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Range-wide Microsatellite Analysis of the Genetic Population Structure of Prairie Voles (Microtus ochrogaster)

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ABSTRACT.—Understanding population genetic structure provides insight into the evolutionary past, present, and future of a species. In this study, we examine the range-wide population genetic structure of the prairie vole, *Microtus ochrogaster* (n = 170). Early work divided *M. ochrogaster* into seven subspecies using morphological characteristics. We hypothesized polymorphic microsatellite data would reveal a genetic structure roughly congruent with the current classification of subspecies based on their geographic boundaries. We predicted populations within the geographic range of one subspecies would be genetically distinguishable from populations within the geographic range of another subspecies. Microsatellite data from the seven putative subspecies suggested ~90% of molecular variation was within populations. A STRUCTURE cluster analysis had a best supported $k = 3$, but most individuals were admixed for the three genetic clusters, and only individuals of *M. o. ohionensis* were distinctive in being essentially represented by a single cluster. Therefore, our molecular data showed evidence of relatively high gene flow and little geographic differentiation throughout the range of the six contiguous subspecies. The subspecific classification of *M. ochrogaster* should be re-evaluated using a comprehensive taxonomic approach that combines molecular, morphometric, and other data.

INTRODUCTION

The contemporary genetic structure of populations reflects the distribution of genetic variation among groups of individuals within a species and is shaped by extrinsic factors such as geophysical events (*e.g.*, glaciation) and anthropogenic habitat alterations, as well as intrinsic properties of the organism (*e.g.*, mobility, sex-biased dispersal, mating system; Laurence *et al.*, 2011). Uncovering the geographic pattern of genetic variation across a species' range can provide information fundamental for understanding past and current factors affecting a species' history and its future evolutionary potential. As connectivity among populations decreases, gene flow also reduces, and populations tend to evolve along independent trajectories. These independent trajectories lead to greater genetic differentiation among populations such that geographic partitioning of genetic variability can play an important role in the formation of new species (Shaffer, 2014).

Twenty endemic *Microtus* (Rodentia: Cricetidae) species have evolved in North America (Jaarola *et al.*, 2004) in the last 1–2 million years (Chaline *et al.*, 1999), suggesting this genus has a relatively rapid rate of speciation (Fink *et al.*, 2010; Triant and DeWoody, 2006). The prairie vole, *M. ochrogaster* (Wagner, 1842), is a socially monogamous species that is broadly

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distributed across the central United States and south-central Canada. This species is thought to have originated in the "True Prairie" (tall grass prairie), which is located in the central United States (*i.e.*, the Great Plains) and represents approximately the central region of *M. ochrogaster*'s current distribution (Choate and Williams, 1978).

As of 1981, seven nominal subspecies were described within *M. ochrogaster* based primarily on differences in body size and pelage color and texture associated with different geographical ranges (Hall, 1981). *Microtus ochrogaster ochrogaster* (Wagner, 1842) was described as being dark in color with "grizzled" dorsal pelage and characteristic "buffy" ventral pelage (Bole and Moulthrop, 1942). *Microtus ochrogaster haydenii* (Baird, 1858) was characterized by being large in size and very pale in terms of pelage, although it too had "grizzled" dorsal and "buffy" ventral pelage (Bailey, 1900; Bole and Moulthrop, 1942). *Microtus ochrogaster minor* (Merriam, 1888) was thought to be smaller than the other subspecies (Bailey, 1900; Bole and Moulthrop, 1942). This subspecies had similar coloration to *M. o. haydenii* but even more "grizzled" and more "buffy" pelage (Bole and Moulthrop, 1942). *Microtus ochrogaster ohionensis* (Bole and Moulthrop, 1942) was first described as the only subspecies that was not "buff-bellied" but had white ventral pelage (Bole and Moulthrop, 1942). This subspecies was also thought to have dark dorsal pelage and to look exceedingly like *M. pennsylvanicus* (meadow vole), especially to individuals not familiar with the differences in pelage texture of the two species (Bole and Moulthrop, 1942). *Microtus ochrogaster ludovicianus* (Bailey, 1900) had the following distinguishing characteristics: dark pelage, narrow skull, "pinkish" dorsal pelage, large molars, and incisors dark in color (Bole and Mouthrop, 1942). *Microtus ochrogaster taylori* (Hibbard and Rinker, 1943) was thought to have darker pelage than *M. o. haydenii* and to be larger than *M. o. ochrogaster* (Choate and Williams, 1978). When first described, *M. ochrogaster similis* (Severinghaus, 1977) was compared to the other subspecies as follows: smaller than *M. o. haydenii* and *M. o. taylori* based on external and cranial morphometric measurements, larger and with lighter dorsal pelage than *M. o. ludovicianus, M. o. minor* and *M. o. ohionensis*, and a larger hind foot and lighter dorsal pelage than *M. o. ochrogaster*.

All subspecies, except *M. o. ludovicanus,* make up a continuous distribution (Fig. 1); *M. o. ludovicianus* occurs in the coastal tall grass prairies of southeastern Texas and southwestern Louisiana (Lowery, 1974). Originally, this subspecies was described as a distinct species, *M. ludovicianus,* given its disjunct range until Lowery (1974) deemed it a subspecies of *M. ochrogaster* due to its morphological similarity. However, since the original individuals were found in 1900 (Calcasieu Parish, Louisiana) and then again in 1905 (Hardin County, Texas) no other evidence of *M. o. ludovicianus* has been found. After failing to trap any individuals for approximately 30 y, Lowery (1974) concluded the subspecies was very rare, if not extirpated.

