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RESEARCH

Genetic diversity and differentiation in the leaf litter weevil *Geochus politus* across an urban-rural gradient

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Abstract: Urban reserves have the potential to retain relatively high biodiversity. However, populations of the taxa within them can have reduced genetic diversity and, if gene flow between populations is inhibited by urbanised surroundings, can become genetically differentiated. Here we determine whether differences in population genetic differentiation and diversity can be observed in the leaf litter inhabiting weevil *Geochus politus* along the urban-rural gradient spanning Waitākere Ranges Regional Parkland and suburbs of West Auckland, New Zealand. Nine microsatellite markers were developed and screened across 300 individuals from nine sampling locations. Pairwise F_{ST} values, a principal coordinates analysis, and Bayesian estimates of population structure all demonstrated that the most urban site was strongly differentiated from the others. This site also had the lowest heterozygosity and highest F_{IS} values, potentially indicating a loss of genetic variation and a greater degree of inbreeding, although not to a dramatic extent. Differentiation was also observed among sampling locations within continuous forest, suggesting that both urbanisation and other landscape variables are influencing gene flow between these locations. This study highlights the potential for urban reserves to harbour significant diversity and emphasises the importance of maintaining these sites.

Keywords: gene flow, genetic diversity, population structure, urban reserves

Introduction

Habitat modification is a major contributing factor to modern day biodiversity loss (Tilman 2000; Alvey 2006; Lee & Lee 2014). The negative effects are exacerbated in landscapes where the environment is severely modified and natural habitats are marginalised in favour of urban development (McKinney 2002; Shochat et al. 2010; Faeth et al. 2011). While it is generally accepted that urban areas harbour comparatively less biodiversity than that of intact natural rural regions (McKinney 2002; Alvey 2006), urban reserves have the potential to retain native taxa to a significant degree (Kuschel 1990; McGeoch & Chown 1997; Watts & Larivière 2004, Ives et al. 2016). This is often because urban reserves are residual fragments of historically continuous areas that have been unnaturally subdivided into smaller isolated patches.

Habitat fragmentation generally has negative effects on populations, genetic diversity, and gene flow; however the nature and magnitude of these effects often differ considerably in different taxa (Keller et al. 2004; Noël et al. 2007; Vandergast et al. 2007), even for similar species within the same landscape (Bates 2002; Lai & Pullin 2005). Urbanised native species present in habitat fragments are thought to be vulnerable to extinction due to declines in their original population size (Haddad et al. 2015). Furthermore, there is often a lack of connectivity between reserve sites bounded by unsuitable habitat that usually accompanies habitat disturbance (Vandergast et al. 2007; Delaney et al. 2010). Gene flow between fragments is therefore typically reduced or absent, and the resulting isolated populations then become differentiated from each other and are prone to higher inbreeding and a loss of genetic diversity (Delaney et al. 2010). The degree to which taxa are affected is heavily influenced by the level of habitat connectivity (Coulon et al. 2004), their dispersal capability (Uezu et al. 2005), and their population size (Lai & Pullin 2005).

Population genetic responses of insect populations that have been separated from their parental populations due to urbanisation has received limited study. Habitat patchiness presents a barrier to gene flow among insects with poor dispersal capabilities, which is evident in the high genetic structuring observed in fragmented landscapes e.g. Keyghobadi et al. 1999, 2005 (*Parnassius smintheus*, Lepidoptera: Papilionidae); Knutsen et al. 2000 (*Bolitophagus reticulatus*, Coleoptera: Tenebrionidae). Some studies have attributed genetic differentiation among populations of invertebrate species to anthropogenic structures and urban development, e.g. Desender et al. 2005 (*Pterostichus madidus* and *Abax ater*, Coleoptera: Carabidae); Vandergast et al. 2007 (*Stenopelmatus* sp., Orthoptera: Stenopelmatidae). Included among these are

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studies of the flightless ground beetles *Carabus violaceus* Linnaeus and *Abax parallelepipedus* Piller & Mitterpacher near Bern in Switzerland, with significant genetic differentiation being observed between populations in forest fragments separated by major roads (Keller & Lagardier 2003; Keller et al. 2004).

The city of Auckland, New Zealand is located on the Auckland isthmus and spreads northward and southward occupying approximately 1086 km². Indigenous Māori were present in the Auckland isthmus around 600 yrs ago when land started to be developed for agriculture (Hayward 2017). The region was deforested at a faster pace when European colonists arrived and settled in the area in 19th century, and by the 1860s the rural landscape of Auckland was changed significantly (Cameron et al. 2008). The original forests were composed of podocarp and broadleaved species, with a dominance of kauri (*Agathis australis*) that was further removed for building materials (Cameron et al. 2008). The Auckland region was converted from forest to pasturelands then fully urbanised as human growth spread from areas of dense housing around port areas outward to encapsulate much of the Auckland isthmus. Many of the type localities where the first native insects were described from, such as Grafton, Howick, and Clevedon, were either completely extirpated of native habitat or at least severely impacted (Watt 1977), and what remains are vestiges of native habitats that were once forested corridors that connected northern Waikato and southern Northland through the isthmus. While the effects of urbanisation on the extent of native habitats are visually obvious, we have little knowledge about the environmental impact of urbanisation on the population genetic structure of Auckland's native insect species.

