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Commensal ecology, urban landscapes, and their influence on the genetic characteristics of city-dwelling Norway rats (*Rattus norvegicus*)

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Abstract

Movement of individuals promotes colonization of new areas, gene flow among local populations, and has implications for the spread of infectious agents and the control of pest species. Wild Norway rats (*Rattus norvegicus*) are common in highly urbanized areas but surprisingly little is known of their population structure. We sampled individuals from 11 locations within Baltimore, Maryland, to characterize the genetic structure and extent of gene flow between areas within the city. Clustering methods and a neighbour-joining tree based on pairwise genetic distances supported an east–west division in the inner city, and a third cluster comprised of historically more recent sites. Most individuals (~95%) were assigned to their area of capture, indicating strong site fidelity. Moreover, the axial dispersal distance of rats (62 m) fell within typical alley length. Several rats were assigned to areas 2–11.5 km away, indicating some, albeit infrequent, long-distance movement within the city. Although individual movement appears to be limited (30–150 m), locations up to 1.7 km are comprised of relatives. Moderate F_{ST} , differentiation between identified clusters, and high allelic diversity indicate that regular gene flow, either via recruitment or migration, has prevented isolation. Therefore, ecology of commensal rodents in urban areas and life-history characteristics of Norway rats likely counteract many expected effects of isolation or founder events. An understanding of levels of connectivity of rat populations inhabiting urban areas provides information about the spatial scale at which populations of rats may spread disease, invade new areas, or be eradicated from an existing area without reinvasion.

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This work is part of a study examining ecological interactions and infectious pathogens within populations of rodents. Lynne Gardner-Santana is an ecologist studying mammalian population genetics. Douglas Norris is an entomologist researching population genetics of arthropod vectors and their pathogens. Christen Fornadel is a PhD candidate studying arthropod vector biology. Ella Hinson is a PhD candidate studying host immunity to viral infections. Sabra Klein is an immunologist researching endocrine-immune responses of rodents to infection. Gregory Glass researches reservoir and vector populations and the environmental variables contributing to the persistence and emergence of infectious diseases.

Keywords

assignment; habitat fragmentation; population genetics; population structure; urban wildlife; zoonotic diseases

Introduction

Key aspects of population ecology, such as recruitment and survivorship, are affected by gene flow across landscapes. Landscape characteristics, including levels of connectivity (Hamilton *et al.* 2006), influence individual movement (Manel *et al.* 2003), alter patterns of gene flow (Aulsebrook 1995), and may determine the distribution and spread of zoonotic pathogens. In particular, urban landscapes are highly fragmented, which can lead to a decrease in genetic diversity (Mossman & Wasser 2001; Noël *et al.* 2007) due to restricted gene flow. Founder events during colonization may also decrease genetic diversity (Nei *et al.* 1975), which has important ramifications for offspring viability (Briskie & Mackintosh 2004), the ability of individuals to resist infection (Spielman *et al.* 2004), individual fitness, and population sustainability (Hansson & Westerberg 2002).

Norway rats (*Rattus norvegicus*) are commensal rodents found in urban areas throughout the world and are pervasive in urbanized areas of Baltimore, Maryland (Childs *et al.* 1991b). Baltimore is located on the eastern coast of the USA and was founded in the early 18th century (Chapelle 2000). Baltimore's port was a major depository for grain during the American Revolution (Chapelle 2000), likely the time period during which Norway rats were introduced and invaded the city (Avery 1985). Commensal rodents exhibit specific ecological and behavioural characteristics stemming from their cohabitation with humans (Pocock *et al.* 2004; Huck *et al.* 2008) and are generally concentrated into high-density populations (e.g. Fedriani *et al.* 2001). Rats inhabit areas of central Baltimore (Childs *et al.* 1991b) near anthropogenically produced food sources, such as garbage, and inhabit areas with increased physical structuring (e.g. Gray *et al.* 2000) that provide harbourage (Emlen *et al.* 1948; Orgain & Schein 1953).

Most mammals are characterized by female philopatry and male natal dispersal (Greenwood 1980). Philopatry in commensal Norway rats is expected, as female rats establish burrow systems in backyards or underneath structures, which grow as litters are born (Calhoun 1962). Observational studies in city environments indicate that rats have smaller activity areas (25–150 m; Davis *et al.* 1948; Glass *et al.* 1989; Traweger *et al.* 2006) than those radio-tracked in rural landscapes (> 260–2000 m; Taylor 1978; Taylor & Quay 1978; Macdonald & Fenn 1995). Despite these small activity areas, social factors, such as aggression (Davis 1951a, b), may facilitate movement to new areas, and rats can travel long distances after large disturbances in their environment (Taylor 1978).

Norway rats are reservoirs for many known (Childs *et al.* 1985, 1987, 1988, 1991a; Hinson *et al.* 2004; Easterbrook *et al.* 2005, 2007) and suspected (Favorov *et al.* 2000) zoonotic pathogens, several of which are found in rats in Baltimore (Easterbrook *et al.* 2007). High densities of urban rats promote zoonotic disease transmission. Extensive research on urban rat ecology has occurred in Baltimore since the mid-1940s (Davis 1987), but, despite their

profound impact on public health, rat populations have not been genetically characterized. Understanding population structure and individual movement in urban environments is valuable for both pest control efforts (Traweger *et al.* 2006), by aiding in delineation of eradication units (Abdelkrim *et al.* 2005, 2007), and in helping characterize and control outbreaks of disease spread by commensal rodents.

