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Source: Journal of Mammalogy, 93(2) : 447-455

Published By: American Society of Mammalogists

URL: https://doi.org/10.1644/10-MAMM-A-410.1

Journal of Mammalogy, 93(2):447–455, 2012

Microsatellite analysis of raccoon (*Procyon lotor*) population structure across an extensive metropolitan landscape

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Understanding population structure can lend insight into the spread of animal-borne disease, and the effects of anthropogenic land use on habitat. Raccoons are highly adaptive to human land development and can persist in a wide range of habitat types, making them ideal subjects for investigating the level of population structure in a highly fragmented area. A total of 323 raccoons were livetrapped from 7 locations encompassing 3 distinct habitat types (agriculture, urban forest preserves, and residential) across the Chicago metropolitan region (maximum distance between 2 sites was 128 km). Genetic analyses of 14 microsatellite loci indicate that although raccoon populations across the region share up to 50% of the allelic diversity, they segregated into at least 2 distinct subpopulations, dividing the Chicago metropolitan region into northern and southern groups with further structure occurring within these larger groups. Incorporating sample sites between the identified north–south groups may provide greater resolution as to where this split occurs. Although there is evidence of population structure between all sample sites, migrant analysis suggests there is enough gene flow to preserve genetic diversity throughout the population.

Key words: fragmentation, microsatellite, molecular techniques, population genetics, *Procyon lotor*, rabies, raccoon, urban habitat

DOI: 10.1644/10-MAMM-A-410.1

Habitat fragmentation via anthropogenic activity such as road building and land development continues to impact mesopredator populations to varying degrees (Crooks 2002; Ditchkoff et al. 2006; Prange et al. 2003; Riley et al. 2006). Understanding how population structure, dispersal, and social behaviors change in response to habitat alterations can serve to illustrate the extent of human impact on natural systems. Although traditional field studies are critical to understanding a species, it can be difficult to obtain comprehensive data for nocturnal, arboreal, and forest-dwelling animals (Cullingham et al. 2008). However, as molecular techniques such as microsatellite genotyping have been developed and extended over the past 25 years, collecting data for cryptic species has become possible. Associated with those technologies, sophisticated statistical methods have been developed to determine gene flow within and among populations (Christian and George 2008; Molenberghs 2005). This information can be used to infer population structure and individual behavior with greater fidelity, even with animals that are difficult to observe.

Raccoons (*Procyon lotor*) in the Chicago area are ideal candidates for a molecular population study of their ecology, which will aid in the understanding of an important reservoir of zoonotic pathogens in the urban environment. Raccoons are abundant throughout the Chicago area, which is highly fragmented by both natural and artificial barriers. The Chicago River bisects the region, and there are at least 9 interstate highways, 6 United States highways, and 15 state highways that cut through the area. Habitat fragmentation also occurs through land alteration for agriculture, industry, and housing. Because raccoons are highly adaptable (Crooks 2002; Cullingham et al. 2008), they can be observed in nearly all habitat types that occur within the highly fragmented landscape of the Chicago area (Prange et al. 2004; Randa and Yunger 2006). Although their ubiquitous distribution from rural to urbanized sites suggests that they successfully disperse throughout their range, studies of other mid- to large-sized carnivores show that habitat fragmentation can hinder movement. For example, vehicular traffic associated with a

single highway has been shown to reduce gene flow in coyotes (*Canis latrans*), bobcats (*Lynx rufus*), and grizzly bears (*Ursus arctos horribilis*—Millions and Swanson 2007; Proctor et al. 2002; Riley et al. 2006; Sacks et al. 2004).

Raccoon population structure and dispersal patterns can influence the way a zoonotic disease moves through a landscape. In an area with human populations, those patterns can have significant impacts on human health. Raccoons are host to dozens of pathogenic agents that are communicable to humans (leptospirosis, roundworm, and rabies) and other animals (canine distemper, pneumonia, and rabies) alike (Page et al. 2008; Rosatte et al. 2010). These highly adaptable animals often exploit human structures and trash for shelter and food (Bozek et al. 2007; Prange et al. 2004), increasing their densities in urban forest preserves and residential neighborhoods (Graser 2008; Prange et al. 2003). Such increased densities of wild animals are often correlated with epizootics and an increased risk to human health (Page et al. 2005; Riley et al. 1998).

Raccoons from the Midwest have been the subject of many field studies over the past 15 years (e.g., Hauver et al. 2010; Prange et al. 2004; Stevens et al. 1995). Nevertheless, few have examined the effects of urbanization on raccoons over large distances, and none have used genetic tests on this scale. Because raccoons are habitat generalists, they make an ideal model species on which to study epizootics in a fragmented environment. Understanding gene flow across a large region provides valuable insight as to how disease will travel through a population (Recuenco et al. 2008; Rees et al. 2009; Rosatte et al. 2006). This study specifically addresses the following questions: Are raccoons in the Chicago area panmictic, or is there hidden population structure? Does habitat size or habitat type affect genetic parameters? This study represents a key component to understanding the ecological role raccoons play in a highly developed region.

