EFFECTS OF URBANIZATION ON POPULATION GENETIC ATTRIBUTES OF RACCOONS (*PROCYON LOTOR*)

By

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Bachelor of Science
Oklahoma State University
2001

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2003

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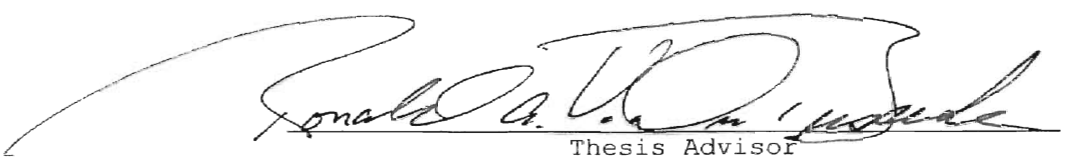
Thesis Approved:



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ACKNOWLEDGMENTS

I would like to thank my advisor Dr. Ron Van Den Bussche for the help and support he gave me throughout this study. I would also like to thank my two other committee members Dr. Anthony Echelle and Dr. Meredith Hamilton for their help in improving my writing abilities through their edits and comments. I am very grateful to Max McGraw Wildlife Foundation (MMWF) for funding this project and collecting the tissue samples I used. Dr. Stan Gehrt of MMWF collected the tissues, outlined the overall project, and helped guide me to look at the bigger picture of this study. I thank him for all his help.

I thank fellow lab mates in Dr. Ron Van Den Bussche's lab that helped me throughout this project. Special thanks go to Dr. Dave Onorato for guidance, support and help from analyses to finishing writing. Others that have helped me throughout my development include Emily Ackland, Dr. Steve Hoofer, Dr. Greg Wilson, Eric Hansen, Derrick Chappell, and Sarah Moore. They would not let me give up or do less than my best and I thank them sincerely.

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EFFECTS OF URBANIZATION ON POPULATION GENETIC ATTRIBUTES OF RACCOONS (*PROCYON LOTOR*)

Abstract: Many wildlife populations have been affected as a result of increasing human population size and increasing urbanization. Urbanization destroys and fragments wildlife habitat which may alter feeding behavior, home range size and use, population densities, and genetic structure of many species. Genetic variation, inbreeding, gene flow (i.e. genetic differentiation) and relatedness of an urban, suburban, and rural population of raccoons (*Procyon lotor*) were examined using 9 microsatellite loci. Significant genetic differentiation (F_{ST}) was detected between all populations. Genetic variation as defined by (H_E) was not significantly different between the three populations. Inbreeding defined by F_{IS} was significantly higher in MMWF than in BUSSE but not in any other pairwise comparisons. Due to male-biased dispersal and female philopatry, females within BUSSE and GP were more related than males. Fragmentation caused by increasing urbanization alters feeding behavior, densities, and home range sizes of raccoons but not genetic attributes.

Introduction

Habitat loss and fragmentation result in small populations at risk of losing genetic variation due to stochastic, demographic, environmental, and genetic processes that can affect both short- and long-term persistence of populations, thus, contributing to the endangerment of fragmented populations (Frankel and Soulé 1981; Shaffer 1981, 1987; Srikwan and Woodruff 2000). Due to anthropogenic factors associated with urbanization (habitat destruction and fragmentation), numerous species of plants and animals either currently face extinction or have declined drastically during the past century (Frankham et al. 2002). For example, river otter (*Lutra canadensis*), wolverine (*Gulo gulo*), sika deer (*Cervus nippon*), African buffalo (*Syncerus caffer*), gray wolf (*Canis lupus*), several species of reptiles (*Ambystoma californiense*),

and birds (Blue-gray gnatcatcher [*Polioptila caerulea*], Western Wood-Peewees [*Contopus sordidulus*], and Dark-eyed Juncos [*Junco hyemalis*]) have declined due to the effects of urbanization (Barry and Schaffer 1994; Blair 1996; O'Ryan et al. 1998; Gering and Blair 1999; Mitchell et al. 1999; Goodman et al. 2001; Sorace 2001; Walker et al. 2001; Blundell et al. 2002; Suarez and Case 2002; Valière et al. 2003).

In addition to the potentially negative genetic consequences, habitat fragmentation associated with urbanization decreases overall biodiversity in fragmented landscapes by allowing some species to increase at the expense of others (McDonnell and Pickett 1990; Gering and Blair 1999; Savard et al. 2000; Sorace 2001). Species such as raccoon (*Procyon lotor*), fox (*Urocyon cinereoargenteus, Vulpes vulpes*), skunk (*Mephitis mephitis*), deer (*Odocoileus virginianus*), hedgehog (*Erinaceus europaeus*), coyote (*Canis latrans*), and some birds (e.g. House Sparrows [*Passer domesticus*], and European Starlings [*Sturnus vulgaris*]) thrive in urban areas (Harrison 1997; Gering and Blair 1999; Rosenblatt et al. 1999; Savard et al. 2000; Grinder and Krausman 2001; Sorace 2001; Crooks 2002; Wandeler et al. 2003).

Although it has been documented that wildlife prospering in urban areas modify their behavior and ecology through alterations in habitat use and selection, increase or decrease home range size and use, and undergo changes in population densities (Harrison 1997; Rosenblatt et al. 1999; Grinder and Krausman 2001; Crooks 2002; Rubin et al. 2002), few studies have examined the genetic attributes of species thriving in fragmented, urban landscapes. Many such species appear to be thriving in these habitats, but, they may eventually suffer the genetic consequences of small populations (increased levels of inbreeding and the concomitant loss of genetic variation). Thus, not only is it important to understand the effects of urbanization on declining

populations, it is critical to understand the genetic attributes of species that modify their demographic characteristics to exploit urban areas (Roscoe 1993; Daszak and Cunningham 2000; Daszak et al. 2001; Wandeler et al. 2003).

Raccoons are medium-sized carnivores of the family Procyonidae. Their distribution is widespread throughout North America, extending from Panama to southern Canada (Rue 1981) and they have been introduced onto the islands of southeast Alaska (Scheffer 1947), the Queen Charlotte Islands of British Columbia (Hartman and Eastman 1999), Germany (Lutz 1984, 1995), and France (Leger 1999). Raccoons are generalists that exploit a variety of habitats ranging from wetlands and bottomland forests to areas dominated by industry. Additionally, their behavior and population demographics change across this rural to urban gradient. Population densities of raccoons generally are higher in riparian habitats and forests (Yeager and Rennels 1943; Fritzell 1978). Landscapes with woodlots, such as woodland parks typical of many urban areas, are also used heavily by raccoons (Stuewer 1943; Twichell and Dill 1949; Ellis 1964; Rosatte et al. 1992). However, with increased urbanization, raccoons may achieve higher densities in urban and suburban areas compared to rural areas (Hoffman and Gottschang 1977; Gehrt, S. D. 1999. Raccoon investigations. Annual report for Max McGraw Wildlife Foundation, Illinois, USA; Gehrt 2002). Along with increases in population densities, urbanization results in changes in behavioral attributes of raccoons such as switching from dependence upon natural resources to artificial food sources (dumpsters and picnic areas) and reduced movements (Hoffman and Gottschang 1977; McDonnell and Pickett 1990; Gehrt, S. D. 1999. Raccoon investigations. Annual report for Max McGraw Wildlife Foundation, Illinois, USA).

The purpose of this study is to elucidate the genetic diversity of urban, suburban, and rural populations of raccoons and to compare the genetic attributes (i.e. genetic variation, inbreeding, gene flow, and relatedness) of these populations across this gradient of human encroachment and habitat alteration. More specifically, I predict that levels of within-population genetic variation and among-population gene flow will decrease with increasing urbanization. This within-population decrease in genetic diversity is hypothesized due to the increased barriers to gene flow, such as roads, resulting in habitat patches and the concomitant reduced movements and associated with urban areas (Wayne et al. 1992; Hitchings and Beebee 1998; Vos et al. 2001). I predict that inbreeding will increase with urbanization adding to the decrease in within-population genetic variation.

STUDY AREA

The first study area is the 1,499-ha Busse Woods Forest Preserve (BUSSE), located approximately 20 km northwest of Chicago in Cook County, Illinois and represents an urban population of raccoons (Figure 1 & 2). This area comprises 39% mature forest, 45% open (including old field, grassland, and mowed areas), and 16% water (including lakes and streams; Gehrt 2002). The site is bordered by or bisected by two eight-lane highways and four state highways. BUSSE is open to the public, has numerous picnic areas, and receives >1 million visitors per year but visitation is highly seasonal because portions of the preserve are closed part of the year (Gehrt 2002). People primarily use the preserve for picnicking but it is also used for hiking, bird watching, and cross-country skiing. Artificial food supplements used by raccoons come from the picnic areas. During 1995-1997, raccoon densities ranged from 36.6-72.6 raccoons/km^2 (Gehrt 2002).

The second study site is the 526-ha Max McGraw Wildlife Foundation (MMWF). This privately owned land is located approximately 40 km northwest of Chicago in Kane County, Illinois (Figure 1 & 3). This site is composed of 30% woodland, 57% open field, and 45% water (Gehrt 2002). This is a suburban area with a mix of residential, commercial development, and undeveloped sites. Supplemental food for raccoons is provided from a miniature golf course, a restaurant, and a small shopping plaza located at the edge of the property. Access to MMWF is restricted because it is privately owned; however, two four-laned roads border and bisect the land with traffic volume ranging from 17,600 to 57,900 vehicles per 24 hours (Gehrt 2002). From 1995-1997 densities ranged from 41.1-93.0 raccoons/km^2 (Gehrt 2002).

The third study site is the 1,214-ha Glacial Park (GP) located approximately 60 km northwest of Chicago in McHenry County, Illinois and represents a rural population of raccoons (Figure 1 & 4). This study site is composed of 20% woodland, 70% open, and 10% water (Gehrt 2002). Agriculture is the primary land use of the surrounding area. Major uses by people include hiking, bird watching, canoeing, cross-country skiing and snow mobiling on specified trails, and nature observation (Prange et al., In Press). There are virtually no artificial food sources at this location. Densities here are much lower than the other two study sites with estimated densities between 1995 and 1997 ranging from 3.1-14.6 raccoons/km^2 (Gehrt 2002).

METHODS

Blood or tissue samples of 536 raccoons from three study areas in northeastern Illinois were obtained from a previous demographic study conducted between 1995 and 2001 in which populations were sampled in the spring and autumn of each year (S. D. Gehrt, Max McGraw Wildlife Foundation, personal communication). DNA was isolated from the samples

following standard protocols (Longmire et al. 1997) and an aliquot of DNA from one individual was used for the construction of a small insert-size genomic library to be used in the isolation of microsatellite loci (Strassmann 1996). Genomic DNA was digested with the restriction enzymes *Alu*I, *Hae*III, and *Rsa*I and the vector pBluescript II sk+ was cut with the restriction enzyme *Eco*RV. Cut genomic and vector DNA was loaded into an agarose gel, electrophoresed and visualized with UV light to excise genomic DNA between 100 and 300 base pairs in length. Extracted DNA was purified from the agarose gel using the QIAquick gel extraction kit (QIAGEN). Linerized vector DNA was extracted and purified using the same approach. After extraction, genomic DNA was dephosphorylated, ligated with the vector and transformed into competent *E. coli* cells. As a positive control, uncut pBluescriptII sk+ vector was also transformed. Transformations were plated on large (245 X 245 X 20 mm) LB-AMP agar plates with Xgal and IPTG for visual identification of transformations containing recombinant vectors. Agar plates containing recombinant colonies were lifted with Hybond XL membrane (Amersham Pharmacia Biotech, Piscataway, NJ) for hybridization. Membranes were hybridized with a $(GT)_n$ oligomer radioactively labeled with α^{32}P-dCTP. Hybridized membranes were washed of excess radiation via 7 post-hybridization washes and exposed to autoradiographic film for identification of colonies containing $(GT)_n$ microsatellite repeats. Positive colonies were picked from the large agar plates and plated on small (100 X 15 mm), gridded plates as slashes. Colonies were grown overnight at 37°, plate lifted, and hybridized with a radioactively labeled $(GT)_n$ probe to confirm the presence of $(GT)_n$ microsatellites. DNA was extracted from the *E. coli* cells and amplified via PCR using primers that flanked the insert.

Double-stranded amplicons were purified using the Wizard PCR Prep DNA Purification System (Promega, Madison, WI) and sequenced in both directions using Big-Dye chain terminators and a 377 automated DNA sequencer (Applied Biosystems, Foster City, CA).

In addition to the microsatellite loci identified in this study, I used previously reported microsatellite loci from kinkajous (*Potos flavus*; Kays et al. 2000) and bears (*Ursus americanus*; Paetkau and Strobeck 1994, Paetkau et al. 1995-Table 1). PCR amplifications for microsatellites were conducted in 15 µl volumes containing 9 µl True Allele Premix, 3.8 µl ddH_20, 0.17 µM of each primer (forward and reverse), and 50-100 ng DNA. The thermal profile consisted of a 12 minute denaturation and enzyme activation cycle at 95°C; 10 cycles of 94°C denaturation for 15 seconds, 55°C for 60 seconds, and 72°C for 30 seconds; followed by 25 cycles of 89°C for 15 seconds, 55°C for 60 seconds, and 72°C for 30 seconds. A final 72°C incubation for 30 minutes was used to ensure that all reactions go to completion.

PCR products of three loci from a single individual were mixed together to obtain a 1:2 µl dilution and run in a single lane. Allele size and microsatellite source (e.g., raccoon, bear, or kinkajou) determined which loci were run together. One microliter of this cocktail of loci was combined with 3 µl of loading mix (2.5 µl of formamide, 0.5 µl of ROX size standard, 0.25 µl of loading buffer containing blue dextran). PCR loading mixtures were denatured at 95°C for 5 minutes and 1.5 µl were loaded into a single lane of a 5% Long Ranger polyacrylamide gel. Microsatellite variation was visualized using a Perkin-Elmer Applied Biosystems 377 Automated DNA Sequencer with GENESCAN® 400 HD [ROX] internal size standards. All gels were

analyzed and scored using GENESCAN ANALYSIS 2.02 and GENOTYPER 2.0 software.

DATA ANALYSIS

Calculation of allele frequencies, mean number of alleles per locus, deviations from Hardy-Weinberg expectations (HWE), genotypic linkage disequilibrium, and population differentiation (F_{ST} and F_{IS}) were assessed using Arlequin 2.0 (Schneider et al. 2000). Deviations from Hardy-Weinberg expectations and pairwise tests for genotypic disequilibrium among loci were tested with the Bonferroni adjustment for multiple comparisons (Rice 1989). Heterozygosity was measured as the mean observed heterozygosity (H_O) and mean expected heterozygosity (H_E) based on Hardy-Weinberg assumptions. The latter measure, H_E, is based on allele frequencies and is generally considered a better measure of genetic variability (Nei and Roychoudary 1974). Differences among populations in number of alleles per locus and H_E were assessed using Wilcoxon's signed rank tests. Private alleles, defined as those occurring in only one population, were identified in each population.

The program RELATEDNESS 5.0 (Queller and Goodnight 1989) was used to calculate levels of relatedness for all individuals genotyped, for individuals within each population, and for males and females separately within each population. This index of relatedness ranges from -1 to 1, and in populations at Hardy-Weinberg equilibrium, values for parent-offspring or full siblings should approach 0.5 (Queller and Goodnight 1989). Mean relatedness for each of the categories described above was evaluated with a two-sample randomization test. Observed mean difference was compared with the means of 10,000 random samplings of the same data.

Probability of identity (P_{ID}) and population assignments were calculated using the web available program Doh!

(http://www.biology.ualberta.ca/jbrzusto/Doh.php). An overall value was obtained from the P_{ID} value calculated for each locus. Doh! calculates the unbiased estimator of P_{ID} as defined by Paetkau et al. (1998).

RESULTS

Twenty-four clones that hybridized with the $(GT)_n$ probe were isolated and sequenced. Only 5 of the clones that contained the GT motif were clean enough to develop primers. Out of these 5, only three sets of primers amplified the target DNA (Table 1).

Data consisted of 536 raccoons genotyped at 9 loci. Eighteen exact tests for HWE were performed, and HWE was rejected at $P < 0.0019$ for all loci in the MMWF and BUSSE populations and 4 loci in the GP population (Table 2). Statistical test for linkage disequilibrium were computed for all pairs of loci for each population. Four, 11, and 18 of 36 tests revealed significant results ($P < 0.0019$) in GP , MMWF, and BUSSE populations respectively. None of the microsatellites I developed exhibited significant results ($P < 0.0019$) in all populations so it is unlikely that they are physically linked. Loci PL35 and G10B were significant in all populations. Therefore, due to possible linkage of the two loci, I excluded G10B from the analyses. The probability of identity was 1.662×10^{-9}, which correlates to a probability of two randomly chosen individuals sharing the same genotype over all 8 loci occurring once for every 601,506,639 raccoons sampled.

Mean observed heterozygosity was highest in BUSSE (0.544) followed by GP (0.491) with the lowest value in the suburban area MMWF (0.464-Table 3). Expected heterozygosity (H_E) was highest in BUSSE (0.713) followed by MMWF (0.699) with the lowest value occurring in GP (0.655); however, none of these values are significantly different from

one another with *P*-values ranging from 0.1953 to 0.5469 for the pairwise comparisons. Conversely, GP had significantly fewer alleles per locus than MMWF ($P = 0.0313$) or BUSSE ($P = 0.0078$). There was no difference between MMWF and BUSSE for comparisons of mean number of alleles ($P = 0.2344$). Private alleles were found for 8 loci (Table 4). MMWF had the highest number of private alleles (24), followed by BUSSE (9), then GP (4).

Pairwise F_{ST} scores revealed that MMWF, GP, and BUSSE populations exhibit significant genetic differentiation (Table 5). Mantel tests revealed this is not likely due to isolation by distance ($P = 0.352$). F_{IS} was highest in suburban (MMWF) areas with a value of 0.01296. The urban (BUSSE) and rural (GP) populations exhibited the lowest F_{IS} values of -0.00007 and -0.00017 respectively (Table 3). MMWF and BUSSE F_{IS} values are statistically significant from one another at $P < 0.05$ ($P = 0.0027$). F_{IS} did not differ significantly in the other pairwise comparisons with *P*-values of 0.1771 and 0.9605. F_{IS} values for GP and BUSSE are not significantly different from 0 which is indicative of no inbreeding.

I also examined relatedness within each population (Table 6). GP was the most intra-related population (R = 0.0163) and BUSSE was the most unrelated (R = -0.0054). The relatedness of raccoons in MMWF fell right in between with an R-value of 0.0044. These values are not significantly different from each other and are essentially zero. Female-female dyads in GP and BUSSE exhibit higher levels of relatedness than male-male dyads. In MMWF the relatedness values between female-female and male-male dyads were the same (Table 6). Results from the genotype assignment test revealed between 60% and 70% of individuals were assigned to their population of capture (Table 7 and Figure 5). The majority of cross assignments were between BUSSE

and MMWF (26% and 25%) which are closer geographically than either is to GP (Figure 1, Figure 5).

DISCUSSION

All three populations showed significant heterozygote deficiencies at multiple loci (Table 2). There are two probable explanations for this result: (1) Population substructure exists within the study areas, and (2) these populations are not closed and there is immigration or emigration occurring. Pairwise tests for linkage disequilibrium were rejected just over 11% in GP, 30% in MMWF, and 50% in BUSSE. I expected to see a decrease in genetic variation and gene flow but an increase in inbreeding and relatedness when going from a rural population to an urban population of raccoons to correspond with the gradient seen in the ecology of this species and other animals (Yeager & Rennels 1943; Hoffman & Gottschang 1977; Fritzell 1978; McDonnell & Pickett 1990; Gehrt, S. D. 1999. Raccoon investigations. Annual report for Max McGraw Wildlife Foundation, Illinois, USA).

Genetic variation was highest in the urban population and lowest in the rural population which is opposite of my predictions. One possible explanation for this reversal is the increase in density with the increase in urbanization. Greater number of raccoons in urban areas may buffer against effects of decreased gene flow. These levels of variation, with a mean of 68.9%, are much higher than previously reported for raccoons. White et al. (1998) detected a mean level of heterozygosity of 4.2% while Beck and Kennedy (1980) reported a mean level of 1.4%. Low levels of diversity were also reported by Dew and Kennedy (2.8%; 1980) and Hamilton and Kennedy (1987; H = 0.9%). The high level of heterozygosity in this study is most likely due to the

use of microsatellite loci that are more polymorphic than the allozyme loci used in previous studies.

Pairwise F_{ST} scores revealed that all populations are statistically significant from one another. One possible explanation for this is lack of gene flow due to barriers surrounding BUSSE and MMWF. These barriers include highways that border and bisect the area resulting in road kills as a major mortality factor (S. D. Gehrt, Max McGraw Wildlife Foundation, personal communication). Also, there are no contiguous streams connecting the populations that might act as corridors for dispersal. Raccoons must disperse through several drainage systems to move between the populations.