We used microsatellites to genetically describe individual rats trapped in 11 different locations throughout highly urbanized residential areas of Baltimore. If Norway rats in different areas of Baltimore were established from a small number of founders with limited activity ranges, rats should exhibit reduced genetic diversity, heterozygote deficiency, high levels of pairwise relatedness, and pronounced population structuring throughout the city. This isolation may be exacerbated by the availability of high-quality habitats (Orgain & Schein 1953; Schein & Orgain 1953). However, rats possess short gestation times, are highly fecund, exhibit dominance hierarchies, and have the ability to move long distances. Therefore, the ecology of this species may counteract isolation or founder events. Specifically, our objective was to examine genetic characteristics (e.g. levels of diversity, relatedness, and population structure) of rats trapped in several different highly urbanized areas of Baltimore to elucidate population genetic structure and understand the extent of gene flow in city-dwelling Norway rats.

Materials and methods

Rats were collected using Tomahawk live traps in 11 areas of Baltimore, Maryland (Fig. 1), as previously described (Glass *et al.* 1988, 1989). Sites were from 0.07–13.4 km apart (Table 1) and showed abundant signs of rat activity, such as burrows, signs of gnawing, and faecal matter. Up to 60 traps were nonrandomly distributed throughout each area, placed next to runways created by repeated rat movements. Areas were trapped several nights, ensuring full-alley coverage for rat density estimates. Traps were baited with peanut butter, set at dusk, and retrieved at sunrise the following morning.

DNA was extracted and individuals were genotyped for 10 (CA)_n dinucleotide microsatellite loci from different linkage groups (D1Cebr3, D1Cebr9, D2Cebr1, D3Cebr3, D4Cebr2, D5Cebr1, D6Cebr1, D10Cebr1, D11Cebr1, and D20Cebr1; Giraudeau *et al.* 1999) following protocols outlined in Hinson *et al.* (2006). Loci with high frequencies of null alleles, allelic dropout, and scoring error were identified (Micro-Checker 2.2.3; Van Oosterhout *et al.* 2004, 2006). We eliminated two problem loci with high null allele frequencies (D5Cebr1, 0.11; D11Cebr1, 0.15) from the data set, using only the eight loci with < 0.09 null allele frequencies in further analyses. Where possible, we employed analyses robust to presence of null alleles, including Structure (Pritchard *et al.* 2000; Falush *et al.* 2003), Geneland (Guillot *et al.* 2005a, b, 2008), and chord distance (D_C; Cavalli-Sforza & Edwards 1967) (Chapuis & Estoup 2007; Chapuis *et al.* 2008). Probability of identity (PI) (Paetkau & Strobeck 1994) and PIsibs (Evetts & Weir 1998) were estimated (GeneCap 1.1; Wilberg & Dreher 2004).

To genetically characterize the 11 areas, each locus was tested for linkage disequilibrium (Weir 1996, pp. 126–128) (GenePop 4.0, Raymond & Rousset 1995; Rousset 2008) using Markov chain Monte Carlo (MCMC) parameters of 1000 dememorizations with 100 batches

and 1000 iterations per batch. We calculated allelic diversity (A), expected (H_E) and observed (H_O) heterozygosities, and the conformity of each locus to Hardy–Weinberg equilibrium (Guo & Thompson 1992) (Arlequin 2.0; Schneider *et al.* 2000). Because multiple comparisons artificially inflate type I errors, a sequential Bonferroni correction (Rice 1989) was performed on P values resulting from tests of Hardy–Weinberg equilibrium and pairwise linkage equilibrium to determine significant differences at the experiment-wise type I error rate of 5%. To test the hypothesis that areas experienced historical bottlenecks due to founder events, we used a one-tailed Wilcoxon signed-rank test (Piry *et al.* 1999) for excess heterozygosity using the two-phase model (TPM; 70% SMM:30% IAM; Barker 2005) and examined the results of the mode-shift test to assess presence of cryptic bottlenecks in each site (Bottleneck 1.2.02; Cornuet & Luikart 1996). While the excess heterozygosity test identifies significant reductions in effective population size, mode shift tests examine the loss of rare alleles during more recent or cryptic genetic bottlenecks (Luikart *et al.* 1998). Population genetic structure was initially evaluated with F statistics (Rousset 1997) (SPAGeDi; Hardy & Vekemans 2002), and pairwise N_m was calculated as $F_{ST} \sim 1/(4N_m + 1)$ (Wright 1943). F_{IS} (Weir & Cockerham 1984) was determined based on allele frequencies for each site (GenePop 4.0, Raymond & Rousset 1995; Rousset 2008).

Genetic distances between sample sites were calculated using Cavalli-Sforza & Edwards' (1967) chord distance (DC) (Microsat 1.5b; Minch 1998). We produced 5000 distance matrices from resampled data and analysed them in Neighbor (PHYLIP 3.68; Felsenstein 1989). We then used the Consense package (PHYLIP 3.68) to construct an unrooted majority-rule consensus neighbour-joining (NJ) tree with bootstrap values that was generated using TreeView (Page 1996).

