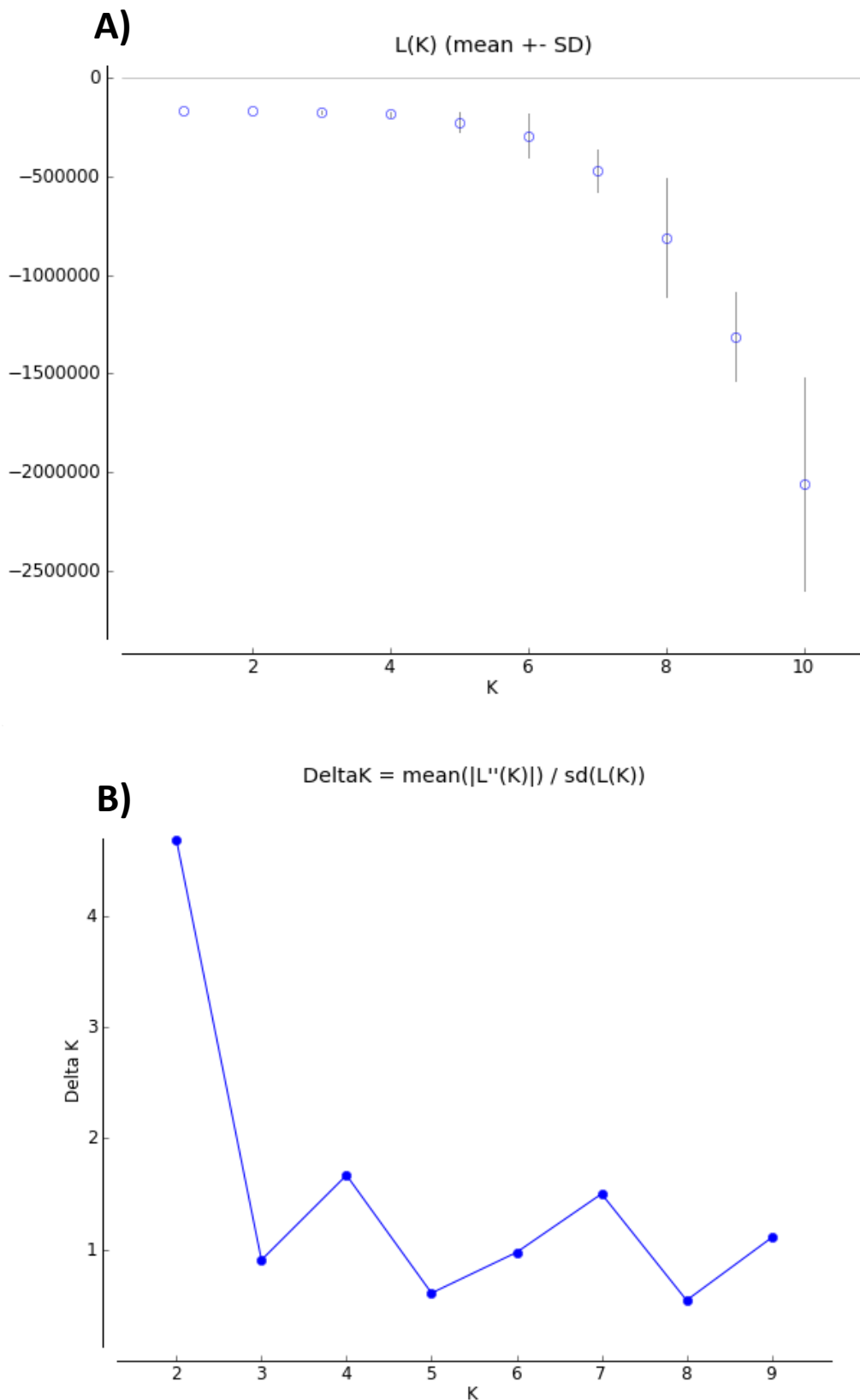


Figure 5: The value of K that has the highest likelihood (L(K)) in STRUCTURE and STRUCTURE harvester was K=1. **A)** Mean L(K) indicating K = 1 is most likely. **B)** The highest deltaK indicate the most likely value of K (minimum of K = 2).

Effect of Habitat Type on Genetic Variation

Across the Greater Sydney region, genotypic similarity (r) was significantly greater than the null hypotheses of $r = 0$ at distances 0-5 km ($r = 0.054$, $p < 0.01$), 6-10 km ($r = 0.021$, $p < 0.01$), 11-20 km ($r = 0.012$, $p < 0.01$), and 21-30 km ($r = 0.003$, $p < 0.05$) (Figure 6A) with the $r = 0$ intercept occurring at 35.907 km. A heterogeneity test supported a pattern of isolation-by-distance (IBD) across the entire Greater Sydney region ($\omega = 99.249$, $p < 0.01$), (Figure 6A and Appendix B). There was no significant evidence of spatial structure within rural habitats ($\omega = 20.195$, $p > 0.01$) suggesting unimpeded gene flow (Figure 6B and Appendix B). Peri-urban and urban habitats had evidence of significant positive spatial structure (peri-urban $\omega = 37.633$, $p < 0.01$, urban $\omega = 56.905$, $p < 0.01$) with genetic relatedness significantly greater distances of 0-5 km for peri-urban ($r = 0.027$, $p < 0.01$, $r = 0$ intercept = 21.969 km) (Figure 6C) and 0-5 km for urban ($r = 0.027$, $p < 0.01$, $r = 0$ intercept = 16.444 km) (Figure 6D) habitats, again suggesting IBD within these habitats (Figures 6C, 6D, and Appendix B).