F_{IS} values indicated inbreeding was highest in MMWF. I expected a decrease in gene flow with an increase in urbanization which would result in BUSSE having the highest F_{IS} value. MMWF's value could be indicative of further population subdivision. I also predicted that as home ranges and movement decreased with increased urbanization, relatedness of individuals within the population would increase. This trend was not statistically supported by my findings. GP had the highest R value (R = 0.0163) while BUSSE had the lowest (R = -0.0054). This is probably an artifact of sampling area and the difference in home range size of urban versus rural raccoons. Home ranges and movement decrease with urbanization around artificial food sources. Since females are philopatric (Greenwood 1980; Dobson 1982; Clark et al. 1989; Gehrt and Fritzell 1998), matrilines will form around such food sources. In BUSSE, food sources are dispersed throughout the park in the form of picnic areas and dumpsters. Each picnic area could support a different family group therefore resulting in lower levels of relatedness when looking at the population as a whole. Raccoons at GP had larger home ranges because of the absence of artificial food

sources. Because of this increase in home range size and decrease in overlap, sampling an equivalent area as BUSSE will sample fewer family groups. This would result in a higher coefficient of relatedness for GP. To compensate for the increased home range size, sampling in a rural area needs to encompass a larger geographical area than an urban setting to more accurately assess population attributes. I also examined relatedness among males and females within each population. Raccoons exhibit male-biased dispersal and female philopatry like many other mammals (Greenwood 1980; Dobson 1982; Clark et al. 1989; Gehrt and Fritzell 1998). Therefore, I expected males within a population to be less related than the females within a population. This trend was exhibited in GP and BUSSE but not in MMWF. Male-male and female-female dyads in MMWF had the same coefficient of relatedness.

Urbanization fragments raccoon populations and although raccoons are highly vagile, barriers to dispersal in urban areas appear sufficient to produce population substructure. Corridors such as streams, if present, could play a major role for raccoons in overcoming urban barriers by allowing dispersal among populations. Similar corridors may aid in reducing fragmentation of other vagile species such as birds, opossums, and fox. Future research needs to focus on landscapes that have been fragmented due to urbanization for a greater period of time. Corridor use in a landscape fragmented by urbanization and resulting effects on population genetic attributes need to be assessed also.

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TABLE 1. Observed number of alleles (A), observed heterozygosity (H_O), expected heterozygosity (H_E), probability of identity (P_{ID}), and primer sequence (Forward primer on top) for each locus.

PRIMER	PRIMER SEQUENCE	A	H_O	H_E	P_{ID}
Pl35	6FAM-CTAGGGCATGTGTACTGGAC	16	0.244	0.437	0.459
	CTTCTCCCTCTGACTTCTCC				
Pl40	HEX-ACCAGGCAATGGTAATACAG	20	0.302	0.399	0.402
	CCAGGAGGACTTGTCAGAT				
Pl61	6FAM-CTGTCATTCTCCAGTGTGTG	19	0.500	0.703	0.128
	CTAACCCCTAAACATCTCCC				
G10C[a]	AAAGCAGAAGGCCTTGATTTCCTG	15	0.765	0.864	0.037
	6FAM-GGGGACATAAACACCGAGACAGC				
G10X[a]	CCACCTTCTTCCAATTCTC	13	0.345	0.515	0.298
	HEX-TCAGTTATCTGTGAAATCAAAA				
PFL4[b]	6FAM-AGGGAATGTTGCTTCTAATCC	29	0.478	0.942	0.007
	GCAGCCAAACAAACTAAAGTCC				
PFL9[b]	6FAM-GCCTTCATTTAGTTGAGGTCAG	14	0.670	0.862	0.038
	GCATTCTGTCAGTGGCTTTCAC				
PFL11[b]	HEX-CATGCAAATAACACGCAC	15	0.698	0.893	0.024
	CTGAACAAGGTAGGAAAGTCACTC				
MEAN		17	0.500	0.702	1.662×10^{-9}

[a]Primers from Paetkau and Strobeck 1994, Paetkau et al. 1995.
[b]Primers from Kays et al. 2000.

Table 2. Probability values from the exact test to assess Hardy-Weinberg equilibrium. Exact test was performed with the Markov chain parameters set at a forecasted length of 100,000 and 1,000 dememorization steps.

Pop.	P135	P140	P161	G10C	G10X	PFL4	PFL9	PFL11
GP	0.30547[+]	0.30712[-]	<0.0001[a-]	<0.03295[-]	<0.0001[a-]	<0.0001[a-]	<0.00281[-]	<0.0001[a-]
MMWF	<0.0001[a-]	<0.0001[a-]	<0.0001[a-]	<0.0001[a-]	<0.0001[a-]	<0.0001[a-]	<0.0001[a-]	<0.0001[a-]
BUSSE	<0.0001[a-]	<0.0001[a-]	<0.0001[a-]	<0.0001[a-]	<0.0001[a-]	<0.0001[a-]	<0.0001[a-]	<0.0001[a-]

[a] Statistically significant at $P < 0.0019$ with Bonferroni correction.
[+] Denotes excess heterozygotes
[-] Denotes deficiency in heterozygotes

TABLE 3. Number of alleles (A), sample size genotyped out of total samples (n), observed heterozygosity (H_O), and expected heterozygosity (H_E) averaged over all nine loci for each population. Inbreeding coefficient (F_{IS}) for each population.