Materials and Methods

Study area.—Rush Creek (RC; 235 ha) and Coral Woods (CW; 166 ha) lie to the northwest, and are the 2 farthest locations from Chicago (Fig. 1; Table 1). These McHenry County conservation district spaces are currently being restored from agricultural use. Both are characterized by oak–hickory forest, RC contains sedge meadow (Spencer 2007), and CW contains maple groves (Davis 2003). They are primarily undeveloped land, in a rural setting (Graser 2008).

Busse Woods (BW) and Steger Woods (SW) are preserves, characterized as patches of protected, remnant habitat surrounded by residential development (Fig. 1). BW lies northwest of Chicago and SW is located to the south. Both sites exhibit a variety of habitats including prairie, marsh, and woodlands (Bender 1999; Mechanic 2006). BW is a 178-ha subset of the larger, 1,497-ha Ned Brown Forest Preserve. SW is inclusive of both the 259-ha Sauk Trail woods and the 364-ha Thorn Creek.

Oak Lawn (OL), Blue Island (BI), and Evergreen Park (EP) are residential neighborhoods in close proximity to one another due south of Chicago (Fig. 1; Table 1). All 3 sites are 50–80% covered by impervious surfaces, qualifying them as urban areas (Graser 2008). They are 1,380, 640, and 510 ha, respectively; and all have the greatest human population density of the sample sites (Table 1).

Field methods and sample collection.—Field methods and sample collection followed the description given in Hauver et al. (2010). Briefly, raccoons were trapped throughout Cook and McCain counties (Fig. 1) during the summer months, in Tomahawk Live Traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin) baited with commercial canned cat food, and sedated with Telozol (Fort Dodge Animal Health, Fort Dodge, Iowa) according to the Animal Care and Use Protocols of The Ohio State University (ILACUC#2003R0062) and the American Society of Mammalogists (Sikes et al. 2011). Morphological data, mark–recapture data from ear-tagging, and blood samples were taken from all animals. The blood samples were collected in clot tubes, frozen, and sent to the Brookfield Zoo Genetics Lab where they were stored at −74°C. Once they were fully recovered, trapped animals were released at the trap site.

Microsatellite analysis.—We digested the blood clots overnight with proteinase K and extracted DNA using phenol, phenol–chloroform methods (Sambrook and Russell 2001). DNA extraction and polymerase chain reaction amplification of the 14 microsatellite loci are described in Cullingham et al. (2006—Plo3-86, Plo-M17, Plo-M3, Plo2-14, Plo-M20, Plo-M2, Plo2-123, Plo-M15, Plo2-117, and Plo3-117×), Kays et al. (2000—PFL9 and PFL11), and Van Den Bussche (in litt.—P140 and P161; Table 2). We analyzed polymerase chain reaction products using a Beckman/Coulter CEQ 8000XL automated capillary electrophoresis genotyping system (Beckman/Coulter, Inc., Fullerton, California) and determined fragment sizes using System Software version 8.0 (Beckman/Coulter, Inc.). In order to validate genotype data, we used 3 approaches. First, graphic binning in Microsoft Excel (Microsoft Corp., Redmond, Washington) of allele sizes, using procedures and a database common to our laboratory, ensured consistency of allele calls. Second, MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004), set for 10,000 iterations and a 95% confidence interval, checked for possible scoring errors and null alleles. Third, we amplified and reran 30% of the total sample set to clarify ambiguous signals, and to ensure precision through duplication.

Genetic differentiation within populations.—We estimated expected (H_E) and observed (H_O) heterozygosity and average number of alleles per locus (A) using MSTOOLS (Park 2001). We tested all loci for deviations from Hardy–Weinberg equilibrium and for linkage disequilibrium within each sample location using Fisher's exact tests in GENEPOP version 4.0.10 (Raymond and Rousset 1995; available at http://GENEPOP.curtin.edu.au/, accessed 5 August–10 November 2009). Markov chain parameters included a dememorization of 10,000 for 1,000 batches at 10,000 iterations per batch. This provided a low standard error ($SE < 0.01$), as recommended