To understand the extent of gene flow and provide a comprehensive perspective of the genetic dynamics across Baltimore, we examined isolation by distance. Mantel tests were conducted with pairwise kinship coefficients (Loiselle *et al.* 1995) and the natural logarithm of pairwise geographical distance using 10 000 randomizations (F_{STAT} 2.9.3.2; Wright 1943; Goudet *et al.* 2002). We also compared mean pairwise kinship coefficients of each distance class to the kinship of random pairs of individuals using a one-tailed t -test to examine the hypothesis that related individuals were spatially aggregated. Finally, we calculated the pairwise relatedness coefficient (R ; Queller & Goodnight 1989) to determine levels of relatedness of individuals captured in the same area (SPAGeDi; Hardy & Vekemans 2002).

Movement between sampling areas was elucidated using an assignment test with the leave-one-out procedure (GeneClass 2.0; Piry *et al.* 2004). A partial Bayesian approach (Rannala & Mountain 1997) assigned individuals to the 11 areas. We identified first-generation migrants using a likelihood computation (Paetkau *et al.* 2004) with 1000 simulated genotypes ($P = 0.01$) and the $L = L_{home}$ option (Piry *et al.* 2004), as not all source populations were sampled. We performed both one-tailed and two-tailed tests for sex-biased dispersal and philopatry based on several estimators (AI_C , vAI_C , F_{ST} , F_{IS} , R , H_O , and H_S ; Goudet *et al.* 2002).

We estimated neighbourhood size (N_b) to understand the geographical extent of an individual's genes using $N_b = (1 - F_1)/b$ (Hardy 2003; Vekemans & Hardy 2004), where F_1

equals the mean pairwise kinship coefficient (Loiselle *et al.* 1995). The slope (b) of the regression between the log-transformed pairwise geographical distance and the pairwise kinship coefficient was calculated for an area within the distance equal to the x -intercept of the regression. Axial dispersal distance (3σ) was calculated as $Nb = 4\pi\sigma D$ (Wright 1969; pp. 302–307), where D is the mean density of individuals. Neighbourhood area (No) was calculated by multiplying the inverse of density by Nb (Aspi *et al.* 2006). The estimate of mean density was based upon capture rates and geographical extents (Caughley 1977; pp. 20–21) of six locations throughout Baltimore. This method estimates absolute density of an area by determining the presence or absence of animals per trap. We calculated density of rats per area, $\bar{x}$, using $1-f = e^{-\bar{x}}$, where f is mean frequency per trap (Caughley 1977; p. 21). Proportion of traps catching no animals ($1 - f$) was determined, and we multiplied absolute density by area trapped to produce density estimates.

Because the extent of gene flow in urban rats is unknown, we investigated genetic structuring in rats using two Bayesian approaches that perform at > 90 % accuracy in individual assignment and identify genetic structure at moderate F_{ST} values (Latch *et al.* 2006; Chen *et al.* 2007). We first used the clustering method employed in Structure 2.1 (Pritchard *et al.* 2000; Falush *et al.* 2003) to assign genotypes to K genetic clusters based on allele frequencies without knowledge of spatial coordinates of the trapping area, as it is the standard program used in these types of population genetic studies. The use of several clustering analyses can provide information regarding both population substructure and changes in allele frequencies across a landscape (Chen *et al.* 2007), and using multiple analyses is valuable in verifying results (Latch *et al.* 2006). Although we hypothesized that $K = 11$ based upon isolation and founder events, we evaluated results for $K = 1$ to $K = 20$ with 10 repetitions, a burn-in period of 50 000, and MCMC lengths of 100 000 using the admixture model to produce an estimate of K . We chose these values for K based upon either no genetic structuring within the city ($K = 1$) or levels of substructure within trapping areas ($K = 20$). We also examined the ΔK statistic that identifies the largest change in estimates of K produced by Structure, as ΔK may provide a more realistic estimation of K (Evanno *et al.* 2005). We calculated q -intervals which assign a portion of each individual's genotype to clusters during simulations. We based individual assignment to a cluster on the largest average proportion of their genotype assigned to a cluster over 10 runs.

We also used the MCMC algorithm approach of Geneland 3.1.3 (Guillot *et al.* 2005a, b, 2008) and R 2.8.0 (Ihaka & Gentleman 1996) to detect genetic discontinuities in the study area landscape. Specific geographical coordinates (UTMs) of each genotype were incorporated into the analysis to more accurately portray landscape-level genetic discontinuities than when using nonspatial analyses such as Structure (Guillot *et al.* 2005a, b, 2008). The MCMC analysis was run five times without *a priori* knowledge of population subdivision for 100 000 iterations with 10 m uncertainty of geographical coordinates, minimum $K = 1$, maximum $K = 10$, using the Dirichlet distribution model of independent allele frequencies and incorporating null alleles into the model (Guillot *et al.* 2008). A second MCMC algorithm was run 10 times for 100 000 iterations with a fixed K equal to that of the modal number of populations found for the previous five runs.

Pairwise F_{ST} values for clusters were calculated (Geneland 3.1.3; Guillot *et al.* 2005a, b, 2008). Because trap sites are artificially derived population boundaries, the report of F_{ST} values for overall clustering is valuable in understanding connectivity across the city, while pairwise F_{ST} values between areas are valuable in understanding connectivity within the city. Data input files for the Arlequin 2.0, GenePop 4.0, Bottleneck 1.2.02, Microsat 1.5b, and Structure 2.1 software were created using Convert 1.3 software (Glaubitz 2004).