Pop.	A	n	H_O	H_E	F_{IS}
GP	10	94	0.491	0.655	-0.00017
MMWF	15	231	0.464	0.699	0.01296
BUSSE	13	211	0.544	0.713	-0.00007

TABLE 4. Unique alleles defined by alleles only found in one population.

Primer	GP	MMWF	BUSSE
PL35	220	234,266,270,294,296	252,262,278,280,282
PL40	139	111,113,117,129,131,135	
PL61		153,159,161,175,179	
G10C		127	91
G10X	119	113,121,131,133	137
PFL4	186	172,182	
PFL9		230	

TABLE 5. Genetic differentiation as defined by pairwise F_{ST} values (below diagonal) and probability values (± SD) above diagonal.

	GP	MMWF	BUSSE
GP		0.01802±0.0121	0.00000±0.0000
MMWF	0.00009[a]		0.01802±0.0121
BUSSE	0.00012[a]	0.00004[a]	

[a]Statistically significant at $P < 0.05$ level.

TABLE 6. Queller and Goodnight (1989) coefficients of relatedness for female-female dyads, male-male dyads and all possible dyads (overall) for each population.

Pop.	Female-Female	Male-Male	Overall
GP	0.013	-0.0249	0.0163
MMWF	-0.0002	-0.0002	0.0044
BUSSE	0.0015	-0.0045	-0.0054

TABLE 7. Population assignments from the genotype assignment test in DOH!. Number of genotyped individuals assigned from pop. I (row) to pop. j (column).

Pop.	GP	MMWF	BUSSE
GP (n=94)	66	14	14
MMWF (n=231)	34	138	59
BUSSE (n=211)	25	56	130

Figure Legends

FIG. 1. Location of study areas.

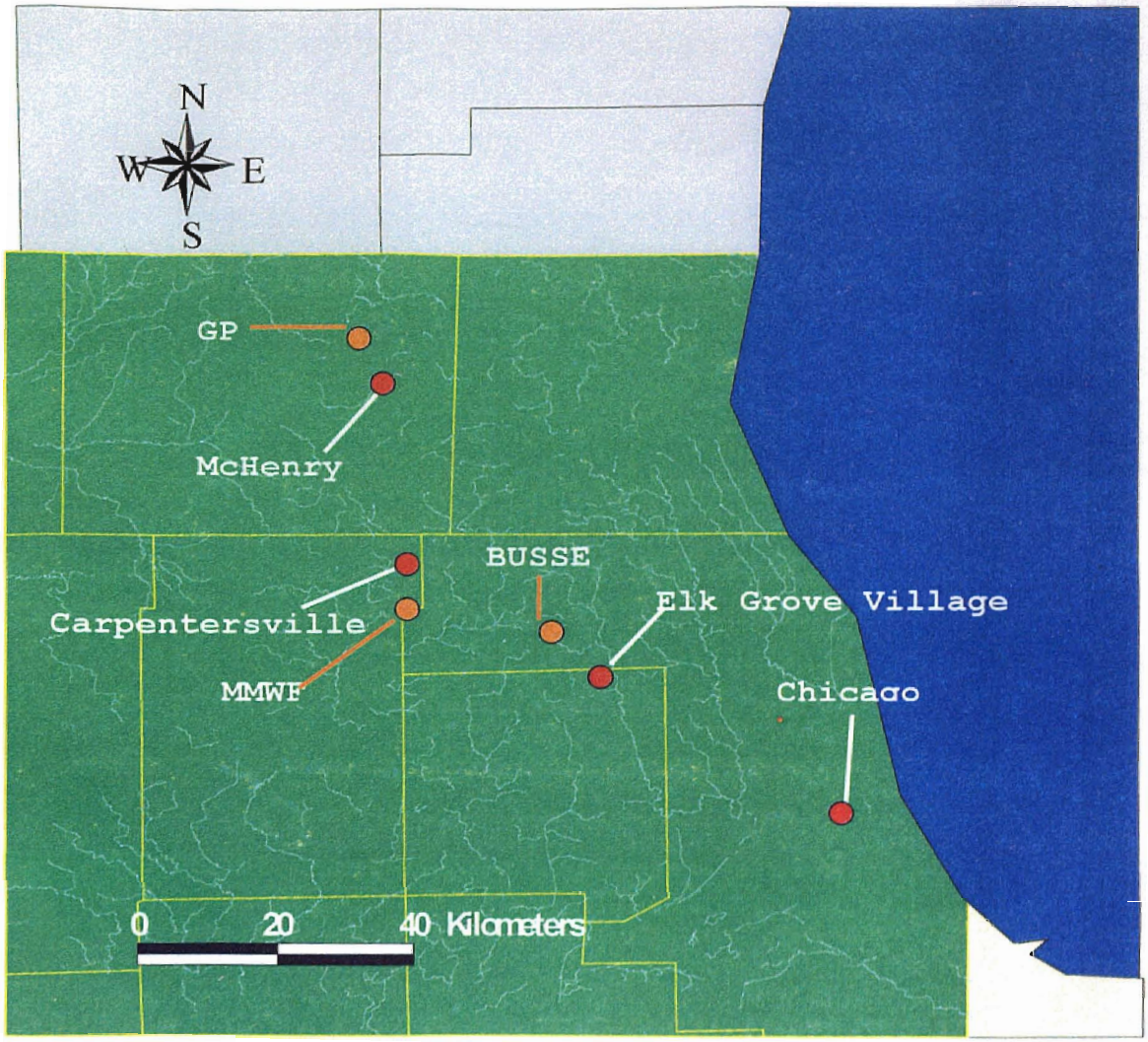
Fig. 2. Aerial photograph of Busse Woods Forest Preserve and surrounding area (http://www.isgs.uiuc.edu/nsdihome/webdocs/doqs/graphic.html).