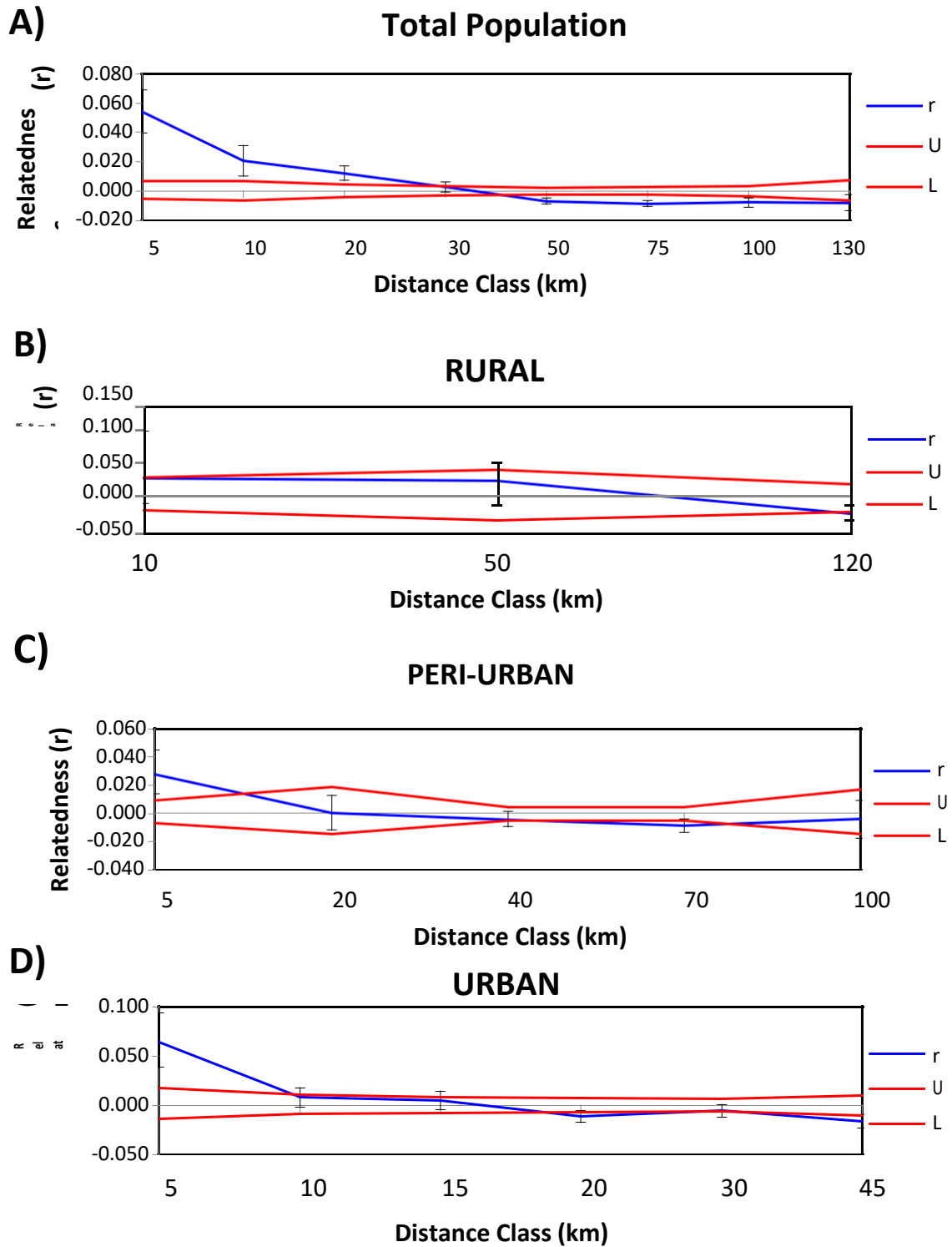


Figure 6: Spatial correlogram displaying mean genetic relatedness (r) at different distance class bins. **A)** total region, **B)** rural habitat, **C)** peri-urban habitat, **D)** urban habitat. Error bars show the 95% confidence intervals of each distance class as determined by 9999 iterations. Upper (U) and lower (L) 95% confidence intervals of the null hypothesis ($r = 0$) as determined by 9999 iterations are also shown (red dashed lines).

Sex-Biased Dispersal of Red Foxes in the Greater Sydney Region

There was significant evidence for positive spatial structure in both male or female red foxes across the Greater Sydney region (heterogeneity tests: males $\omega = 55.774$, $p < 0.01$, females $\omega = 42.043$, $p < 0.01$). Genetic relatedness was significantly greater than the null hypotheses for males at distances 0-5 km ($r = 0.05$, $p < 0.01$) and 6-20 km ($r = 0.017$, $p < 0.01$), with an $r = 0$ intercept of 37.187 km (Figure 7A). For females, genetic relatedness was significantly greater than the null hypothesis at distances 0-5 km ($r = 0.035$, $p < 0.001$) and 6-20 km ($r = 0.009$, $p < 0.05$) with an $r = 0$ intercept of 36.135 km (Figure 7B). The similarity of male and female average dispersal distances across the Greater Sydney region indicates a lack of evidence for sex-biased dispersal across the total region (Figures 7a, 7b, and Appendix B).

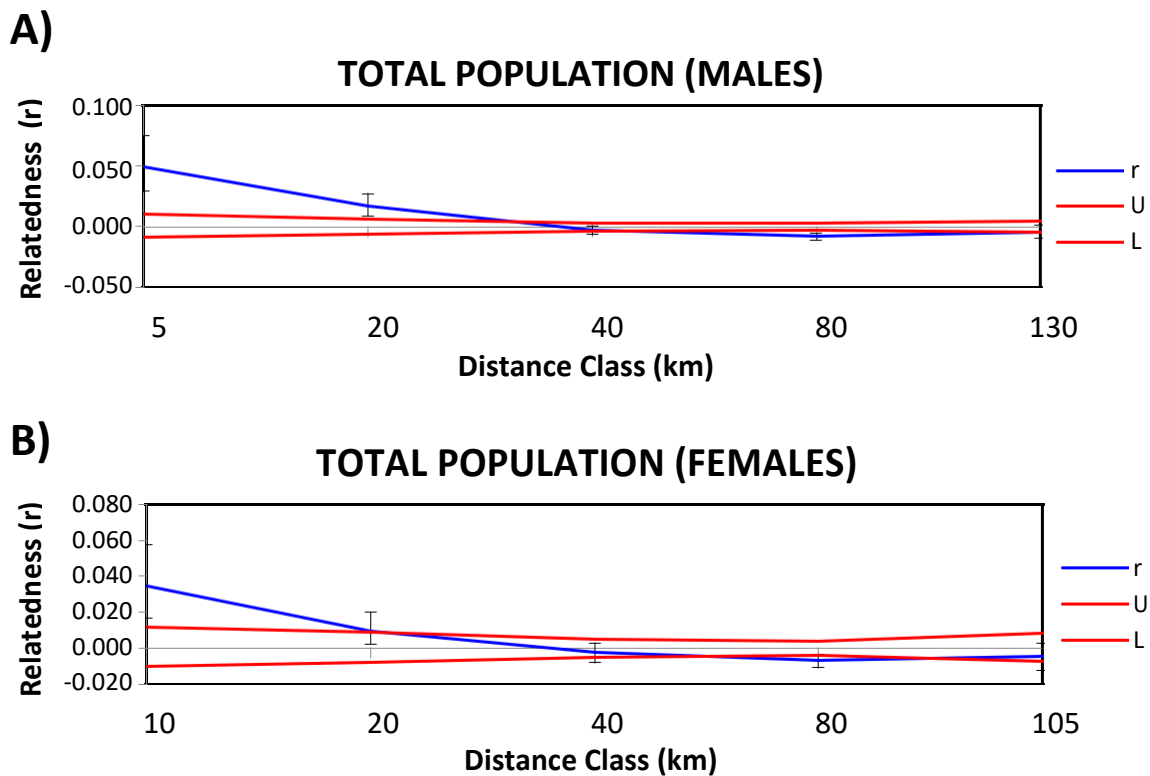


Figure 7: Spatial correlogram displaying mean genetic relatedness (r) within the total region at different distance class bins. **A)** males and **B)** females. Error bars show 95% confidence intervals for each distance class as determined by 9999 iterations. Upper (U) and lower (L) 95% confidence intervals of the null hypothesis ($r = 0$) as determined by 9999 iterations are also shown (red dashed lines).