Although *M. ochrogaster* has become an important laboratory model organism for studying the neurobiological mechanisms associated with aspects of social behavior such as attachment and parental care (*e.g.*, Young and Wang, 2004; McGraw and Young, 2010), the population genetics of this species has been largely ignored. No molecular work has been conducted to examine the broad scale population genetic structure of *M. ochrogaster* throughout its range. We sought to remedy this information gap using polymorphic microsatellite loci to describe range-wide variation within this species. Because seven subspecies of *M. ochrogaster* have been described, we hypothesized the microsatellite data would reveal a genetic structure roughly congruent with the current classification of subspecies based on the geographic boundaries of these subspecies (Fig. 1). We predicted populations within the geographic range of one subspecies would be genetically distinguishable from populations within the geographic range of another subspecies.

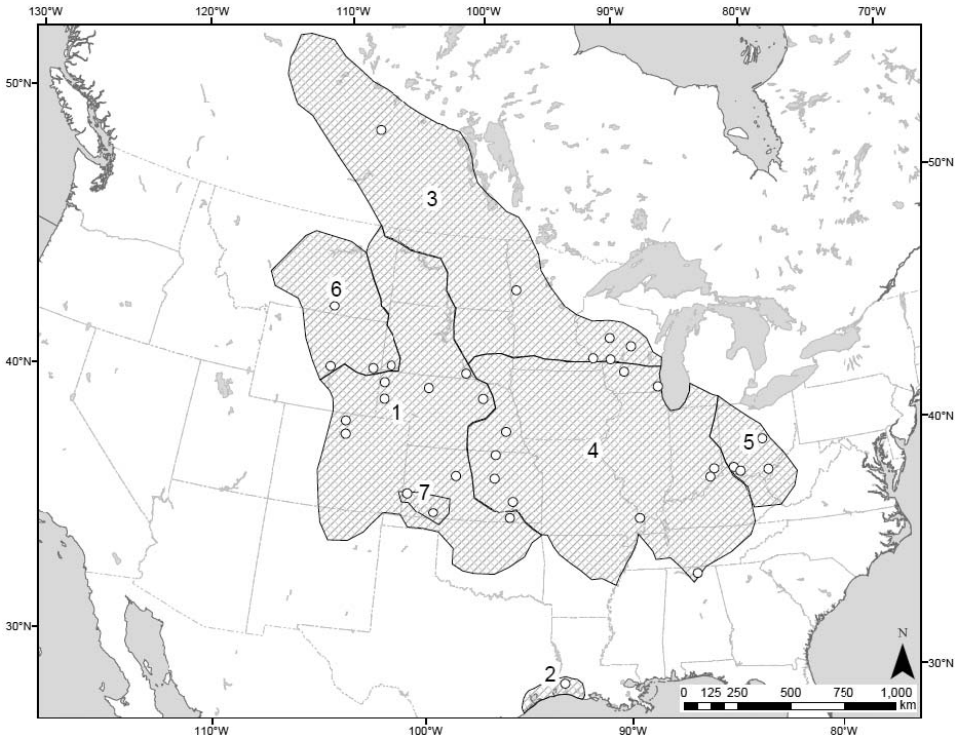

FIG. 1.—Map of North America indicating the approximate distributions of the seven putative subspecies of prairie vole, *Microtus ochrogaster*, modified from Hall (1981), and sampling sites (○). The labels on the figure correspond to the following putative subspecies designations: 1 = *Microtus ochrogaster hyadenii*, 2 = *M. o. ludovicianus*, 3 = *M. o. minor*; 4 = *M. o. ochrogaster*, 5 = *M. o. ohionensis*, 6 = *M. o. similis*, and 7 = *M. o. taylori*

MATERIALS AND METHODS

Tissue samples were collected from a total of 170 individual *M. ochrogaster* (Appendix I) for DNA extraction; 141 were obtained from museum study skins and 29 from liver, heart, spleen, and/or muscle tissue preserved in ethanol. For skin samples, a 2 mm square section of tissue was cut from the ventral side of the individual. Samples were collected from animals trapped between 1895 and 2010 and included individuals from all seven putative subspecies: *M. o. haydenii* (n = 52), *M. o. ludovicianus* (n = 2), *M. o. minor* (n = 30), *M. o. ochrogaster* (n = 42), *M. o. ohionensis* (n = 16), *M. o. similis* (n = 15), and *M. o. taylori* (n = 13). *A priori* putative subspecies assignments were given to samples based on the county in which they were trapped and the geographic subspecies boundaries in Hall (1981). Samples represented 36 counties (or parishes with respect to Louisiana) within 13 states across the United States and one Canadian province (Fig. 1; Appendix I). Individuals trapped in the same county/parish were considered to be a single population (for a total of 36 populations) and the geographic center of a county was used to calculate inter-population distances. Museum specimens were an ideal resource for this study in terms of high sample size and variation in source locations

because the study organism has a wide geographical range that is difficult to trap effectively for a broad study.

Total genomic DNA was extracted from tissue using DNeasy animal tissue kits (Qiagen, Valencia, CA) and a modified protocol from Rowe *et al.* (2011). Negative controls (water) were used during the DNA extraction process. Polymerase chain reaction (PCR) was performed to amplify six microsatellite loci (AV13, MOE2, MSMM2, MSMM3, MSMM5, and MSMM6) as described in Keane *et al.* (2007). Initial PCR contained the following: 10× PCR buffer, dNTPs (0.2 mM), $MgCl_2$ (1.0 mM—AV13, MSMM2, MSMM3, MSMM5, MSMM6; 1.5 mM—MOE2), forward and reverse primers (0.67 μM), GoTaqFlexi (0.375 units; Promega, Madison, WI), DNA (30—150 ng), and water in a total reaction volume of 15 μL. One primer of each primer pair was labeled fluorescently with either HEX, 6-FAM, or NED phosphoramidite (IDT DNA Technologies, