Spatial variation in the parasite communities and genomic structure of urban rats in New York City

L. P. Angley1 | M. Combs2 | C. Firth3,4 | M. J. Frye5 | I. Lipkin3 | J. L. Richardson1 | J. Munshi-South2

1Department of Biology, Providence College, Providence, RI, USA
2Louis Calder Center and Department of Biological Sciences, Fordham University, Armonk, NY, USA
3Mailman School of Public Health, Columbia University, New York, NY, USA
4School of BioSciences, The University of Melbourne, Parkville, VIC, Australia
5New York State Integrated Pest Management Program, Cornell University, Geneva, NY, USA

Correspondence
Jonathan L. Richardson, Department of Biology, Providence College, Providence, RI, USA.
Email: jrichardson4@gmail.com

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Summary
Brown rats (Rattus norvegicus) are a globally distributed pest. Urban habitats can support large infestations of rats, posing a potential risk to public health from the parasites and pathogens they carry. Despite the potential influence of rodent-borne zoonotic diseases on human health, it is unclear how urban habitats affect the structure and transmission dynamics of ectoparasite and microbial communities (all referred to as "parasites" hereafter) among rat colonies. In this study, we use ecological data on parasites and genomic sequencing of their rat hosts to examine associations between spatial proximity, genetic relatedness and the parasite communities associated with 133 rats at five sites in sections of New York City with persistent rat infestations. We build on previous work showing that rats in New York carry a wide variety of parasites and report that these communities differ significantly among sites, even across small geographical distances. Ectoparasite community similarity was positively associated with geographical proximity; however, there was no general association between distance and microbial communities of rats. Sites with greater overall parasite diversity also had rats with greater infection levels and parasite species richness. Parasite community similarity among sites was not linked to genetic relatedness of rats, suggesting that these communities are not associated with genetic similarity among host individuals or host dispersal among sites. Discriminant analysis identified site-specific associations of several parasite species, suggesting that the presence of some species within parasite communities may allow researchers to determine the sites of origin for newly sampled rats. The results of our study help clarify the roles that colony structure and geographical proximity play in determining the ecology of R. norvegicus as a significant urban reservoir of zoonotic diseases. Our study also highlights the spatial variation present in urban rat parasite communities, indicating that rats across New York City are not reservoirs for a homogenous set of parasites and pathogens. As a result, the epidemiological risks may be similarly heterogeneous for people in urban habitats.

Keywords
bacteria, disease ecology, epidemiology, public health, urban ecology, viruses, zoonotic disease
INTRODUCTION

The brown rat (Rattus norvegicus, AKA the Norway or city rat) is a human commensal that is globally distributed throughout urban landscapes. This species is well adapted to living in proximity to humans, and the high urban densities of rats result from their ability to exploit human resources (Aplin, Chesser, & Ten Have, 2003; Feng & Himsworth, 2014; Himsworth, Jardine, Parsons, Feng, & Patrick, 2014). Brown rats are a ground-burrowing species (Himsworth, Bidulka et al., 2013), and the extensive sewer, subway and park infrastructure throughout cities provide them with suitable substrate for burrowing (Childs et al., 1998; Johnson, Bragdon, Olson, Merlino, & Bonaparte, 2016). Furthermore, the growth of cities has led to an increase in both human and rat populations in urban areas (Himsworth et al., 2014).