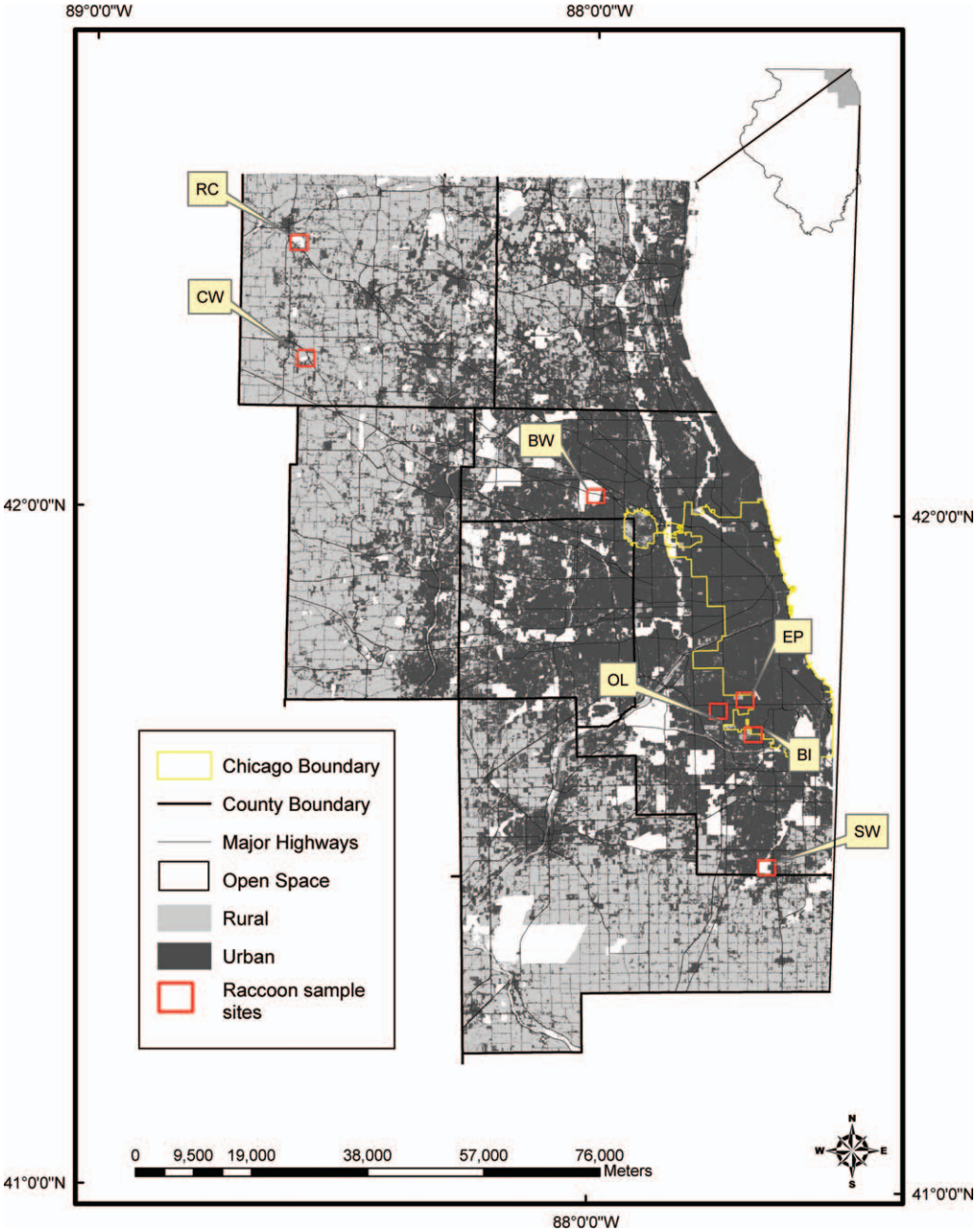

Fig. 1.—Locations of sample sites of raccoons (*Procyon lotor*) in the greater Chicago area: RC = Rush Creek, CW = Coral Woods, BW = Busse Woods, OL = Oak Lawn, BI = Blue Island, EP = Evergreen Park, SW = Steger Woods.

by Raymond and Rousset (1995). Significance levels were adjusted using a strict Bonferroni correction applied for multiple comparisons (k = 98, $\alpha = 0.00051$—Rice 1989). We determined private allelic richness (P_R) using HP-RARE 1.1 via a rarefication method (Kalinowski 2005). We estimated allelic richness (A_R) and F_{IS} using FSTAT version 2.9.3.2 (Goudet 2001).

Genetic differentiation and structure among populations.—We investigated genetic differentiation and substructure among sample sites throughout the Chicago area. We calculated F_{ST} values according to Weir and Cockerham (1984) between each pair of sample sites based on 10,000 permutations for $\alpha = 0.05$ using Arlequin version 3.1 (Excoffier et al. 2005). We also used Arlequin to perform a hierarchical analysis of molecular variance (AMOVA; $\alpha = 0.05$—Excoffier et al. 1992) to determine significance of genetic variation between sample sites and when grouped by habitat type and geographic location. Finally, we tested the correlation of physical and genetic distance with a partial Mantel test using MANTEL! (Liedloff 1999) set for 10,000 iterations.

We used 2 Bayesian clustering analyses to determine hidden population structure of raccoons throughout the Chicago area; 1 nonspatial (STRUCTURE version 2.2—Pritchard et al. 2000), and 1 spatially sensitive (TESS version 2.3—Chen et al. 2007). Bayesian clustering analysis assigns

Table 1.—Characteristics of locations where raccoons (*Procyon lotor*) were sampled in the greater Chicago area ($n = 323$): Site, site code, sample size (n), habitat type—rural (R), forest preserve (F), urban–residential (U), the nearest town (Town), distances to town were measured from the center of the sample site to the center of the nearest town in kilometers, town population size, human population density (per km^2), and housing density (per km^2). Demographic data were taken from 2000 United States Census data from United States Census Bureau; http://www.census.gov.