In urban habitats, heterogeneity tests indicated isolation by distance for females ($\omega = 26.527$, $p < 0.01$) but not males ($\omega = 16.293$, $p > 0.01$). Genetic relatedness in females was greater than 0 from 0-11 km ($r = 0.027$, $p < 0.01$, $r = 0$ intercept = 17.650 km) (Figure 8A). The lack of evidence for spatial structure in urban males may be due to insufficient sampling as genetic relatedness was greater than 0 from 0-9km ($r = 0.017$, $p < 0.05$, $r = 0$ intercept = 21.311 km) (Figure 8B).

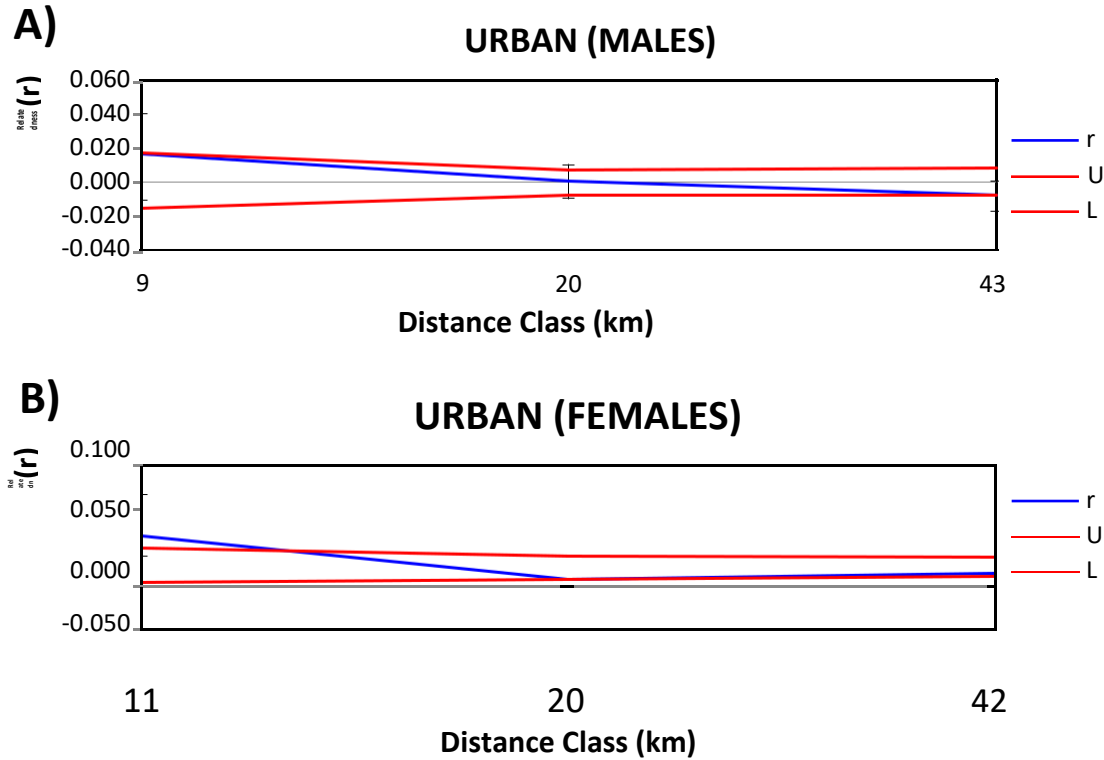


Figure 8: Spatial correlogram displaying mean genetic relatedness (r) within the urban habitat at different distance class bins. **A)** males and **B)** females. Error bars show the 95% confidence intervals for each distance class as determined by 9999 iterations. Upper (U) and lower (L) 95% confidence intervals for the null hypothesis ($r = 0$) as determined by 9999 iterations are also shown (red dashed lines).

Effect of Urbanisation on Dispersal

The MLPE comparing the correlation between urbanisation landcover resistance and geographic distance to observed genetic distance matrices indicated an effect of urbanisation on dispersal. The resistance models with a $\Delta AICc < 5$ shown in Table 2. All model results are provided in Appendix C. Observed genetic variation in the Greater Sydney region was strongly correlated with all models that included geographic distance combined with land cover resistance, as well as geographic distance alone (Table 2). Current maps are given showing the cumulative least-cost pathway dispersal between each sample in the Greater Sydney region when the “resistance 3” model was

applied (as this showed the strongest correlation to genetic variation based on $\Delta AICc$) (Appendix D).

Table 2: Urbanisation models with delta AICc values of less than 5 for straight line distance and least cost distance models. Table is ordered by lowest to highest delta AICc.

Method	Model	$\Delta AICc$	BIC	Weight
Straight Line Distance	GD ~ GGD + Resistance 3	0	41758.93	0.547254
	GD ~ GGD	2.2753	41755.01	0.175434
	GD ~ GGD + Resistance 4	4.191505	41763.12	0.0673
	GD ~ GGD + Resistance 5	4.199312	41763.13	0.067038
	GD ~ GGD + Resistance 1	4.202979	41763.13	0.066915
	GD ~ GGD + Resistance 2	4.266365	41763.2	0.064828
Least Cost Distance	GD ~ GGD + Resistance 3	0	41650.89	0.300844
	GD ~ GGD	0.233149	41644.92	0.267741
	GD ~ GGD + Resistance 1	1.793711	41652.68	0.122699
	GD ~ GGD + Resistance 4	2.159892	41653.05	0.102171
	GD ~ GGD + Resistance 5	2.177506	41653.06	0.101275
	GD ~ GGD + Resistance 2	2.238363	41653.13	0.09824

Within the total population a combination of geographic distance and transport infrastructure (roads and railways) was more strongly correlated with genetic variance compared to either geographic distance or transport infrastructure alone (Table 3). Waterways were more strongly correlated with genetic variance when compared to either geographic distance alone or a combination of geographic distance and waterway resistance (Table 3). Current maps showing the cumulative least-cost pathway dispersal between each sample in the Greater Sydney region when roads and railways, and waterways were acting as dispersal barriers can be seen in Appendix D.

Table 3: Road/railway and waterway models with delta AICc values of less than 5 for straight line distance and lease cost distance models. Table is ordered by lowest to highest delta AICc.