Fig. 3. Aerial photograph of Max McGraw Wildlife Foundation and surrounding area (http://www.isgs.uiuc.edu/nsdihome/webdocs/doqs/graphic.html).

Fig. 4. Aerial photograph of Glacial Park and surrounding area (http://www.isgs.uiuc.edu/nsdihome/webdocs/doqs/graphic.html).

FIG. 5. Population assignment analysis results showing sample size (n), expected heterozygosity (H_E), average number of alleles (A), F_{IS}, and proportion (Prop.) of genotyped individuals assigned to the population of capture for each population, and pairwise F_{ST} with *P*-values. Proportion of individuals captured in one population but cross assigned to another population are by the arrows showing the direction of the cross assignment.

Figure 1



=Study area

=City

Figure 2

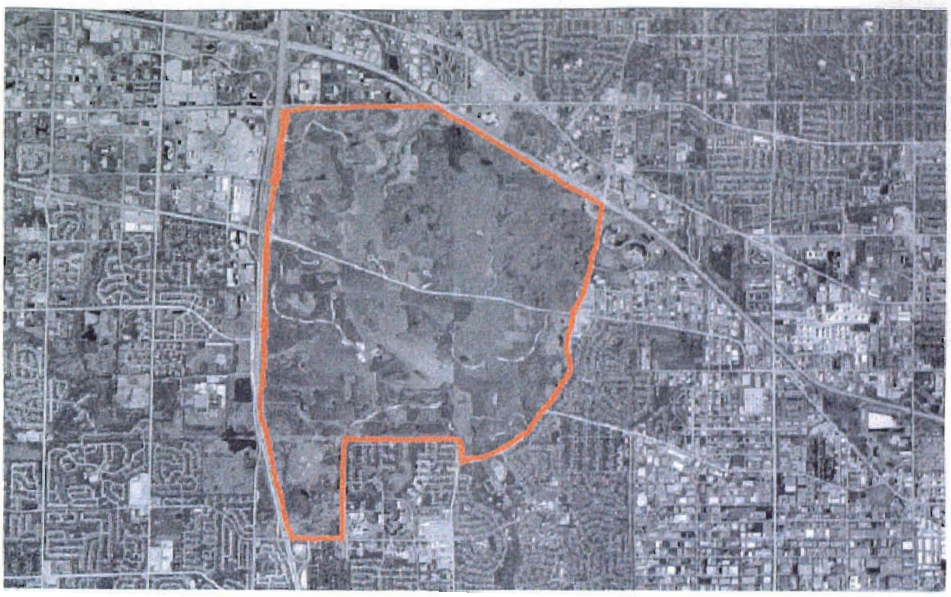


Figure 3

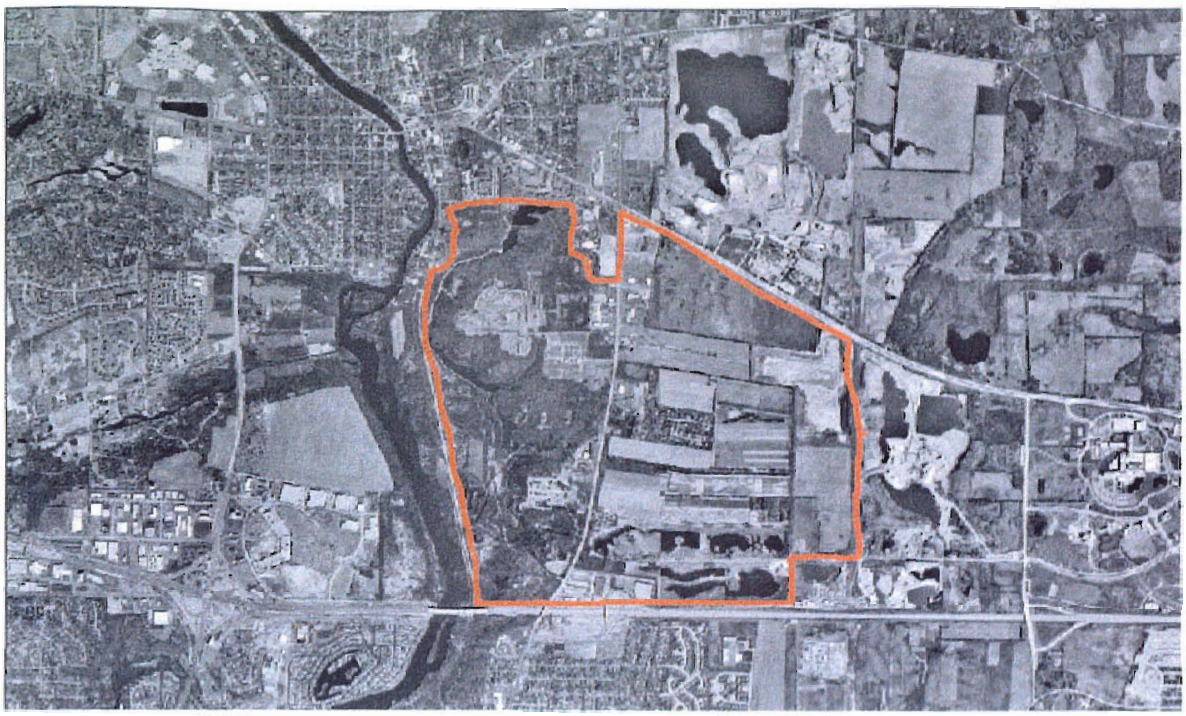
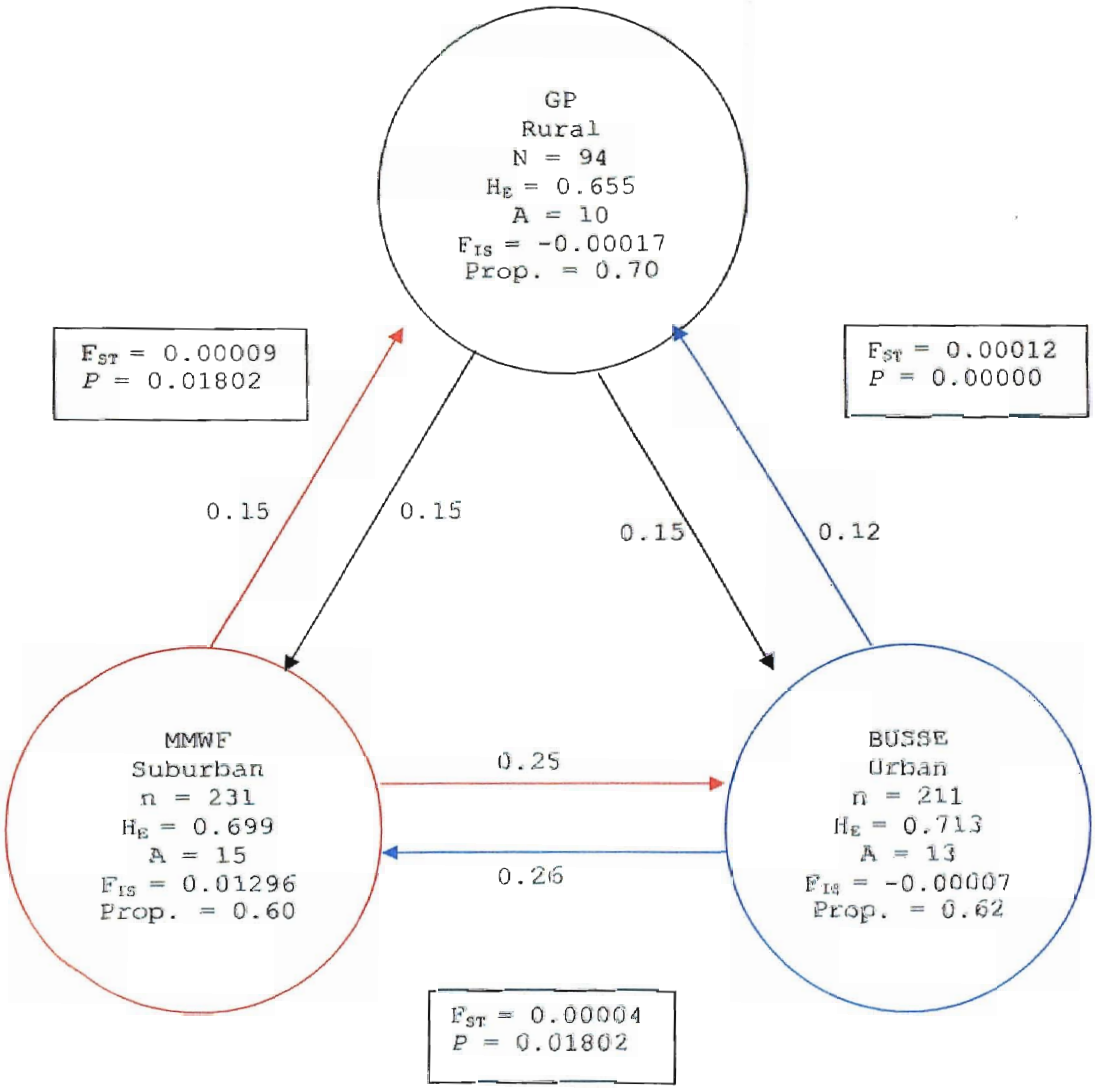


Figure 4

Figure 5


GP
Rural
N = 94
H_E = 0.655
A = 10
F_{IS} = -0.00017
Prop. = 0.70
F_{ST} = 0.00009
P = 0.01802
F_{ST} = 0.00012
P = 0.00000
0.15
0.15
0.15
0.12
MMWF
Suburban
n = 231
H_E = 0.699
A = 15
F_{IS} = 0.01296
Prop. = 0.60
0.25
0.26
BUSSE
Urban
n = 211
H_E = 0.713
A = 13
F_{IS} = -0.00007
Prop. = 0.62
F_{ST} = 0.00004
P = 0.01802

VITA (2)

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