The weevil, *Geochus politus* Broun, is a small (approximately 1.0 mm length), apterous (wingless) species (Fig. 1), and is one of four *Geochus* species known from the Auckland area (Kuschel 1990). The tiny, flattened larvae are known to be dead-leaf miners and have been reared from *Weinmannia* leaves (May 1992). Adults of *Geochus* species are abundant in leaf litter throughout New Zealand including forest fragments in the Auckland region (Kuschel 1990; May 1992; Leschen et al. in press). *Geochus politus* is found in the forested Waitākere Ranges in West Auckland and eastward to Wattle Bay Reserve. The flightlessness of *G. politus* would likely limit dispersal between forest fragments while populations persist within them (Driscoll & Weir 2005). Poor vagility could also impede gene flow between populations within contiguous habitat, where distance or less conspicuous barriers might inhibit dispersal, resulting in high genetic differentiation (Garrick et al. 2004). Conversely, there is potential for genetic structuring to be limited or absent if the spatial scale under consideration is only small, and the population sizes within each patch are large and therefore robust to drift over the given timescale (Anderson et al. 2010).

Here, we investigate the extent to which genetic structuring can be observed in *G. politus* populations across an approximately 20 km distance along an urban-rural gradient in West Auckland. By sampling from locations that include urban reserves and sites within continuous forest we determine if there are differences in genetic differentiation and diversity between the assigned landscape type categories. We also test

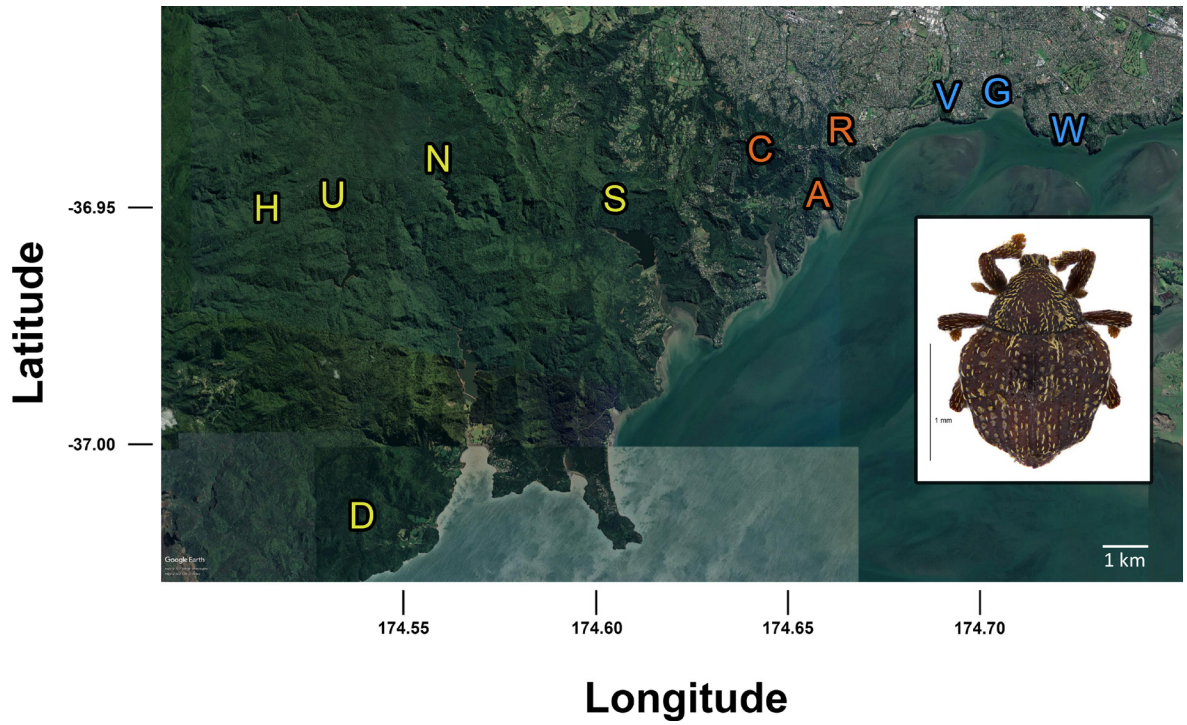

Figure 1. Map of sampling locations in West Auckland spanning Waitakere Ranges Regional Parkland and western suburbs, with a photograph of a *Geochus politus* specimen and 1 mm specimen scale bar. The letters correspond to the following sites: D = Donald Mclean Summit Track, H = Home Track, U = Upper Huia Dam Track, N = Upper Nihotupu Dam Walk, S = Slip Track, C = Clark Bush Track, A = Atkinson Park, R = Rahui Kahika Reserve, V = Craigavon Park, G = Gittos Domain, and W = Wattle Reserve. Letter colours correspond to the three landscape type categories: yellow = heavily forested, orange = semi-urban, and blue = urban. Aerial photographs taken from Google Earth, Images © 2021 Maxar Technologies and CNES / Airbus. Specimen image credit to Birgit Rhode, Manaaki Whenua – Landcare Research. White scale bar in lower right-hand corner indicates 1 km.

the hypothesis that genetic diversity declines and inbreeding increases along a transect from contiguous forested areas into isolated urban reserves and assess whether urban reserves still retain substantial genetic diversity.