Site	Code	n	Habitat type	Town	Distance to town (km)	Population size	Human density	Housing density
Rush Creek	RC	54	R	Harvard	2.4	8,000	578	197
Coral Woods	CW	32	R	Marengo	3.2	7,000	616	240
Busse Woods	BW	99	F	Schaumburg	1.5	50,000	1,532	672
Oak Lawn	OL	26	U	Oak Lawn	0.0	55,245	2,481	1,026
Blue Island	BI	10	U	Blue Island	0.0	23,463	2,248	875
Evergreen Park	EP	34	U	Evergreen Park	0.0	20,821	2,536	928
Steger Woods	SW	68	F	Chicago Heights	4.0	12,831	1,321	431

individuals to groups, in order to minimize Hardy-Weinberg and linkage disequilibrium. For the nonspatial STRUCTURE analysis, we evaluated 10 repetitions for each value of K, for $K = 1$–10 subpopulations, with Markov chain Monte Carlo resampling using 200,000 repetitions after a burn-in of 100,000. Because significant gene flow was expected, we assigned an admixture model to the program. We determined the most likely number of clusters by calculating the change in K (ΔK) as described in Evanno et al. (2005). We assigned individuals to a cluster if they had an association of at least 0.80, as suggested by Crawford et al. (2009) and Cullingham et al. (2008). The spatially sensitive analysis, TESS, uses the same principles as STRUCTURE but assigns unique x and y geographic coordinates to each individual. This program can vary spatial interaction parameters, which will vary the degree to which geographic information influences individual cluster assignment. As this value increases, so does the influence of sample location on cluster assignment (Chen et al. 2007). For example, a value of 0 uses only genetic data mimicking the assumptions made in STRUCTURE, whereas a value of 0.99 discounts the genetic data and bases the clustering analysis entirely on geographic proximity. Because TESS is spatially sensitive, it places individuals into the most likely groupings regardless of the number K programmed into the analyses. We set TESS to $K = 9$ with a burn-in of 100,000 and 300,000 sweeps for each of 10 runs at 3 different values for the spatial interaction parameter: 0, 0.50, and 0.99 (Crawford et al. 2009).

Gene flow among populations.—We estimated the number of migrants between all pairs of sample sites using the number of genetic migrants (*Nm*) method (Barton and Slatkin 1986) in GENEPOP and Bayesian analysis in BAYESASS (Wilson and Rannala 2003), which utilizes Markov chain Monte Carlo resampling techniques to determine migration rates. One advantage of this latter method is that it relaxes the necessity for all loci to be in Hardy–Weinberg equilibrium. Here, it was run with 3,000,000 iterations at a sampling frequency of 2,000 and a burn-in of 999,999 as recommended by Wilson and Rannala (2003).

Results

Genetic differentiation within populations.—A total of 323 raccoons from 7 locations were genotyped at 14 loci. The number of alleles for each locus (A_N) ranged from 9 to 37 with an average over all loci and all sample sites of 17.71. A_R was uniformly lower, ranging from 4.06 to 11.58 with an average of 7.67. Over all loci, the average number of alleles per sample site (A) varied from 7.29 (BI) to 13.07 (BW) with an average of 10.60 (Table 3). A_R ranged from 6.19 (EP) to 7.37 (BW), and P_R ranged from 0.25 (EP) to 0.67 (BI; Table 3). Although BI had the smallest sample size and lowest number of alleles, it showed the highest P_R. Nearby residential sites, EP and OL, with intermediate sample sizes, had a low number of alleles, the lowest number of private alleles (N_P), and the lowest A_R. Levels of heterozygosity were similar in all sample sites ranging from $H_O = 72\%$ in RC to 78% in BW. We found significant deviation from Hardy–Weinberg equilibrium in 5 of 91 locus–sample site comparisons after Bonferroni correction ($P \leq 0.00055$). However, there were no sample sites or loci that were consistently significant; and Cullingham et al. (2009) reported all loci in Hardy–Weinberg equilibrium, Dharmarajan et al. (2009) found 2 comparisons significant, and Hauver et al. (2010) found 1. We found no consistent evidence of linkage disequilibrium among any pair of loci across all sample sites ($P > 0.00051$ after Bonferroni correction), which is consistent with findings from other studies (Cullingham et al. 2006; Roy Nielson and Nielson 2007). Null alleles were detected in 7 of 91 sample site–locus comparisons (excluding the x-linked Plo3-117×; $P \leq 0.001$); however, there was no consistency in the positive results, and Hauver et al. (2010) found no evidence of null alleles for the same markers. Because there was no consistent evidence for linkage disequilibrium or null alleles across all populations, all loci were included in further analyses. F_{IS} values indicated significant heterozygote deficiencies for 4 sites: RC, CW, BW, and SW.