Results

A total of 277 *Rattus norvegicus* were sampled from 11 locations throughout residential neighbourhoods of Baltimore (Fig. 1). Areas were characterized by row houses with small backyards comprised of concrete parking pads and small garden areas often occupied by rat burrow systems. Several areas underwent extensive restoration, transitioning from poorly maintained, often abandoned buildings to newly renovated housing. Many areas in Baltimore do not support large Norway rat populations. Therefore, prior to trapping, areas were surveyed for signs of rat activity (Easterbrook *et al.* 2005). Mean pairwise Euclidean distance between sampling sites ($\pm$ SD) was 4.2 ± 2.9 km (Table 1). However, two areas had sites adjacent to one another across individual streets. Sample sizes ranged from 20–29 rats per location (Table 2).

No incidence of false alleles or allelic dropout was detected (Micro-Checker 2.2.3; Van Oosterhout *et al.* 2004, 2006). Null alleles were found at three loci [D2Cebr1 ($P = 0.06$); D10Cebr1 ($P = 0.073$); and D20Cebr1 ($P = 0.084$)]. However, our mean null allele frequency of 0.04 ± 0.03 was below the threshold of 0.19 found to significantly underestimate H_E (Chapuis *et al.* 2008). Null alleles may be present because markers initially developed for laboratory *R. norvegicus* (Giraudeau *et al.* 1999) were applied to wild populations (Paetkau & Strobeck 1995). Lack of adjacent-allele heterozygotes, an indicator of scoring error due to stuttering, was found at three loci (D2Cebr1, D10Cebr1, and D20Cebr1), which were re-scored prior to analyses. At eight loci, this study had a $PI_{sibs} = 2.2 \times 10^{-4}$ and a $PI = 9.3 \times 10^{-12}$, sufficiently low to elucidate unique genotypes (Waits *et al.* 2001). Single-locus PI_{sibs} for the eight loci were 0.30–0.47.

Although overall linkage disequilibrium was found in two pairs of loci (D1–9; D6 and D2; D6, $P < 0.0001$), it occurred only in three trap sites (Table 2). Allelic diversity was 12.1 ± 3.2 alleles per locus (range 7–16; $n = 97$). The overall sample was in Hardy–Weinberg equilibrium ($\chi^2 = 6.6$, d.f. = 10, $P > 0.5$); however, four sites exhibited heterozygote deficiency due to null alleles (Table 2). Bottlenecks were detected in nine of the 11 sites, with mode-shifts detected in four of these nine sites (Table 2). Pairwise F_{ST} and corresponding pairwise N_m values ranged widely (Table 1) with a mean overall $F_{ST} = 0.07 \pm 0.005$.

The NJ tree separated the farthest eastern populations (Northeast Market, Ashland, and the two Jefferson trap sites) from mainly western populations (Winston-Govans, Hugo, Druid, McKewin, West Baltimore, Ostend, and Brooklyn) (Fig. 2). Mantel tests indicated that rats captured in close proximity were more related to each other ($r = 0.31$; $P = 0.021$), and identified global isolation by distance ($r = 0.24$; $P < 0.0001$) with an x -intercept of 1.7 km

(Fig. 3). Mean pairwise kinship coefficient of individuals captured in the same sites (0.067) was higher than the population mean (0.0002) ($t_{calc} = 32.8$, d.f. = 3797, $P < 0.0001$; Fig. 3). With the exception of the fourth distance class (8.1, pairwise kinship = -0.0024), all distance classes possessed average pairwise kinship coefficients that were significantly different from the population mean of 0.0002 (Fig. 3).

Assignment tests typically associated individuals (95.3%) with their site of capture (GeneClass2; Piry *et al.* 2004) (Table 3), but 13 of 277 (4.7%) individuals were misassigned, with no sex or age bias (5 M: 8 F; $\chi^2 = 2.62$, d.f. = 1, $P > 0.1$). These individuals consisted of five juveniles (2 M; 3 F) and eight sexually mature (> 200 g) rats [3 M; 5 F (2 pregnant)]. Of these, approximately half were assigned to areas within 400 m, the other half were assigned to areas 2–6 km away, and one individual was assigned to a site 11.5 km away. Eighteen (6.5%) first-generation migrants were assigned to areas 0.07–8.1 km away from that of capture with no sex or age bias [7 M; 11 F (2 pregnant); $\chi^2 = 0.89$; d.f. = 1; $P = 0.35$]. About one-third of migrants moved within ~800 m (mean = 568 m), while two-thirds of the individuals moved from 3–8 km (mean = 5 km), and one individual was unlikely to originate from any of the sampled sites. There was one individual identified both as a first-generation migrant and as misassigned. No significant differences existed in dispersal based on sex (all tests $P > 0.3$). *Nb* of city rats was 44 individuals, and mean *Na* was 5580 m² (range 2231–31 811 m²), with a mean axial dispersal distance of 63.2 m (range 40.0–150.9 m). Mean density of rats (0.007 ± 0.005 rats/m) resulted in estimates of 2–217 rats per alley (mean = 49.5 rats/ alley).