In addition to the effects of rats on infrastructure and food supply (Doherty, Glen, Nimmo, Ritchie, & Dickman, 2016), rat infestations also pose health risks to humans (Himsworth, Parsons, Jardine, & Patrick, 2013; Himsworth, Bidulka et al., 2013; Ko, Reis, Dourado, Johnson, & Riley, 1999; Meerburg, Singleton, & Kijlstra, 2009). Brown rats are known hosts of several human pathogens that cause important zoonotic diseases, including Seoul hantavirus, leptospirosis and bubonic plague (Costa et al., 2014; Himsworth et al., 2014; Leibler, Zakhour, Gadhoke, & Gaeta, 2016). High rat densities increase the likelihood of human–rat contact, posing an acute risk to public health because zoonotic pathogens or ectoparasite vectors can be transmitted during rodent encounters (Frye et al., 2015; Himsworth, Parsons et al., 2013; Rogalski, Gowler, Shaw, Hufbauer, & Duffy, 2016). For example, a recent review of published epidemiologic studies of zoonotic and vector-borne infections among urban homeless reported Bartonella spp. as the most frequently identified vector-borne infection (Leibler et al., 2016). Other zoonotic pathogens that were commonly detected among the people sampled included Seoul hantavirus, Leptospira interrogans and Rickettsia typhi (Leibler et al., 2016). As the percentage of people residing in urban areas continues to rise, the incidence of zoonotic diseases is also expected to increase (Himsworth et al., 2014; Patz et al., 2004).

Previous studies suggest that the degree of ectoparasite and microbial pathogen (all called “parasites” hereafter for simplicity) transmission between hosts of the same species depends on the level of connectivity between host populations in the landscape (Godfrey, 2013; Kerth & Van Schaik, 2012; Leu, Kappeler, & Bull, 2010), and the social structure of the host species (Godfrey, 2013; Leu et al., 2010). A highly connected set of populations or colonies would likely share many dispersing individuals, creating ample opportunity for parasite transmission (Altizer et al., 2003; Kerth & Van Schaik, 2012). Thus, highly social species, such as rats, are expected to exhibit a higher probability of microbial and ectoparasite transmission (Altizer et al., 2003; Møller, Dufva, & Allander, 1993). For example, colonial social systems with shared nesting sites or mate competition and aggression are known to enhance the transmission of parasites between individuals and increase disease prevalence (Brown & Brown, 2017; Durrer & Schmid-hempel, 2017; Leu et al., 2010). Accordingly, a better understanding of the ecology and distribution of urban rats, and their parasite communities, can help identify conditions that foster zoonotic pathogen transmission.

Brown rats have small home-range sizes and can be territorial, which can promote the separation of rat colonies without complete physical isolation, and influence the spread of parasites between individuals (Davis, Emlen, & Stokes, 1948; Heiberg et al., 2012; Himsworth et al., 2014). Variation in the availability of food throughout urban landscapes also influences colony sizes and parasite abundance (Firth et al., 2014; Frye et al., 2015; Himsworth, Bidulka et al., 2013). Members from large colonies may also engage in more social interactions, so direct parasite transmission in larger rat colonies may be more prevalent (Leu et al., 2010; Stanko, Miklisová, De Bellocq, & Morand, 2002). For example, as ectoparasites are transmitted mostly through direct contact between rat hosts (Frye et al., 2015), colony size and distribution can play an important role in the spread of ectoparasites (Leu et al., 2010). In contrast, some internal microbial pathogens are shed via urine or faeces and may remain viable outside of the body for up to several months (Karaseva, Chernukha, & Piskunova, 1973). When these pathogens are present in the environment, the risk of transmission may increase substantially, even without direct contact between rats (Gedeon, Bodelón, & Kuenzi, 2010; Hilton, Willis, & Hickie, 2002).

While social dynamics may promote more insular rat colonies and parasite communities, dispersal of rats can connect colonies and influence parasite dynamics. Consequently, geographical distance may influence the similarity of parasite communities among rat colonies or populations. Very little is known about the movement and dispersal of brown rats in urban landscapes, but recent studies using tracking methods and genetic data indicate that brown rat movements are modest (mean of 100-200 metres), with some instances of long-distance dispersal greater than 500 metres (Gardner-Santana et al., 2009; Glass, Klein, Norris, & Gardner, 2016; Heiberg et al., 2012; Richardson et al., 2017). This pattern suggests that limited movement and dispersal of brown rats could...
result in localized parasite communities that become less similar over short geographical distances (Diagne et al., 2017). However, transmission through indirect contact is also possible and may be facilitated by spatial overlap in rat home ranges without direct host–host interactions (Leu et al., 2010), or through contact with excreta (Felzemburgh et al., 2014). This mode of transmission makes it less likely that geographical separation alone leads to divergent parasite communities. However, the relationship between geography, dispersal and parasite communities is unknown in urban rats.