Methods

Sampling locations and specimen collection

The urban-forest gradient spanning Waitākere Ranges Regional Parkland and suburban West Auckland was chosen as the area of study. The native forest in the Waitākere Ranges is comprised of a large proportion of kauri (*Agathis australis*), podocarp and broadleaf forest, with mānuka (*Leptospermum scoparium*), kānuka (*Kunzea ericoides*), and broadleaf scrub based native forest types also common (Griffiths et al. 2021). Sites, or sampling locations, were selected along an approximate east-west transect across the region, with some sites within contiguous native forest and others in increasingly urban regions. A total of 11 sites were selected and categorised by landscape type as either heavily forested, where most sites were connected by continuous forest, semi-urban, where sites were in a suburban region which retains a large degree of native forest cover, or urban, where sites represented the only areas of forest present within otherwise entirely developed areas. The following sites were selected: Donald Mclean Summit Track, Home Track, Upper Huia Dam Track, Upper Nihotupu Dam Walk, and Slip Track (heavily forested); Clark Bush Track, Atkinson Park, and Rahui Kahika Reserve (semi-urban); and Craigavon Park, Gittos Domain, and Wattle Reserve (urban) (Fig. 1, coordinates included in Appendix S1 in Supplementary Material). The sites were roughly equidistant along the transect to minimise the factor of distance confounding that of potential barriers. Sites within the Waitākere Ranges were chosen such that none were separated by roads, but there were walking tracks between sites as their abundance in the area made their presence between sites difficult to avoid. One site was selected in Auckland Centennial Parkland (the Home Track), which is separated from Waitākere Ranges Regional Parkland by a single, but frequently used, road. An additional site within the Waitākere Ranges that deviated from the transect was also chosen (Donald Mclean Summit Track). The urban sites Gittos Domain and Craigavon Park had a higher proportion of non-native plants, including mature pine trees (*Pinus* spp.).

Sampling took place during summer and autumn, between December 2015 and April 2016 inclusive. Specimens of leaf litter invertebrates were mass-collected using a leaf litter sifter. Leaf litter samples were collected ad-hoc along the tracks at each sampling location and were sifted to reduce the size of the samples. The resulting samples from the collection bag were then transferred to Berlese funnels for invertebrate extraction, a method proven to be effective for the collection of beetles (Owens & Carlton 2015). Invertebrates were collected in Agee jars containing 95% ethanol as the leaf litter sample dried out under light. This process took 6–22 days depending on sample size and humidity. *Geochus* specimens were removed from the concentrated invertebrate samples and identified to species using a key (G. Kuschel, unpubl.) and by comparison to previously identified specimens in the New Zealand Arthropod Collection (NZAC). Specimens were stored in 95% ethanol. The target sample size for each site was 20 specimens. Sites where fewer than 20 *G. politus* individuals were initially obtained were revisited until a total of 20 specimens was reached. If no specimens were obtained at a sampling location, then the site was not revisited.

Of the four *Geochus* species previously known from the Auckland region prior to this study, *Geochus inaequalis* Broun, *G. politus*, *G. similis* Broun, and *Geochus* sp. 1 (Kuschel 1990), the three named species were collected (Appendix S2). *Geochus politus* was found at the highest number of sites and was therefore chosen as our study species. No *Geochus* species were collected in Craigavon Park, and *G. politus* was not collected in Gittos Domain. This left nine sampling locations remaining in the analysis, and only one in the strictly urban category.

Microsatellite discovery and screening

Genomic DNA was destructively extracted from 15 *G. politus* specimens crushed in a single digest mixture to obtain DNA for microsatellite development. Specimens from the Rahui Kahika reserve collection site were used, as this sample contained the highest number of specimens. The DNeasy Mericon Food Kit (Qiagen) was used following the manufacturer's instructions. The extracted genomic DNA was run on a 0.8% agarose gel with 1x TAE buffer using a GelRed stain and was quantified using the Quantifluor^{TM} (Promega). After sufficient quality and quantity was established, the sample was sent to New Zealand Genomics Limited (NZGL) for library preparation and sequencing on an Illumina MiSeq.

Microsatellite development was guided by the recommendations of Gardner et al. (2011). Microsatellite discovery and primer design was conducted using msatcommander (Faircloth 2008) with incorporated Primer3 software (Untergasser et al. 2012), searching for di- through to penta-nucleotide motifs with a minimum repeat size of eight, and with msatexplorer 2.0.0.5 (https://code.google.com/archive/p/msatexplorer/). Primers were initially tested using M13 tags with fluorescent FAM labels (Schuelke 2000) on individuals spanning a wide geographic range across the sampling locations. Markers that amplified successfully and were polymorphic were selected for multiplexing, with two multiplexes designed using Multiplex Manager v 1.2 (Holleley & Geerts 2009).

For genotyping, genomic DNA was extracted from individual *G. politus* specimens using the X-tractor Gene™ (Corbett Life Sciences) and the associated reagents. Digests were incubated for 20–30 hours, with gDNA then being extracted following the manufacturer's instructions. Samples were eluted in 70 µl of elution buffer.

Multiplex PCR reaction volumes consisted of 1 µl genomic DNA, 5 µl of Type-it Multiplex PCR Master Mix (Qiagen), 1 µl of primer pair mix (final concentrations of each primer pair given in Appendix S3), and 3 µl of PCR grade water. PCR conditions were as follows: an initial denaturation cycle at 95°C for 5 minutes; then 35 cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 1 minute and 30 seconds, and extension at 72°C for 1 minute; then a final extension cycle at 60°C for 30 minutes. PCR products were visualised on a 2% agarose gel with 1x TAE buffer and GelRed™ stain, electrophoresed using a 3500xL Genetic Analyser (Applied Biosystems by Life Technologies) and analysed using the program GeneMapper v 5.0 (Applied Biosystems by Life Technologies). The dataset was checked for allelic dropout, scoring errors due to stutter, and the presence of null alleles using Micro-Checker v 2.2.3 (Van Oosterhout et al. 2004), with a standard Bonferroni-adjusted 95% confidence interval and 10 000 repetitions.