Runs in Structure software indicated the largest mean log likelihood over 10 runs (-7302.68) at $K = 5$ clusters (Fig. 4). These clusters were: (i) bisection of the city across an east–west corridor [West Baltimore, 60% of Ashland and Brooklyn, 25% of Hugo, 25% of Jefferson (Luzerne), 40% of Northeast Market, and 30% of McKewin]; (ii) a site in the southeastern and most recently settled area of the city (30% Brooklyn); (iii) sites in the historically more contemporary areas of the city (Winston-Govans, 60% of Druid, 65% of McKewin, 60% of Hugo, and 75% of Ostend); (iv) sites in eastern Baltimore inner-city area [50% of Northeast Market, 85% of Jefferson (Rose), 75% of Jefferson (Luzerne), and 30% of Ashland]; and (v) three miscellaneous individuals. In contrast, the number of clusters in the data estimated by using the K statistic (Evanno *et al.* 2005) was $K = 3$ clusters (-7415.32) consisting of: (i) West Baltimore, 60% of Ashland, 45% of Druid, 40% of Hugo and Brooklyn, 30% of Northeast Market, 25% of McKewin and Jefferson (Luzerne), 20% of Ostend, and 10% of Winston-Govans; (ii) 90% of Winston-Govans, 80% of Ostend, 75% of McKewin, 70% of Northeast Market, 55% of Druid, and 40% of Ashland; and (iii) two miscellaneous individuals (Fig. 4).

Geneland produced a mode at $K = 3$ clusters. The two highest log likelihoods were run 6 (-7054.82) and run 1 (-7056.91). Run 7 (-7093.73) also produced a similar pattern of geographical clustering: (i) a group of sites in the east Baltimore inner-city area (Ashland, Northeast Market and the Jefferson trap sites); (ii) sites in the west Baltimore inner-city area (Ostend and West Baltimore); and (iii) sites in the more recent areas of the city (Winston-Govans, Druid, McKewin, Hugo, and Brooklyn) (Fig. 1). Runs 8 (-7079.92) and 9 (-7081.81) produced a similar pattern, but clustered the Ostend trap site with the historically

more recent trap sites. Pairwise F_{ST} values calculated by Geneland for the three clusters had similar ranges for run 6 (0.0327–0.0494) and runs 1 and 7 (0.0314–0.0488). Runs 8 and 9 also produced similar, although slightly inflated, pairwise F_{ST} values (0.0445–0.0652).

Discussion

High levels of habitat fragmentation characteristic of urban areas should limit individual rat movement between areas, providing the basis for our initial hypothesis that city rat populations would be geographically isolated and genetically structured. The findings of this study strongly support this hypothesis. However, the biology and ecology of commensal Norway rats temper the genetic isolation and serve to homogenize the global population to a limited geographical extent.

Structure within the Baltimore rat population is clearly evident, with related individuals present in a distance spanning approximately 11 city blocks (1.7 km; Fig. 3). This is further supported by our estimated neighbourhood area ($Na = 5580 \text{ m}^2$), which spans an area only 1.2 times that of the average alley area, including backyards, of 4873 m^2 . As pairwise distance between trapping areas increases, the genetic similarity between rats decreases (Favre *et al.* 1997), which is illustrated by pairwise F_{ST} values supporting a pattern of isolation by distance (Table 1). Isolation by distance indicates that individual rats within the city conform to the stepping-stone model of gene flow (Kimura 1953), which results in an aggregation of related individuals. This aggregation effectively increases the proportion of alleles shared via co-ancestry, encouraging inbreeding and low heterozygosity similar to that of insular rat populations ($H_E = 0.42$; Abdelkrim *et al.* 2005). Moreover, rats in resource-rich areas may not disperse (Lin *et al.* 2006), facilitating aggregation of related individuals (Verdolin & Slobodchikoff 2009) and discouraging colonization. Thus, isolation by distance, short axial dispersal distances falling within the length of a city block (40–151 m), and an Na just slightly greater than a city block support a geographical limit to rat movement that is further corroborated by site fidelity (95.3% of rats assigned to capture area).

Ultimately, dispersal patterns and social structure determine levels and distribution of genetic variation in populations (Chesser 1991b; Matocq *et al.* 2000). Demographic characteristics of most mammalian species include male-biased dispersal and female philopatry resulting from inbreeding avoidance, male access to mates, and a disproportionate female investment in reproduction (Greenwood 1980). Philopatry can increase levels of isolation and genetic structuring (Brouat *et al.* 2007), which could decrease local effective population sizes in Baltimore (N_e ; Wright 1931) due to generational increases in co-ancestry between pairs of females in the same location. An increase in co-ancestry could also occur if we trapped animals prior to emigration from the natal site (Chesser 1991b), although given the body size of the trapped rats, this seems less likely. Positive F_{IS} values in 55% of sites (Table 2) indicate possible increased co-ancestry between offspring (Storz *et al.* 2001), providing further support for isolation by distance in city rats. Although density can have a sampling effect on F_{IS} values, regression analysis indicated no such relationship ($r = -0.222$; d.f. = 1; $P = 0.777$).