Method	Model	$\Delta AICc$	BIC	Weight
Straight Line Distance	GD~GGD+RR	0	41656.32	0.999923
	GD~RR	18.93609	41669.06	7.73E-05
	GD ~ GGD	104.8802	41755.01	1.68E-23
Least Cost Distance	GD~GGD+RR	0	41638.42	0.998257
	GD ~ GGD	12.70115	41644.92	0.001743
	GD~RR	36.84125	41669.06	9.98E-09
Straight Line Distance	GD~WW	0	41642.6	0.554204
	GD~GGD+WW	0.435339	41649.24	0.445796
	GD ~ GGD	112.4017	41755.01	2.17E-25
Least Cost Distance	GD~WW	0	41642.6	0.530904
	GD~GGD+WW	1.125278	41649.93	0.302458
	GD ~ GGD	2.317505	41669.06	0.166639

The Effective Population Size

The initial estimate for N_e using a linkage disequilibrium method was 184.3 (95% Cis = 181.6, 187.2). After correcting for physically linked loci, using the number of chromosomes for the red fox, the estimated of $N_{e(adj1)}$ was 211.7726 (95% Cis = 208.6702, 215.1049). Density estimates suggest around 12 foxes per square km in urban Sydney and Sydney urban extent is predicted to be 1625.27 km² (Angel, Blei et al. 2012), resulting in an estimated 19,503 foxes in urban Sydney. Even if this estimate was halved the estimated N_e is still extremely low.

DISCUSSION:

The average inferred dispersal of red foxes within urban habitats in the Greater Sydney region was significantly lower than in peri-urban and rural habitats. I found that medium to high level of urban landscape features combined with geographic distance, roads, railways and waterways limited dispersal of red foxes across the total Sydney region. I discovered no evidence for sex-biased dispersal of red foxes within the total region or within urban habitats. The effective population size (N_e) of red foxes within the total region was unusually low and may imply reduced adaptive potential.

Based on the genetic variation observed in red foxes from the greater Sydney region there was sufficient gene flow to prevent significant localised differences in allele frequencies.

However, genotypic data infers that dispersal of red foxes in urban habitats was significantly lower than surrounding peri-urban and rural habitats.

Previous research suggests red fox dispersal distances are on average double their home range (Trehwella, Harris et al. 1988, Šálek, Kreisinger et al. 2010, Hradsky, Kelly et al. 2019). The reduced dispersal distances of red foxes in urban compared to peri-urban and rural Sydney habitats seen here may be the result of reduced urban home ranges. Reduced dispersal and potentially smaller home ranges within urban areas support the estimated higher density of red foxes in urban Sydney compared to surrounding rural habitats (Saunders and McLeod 2007).

Reduced genetic variation and dispersal distances of red foxes in urban habitats is also found in other Australian cities and around the world. Studies on red foxes in Melbourne (Marks and Bloomfield 1999, Robinson and Marks 2001) found that in urban habitats genetic variability and average dispersal distances were lower than surrounding rural areas. Similar evidence from European, English, American, and Japanese cities identified reduced dispersal of red foxes in urban habitats (Simonsen, Pertoldi et al. 2003, Wandeler, Funk et al. 2003, Atterby, Allnutt et al. 2015, Šálek, Drahníková et al. 2015, Kato, Amaike et al. 2017). Further studies on red fox dispersal within urban areas are limited, restricting our current understanding of the effects of urbanisation on this aspect of Australian red fox population genetics.

Density estimates of red foxes within Australian cities are between 3 and 16 foxes per km² (Marks and Bloomfield 1999), however it is important to note that these estimates are based on a study from 20 years ago in Melbourne and may have changed with continued urban expansion. While further studies of red fox densities in Australian cities are needed, observed densities of red foxes in cities around Europe, England, and North America are similarly much higher within urban habitats

than rural habitats, reaching up to 37 foxes per km² in some areas (Baker, Funk et al. 2000, Wandeler, Funk et al. 2003, Baker, Dowding et al. 2007, Šálek, Drahníková et al. 2015).

Estimates of home ranges of foxes in Australia range from 0.23 km² (Marks and Bloomfield 2006) within urban habitats to 28 km² (Burrows, Algar et al. 2003) in rural habitats. Low home ranges within cities have also been found in red fox populations in England, Europe, and North America (Harris 1981, Baker, Funk et al. 2000, Baker, Dowding et al. 2007, Šálek, Drahníková et al. 2015) indicating a significant effect of urbanisation on home ranges of urban red foxes.

Red foxes are not the only predators that experience increased density and reduced home range within urban environments. Similar patterns have been found for grey foxes (*Urocyon cinereoargenteus*), coyotes, bobcats (*Lynx rufus*), raccoons (*Procyon lotor*), striped skunks (*Mephitis mephitis*), Eurasian badgers, and stone martins (*Martes foina*) (Rodewald and Gehrt 2014, Šálek, Drahníková et al. 2015, Poessel, Gese et al. 2017). Together with the results presented here, this body of previous research suggests that urbanisation has significant effects on population dynamics and distribution patterns of urban predators. While anthropogenic resources are known to significantly benefit red fox survival within urban habitats (Harris 1981, Stepkovitch 2017, Stepkovitch, Martin et al. 2019), urban landscape features may also be contributing to increased density and reduced dispersal and home ranges within urban environments. Red foxes in Sydney's urban habitats may disperse comparatively low distances due to barriers created by different land cover types. My results indicated that medium to high urbanisation combined with geographic distance provided a dispersal barrier to red foxes within Greater Sydney. The "urbanisation level three" land cover variable (public services areas, government buildings and facilities, recreation and culture areas, urban defence facilities, and research facilities) combined with geographic distance provided the strongest correlation with the observed genetic variation of red foxes in the Greater Sydney region. These types of landcover comprise concrete and fencing which may explain their limiting effects on red fox dispersal. A higher occurrence of these types of landscape features in urban Sydney (Figure 2) could contribute to reduced dispersal of red foxes within urban habitats. In Robinson and Marks (2001) study of red foxes in urban Melbourne, it was posited that the reduced genetic variability between foxes in urban habitats compared to rural habitats was due to dense urban residential housing acting as a barrier to dispersal. However, these findings and my own findings differed from studies in North American (Sacks, Brazeal et al. 2016) and European (Mullins, McDevitt et al. 2014) cities which suggested urbanisation did not limit red fox dispersal (Mullins, McDevitt et al. 2014, Sacks, Brazeal et al. 2016). This discrepancy may result from potential behavioural differences between Australian red fox populations and American and

European red fox populations, or possibly from different physical components comprising the measures of urbanisation used in the different studies.

My results also suggest that roads, railways, and waterways limit dispersal for red foxes in the Greater Sydney region. Support for these findings come from similar studies in Zurich (Switzerland) (Wandeler, Funk et al. 2003) and Sapporo (Japan) (Kato, Amaike et al. 2017) which identified large waterways as significant barriers to gene flow between sub-populations of red foxes in urban habitats. Likewise, Kato et. al. (2017) found large roads and railways to be significant barriers to gene flow. Other urban predators including coyotes (Riley, Sauvajot et al. 2003, DeCandia, Henger et al. 2019) and mountain lions (*Puma concolor*) (Riley, Serieys et al. 2014) within and around urban areas are significantly impacted by urban landscape features such as major roads and highways. These findings, together with my own, raise concerns that continued urban expansion could result in habitat fragmentation for urban predators.