Genetic differentiation and structure among populations.—There were significant genetic differences (F_{ST}) between all pairs of sample sites ($F_{ST} = 0.016$–0.078; $P < 0.003$). The AMOVA found significant differentiation among sample sites within groups regardless of how they were partitioned (Vb = 0.16; $P \leq 0.001$). There was a significant difference among groups when sample sites were subdivided into 2 groups based on geographic location: northwest (NW: RC, CW, and BW) and southeast (SE: OL, BI, EP, and SW; Va = 0.04; $P \leq 0.05$). However, when sample sites were grouped by habitat

Table 2.—Loci used for population substructure analysis of raccoons (*Procyon lotor*) in the greater Chicago area, size ranges (bp), number of alleles (A_N), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosities for raccoon populations in the greater Chicago area. Significant differences between observed and expected heterozygosity are indicated with Hz deficiency *P*-values (*P*). Asterisks (*) indicate significant deviation from Hardy–Weinberg equilibrium ($P \leq 0.00051$).

Locus	Size range (bp)	A_N	A_R	H_O	H_E	*P*
Plo3-86	320–437	37	11.19	0.75	0.88	0.00*
Plo-M17	169–234	11	5.35	0.81	0.75	0.93
Plo-M3	263–289	9	6.05	0.76	0.79	0.71
Plo2-14	227–327	29	8.89	0.83	0.87	0.00
Plo-M20	175–231	15	7.58	0.83	0.82	0.18
Plo-M2	282–336	17	8.42	0.81	0.84	0.21
Plo2-123	558–620	16	7.36	0.83	0.85	0.27
Plo-M15	159–198	17	8.18	0.76	0.84	0.00*
Plo2-117	274–353	31	11.58	0.91	0.90	0.00
Plo3-117×	260–387	13	6.30	0.37	0.74	0.00*
PFL11	142–177	19	8.78	0.84	0.84	0.01
P161	123–151	9	4.06	0.37	0.41	0.13
PFL9	201–231	13	7.26	0.77	0.79	0.06
P140	166–190	12	6.42	0.73	0.73	0.14
Overall		17.71	7.67			

type (residential, forest preserve, or agricultural), there were no significant differences among groups. Finally, we detected no correlation between geographic and genetic distance using the Mantel test ($G = 2.63$, $P = 0.4557$).

Bayesian population structure.—The ΔK values were highest when only 2 genetically distinct clusters were identified. These clusters corresponded to NW and SE (Fig. 2a). Of 323 individuals, 146 (45%) were definitively assigned to NW, and 106 (33%) to SE. Seven individuals identified as belonging to the NW cluster were sampled from SE, and 6 individuals were identified as belonging to the SE cluster were sampled from NW (Fig. 2c). The remaining 71 individuals (22%) could not be assigned to either cluster. Of these, 44 (62%) were sampled from NW and 27 (38%) were sampled from SE.

Results from TESS for all 3 spatial interaction parameters (0, 0.50, and 0.99) paralleled those from STRUCTURE (Figs. 2a and 2b). However, TESS identified additional structure within these subpopulations that was undetected by STRUCTURE. The RC and CW sites from NW were clustered together, whereas BW was isolated. In the SE cluster, the raccoons from SW and OL had a genetic signature consistent with SE, but a portion of the individuals from EP and BI contained a 2nd, distinct proportion of alleles (shown in dark green in Fig. 2). This additional differentiation within larger clusters was the same regardless of the spatial interaction parameter.

Gene flow between populations.—Two methods were used to estimate gene flow and migration rate between sample sites. The Bayesian analysis suggested raccoons from 4 of the locations (RC, BW, EP, and SW) stayed in their natal sites more than 95% of the time. Of those locations that showed migration signal, raccoons from CW migrated to RC, and those from OL and BI dispersed to EP (Table 4a). The *Nm* method detected 7 migrants per generation when the entire data set was analyzed and corrected for sample size variation, with at least 1 migrant between every pair of sites. More migrants were detected between the agricultural sites to the north (RC and CW) and those involving SW (Table 4b).

Table 3.—Relative polymorphism of raccoon (*Procyon lotor*) sample locations from the greater Chicago area (Site). Included are: sample size (*n*), average number of alleles (A), allelic richness (A_R), number of private alleles (N_P), private allelic richness (P_R), observed (H_O) and expected (H_E) heterozygosities, and F_{IS} values. Significant differences between observed and expected heterozygosities are indicated with Hz deficiency *P*-value (*P*). Asterisks (*) indicate significant F_{IS} values ($P \leq 0.00051$).

Site	*n*	A	A_R	N_P	P_R	H_O	H_E	F_{IS}	*P*
RC	54	11.93	7.10	10.00	0.59	0.72	0.79	0.08*	0.00*
CW	32	11.00	7.18	6.00	0.54	0.73	0.81	0.10*	0.00*
BW	99	13.07	7.37	14.00	0.57	0.78	0.82	0.05*	0.00*
OL	26	9.43	6.72	3.00	0.30	0.74	0.78	0.05	0.01
BI	10	7.29	7.01	4.00	0.67	0.75	0.80	0.07	0.03
EP	34	8.86	6.19	3.00	0.25	0.74	0.73	−0.02	0.82
SW	68	12.64	7.25	15.00	0.58	0.73	0.81	0.10*	0.00*

Discussion

The ubiquity of raccoons in metropolitan landscapes may give the appearance of a lack of population structure; however, our results from the Chicago area indicate that both subpopulation structure and semi-isolated populations likely exist in highly fragmented urban environments. The substructure observed in this study could be due to several factors working together that include habitat type (Moodley and Harley 2005; Tatarenkov and Johannesson 1994), distance (Dharmarajan et al. 2009), and physical barriers (Cullingham et al. 2009; Riley et al. 2006). Clustering according to habitat type was not significant in this study, suggesting there is no evidence for genetic adaptation to habitat type in this data. There also was no significant correlation of genetic distance with geographic distance. Although habitat and distance may have a small effect on structure, physical barriers and behavior may play a more significant role in the formation of population structure in this species.