Despite indications of high levels of co-ancestry and its potential effects on reducing local and inflating global N_e (Chesser 1991a; Nunney 1999), genetically based tests were unable to elucidate philopatry, sex-biased dispersal, or a difference in pairwise R of females and males ($R_f = 0.17$; $R_m = 0.14$; $P = 0.19$). Therefore, our results suggest that an atypical mammalian structure exists in commensal *R. norvegicus*. The majority of misassigned individuals and first-generation migrants identified were sexually mature adults, indicating that natal male dispersal may be absent in city rats. If males fail to hold and defend territories in areas of high density (Macdonald & Fenn 1995), such as those found in commensal Baltimore rats (Childs *et al.* 1991b), the expected spatial distribution of both sexes would change, altering movement patterns, local population demographic features, and local population genetic characteristics. Our findings, including high levels of genetic variation ($H_E = 0.73$), allelic diversity (mean = 12.1 alleles/locus), and a moderate $F_{ST} = 0.07$ indicate a homogenizing effect of regular gene flow and suggest altered behaviour and ecology in commensal rats.

Strong site fidelity and limited movement in rats should result in high levels of genetic structuring observed in Baltimore city rats. However, Bayesian modelling indicated only broad-scale structuring at low levels consistent with the major landscape features in Baltimore. Moderate sub-structuring existed, with only 3–4 distinct clusters in an area of approximately 200 km². Structure, K , Geneland, and the NJ tree analyses (Fig 1, Fig 2 and Fig 4) indicated high levels of admixture between trapping areas. Structure detected the offspring of immigrant parents (Pritchard & Wen 2004), which supports our hypothesis that single or multiple historical admixture events occurred in trapping areas exhibiting bottleneck events, linkage disequilibrium, or heterozygote deficiencies. If rats introduced at the harbour via shipping founded populations that subsequently expanded into other areas, admixture may have created unique gene pools serving as pockets of high diversity (Rollins *et al.* 2006) within Baltimore. If individual founder events conform to the migrant pool model of colonization history, contribution of alleles from several gene pools would increase diversity (Frankham 1997, 2005; Balloux & Lugon-Moulin 2002; Kolbe *et al.* 2004). Structure, however, may produce biased ancestral populations that contain predominantly admixed individuals (Pritchard & Wen 2004), as was found in our data. Thus, Geneland may more accurately model local differentiation because it incorporates spatial information (Guillot *et al.* 2005a, b, 2008) and exhibits power to identify contact zones at the landscape level with little or no recent migration (Chen *et al.* 2007).

The east–west differentiation of the populations occurs along the Jones Falls, a rapidly flowing waterway that originates to the north in Baltimore County and travels south through the city before it empties into the harbour. Both programs also clustered pairs of populations together that were not spatially proximal to each other, but instead were located in more recent and peripheral areas of the city, suggesting a temporal factor in overall population substructure. Dispersal of rats from the harbour area where the city was established could produce a radiating pattern in differentiation. Half of the bottlenecks detected were characterized by mode shifts, indicating very recent genetic constraints (20–80 generations; Luikart *et al.* 1998) in populations that inhabit the more contemporary areas of the city.

Bottlenecks were detected in most trap sites and heterozygote deficiency was found in one trapping area (Brooklyn), yet no prolonged deleterious effects of isolation are reflected in levels of genetic variation or diversity. Local sampling effects on allele frequencies could occur based upon the large variation in population densities between sites. However, we found no evidence that density of rats was related to levels of allelic diversity ($r = 0.19$; d.f. = 5, $P = 0.21$) or heterozygosity (H_E : $r = -0.12$, d.f. = 5, $P = 0.53$; H_O : $r = -0.13$, d.f. = 5, $P = 0.55$). Instead, patterns of dispersal, social structure and interaction, population history, and landscape characteristics all contribute to high diversity and moderate rather than severe levels of substructuring in city rats. For example, multiple paternity may contribute to high levels of genetic variance and the moderate differentiation ($F_{ST} = 0.07$) that we found in city rats. While multiple paternity has been demonstrated in laboratory *R. norvegicus* (Hinson *et al.* 2006), no studies of paternity have been conducted in wild Norway rats. Macdonald *et al.* (1999) observed an oestrus female followed by a string of several males in a farm population of Norway rats, and similar observations were reported in Baltimore (Glass *et al.* 1989). Although males exhibit a dominance hierarchy (Berdoy *et al.* 1995), observational studies indicate that subordinate males often procure relatively equal numbers of matings (Macdonald *et al.* 1999; Hinson *et al.* 2006). Movement of pregnant females could also alter levels of genetic structuring, and rates of gene flow between areas would amplify if females were pregnant prior to migration. However, the degree of impact on homogenization at the global level is highly dependent upon local N_e and existing levels of inbreeding in the subpopulation.