Data analysis

GenAlEx v 6.503 (Peakall & Smouse 2006) was used to generate the mean number of alleles per marker, observed and expected heterozygosities, and to undertake chi-square tests of Hardy-Weinberg equilibrium. Tests for linkage disequilibrium using default parameters, a pairwise population F_{ST} matrix, G-tests for pairwise differentiation, and estimates of the inbreeding coefficient F_{IS} were implemented in Genepop v 4.2 (Rousset 2008). Measures of allelic richness, an allelic diversity measure that corrects for variable sample sizes, were calculated in FSTAT v 2.9.3 (Goudet 1995).

The program Structure v 2.3.4 (Pritchard et al. 2000) was used to approximate the number of genetically distinct populations within the dataset. The model parameter set assumed admixture and independence of allele frequencies between groups, with the "locprior" option set to 0. A burn in of 100 000 steps and a chain length of 1 000 000 steps was used. Eight replicates for K values from 1 to 10 were conducted. The most likely K value was estimated using the plot of ln Pr (X|K) vs K and Evanno's method (Evanno et al. 2005) as implemented in Structure Harvester v 0.6.94 (Earl 2012). The program CLUMPAK (Kopelman et al. 2015) was used to generate bar plots, and to identify major clusters where there were different outcomes across replicate runs for values of K.

A principal coordinates analysis (PCoA) of the genetic variation present among samples, along with an analysis of spatial autocorrelation with bootstrapping using the methodology of Smouse and Peakall (1999) was performed in the R (R Core Team, 2016) package PopGenReport v3.0.4 (Adamack & Gruber 2014). This PCoA was performed on a pairwise distance genetic distance matrix between each individual sampled. PopGenReport was also used to compare the Smouse pairwise genetic distance between all individuals, and to plot this versus the geographic straight-line distance between each sample.

Results

Development and screening of microsatellite markers

A total of 1881557 reads were obtained from the Illumina MiSeq run after trimming. Of the 649 microsatellite markers generated by msatcommander, 30 were selected for further testing. Twenty-three of these were found to amplify successfully, with 12 displaying polymorphism when peaks were visualised. All 12 of these markers were utilised for multiplex development, though two were removed from the final multiplex reactions due to significant stutter, and one from the final dataset due to extensive missing data (22.5%). The marker GP27 was kept in the dataset despite having 14% missing data throughout the dataset, to retain information and because the missing data was concentrated in only a few populations. Micro-Checker v 2.2.3 indicated the potential for the presence of null alleles in these populations, and this was taken into consideration during the analyses. Ultimately nine markers were used for genotyping the final dataset (Appendix S3). Tests for linkage disequilibrium found no significantly linked pairs of markers in more than a single population, suggesting that no markers were physically linked. Individuals that had missing data for four or more loci were removed from the dataset. The final dataset included 300 individuals, with the sample size per sampling location (*n*) given in Table 1 (dataset publicly available at datastore.landcareresearch.co.nz/dataset/geochus-politus-dataset).

Summary statistics

Summary statistics are presented in Table 1. Observed heterozygosity values were largely lower than expected by Hardy-Weinberg equilibrium across all sites, but this was only significant among Wattle Reserve individuals. Allelic richness values ranged from 5.20 (Clark Bush Track) to 8.12 (Upper Nihotupu Dam Walk). These were relatively similar across populations. Inbreeding coefficient values were all positive. Wattle Reserve had the highest mean F_{IS} value (0.21), but there were no clear trends in F_{IS} values across landscape type categories among the other sites.

Genetic structuring

The matrix of pairwise F_{ST} values indicates moderate to strong differentiation between most sampling locations, with G-tests indicating that all were highly significant at the 1% level (Fig. 2). The Wattle Reserve site shows the most consistently strong differentiation from the other sampling locations.

The Structure Harvester analyses indicated that the optimal value of K value is five (Appendix S4). The K = 5 Structure plot shows three pairs of sampling locations forming clusters: Home Track and Upper Huia Dam Track, Slip Track and Clark Bush Track, and Atkinson Park and Rahui Kahika Reserve (Fig. 3), which is consistent with the proximity of the sites to

Table 1. Summary statistics across sampling locations: number of individuals (*n*), mean allelic richness (A_R) and standard deviations (SD), mean Wright's inbreeding coefficient (F_{IS}), observed and expected heterozygosities (H_O, H_E) and standard errors (SE), and the number of markers that deviate from Hardy-Weinberg equilibrium (HWE) at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Sampling Location	*n*	Mean A_R (SD)	Mean F_{IS}	H_O (SE)	H_E (SE)	HWE
Donald Mclean Summit Track	46	7.23 (2.17)	0.08	0.701 (0.035)	0.753 (0.028)	2***
Home Track	19	6.61 (3.15)	0.12	0.554 (0.089)	0.624 (0.086)	1***
Upper Huia Dam Track	24	6.44 (2.61)	0.13	0.587 (0.069)	0.664 (0.066)	1*,1***
Upper Nihotupu Dam Walk	18	8.12 (2.43)	0.10	0.716 (0.051)	0.772 (0.025)	2**
Slip Track	27	7.20 (2.11)	0.03	0.703 (0.056)	0.713 (0.040)	0
Clark Bush Track	35	5.20 (2.31)	0.07	0.533 (0.088)	0.567 (0.074)	1***
Atkinson Park	25	7.26 (1.8)	0.15	0.609 (0.093)	0.715 (0.038)	1*,1**,1***
Rahui Kahika Reserve	62	6.44 (2.12)	0.02	0.631 (0.079)	0.641 (0.067)	2***
Wattle Reserve	44	5.89 (1.22)	0.21	0.507 (0.078)	0.655 (0.043)	1**,2***