Recent, similar genetic studies of raccoon populations have been conducted in fragmented agricultural landscapes (Dharmarajan et al. 2009), in agricultural border regions of Canada (Cullingham et al. 2008), and in undisturbed forested habitat (Root et al. 2009). Although sample sizes at individual trapping sites in these studies were similar, the larger geographical area sampled in our urban study (127 km^2 versus 36 km^2) did not appear to influence the genetic pattern. Higher heterozygosity levels were reported in populations in undisturbed habitats ($H_O = 0.83$—Root el al. 2009) than in populations from the agricultural sites ($H_O = 0.75$—Dharmarajan et al. 2009) and the urban raccoons in this study ($H_O = 0.74$). A reduced number of alleles per locus was observed in agricultural raccoons ($n = 645$, $A_N = 13.1$), but not in urban ($n = 323$, $A_N = 17.7$) or undisturbed ($n = 185$, $A_N = 16.2$) populations. Although raccoons in the undevel-

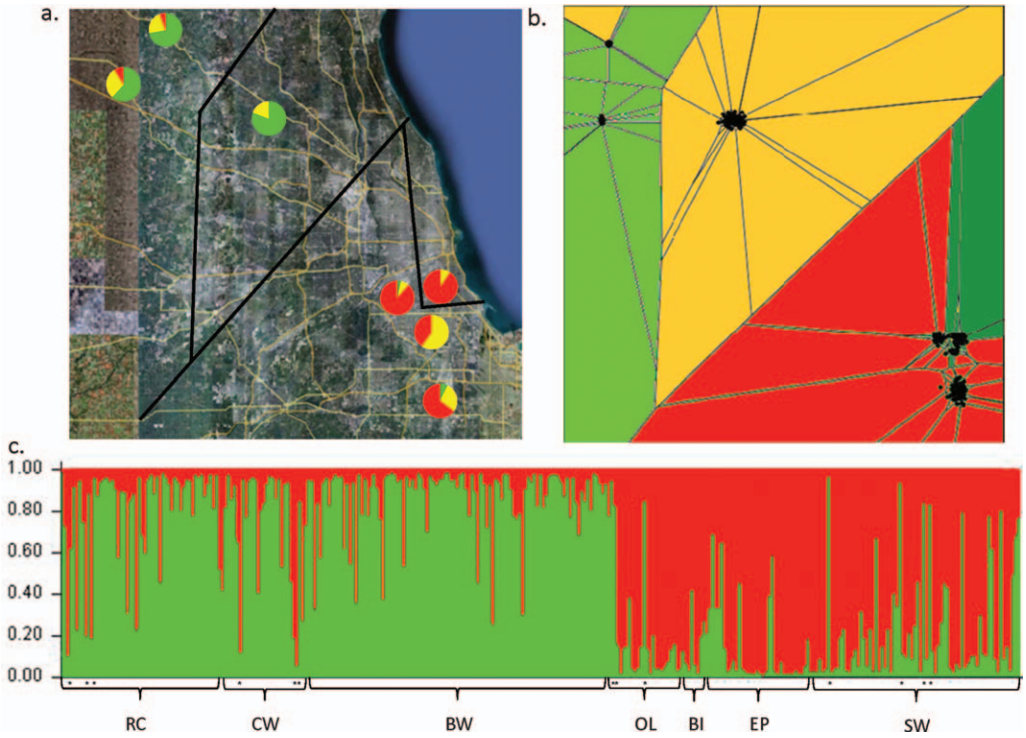

Fig. 2.—Bayesian clustering analysis of raccoons (*Procyon lotor*) from the greater Chicago area as detected by STRUCTURE and TESS. a) A map of the Chicago metropolitan region including western agricultural land with major highways. Each pie chart represents the proportion of raccoons at each sample site that showed characteristic allele suites from 2 geographic groupings at an 80% probability level. Red represents northwest (NW), green southeast (SE), and yellow are individuals that could not be definitively assigned. Dark lines correspond to the Tesselation structure seen in chart b for reference. b) Voronoi diagram generated from TESS of all 7 sample sites with the spatial interaction parameter = 0. c) Q-values from STRUCTURE output. The individuals from each sample location have been bracketed off. Individuals with a genotype signature suggesting migration between subpopulations are indicated with an asterisk (*).

oped area in Pennsylvania appeared panmictic (Root et al. 2009), the study by Dharmarajan et al. (2009) and our study found structure among sample locations in highly altered anthropogenic habitats. Measures of genetic distance (F_{ST}) were significant between all pairs of sample sites in the Chicago study and 41% of the agricultural pairs (Dharmarajan et al. 2009). In contrast, there were no significant F_{ST} comparisons in the undisturbed forest populations (Root et al. 2009). Finally, significant F_{IS} values in the forest preserve and agricultural sites indicate isolation and possible inbreeding in these locations but the 3 residential sites do not show significant F_{IS} values. These results suggest that populations in a modified agricultural or urban landscape have a reduced level of gene flow.