Concerns for disease emergence in large cities, especially through zoonotic transmission, continue to grow as global business, international travel and population sizes increase (Bradley & Altizer, 2007). Large cities, such as New York City (NYC), are centres of global trade and transportation that are susceptible to introductions of novel pathogens (Bradley & Altizer, 2007). For example, an introduction of West Nile virus into NYC in 1999 quickly spread across the country and has become enzootic within parts of the United States (Nash et al., 2001). Two previous studies have looked at the parasite species found on brown rats at five sites in the Manhattan section of New York City. Firth et al. (2014) identified a wide diversity of known and novel microbes within rats tested, including several zoonotic pathogens. Frye et al. (2015) surveyed the ectoparasites on the same rats and found several species relevant for public health, including the tropical rat mite (Ornithonyssus bacoti), the spiny rat mite (Laelaps echidnina/nuttalli), which can both cause skin irritation in humans, the spined rat louse (Polyplax spinulosa), which can cause pruritus of the scalp, and the oriental rat flea (Xenopsylla cheopis), which is a vector of Yersinia pestis, the causative agent of plague. Our study includes the bacterial and viral pathogens known to cause disease, as well as novel microbial species, regardless of pathogenicity. When necessary, we explicitly distinguish ectoparasites from bacteria and viruses.

Here, we generate reduced representation genomic data for the same rats as Firth et al. (2014) and Frye et al. (2015) to evaluate the relationship between connectivity of urban rats and the structure of their parasite communities, including many pathogens of public health concern. Our aim was to evaluate the movement and gene flow of brown rats using genetic data in order to improve our understanding of urban rat ecology, including how colony structure and social behaviours influence parasite dispersal and transmission. We evaluated genomic variation and parasite communities in 133 rats from the Manhattan borough of New York City, an area that has been identified as a persistent reservoir of rat infestation (Childs et al., 1998; Johnson et al., 2016). We apply community ecology and population genomic analyses to a public health issue in order to (i) examine dispersal and gene flow of rats across an urban landscape; (ii) provide novel insights into parasite dispersal among rat colonies; and (iii) facilitate future rodent control and epidemiological interventions.

2 | MATERIALS AND METHODS

2.1 | Study area and parasite sampling

Brown rat and parasite samples were collected according to procedures reported in Firth et al. (2014) and Frye et al. (2015). A total of 133 brown rats were collected across five sites in New York City: three large residential buildings (RH1, RH2 and RH3), one multi-use, indoor location (RS contains transportation, retail and food services) and one outdoor green space (RP). Specific locations are not provided at the request of public officials (but see Figure 1d for map). We calculated great-circle distance between sites using the distance matrix command in the geosphere package of R (Hijmans & Van Etten, 2014). These sites were chosen for their high human density and persistent rat infestations. Ectoparasites were sampled by removing them from rats immediately following euthanasia and identified as described in Frye et al. (2015). Targeted molecular assays were used to identify known bacterial and viral pathogens in all individuals, and high-throughput sequencing and metagenomic methods were used to identify novel viruses as described in Firth et al. (2014). These included 15 human pathogens previously associated with brown rats and 18 novel viruses, many of which are related to known human pathogens.