D	0.000								
H	0.171	0.000							
U	0.149	0.088	0.000						
N	0.078	0.132	0.125	0.000					
S	0.077	0.203	0.189	0.054	0.000				
C	0.168	0.202	0.246	0.123	0.128	0.000			
A	0.136	0.174	0.176	0.070	0.098	0.178	0.000		
R	0.157	0.239	0.230	0.094	0.113	0.185	0.058	0.000	
W	0.223	0.245	0.252	0.189	0.221	0.278	0.195	0.246	0.000
	D	**H**	**U**	**N**	**S**	**C**	**A**	**R**	**W**

Figure 2. Pairwise population F_{ST} matrix across sampling locations, with G-tests indicating that all were highly significant at the 1% level. Green shading increases with increasing F_{ST} value. Sampling locations are as follows: D = Donald Mclean Summit Track, H = Home Track, U = Upper Huia Dam Track, N = Upper Nihotupu Dam Walk, S = Slip Track, C = Clark Bush Track, A = Atkinson Park, R = Rahui Kahika Reserve, and W = Wattle Reserve.

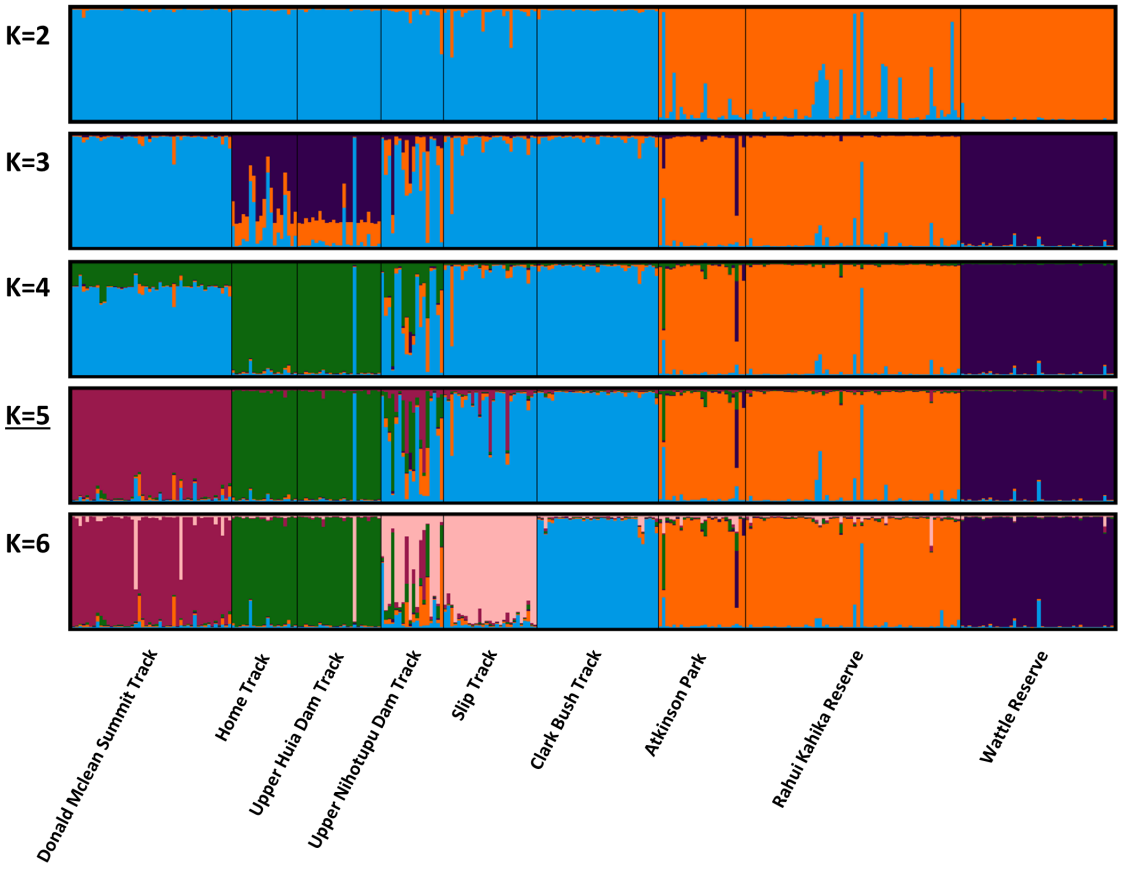

Figure 3. Summary plots of Structure v 2.3.4 analyses as generated in CLUMPAK for K values 2–6, with K = 5 (underlined) being selected as the most likely K value in Structure Harvester v. 0.6.94.

each other along the approximate transect (Fig. 4). The Upper Nihotupu Dam Track individuals do not form a distinct cluster, and instead display membership to four of the genetic clusters associated strongly with other sites (Figs 3, 4). Wattle Reserve maintains its distinctness from all other populations at K values three through six (Fig. 3). The findings from the PCoA largely echo those of the Structure analyses (Fig. 5). The Wattle Reserve individuals separate from those from the other sites on their own principal coordinate (PC1). The remaining sites cluster closer together and form a gradient, with PC2 roughly corresponding to their order along the transect. Both the plot of genetic distance vs. geographic distance, and the spatial autocorrelation plot showed significant spatial autocorrelation, whereby samples that were collected under approximately 4.5 km from each other were significantly more related than those collected further apart (Fig. 6; Appendix S5).