Based on the observed male and female dispersal distances of red foxes identified in my research, there is no evidence to suggest sex-biased dispersal within the Greater Sydney region. This result differs significantly to studies on sex-biased dispersal in other Australian (Marks and Bloomfield 1999, Robinson and Marks 2001, Marks and Bloomfield 2006) and English (Trehwella, Harris et al. 1988, Baker, Funk et al. 2000, Baker, Dowding et al. 2007) cities, which found greater dispersal distances in males. This discrepancy may result from limited female samples, particularly in rural habitats. Increased sampling size would be beneficial to draw stronger conclusions about sex-biased dispersal in red foxes from the Greater Sydney region.

The estimated N_e of the Sydney red fox population is significantly lower than the expected population size in the Greater Sydney region based on destiny estimates. Severe changes in population size caused by factors such as population bottlenecks or founder effects (Frankham 1995, Li and Roossinck 2004, Broquet, Angelone et al. 2010) can result in reduced N_e . Founder effects occur when a small number of individuals from a large population are permanently separated. Even if the population grows post-bottleneck, a large amount of genetic diversity is lost resulting in reduced N_e . The low N_e observed in the red foxes of Sydney could be the result of a founder effect (caused by a small founder population) and a bottleneck resulting from managing red fox populations. The estimated N_e observed in red foxes from the Great Sydney region is small enough to expect the loss of allelic variation through random genetic drift. This process will lower the adaptive potential of foxes within the region (Eizaguirre and Baltazar-Soares 2014, Hoffmann, Sgrò et al. 2017) limiting the ability of foxes to adapt to environmental changes, such as those caused by climate change, and to develop resistance to management practises such as baits. To further understand the population dynamics and dispersal patterns of red foxes in the Sydney area,

future studies would benefit from sampling a greater number of foxes from across the landscape. An increased number of individuals sampled from across an evenly distributed landscape would provide greater statistical power to obtained results.

Further research examining population and landscape genetics of red foxes over a larger spatial scale, such as the entirety of New South Wales or Australia, would provide key population information and could be used to ascertain how different landscapes across the continent affect red fox dispersal. Furthermore, research on Australia's other key invasive predator, the feral cat (*Felis catus*), could also provide important information for conservation management. Recent studies suggest that some species predated upon by red foxes and cats may become more susceptible to cat predation in open habitats after red fox numbers are reduced due to management efforts (Molsher, Newsome et al. 2017). As it is recommended to carry out simultaneous control programs for red foxes and cats to maximise conservation success, knowledge of feral cat population dynamics and dispersal patterns within the same landscape as red foxes could be beneficial in conservation management and decision-making.

Additionally, it would be of interest to develop a larger SNP dataset to investigate for loci under selection within the urban environment (Parisod and Holderegger 2012) especially given that morphological changes have been observed in red foxes in urban Sydney habitats (Stepkovitch, Martin et al. 2019).

MANAGEMENT IMPLICATIONS

One of the challenges in managing Sydney's red foxes is the limited availability of information on population and dispersal. My research addresses this knowledge gap by providing greater insight into the population dynamics and habitat specific dispersal patterns of red foxes within the greater Sydney region. Urbanisation is shown to reduced dispersal of red foxes and medium to high levels of urbanisation, roads, railways, and waterways may act as dispersal barriers with the Greater Sydney region. Current results indicate that urban red foxes may maintain small habitat sizes supporting increased fox density. Increased greening and implementation of native corridors to provide increased habitat for native species within urban regions could lead to increased red fox habitat within these environments. Red foxes exhibit different dispersal dynamics within urban habitats compared to rural habitats in the Greater Sydney region and as such management efforts should be tailored to the specific habitat type.

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APPENDIX A

Table A1: Detailed outline of the filtering process showing the number of SNPs and Individuals retained after each filtering procedure.

Filtering Procedure	SNPs	Individuals
Reproducibility Average (100%)	16,601	90
Call Rate SNP (> 95%)	3,712	90
Call Rate Individuals (> 90%)	3,712	86
Maximum Average Read Depth (average read depth + 4 x $\sqrt{\text{average read depth}}$)	3,601	86
Average Read Depth (< 5)	2,656	86
Duplicate clone IDs	2,628	86
Minor Allele Frequency (> 0.2)	2,337	86
Loci Under Selection	2,337	86
Loci out of HWE	2,078	86