2.2 | Genomic Sequencing

Using tissue samples collected by Firth et al. (2014), we extracted genomic DNA using the DNeasy Blood and Tissue Kit with an RNase treatment (Qiagen, Valencia, CA). We prepared double-digest restriction site associated DNA sequencing (ddRADseq) libraries by first digesting 250 ng of high-quality DNA using the SphI and MluCl restriction enzymes and then cleaning the product with 1.5× volume of Agencourt XP beads (Beckman Coulter, Brea, CA). To identify rats after sequencing, we ligated "P1" primers that included one of 48 unique five base pair (bp) barcodes to the fragmented DNA, pooling products for sets of 48 and cleaning barcoded products with beads as described above. Next, we size-selected DNA fragments using the Pippin Prep, isolating fragments between 376 bp and 412 bp (Sage Science, Beverly, MA). We then conducted several PCR amplifications using 20 ng of size-selected DNA with Phusion High-fidelity PCR reagents and the manufacturer’s recommended conditions (New England Biolabs, Ipswich, MA). This step added Illumina sequencing primers and a second unique "P2" index barcode to each pool of 48 samples, giving each sample an identifiable P1/P2 combined identifier. PCR products were then run on an Agilent 2100 Bioanalyzer to confirm the correct size distribution and concentration. Samples from sites RP and RS were sequenced at the New York Genome Center on one lane of an Illumina HiSeq 2500, while samples from sites RH1, RH2 and RH3 were sequenced at New York University on one lane of a HiSeq 2000.

Following sequencing, samples were demultiplexed and trimmed using the "process_radtags" script in Stacks 1.35 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). We aligned the reads assigned to each rat to the most recent R. norvegicus reference genome, Rnor6.0.
using Bowtie2 under default parameters (Langmead & Salzberg, 2012). Individuals with alignment rates below 50% were removed and the resultant files were used to run the "ref_map" pipeline in Stacks to call SNPs (single nucleotide polymorphisms), using parameters known to work well for brown rats (n = 2, m = 3) (Puckett et al., 2016). Finally, we used the "populations" script in STACKS to filter for SNPs sequenced in samples from all five sites (p = 5), and present in at least 60% of the samples (r = 0.6), with a minor allele frequency above 5%. Summary statistics were reported from Stacks output, and \( G_{ST}^{r} \) differentiation metrics between sites were calculated in the diveRsity package of R (Keenan, McGinnity, Cross, Crozier, & Prodöhl, 2013). \( G_{ST}^{r} \) is an alternative to \( F_{ST} \), estimates of genetic differentiation that accounts for differences in samples size and genetic variation across sites (Hedrick, 2005). To calculate genetic distance between individuals, we used Prevosti distance implemented in the poppr package of R (Kamvar, et al. 2007) to calculate within-family kinship coefficients.

2.3 | Characterizing Parasite Communities

We calculated species richness of the parasite community harboured by each rat and at each sampling site using the number of unique ectoparasite, bacterial and viral species observed in the sample. We also calculated abundance for ectoparasites, where the number of individuals of each species was available. We used the Bray–Curtis ecological dissimilarity index to measure the differences in parasite communities between rats and sites (Bray & Curtis, 1957). This metric is common in ecological studies on species community divergence between locations because it can be used for both abundance and presence-absence data (Legendre & Gallagher, 2001).

2.4 | Effects of spatial proximity on parasite community

We used canonical discriminant analysis (CDA) to summarize variation among parasite communities found at each site. This analysis determines whether specific parasite species can aid in distinguishing the origin of rats among the sampling sites based on occurrence patterns and was performed in the candisc package of R (Friendly & Fox, 2016). We also used Mantel matrix correlations to determine the relationship between Bray–Curtis parasite dissimilarity and Euclidean geographical distance between rats and sites. We log transformed the geographical distance data between rats to meet the assumptions of normality. We predicted that rats located closer together would share a more similar parasite community. Mantel tests account for non-independence among elements in distance matrices using permutations of the matrices for \( p \)-value calculations (Legendre & Fortin, 2010; Mantel, 1967).