The clusters detected by Bayesian analysis fit well with both the sample set and the landscape of Chicago. The 2 sample sites on the border of NW and SE (BW and OL) are separated by the Chicago metropolitan area, which includes barriers such as interstate highways, freight and commuter rail lines, rivers, and patchy habitats including other forest preserve and residential habitats. Considering that a single busy road could reduce successful dispersal, and abundant, predictable food resources (e.g., dumpsters and garbage cans) could lower the need to disperse (Prange et al. 2004; Riley et al. 2006), it is not surprising that these 2 subpopulations were identified and that significant F_{ST} values are evident between the sample sites. Although exact locations of dispersal boundaries are not defined by this study, examination of the data suggests that they exist between the sample sites included in this study.

We were surprised to find genetic differentiation among the residential sites (OL, EP, and BI) that are in close proximity (up to 7.21 km) to each other. The raccoons in EP had the highest genetic distances (F_{ST} = 0.057–0.078) with all locations including BI and OL, but showed a higher rate of immigration from the other residential sites. This could be the result of migrant or translocated individuals having been trapped for this study, but failing to breed at this location. There also were a number of private alleles in each sample site; and BI, with the smallest sample size, had the highest P_R. Given the apparent homogeneous nature of OL, BI, and EP, high-density residential neighborhoods and the limited space separating them, the elevated genetic distances between sample locations are likely due to decreased home ranges and dispersal in the presence of rich food sources such as dumpsters (Bozek et al. 2007; Prange et al. 2004). Furthermore, this habitat is a matrix of heavily travelled streets, which hinders successful dispersal and movement. Population size also might be inadvertently controlled by removal of nuisance individuals rather than natural migration. Finally, a social component such as learned behavior for foraging and nesting sites by juveniles may encourage raccoons to stay in

Table 4.—Migrant analysis of raccoons (*Procyon lotor*) from the greater Chicago area. a) The means of the posterior distribution of migration rates into each population. The rows represent the originating populations, the columns the destination populations. The diagonal describes the proportion of individuals derived from the source population each generation. Standard deviations for all values were <0.05 except one in italics was 0.06. b) Geographic distances between sample sites in kilometers are above the diagonal, the number of migrants per generation between each sample site by the *Nm* method after size correction is below the diagonal.

	Migrant destination						
	RC	CW	BW	OL	BI	EP	SW
a. Migrant source							
RC	0.968	0.002	0.019	0.002	0.002	0.003	0.005
CW	0.276	0.676	0.029	0.004	0.004	0.005	0.005
BW	0.003	0.001	0.991	0.001	0.001	0.001	0.001
OL	0.006	0.005	0.008	0.679	0.028	0.273	0.024
BI	0.024	0.065	0.021	0.014	0.696	*0.161*	0.069
EP	0.003	0.002	0.002	0.002	0.002	0.999	0.005
SW	0.021	0.002	0.004	0.002	0.002	0.01	0.959
	Geographic distance						
	RC	CW	BW	OL	BI	EP	SW
b. Number of migrants							
RC		19.44	62.46	103.12	109.92	103.8	127.79
CW	4.25		51.67	89.18	96.22	91.27	112.08
BW	4.18	3.33		41.9	48.55	43.22	67.77
OL	3.1	2.87	2.28		7.21	4.08	26.45
BI	1.61	2.34	1.54	2.08		5.82	21.28
EP	2.44	2.02	1.97	1.91	1.38		26.95
SW	3.05	2.88	3.32	3.84	2.59	3.71	

their natal area (Dharmarajan et al. 2009; Stamps and Swaisgood 2007).

We were intrigued by evidence of unidirectional gene flow in 2 main locations: CW to RC, and OL/BI to EP. Because RC and EP have relatively larger sample sizes, it is tempting to attribute this signal to statistical artifact. Perhaps these larger sample sizes reflect higher quality habitat with greater food and shelter resources. However, the lack of directionality associated with SW and especially BW, our 2 largest data sets, suggests a real effect, which is supported by field observation. Furthermore, the lack of directionality does not support the theory that the largest sites act as either sources or sinks. We can only speculate on the underlying reasons here: We may be detecting migration toward more desirable habitats. We also may be detecting direct human influence in both the rural and residential areas attributable to differing land management laws and practices, or even a few zealous individuals who trap and relocate pest wildlife. Whatever the underlying cause, it is clear that there is directionality to the gene flow in Chicago-area raccoons.