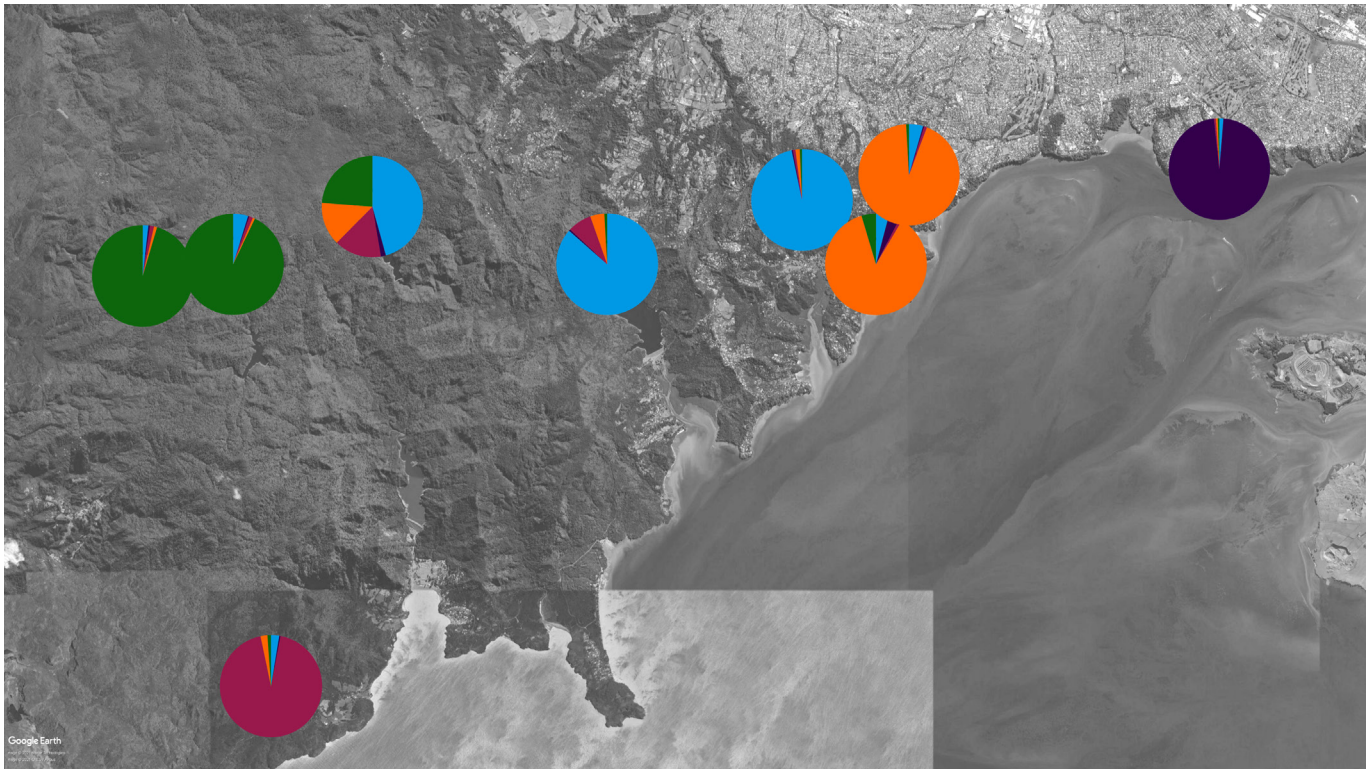

Figure 4. Map of sampling locations with pie charts summarising Structure v.2.3.4 analysis for K = 5, the K value indicated by Structure Harvester v 0.6.94 to be the best fitting value for the data. Aerial photographs taken from Google Earth, Images © 2021 Maxar Technologies and CNES / Airbus.