We evaluated differences in the levels of parasite richness across sites using analysis of variance (ANOVA). Significant differences among sites were determined using Tukey post hoc tests. We used per rat estimates of richness to account for unequal sampling across sites; however, there was also no association between rats sampled and parasite richness in a preliminary test (r² = 0.008, p = .883; Fig. S1). We also used linear regression to evaluate the association between parasite richness at each site and on each rat, after a quantile plot of the mean of the residuals found no severe deviations from the linear assumptions of these tests. We wanted to test the ecological prediction that a rat occupying a site with greater parasite richness is likely to harbour more diverse parasites. We predicted such a pattern in part because of an elevated risk of exposure from the presence of a more diverse parasite community (Sordatto & Kardish, 2014). The analyses were performed using the base package within R.

2.5 | Effects of space on genetic similarity

We used discriminant analysis of principal components (DAPC) to identify genetic structure across our five sampling locations. This analysis, implemented in the adegenet package of R (Jombart, Devillard, & Balloux, 2010), identifies genetic structure by maximizing the genetic variation among sites while minimizing variation within identified groups. DAPC is particularly useful in visually assessing genetic variation along discriminant function axes (Jombart et al., 2010). We also performed a Mantel test between genetic and geographical distances to detect the presence of genetic isolation by geographical distance (Jenkins et al., 2010).

2.6 | Effects of genetic similarity on parasite community

We evaluated the relationship between genetic divergence between rats and parasite community similarity to test the prediction that rats that are more closely related genetically are also more likely to share similar parasites, either through direct contact or dispersal-mediated parasite transmission (Beadle et al., 2004; Wassom, Dick, Arnason, Strickland, & Grundmann, 1986). To test this relationship, we used Mantel matrix correlation between genetic distance and Bray–Curtis distance matrices on both a site and individual level. To determine if there was a role for relatedness in parasite transmission, we also did a principal component analysis on the parasite community data, which summarizes multivariate data in new orthogonal axes. We then overlaid highly related individuals onto the PCA biplot to see if they had more similar parasite communities than other, between-site pairs of rats.

3 | RESULTS

3.1 | Genomic Sequencing

We retained genetic SNP profiles for 100 rats after filtering individuals for sequencing depth and genome alignment rate. SNP calling resulted in 2,555 high-quality nuclear SNPs. Measures of genetic diversity varied across sites and include observed heterozygosity (mean = 0.108), \( \pi \) (0.122), and inbreeding coefficient \( F_{IS} \) (0.086). Genetic differentiation \( (G_{ST}^{r}) \) between the five sites ranged from 0.116 to 0.278.
Effects of spatial proximity on parasite communities

CDA of ectoparasites (Figure 1a), bacteria (Figure 1b) and viruses (Figure 1c) shows the linear combinations of species that have the highest site associations among the five locations. For ectoparasites, site RH3 was strongly associated with the presence of the oriental rat flea, while *O. bacoti* mites were associated with rats from the RS mixed-use location. Rats from RH1, RH2 and RP sites were weakly associated with the presence of the spined rat louse and *Laelaps* rat.
mites (Figure 1a). In contrast, only a few microbes showed strong associations with a specific site. The outdoor RP site was the most distinct from the other sites and was strongly associated with the presence of Campylobacter jejuni, L. interrogans, Shigella/Escherichia coli (EIEC), Seoul hantavirus, orbivirus and calhevirus (Figure 1b). The RH1 site was strongly associated with the presence of human sapovirus, porcine sapovirus, rosavirus and hepatitis E virus, and the RH3 site was strongly associated with the presence of Bartonella spp. (Figure 1c).

There was a strong association between ectoparasite community dissimilarity and geographical distance between sites, with nearer sites sharing more similar ectoparasite communities ($r^2 = 0.7295, p = .001$; Figure 2a). A similar pattern was detected for the bacterial and viral communities, but with much less of the variation in community similarity explained by geographical distance (bacterial: $r^2 = 0.0855, p = .001$; viral: $r^2 = 0.122, p = .001$; Figure 2b,c).