APPENDIX B

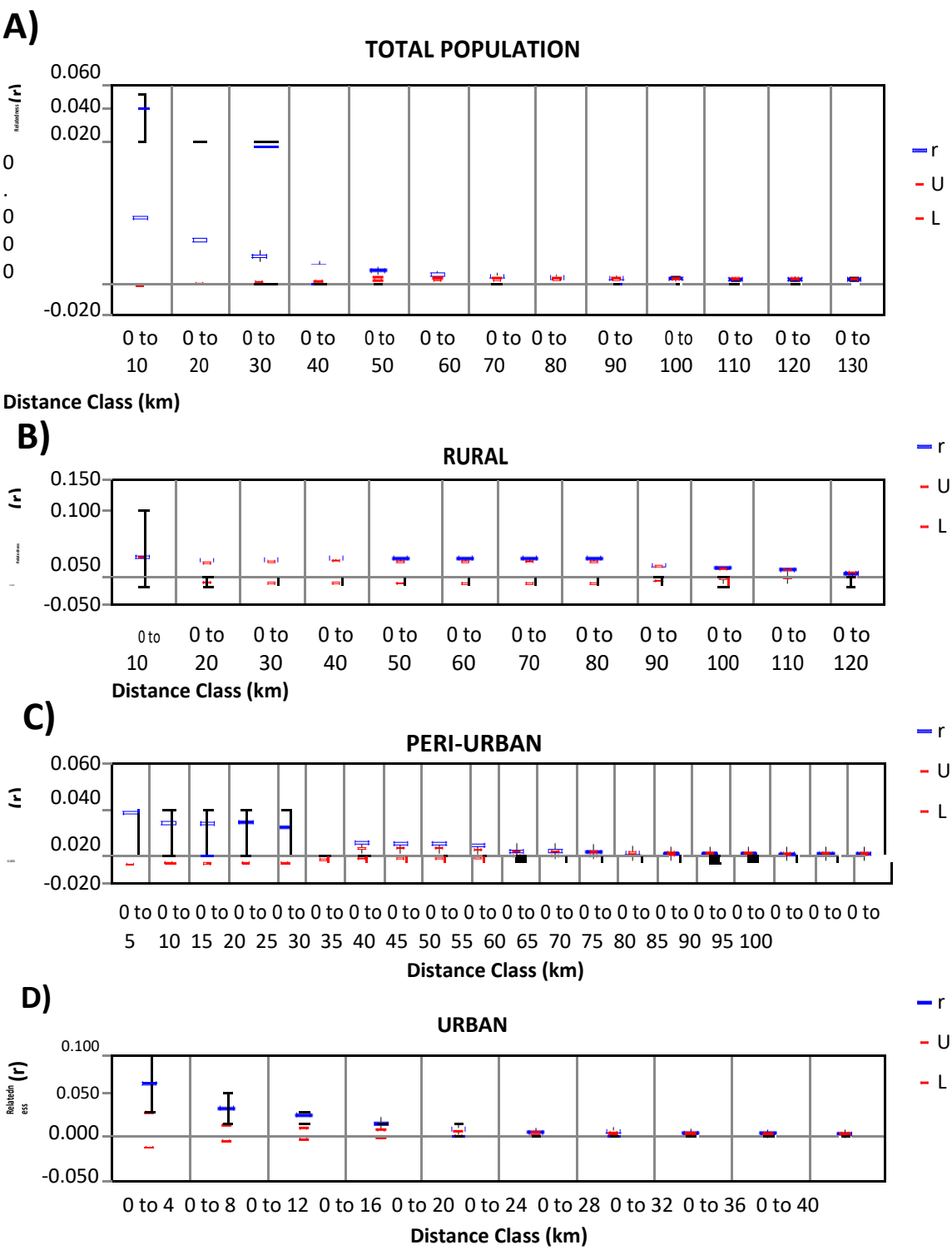


Figure B1: Spatial correlogram displaying mean genetic relatedness (r) at stacked incremental distance class bins. **A)** total region, **B)** rural habitat, **C)** peri-urban habitat, **D)** urban habitat. Error bar showing the 95% confidence intervals of each distance class as determined by 9999 iterations

are present. The upper (U) and lower (L) 95% confidence intervals of the null hypothesis ($r = 0$) as determined by 9999 iterations are also shown.

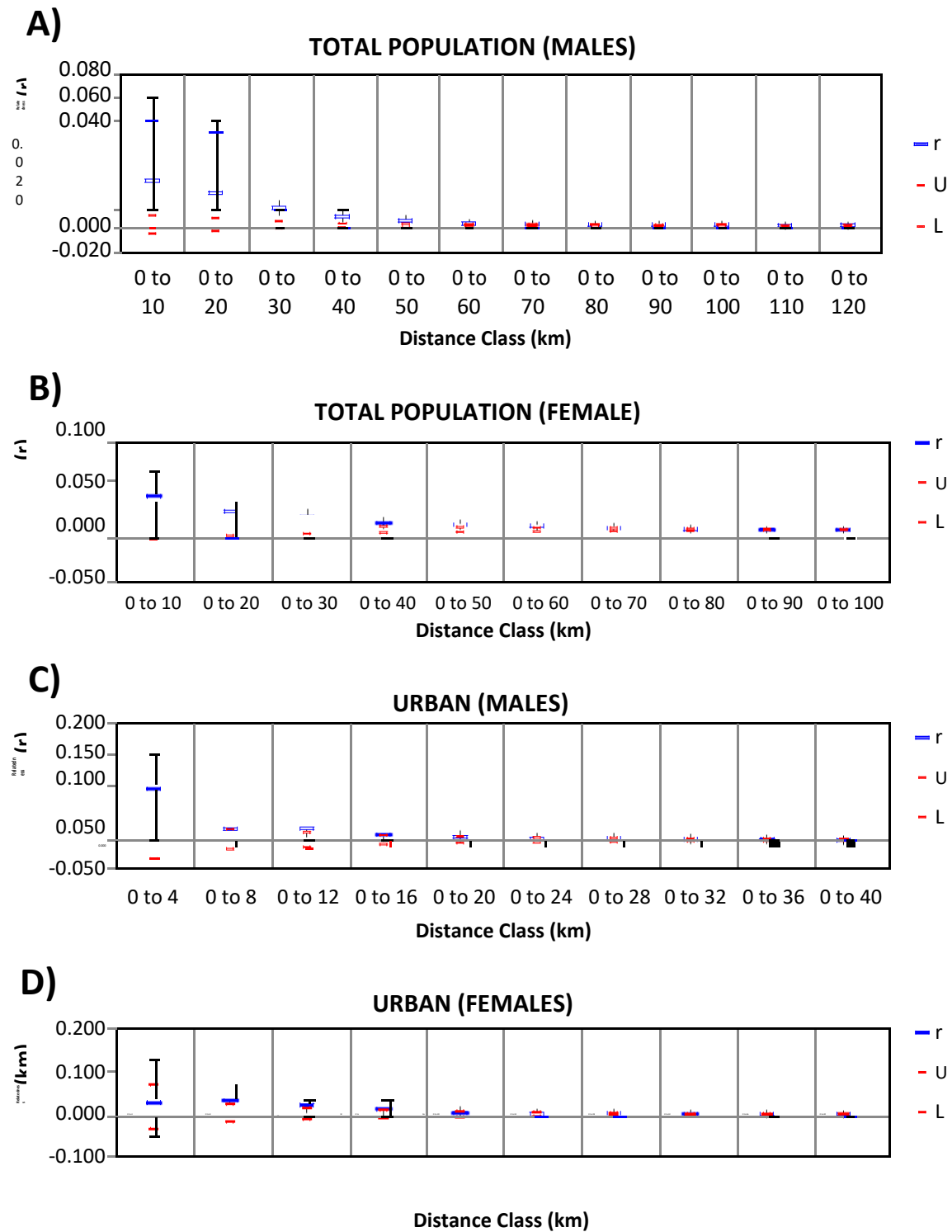


Figure B2: Spatial correlogram displaying mean genetic relatedness (r) at stacked incremental distance class bins. **A)** total population males, **B)** total population females, **C)** urban males, **D)** urban females. Error bar showing the 95% confidence intervals of each distance class as determined by 9999 iterations are present. The upper (U) and lower (L) 95% confidence intervals of the null hypothesis ($r = 0$) as determined by 9999 iterations are also shown.

APPENDIX C

Table C1: All urbanisation models with straight line distance and least cost distance models.

Table is ordered by lowest to highest delta AICc.