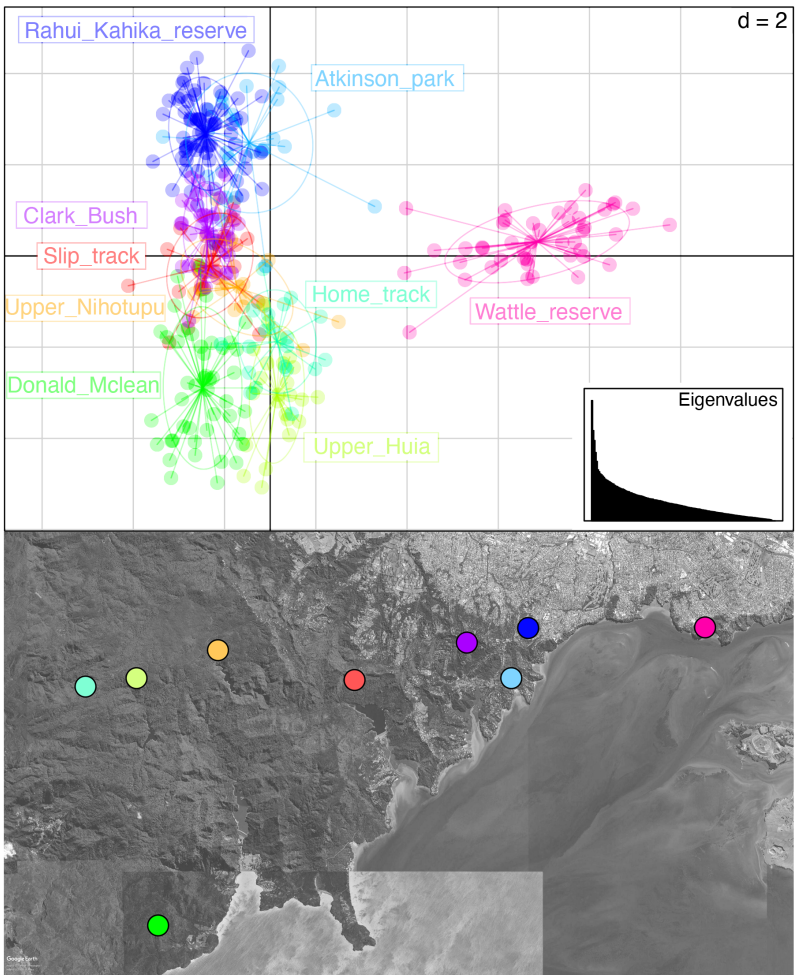

Figure 5. Principle coordinates analysis (PCoA) plot created in R using the PopGenReport package (Adamack & Gruber 2014), with a map of sampling locations marked in matching colours to those used in the PCoA plot. Aerial photographs taken from Google Earth, Images © 2021 Maxar Technologies and CNES / Airbus.

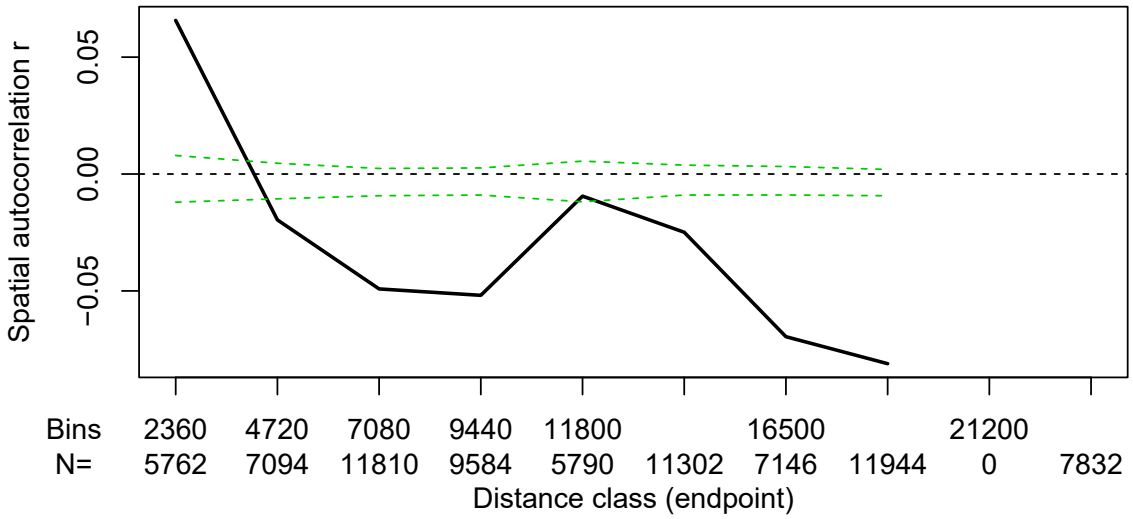

Figure 6. Spatial autocorrelation plot implemented in R using the PopGenReport package (Adamack & Gruber, 2014).

Discussion

Genetic diversity and differentiation

With increasing habitat modification and fragmentation around urban centres, it is important to understand how these factors affect connectivity within populations of native species and their genetic diversity. We found that the sampled *G. politus* populations across the West Auckland region exhibited no significant differences in heterozygosity or allelic richness between the heavily forested sites and the semi-urban sites. There was however a higher degree of inbreeding and potentially lower genetic diversity at the single site in the urban category, Wattle Reserve. This loss of diversity could be the product of isolation contributing to a reduced effective population size, as this site was also the most genetically distinct from all other sampling locations across different analyses, and therefore the population present in this sampling location might be vulnerable to losses of genetic diversity in the future. The conclusion that urbanisation plays a role in the distinctiveness of Wattle Reserve is consistent with other studies of flightless insects (Keller & Largiader 2003; Keller et al. 2004, Vandergast et al. 2007). Regardless of the cause, the high level of differentiation between Wattle Reserve and other sites suggests that there is relatively reduced to no gene flow between them, which is also consistent with the lack of collections from intervening urban sites (Craigavon Park and Gittos Domain).

The genetic differentiation observed across sampling locations indicates the geographic scale (c. 20 km) of this study was sufficient to restrict gene flow between sites. The significant differentiation observed among sites was observed at even finer scales than those of similar studies of flightless insects (Desender et al. 2005; Vandergast et al. 2007). Our results are consistent with studies on carabid ground beetles by Keller and Lagardier (2003) and Keller et al. (2004) who observed differentiation among sampling locations at either side of roads across distances of up to 1.75 km between sites. Observing these genetic differences among *G. politus* populations is also consistent with other studies of the New Zealand leaf litter fauna, which have demonstrated differentiation across scales of 10s of kilometres using mitochondrial DNA markers (Boyer et al. 2007; Marske et al. 2011).

While multiple analyses showed spatial autocorrelation, and therefore demonstrated that isolation by distance is an important factor in structuring the populations, distance alone is unlikely to explain differentiation among the semi-urban sites which are relatively equidistant. The Rahui Kahika and Atkinson Park sites form a cluster with a low level of differentiation between them (Fig. 2), and are distinct from the Clark Bush Track (Figs 3–5). Furthermore, the pairwise F_{ST} values indicate strong differentiation between these two groupings and a low level of differentiation between Rahui Kahika Reserve and Atkinson Park. While there is a large amount of forest cover across this area, two large roads are located between the Clark Bush Track and Atkinson Park, whereas Atkinson Park and Rahui Kahika Reserve are separated by two smaller streets. The less disrupted forest cover between the latter two sites could provide an explanation for the patterns observed here. Alternatively, the *G. politus* individuals at the Clark Bush Track could be remnants of a different source population to those of the other two Semi-Urban sites. Further sampling in the forested areas between these sites coupled with natural history observations of *G. politus* would provide important data that would help to identify specific barriers to dispersal, for instance, the presence of suitable leaf litter from principle host plants.