Total combined parasite species richness per rat differed significantly across the five sites ($F = 6.567, p < .001$; Figure 3). The RS site had significantly lower richness (mean = 3.8 species per rat), followed by RH2 (mean = 5). The RP park site, RH1 and RH3 did not differ from each other and had the highest total richness (means = 6.1, 6.3 and 6.5, respectively). Viral richness differed significantly across the five sites ($F = 5.08, p < .001$). The RS site had the lowest richness (mean = 1.0 virus per rat), followed by RH3 (mean = 2.1). The RH1 and RP sites did not differ significantly from each other and had the highest viral richness (means = 3.6 and 3.1, respectively). Bacterial richness also differed significantly across sites ($F = 7.385, p < .001$). The RH1, RH2 and RS sites had significantly lower richness (mean = 0.6, 0.93, and 0.9). The RH3 and RP sites did not differ from each other significantly and had the highest bacterial richness (means = 1.75, 1.72). Ectoparasite richness also differed across sites ($F = 9.835, p < .001$), with site RH3 showing the highest richness (mean = 2.4 ectoparasite species per rat).

The total count of parasite species richness also differed by site, with RH2 having the lowest and RP having the highest number of species across all rats sampled (RH1 = 21; RH2 = 17; RH3 = 20; RP = 24; RS = 18). Total parasite richness at each site explained 58% of the variation in parasite richness harboured by individual rats (Figure 4), but the relationship was not significant ($r^2 = 0.586, p = .131$).

3.3 Effects of space on genetic similarity

DAPC was conducted to partition the variance within the SNP genotype data set across the five sampling locations (Figure 5). Individuals from both RP and RS were genetically distinct from each other and the three housing areas, which were more genetically similar to each other.
3.4 | Effects of genetic similarity on parasite communities

The relationship between the genetic data set of the 100 sequenced individuals, using the $G'_{ST}$ pairwise genetic distance measure between sites, and the Bray–Curtis dissimilarity of parasite communities at each site was not statistically significant ($r^2 = 0.027, p = .525$). A similar test was run at the individual level, which showed no relationship between pairwise genetic distances between rats and Bray–Curtis parasite dissimilarities ($r^2 = 0.003, p = .09$). A separate principal components analysis with only the parasite community data found similar variation among sites as the CDA (Fig. S2). However, the three pairs of highly related individuals ($r$ coefficient > 0.25) found at different sites did not share more similar parasite communities than indicated by the overall differences among sites (Fig. S2), further suggesting that genetic similarity is not associated with parasite community similarity.

4 | DISCUSSION

The parasite communities harboured by New York City rats are diverse and differ substantially between sites, even across small distances. There was a relationship between the similarities of parasite communities and how far apart the sites were from one another, as well as strong genetic differences among sites. In addition, certain parasite species were strongly associated with particular sites. This demonstrates how parasitic communities can be localized in an urban context and with further sampling of additional locations throughout NYC, and site-specific parasite associations may be useful for determining the site of origin for rats from unknown locations. Despite strong genetic differences among sites, there was no association between genetic divergence and parasite community similarity. The results from this study demonstrate how both parasite communities and host genetic structure may vary dramatically across fine spatial scales in an urban landscape. This variation also highlights how any public health strategy designed to reduce disease risk to humans must consider that the rats across New York City do not carry a homogenous set of parasites and pathogens. As a result, epidemiological risks may be similarly heterogeneous for people in urban habitats.