The eight bottlenecks detected in trapping areas likely resulted from founder events that were rapidly diluted by gene flow within a few generations because of the high fecundity of *R. norvegicus*. High fecundity and generation overlap in Baltimore rats predicts a large N_e because it allows multiple opportunities for more individuals to contribute to the offspring gene pool, thus reducing co-ancestry (Sugg & Chesser 1994). Plasticity in reproductive traits of urban rats, unlike rural populations (McGuire *et al.* 2006; Brouat *et al.* 2007), is associated with year-round reproduction (Davis & Hall 1951; Glass *et al.* 1989). Baltimore rats experience dramatic population fluctuations, with a 90% population turnover within 6 months in marked residential populations (Glass *et al.* 1989), and are characterized by bimodal peaks of density during the early spring and late fall, presumably resulting from high levels of recruitment (Davis & Hall 1951; Glass *et al.* 1989). Despite high intersite variability in density (2–217 rats/alley), separate estimates of overall rat density conducted 50 years apart in Baltimore were similar (~45 000 rats) (Davis & Fales 1950; Easterbrook *et al.* 2005). Therefore, even areas characterized by low densities may rapidly recover (e.g. Abdelkrim *et al.* 2007). Although the global rat population of Baltimore appears to be quite stable, temporal and seasonal fluctuations at the local scale may result in a sampling effect or rapid overall changes in allele frequencies (Balloux & Lugon-Moulin 2002; Gileva *et al.* 2006), and could explain the high allelic diversity observed within local populations (Table 2).

Unlike observational and mark–recapture studies in Baltimore (Davis *et al.* 1948; Emlen *et al.* 1948; Davis 1953; Calhoun 1962; Glass *et al.* 1989), our findings illustrate that large individual dispersal distances can occur, although they are atypical. Most rat movements

were limited within individual city blocks, yet a small percentage of rats moved distances as much as 400 m (approximately 2.7 times the average length of a Baltimore alley), and, rarely, much farther (across the city). Commensalism promotes high density, spatially clustered populations in urban areas of Baltimore (Childs *et al.* 1991b), yet a density-dependent effect on movement has not specifically been found for urban rats. Rather, the complex social system of Norway rats influences individual movement via intraspecific aggression between dominant and subordinate males (Davis 1951a, b), and may result in negative density-dependent effects (Calhoun 1962; Krebs *et al.* 2007). Commensalism by rats results in human-mediated gene flow (e.g. Baker 1994). Large movements can be induced by disturbance (Taylor 1978), such as neighbourhood restoration (Davis *et al.* 1948). Restoration promotes cleaner conditions, reducing and eliminating available food and harbourage and encouraging emigration. Movement in Baltimore rats will maintain or increase gene flow, counteract complete population subdivision (Hartl & Clark 1997, p. 195; Vilà *et al.* 2003), and promote colonization, and thus spread of pathogens to new areas (Gilabert *et al.* 2007).

Understanding aspects of ecology and gene flow, such as the movement of commensal rodents with human expansion in urban landscapes, is critical to understanding the dynamics of rodent-borne pathogens and is valuable for mitigating human disease outbreaks (Mills 1999). Limited movement of urban rats may generate spatial heterogeneity in pathogen distributions and provide manageable control units. However, our findings provide evidence that dividing urban rat populations into management units at the level of city blocks will be ineffective, and that control must occur at a larger scale (> 2 km). Moreover, moderate levels of genetic differentiation may encourage persistence of chronic infection in host reservoirs by some pathogens (Gilabert *et al.* 2007), and effective dispersal [which may underestimate the actual number of dispersers (e.g. Koenig *et al.* 1996)] suggests that pathogen transmission has the potential to occur across larger geographical scales than would be expected based on previous estimates of individual movement within urban environments.

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Fig. 1.

Results of Bayesian clustering of Norway rat genotypes collected in 11 different highly urbanized areas of Baltimore, Maryland. Symbols placed on trapping areas indicate four distinct genetic clusters found using Bayesian clustering methods. Dotted lines indicate the historic expansion of Baltimore from the harbour (1797) to its current boundary (since 1918; Chapelle 2000).

Fig. 2. Unrooted neighbour-joining tree using Cavalli-Sforza & Edwards' (1967) chord distance. Bootstrap values at each node were calculated using a consensus tree derived from 5000 distance matrices. Nodes supported by 50% bootstrapped values are included.

Fig. 3.

Plot of the relationship between the mean natural log of pairwise Euclidean distance and the mean pairwise kinship coefficient (Loiselle *et al.* 1995) of pairs of individuals in distance classes. The trendline crosses the x-axis at 1.7 km, indicating that related individuals are found within this distance in Baltimore rats. With the exception of the fourth distance class, the average pair-wise kinship at all distance classes were significantly different than the population average pairwise kinship coefficient of 0.0002, indicating a pattern of isolation by distance in the data set.

Fig. 4. Plot of the average log likelihood [$\text{LnPr}(X|K) \pm \text{SE}$] of $K = 1$ to $K = 20$ modelled in Structure software showing that the highest log likelihood (-7302.68) occurred at $K = 5$ clusters. The second axis depicts the calculated ΔK value (Evanno *et al.* 2005) for Structure runs, and indicates that the largest ΔK occurred at $K = 3$ clusters.