Unlike flightless carabids that cruise leaf litter and dead wood for prey (Keller & Lagardier 2003; Keller et al. 2004), roading probably does not affect small scale genetic patterns in the saproxylic *G. politus* to the same extent as in some other organisms (Holderegger & Di Giulio 2010). Like other leaf litter or dead wood beetles, especially flightless weevils, *Geochus* species may also be active above the leaf litter zone and in areas with well-established forest canopies, and dispersal over short distances would be possible. Furthermore, crossing roads during periods of flooding would also be expected in leaf litter beetles, which may explain the genetic similarity between individuals from the Home Track and Upper Huia Dam Track despite the presence of a road between them.

Our results are different from those of flight-capable insects (Zytynska et al. 2017; Melosik et al. 2020), and more comparable to those of flightless forest beetles by Keller and Largiader (2003) and Keller et al. (2004), who utilised microsatellite markers in their genetic studies of populations

of forest-inhabiting ground beetles across a network of forests dissected by roads. They also uncovered genetic differentiation among populations that retained very similar allelic richness and heterozygosity to *G. politus*, though Keller and Largiader (2003) observed lower genetic variation in a smaller forest fragment. This suggests common genetic responses among flightless beetle species, though further study would allow this conclusion to be drawn more confidently. Other studies of flightless invertebrates using other forms of genetic data are less comparable to our study (Desender et al. 2005; Mock et al. 2007; Vandergast et al. 2007), but do show a relatively high amount of population divergence at similarly small spatial scales.

The inclusion of only one site in the urban category does limit the conclusions that can be drawn about the impact of urbanisation on genetic diversity and structuring. Additionally, Donald Mclean Summit Track is also differentiated from all other sites, including those within the same forested area. Future sampling along different transects that cover a range of urban reserves and contiguous forest sites would provide a clearer picture of both the differences in genetic patterns among and within landscape type categories, and the effect of urbanisation on the patterns observed.

Species distributions in West Auckland

Geochus species are not fully co-distributed through western Auckland, though more sampling is needed to confirm this pattern (Appendix S2 for numbers of *Geochus* specimens collected per species). We were not able to collect any specimens of *Geochus* sp. 1, previously known from only a single specimen from Wattle Reserve (Kuschel 1990), although our study included only a single collecting season. Our collections of *G. similis* in only heavily forested bush could indicate that the habitats in the more urban sites are less suitable for this species. Another limiting factor controlling the distribution of *Geochus* weevils could be host-plant availability and the type of leaf litter available, as shown in previous studies (Moeed and Meads 1986, 1987). It is possible that the absence of *G. politus* at Gittos Domain and Craigavon Park is related to this factor, with a higher proportion of non-native vegetation at these sites, though further study specifically investigating this relationship would be required.

Implications for management and restoration of urban reserves

Overall, the genetic diversity seen in *G. politus* within semi-urban sites indicate that these populations are not at immediate risk from the negative effects associated with low genetic variation, especially if management of species includes promoting gene flow among severed populations, though the potential for increased inbreeding at the Wattle Reserve site is of slight concern. While this certainly provides a basis for our understanding of the population genetic diversity of leaf litter fauna in urban reserves, extrapolation of these results to other invertebrates that are present in this microhabitat should be done with caution. As *G. politus* is present at these sites in great abundance, this could be a factor contributing to the maintenance of genetic diversity at these sites. Species present in lower numbers might be more affected by genetic drift and subsequently may lose genetic variation at a faster rate.

The presence of strongly detectable genetic differentiation between Wattle Reserve and the other sites indicates restricted gene flow between reserves in urban areas, which are typically sparsely distributed. This casts doubt on the possibility of leaf litter invertebrates with limited dispersal capabilities being able to naturally colonise these areas and sustain both the species and genetic diversity within them. However, species like *G. politus* could be suitable candidates to be actively restored into bush remnants, especially if appropriate leaf litter is available. The implementation of habitat corridors is often suggested under these circumstances, where previously fragmented habitat patches are linked to maintain connectivity and gene flow between them (Soule & Gilpin 1991; Mech & Hallett 2001). While these have been proven to be effective in promoting dispersal between fragments (Gilbert-Norton et al. 2010), their implementation in the Auckland urban area would be far too complex, especially for wingless invertebrates. A corridor of suitable habitat quality for *G. politus* and similar fauna would require leaf litter and potential host-plant coverage to be maintained across the linked habitats, which could not be achieved in this setting.

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Author Contributions

TB and RL conceptualised the study; TBC undertook the research, TBC and AV undertook the analysis; TBC wrote the manuscript; all authors reviewed and edited the manuscript.

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Supplementary material

Additional supporting information may be found in the supplementary material file for this article:

Appendix S1. Sampling locations and their landscape type categories and coordinates.

Appendix S2. Marker information for those included in multiplexes.

Appendix S3. A summary of the number of *Geochus inaequalis*, *G. politus*, and *G. similis* specimens collected.

Appendix S4. Plots of ln Pr (X|K) and DeltaK vs K generated by Structure Harvester v 0.6.94 (Earl 2012) to estimate the most likely K value.

Appendix S5. Plot of genetic distance vs geographic distance.

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