In addition to spatial heterogeneity of the parasitic communities, certain species were strongly associated with particular sites, suggesting site specificity of some members of the parasite community. For example, discriminant analyses found that the oriental rat flea was strongly associated with the RH3 location, and the tropical rat mite with the RS location (Figure 1a). The combined association of RH1, RH2 and RP with the spined rat louse and the spiny rat mite suggests that these ectoparasites are more widely distributed throughout the landscape and may be more prevalent across rat colonies. The presence of certain microbial pathogens also characterized sites. Bartonella spp. were strongly associated with RH3, while L. interrogans, Shigella spp./enteroinvasive E. coli and Campylobacter jejuni were found mostly at the RP park site (Figure 1b). RP was also dominated by Seoul hantavirus, orbivirus and calhevirus, while RH1 has a richer community of other. Individuals from RH1 and RH3 are grouped close together in discriminant function space and also overlapped extensively with RH2, which has more genetic variation than the other two housing areas. Importantly, this genetic differentiation is not consistent with geographical distance as RP and RH1 are closer geographically than RH1 and RH3. In addition, RH2 is located closest to RH3 geographically, but not in terms of genetic similarity. Overall, there was a weak correlation between the genetic data set, using $G'_{ST}$, and the geographical distance between sites ($r^2 = 0.261, p = .092$).
site-specific viruses (Figure 1c). Ecological differences in the habitats across the five sites may drive site specificity among some members of the parasite community. Despite close proximity, parasite communities can differ from one rat colony to the next, thus increasing the potential spread of a wider variety of zoonotic diseases across small areas of the city. By discriminating sites based on their unique parasite communities, and combining this with city data on rat and disease “hotspots”, (e.g., Walsh 2014; Johnson et al., 2016), we may be able to predict the infection risks posed by specific rat colonies due to their small home-range size and limited dispersal. Also, with further sampling of sites in NYC, site-specific parasites may be useful in determining the site of origin in newly sampled rats across a similar geographical scale. The heterogeneity of the parasites examined in this study suggests that some species may not be easily transmitted across colonies and may not persist in some colonies even if introduced, or that interactions between conspecifics in nearby colonies are lower than expected (Godfrey, 2013).

When examining all rats in our study, parasite communities were significantly more similar in rats located near each other. This pattern was driven by ectoparasites (Figure 2a), as there was a weak association between bacterial (Figure 2b) and viral (Figure 2c) similarity and distance between hosts. Within a single colony, ectoparasites are primarily transmitted through direct contact (Leu et al., 2010; Stanko et al., 2002). Shared nesting and communal care of young is common among rodents and may play an important role in the spread of ectoparasites (Hayes, 2000). Therefore, it may be the case that ectoparasite similarities reflect sociality of rats and their proximity within colonies, which promotes direct contact. However, despite the small home-range size of most rats (Davis et al., 1948; Himsworth et al., 2014), ectoparasites can still spread between members of different colonies. This may occur when members leave in search for food resources or refuge (Glass et al., 2016), or when rats from nearby colonies reinvade areas that have been subject to intensive pest control. Also, as rodent populations increase, territories may begin to overlap, which promotes higher contact frequencies and ectoparasite transmission (Altizer et al., 2003; Davis et al., 1948; Kerth & Van Schaik, 2012). Direct contact is not necessary for the transmission of many microbial pathogens (Himsworth, Bidulka et al., 2013; Himsworth, Parsons et al., 2013), which can also be transmitted through urine or faeces and in some cases, remain viable outside of the body for some time (Karaseva et al., 1973). Because indirect transmission is possible in these cases, microbial communities are less likely to remain isolated based on proximity among colonies, which is consistent with our results of a lack of association between distance and microbial community similarity in rats. Yet, we note that some microbial pathogens can also be spread through direct transmission from intermediate hosts or through arthropod vectors (e.g., Bartonella spp. and orbiviruses; Billeter, Levy, Chomel, & Breitschwerdt, 2008). However, the host range is likely very narrow for the microbes and ectoparasites detected in the rats in our study, especially considering the depauperate animal communities within lower Manhattan that could serve as reservoirs. Additionally, none of the microbes detected are known to be vectored by flying insects, suggesting that the maximum potential movement capacity of these parasites is on rats. The movement of these rat reservoirs is most likely driving any spatial patterns (or lack thereof) observed in the parasite communities.