Method	Model	$\Delta AICc$	BIC	Weight
Straight Line Distance	GD ~ GGD + Resistance 3	0	41758.93	0.547254401
	GD ~ GGD	2.275299666	41755.01	0.175434183
	GD ~ GGD + Resistance 4	4.191504623	41763.12	0.067300083
	GD ~ GGD + Resistance 5	4.199311787	41763.13	0.067037884
	GD ~ GGD + Resistance 1	4.20297904	41763.13	0.066915074
	GD ~ GGD + Resistance 2	4.266364862	41763.2	0.064827594
	GD ~ GGD + All Resistances	7.772510787	41791.48	0.011230782
	GD ~ Resistance 1	302.1423932	42054.87	1.35E-66
	GD ~ Resistance 3	329.4151853	42082.15	1.61E-72
	GD ~ Resistance 2	336.029494	42088.76	5.89E-74
	GD ~ Resistance 5	336.1404939	42088.87	5.57E-74
	GD ~ Resistance 4	336.1473232	42088.88	5.56E-74
Least Cost Distance	GD ~ GGD + Resistance 3	0	41650.89	0.300844306
	GD ~ GGD	0.233148765	41644.92	0.267740571
	GD ~ GGD + Resistance 1	1.79371135	41652.68	0.122699368
	GD ~ GGD + Resistance 4	2.159891973	41653.05	0.102170899
	GD ~ GGD + Resistance 5	2.177505638	41653.06	0.101275047
	GD ~ GGD + Resistance 2	2.238363493	41653.13	0.098239771
	GD ~ GGD + All Resistances	7.512801628	41683.18	0.007030038
	GD ~ Resistance 5	410.1844305	42088.87	2.56E-90
	GD ~ Resistance 3	437.4572226	42082.15	3.06E-96
	GD ~ Resistance 4	444.0715313	42088.88	1.12E-97
	GD ~ Resistance 1	444.1825312	42054.87	1.06E-97
	GD ~ Resistance 2	444.1893605	42088.76	1.06E-97

APPENDIX D

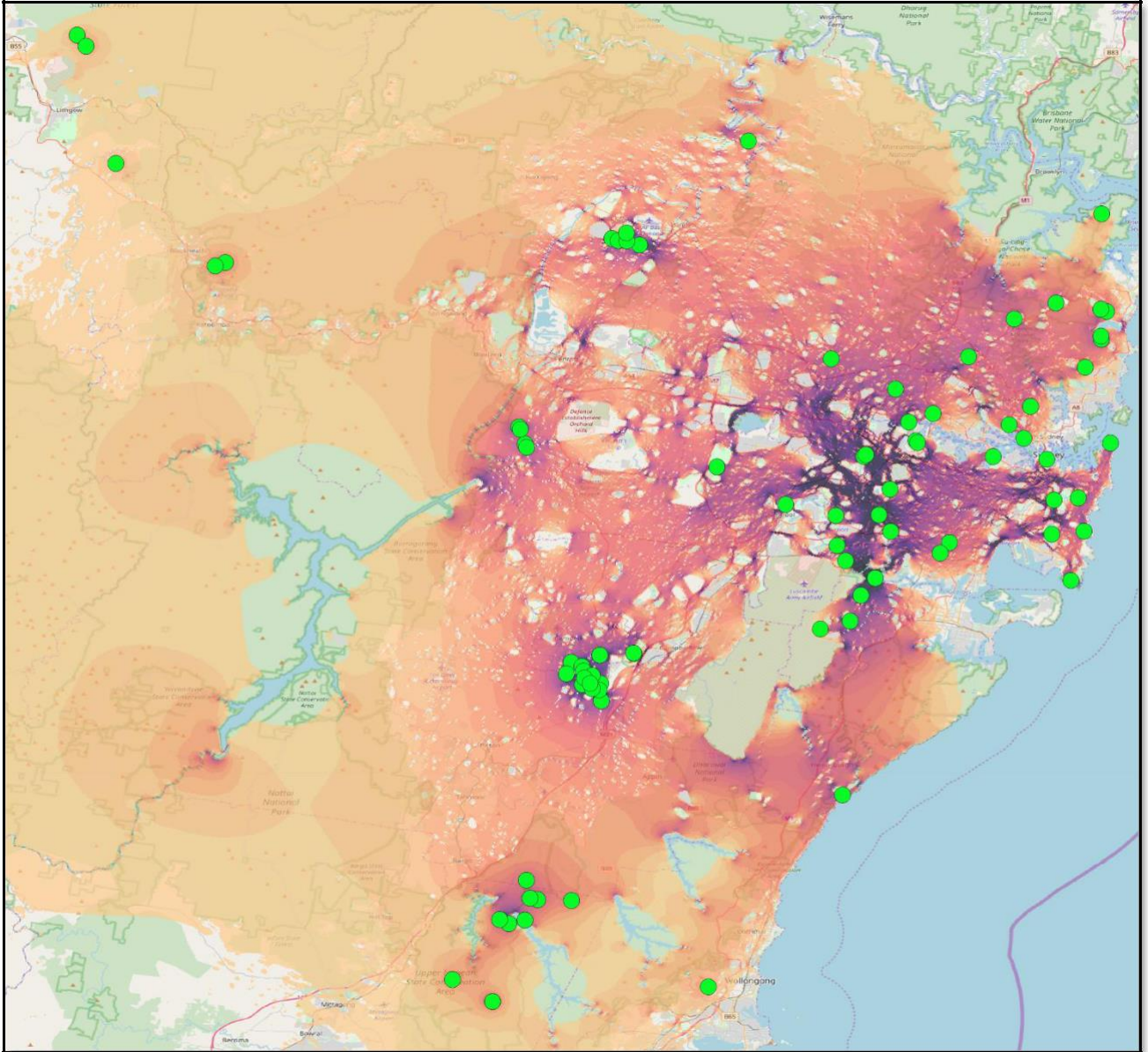


Figure D1: Current map showing the cumulative least-cost distribution pathways between all red fox samples in the Greater Sydney Region with urbanisation level 3 acting as a dispersal barrier. Lighter tones show low dispersal density, while darker tones show high density of dispersal. The locations of the 86 fox samples are also shown in green.