Table 1

Pairwise F_{ST} values and corresponding pairwise N_m values in parentheses (lower diagonal), and pairwise geographic distances (km; upper diagonal) between 11 trapping areas (sites correspond to locations on Fig. 1)

Sampling Site	Jefferson (Luzerne)	Northeast Market	Jefferson (Rose)	Ashland	West Baltimore	Hugo	Winston-Govans	Druid	McKewin	Brooklyn	Ostend
Jefferson (Luzerne)	—	0.619	0.066	0.781	4.811	2.429	6.352	4.997	4.588	7.761	4.784
Northeast Market	0.0361 (6.7)	—	0.555	0.380	4.238	1.972	6.057	4.394	4.241	7.714	4.262
Jefferson (Rose)	0.0359 (6.7)	0.0450 (5.3)	—	0.718	4.757	2.369	6.307	4.931	4.538	7.768	4.736
Ashland	0.0496 (4.8)	0.0240 (10.2)	0.0782 (3.0)	—	4.344	1.661	5.692	4.433	3.888	8.086	4.433
West Baltimore	0.0941 (2.4)	0.0558 (4.2)	0.1114 (2.0)	0.0532 (4.5)	—	4.084	7.257	2.790	5.624	7.004	0.807
Hugo	0.0808 (2.8)	0.0499 (4.8)	0.1097 (2.0)	0.0804 (2.9)	0.0677 (3.4)	—	4.194	2.980	2.337	9.239	4.468
Winston-Govans	0.0635 (3.7)	0.0289 (8.4)	0.0913 (2.5)	0.0555 (4.3)	0.0539 (4.4)	0.0257 (9.5)	—	4.677	1.863	13.369	7.915
Druid	0.0950 (2.4)	0.0601 (3.9)	0.1203 (1.8)	0.0825 (2.8)	0.0973 (2.3)	0.0460 (5.2)	0.0443 (5.4)	—	3.338	9.633	3.561
McKewin	0.1114 (2.0)	0.0769 (3.0)	0.1435 (1.5)	0.1006 (2.2)	0.0605 (3.9)	0.0286 (8.5)	0.0447 (5.3)	0.0830 (2.8)	—	11.514	6.218
Brooklyn	0.1096 (2.0)	0.0724 (3.2)	0.1317 (1.7)	0.0876 (2.6)	0.0834 (2.8)	0.0716 (3.2)	0.0457 (5.2)	0.1008 (2.2)	0.0587 (4.0)	—	6.199
Ostend	0.0795 (2.9)	0.0555 (4.4)	0.1051 (2.1)	0.0717 (3.2)	0.0551 (4.3)	0.0401 (6.0)	0.0393 (6.1)	0.0708 (3.3)	0.0599 (3.9)	0.1042 (2.2)	—

Table 2

The number of Norway rats (N) sampled at each of 11 trapping sites in Baltimore, number of alleles (N_a), number of private alleles (N_p), and average observed (H_O) and expected (H_E) heterozygosities for each sample site. Additional characteristics listed include LE (linkage equilibrium), BN (bottleneck detected), MS (mode-shift detected), and F_{IS} calculated for each site. Asterisks (*) indicate significant P values 0.05

Sampling site	N	N_a	N_p	H_O	H_E	P value	LE	BN P value	MS	F_{IS}
Jefferson (Luzerne)	25	53	0	0.568	0.741	< 0.0001*	Y	0.0125*	N	0.2285
Jefferson (Rose)	25	44	0	0.642	0.666	0.3439	Y	0.0977	N	0.0175
Northeast Market	27	66	1	0.722	0.759	0.0481*	N (D2, D6 & D1-9, D6)	0.0977	N	0.0351
Ashland	25	53	2	0.692	0.759	0.0191*	Y	0.0273*	N	0.0779
Hugo	24	60	5	0.770	0.742	0.0159*	Y	0.0098*	N	0.0554
Winston-Govans	20	59	0	0.742	0.780	0.0014*	Y	0.0273*	Y	-0.0547
Druid	24	55	0	0.614	0.658	0.5308	N (D1-9 & D6)	0.0020*	Y	0.0512
McKewin	29	53	1	0.731	0.699	0.0759	Y	0.0098*	Y	-0.0515
Brooklyn	25	48	1	0.712	0.714	0.0001*	N (D2 & D6)	0.0039*	Y	-0.0141
West Baltimore	23	55	3	0.844	0.774	0.0534	Y	0.0371*	N	-0.1016
Ostend	30	62	1	0.800	0.767	0.1960	Y	0.0039*	N	-0.0464
Mean	25.1	55.3	1.3	0.713	0.734	—	—	—	—	0.01897
Overall	277	97	14	0.713	0.734	0.7590	—	—	—	—

Table 3

Results of assignment tests and identification of first-generation migrants (^M) in GeneClass2. Sample sizes trapped at each site are listed (*N_i*), and individuals assigned to sampling areas other than that of capture with a score ≥ 80% are listed. Individuals denoted by an asterisk (*) possessed *P* < 0.01 of originating in any of the collection sites

Sampling Site	<i>N_i</i>	Jefferson (Luzerne)	Northeast Market	Jefferson (Rose)	Ashland	West Baltimore	Hugo	Winston-Govans	Druid	McKewin	Brooklyn	Ostend
Jefferson (Luzerne)	25	19		3	1 ^M				1, 1 ^M			
Northeast Market	27		23		2, 1 ^M							1 ^M
Jefferson (Rose)	25	1 ^M		23							1 ^M	
Ashland	25				23	1					1 ^M	
West Baltimore	23		1 ^M			20, 1*				1		
Hugo	24						20	1 ^M	2 ^M	1		
Winston-Govans	20							19	1			
Druid	24								20	1 ^M		
McKewin	29				2, 1 ^M					28	1	
Brooklyn	25				1 ^M						24	
Ostend	30					2 ^M	1 ^M					27