The divergence in parasite communities, even at neighbouring sites, can be seen when comparing species richness and species composition across sites. There was a significant difference in total species richness across the five sites collectively (Figure 3). However, on a finer scale, sites that are closest in proximity, such as RP and RH1, had unique parasite communities (Figure 1) but similar species richness (Figure 3). An ANOVA showed that RS had lower species richness compared to RH1, RH3 and RP. The relatively large geographical distance that separates RS from the other sites most likely isolates these rats and their parasitic species from those at the other sampled locations. However, despite the smaller geographical range between the remaining sites, which we may expect to minimize parasite uniqueness, our results show distinct communities across fine spatial scales, as seen in the analyses of spatial proximity on parasite community (Figures 1 and 2).

Analyses of our SNP genomic data for 100 of the 133 rats showed no association between genetic divergence and parasite community divergence among sites or among individuals. Therefore, the genetic structure occurring across the NYC landscape does not seem to influence the parasite communities harboured by rats. The genetic structure of entire colonies at a single site appears to have little effect on the parasites that may be specific to that location. This pattern may be expected if the dominant modes of transmission do not include direct contact between rats (Moller et al., 1993). Alternatively, if rats are able to move between colonies and transmit parasites without breeding (Leu et al., 2010), and hence without gene exchange, this could explain the lack of association between measures of gene flow and parasite community similarity. Lastly, founder effects could also lead to genetic divergence not corresponding to parasite community differences if the parasite community is determined primarily by dispersing individuals founding a new colony and if the gene pool of the new colony experiences a genetic bottleneck resulting in strong genetic differences between the source and new colonies. New parasite species only arrive when rats successfully invade the colony or during movements within the small home ranges of R. norvegicus. However, more research will be needed to identify the reason for this limited association.

Additional analyses of the genomic data showed strong genetic differentiation between the five sites, with $G_{ST}$ values ranging from 0.12 to 0.28. However, genetic differentiation was not strongly associated with geographical distance between sampling locations. For example, despite being close in proximity, rats from nearby sites such as RP and RH1 were genetically isolated (Figure 5). In contrast, the residential sites were farther apart from each other, yet were more genetically similar (RH1, RH2 and RH3; Figure 5). Variation in the ecological characteristics of the sites may be driving the genetic structure among locations. This finding is consistent with growing evidence that nonrandom dispersal resulting from habitat selection, known as “natal habitat preference induction” in rodents, can lead to directed gene flow that promotes phenotypic and genotypic differences between habitat types (Edelaar, Siepielski, & Clobert, 2008; Mabry & Stamps, 2008).
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**CONFLICT OF INTEREST**

The authors have no known conflict of interests related to this work.

**ORCID**

J. L. Richardson [http://orcid.org/0000-0002-3701-2115](http://orcid.org/0000-0002-3701-2115)

**REFERENCES**


**5 | CONCLUSION**

Here, we have provided an evaluation of the relationship between urban rat connectivity and associated parasite communities. These findings provide important insights into how pathogens of public health concern are distributed in the urban core of New York City and can be used to assist future rodent control and epidemiological interventions. The parasite communities harboured by rats were heterogeneous across the study area, which appears to be consistent with modes of contact and transmission between rats. This pattern suggests that the risk of zoonotic infection to humans also varies dramatically across fine spatial scales, creating a challenge to epidemiologists and public health official tasked with monitoring and predicting disease risk. The genetic connectivity of rats in our study indicates that urban habitats are highly heterogeneous over fine spatial scales. Researchers may need to take this heterogeneity into account when predicting likely pathways of rat dispersal, as gene flow was not related to simple distance on the landscape. Resource abundance and dwelling characteristics may drive the direction of dispersal more than spatial distribution. Variation in the parasite communities and the genetic structure of their hosts highlights the importance of strategic approaches when dealing with New York City rats, and likely other cities as well. In order to reduce disease risk to humans, public health officials should consider different colonies of rats as reservoirs of unique sets of zoonotic pathogens. Programs to prevent rodent-borne disease outbreaks in humans should include spatially appropriate field monitoring and adaptive responses to variation in pathogen presence across the cityscape. Understanding this connection between rat ecology and connectivity of these zoonotic reservoirs in an urban landscape is a required first step to predicting zoonotic disease risk to humans in these areas.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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