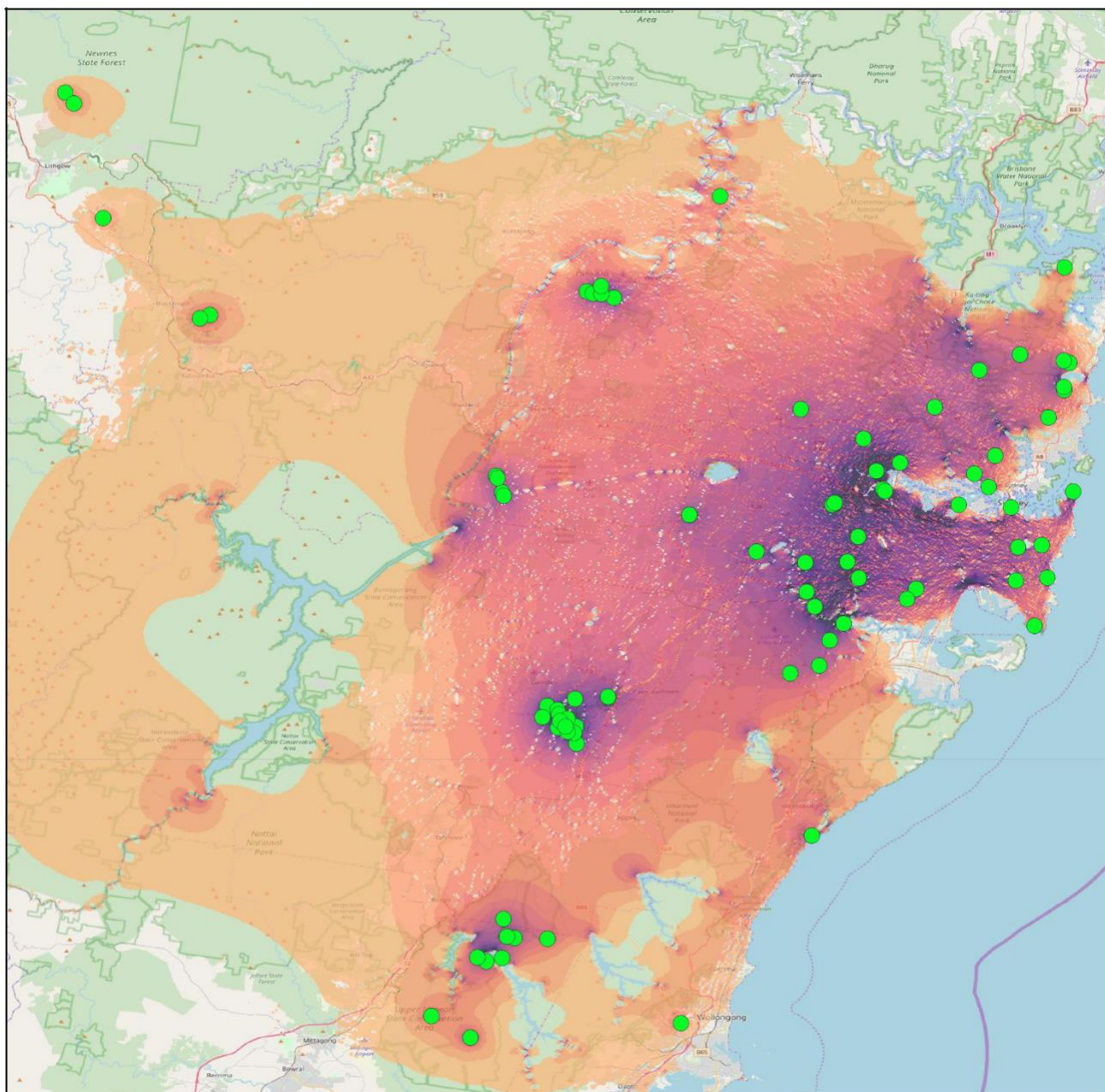


Figure D2: Current map showing the cumulative least-cost distribution pathways between all red fox samples in the Greater Sydney Region with roads and railways acting as a dispersal barrier. Lighter tones show low dispersal density, while darker tones show high density of dispersal. The locations of the 86 fox samples are also shown in green.

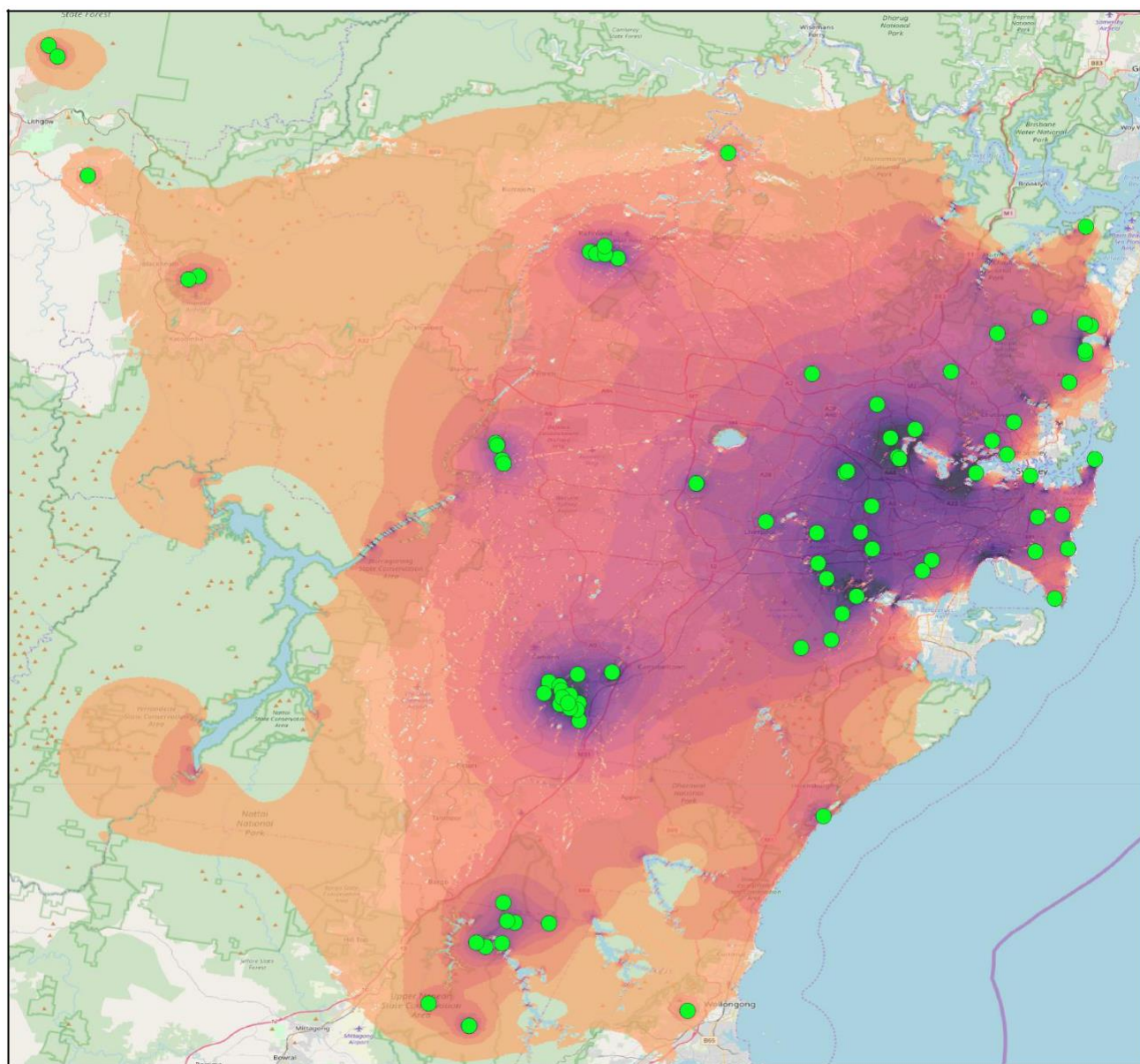


Figure D3: Current map showing the cumulative least-cost distribution pathways between all red fox samples in the Greater Sydney Region with waterways acting as a dispersal barrier. Lighter tones show low dispersal density, while darker tones show high density of dispersal. The locations of the 86 fox samples are also shown in green.