Root causes of population substructure can inform wildlife management efforts. Cullingham et al. (2009) found that large rivers formed dispersal barriers for raccoons, yet their measures of genetic differentiation were nonsignificant. Other factors, such as resource availability, were of greater influence on raccoon dispersal than the rivers themselves. Dharmarajan et al. (2009) found no evidence of structure due to barriers or distance. Instead, they concluded that the significant F_{ST} values were autocorrelated with patch size, agricultural food resources, and percent forest cover that affected social and genetic structure of raccoon populations in their study. In metropolitan areas, there may be little difference to raccoons between preserved habitat and highly developed residential neighborhoods. Both habitats can provide den sites and food resources that may influence raccoon social structure and density (Prange et al. 2003, 2004). The lack of significant structure associated with habitat type in our study may support this conclusion.

This and other studies investigating isolation by barrier in midsized carnivores found similar reductions in migrants. We found at least 1, but no more than 5, effective migrants between any pair of locations (Table 4b). We also found evidence of unidirectional migration among some of the sample sites. Cegelski et al. (2003) found a similar order of magnitude in the number of wolverine (*Gulo gulo*) migrants; and Riley et al. (2006) found migrants between sites on either side of a freeway significantly reduced to 3.7 and 6.5 for bobcats (*L. rufus*) and coyotes (*C. latrans*), respectively. Although major roads and highways might be important barriers for dispersing raccoons, they might not be the most important factors that determine the level and direction of dispersal estimated in this study. Reasons for this could include high mortality on roads, local population densities, or habitat size or quality, and additional studies will be needed to address these questions.

Raccoons are a reservoir for a multitude of pathogens communicable to humans and many other wildlife species; especially rabies, distemper, and roundworms (Rosatte et al. 2010). Physical barriers, such as roads and rivers, have been shown to limit the spread of directly communicable pathogens (Arjo et al 2008; Prange et al. 2004; Recuenco et al. 2008; Russell et al. 2003). The semi-isolated nature of the sample sites in this study suggests that the rate of pathogen transmission throughout a metropolitan area via raccoons will be reduced but not eliminated. It further suggests that raccoons will not spread a pathogen across the area in a wave, but punctuated across patches of the region. It is likely that Chicago-area highways and rivers will limit or divert, or both, the movement of pathogens and separate the northern and southern sections of the region.

Translocated animals can disrupt and expand the pattern of disease propagation through a population (Russell et al. 2003). Several individuals from SE were identified as having an NW-type genotype, or vice versa (Figs. 2a and 2c). These may be translocated individuals, as it was common for nuisance raccoons to be moved from problem areas (Mosillo et al. 1999). In fact, the spread of raccoon rabies on the American East Coast is partially attributed to humans translocating raccoons (Guerra et al. 2003). In 1994, nearly 6,000 raccoons were translocated in Illinois (Bluett 1995). In 2005, Illinois passed regulations curtailing the translocation of nuisance animals (17 IAC 01.525, 2005—Bluett et al. 2003); however, relocation by private parties may still occur. In addition, raccoons are opportunistic and have been known to ''hitch-hike'' on vehicles such as garbage trucks (Wilson et al. 1997), including raccoons from one of our study sites (S. D. Gehrt,

pers. obs.). However, our genetic results suggest that intentional or accidental translocation does not effectively eliminate population structure, either because the majority of translocated raccoons move away from where they were placed or fail to breed there (Mosillo et al. 1999).

Examination of these data suggests there are 3 levels of genetic separation occurring in raccoons in the Chicago area: there is isolation by barrier evident between the 3 northwestern and 4 southeastern sample sites; the open-space sites to the far northwest (RC and CW) are separated from the forested urban site in the near northwest (BW) identified in the TESS analysis, and there is substructure between the residential sites BI and EP from OL and the forested urban site SW; and every site is semi-isolated based on the F_{ST} results. The separation among the residential sites was surprising, but supported by the high private allelic richness at all sites, especially at BI—the smallest sample size. It is evident that the rate of gene flow throughout the region is sufficient to prevent possible inbreeding and loss of variation due to drift, but cannot maintain a panmictic population. The observed level of gene flow would allow a novel disease, such as rabies, to spread among the Chicago-area populations and would require considerable management efforts.

Expanding this study to include additional sites between the locations in this study would allow us to discern whether a region of admixture exists between the 2 identified subpopulations, and to explore if and where boundaries exist. Characterization of additional field studies of residential areas would allow us to measure more detailed movements of individuals within and among residential areas, estimate the effects of road-related mortality, and determine removal rates of nuisance individuals.

Acknowledgments

This work was funded by the National Science Foundation (EID-0425203), Cook County Animal and Rabies Control, and the Max McGraw Wildlife Foundation. We thank D. Parner (deceased) and D. Alexander for their support. We also thank C. Anchor and the Cook County Forest Preserve District for additional support and access to study sites. Fig. 1 was produced by L. White.

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Submitted 8 December 2010. Accepted 30 August 2011.

Associate Editor was Bradley J. Swanson.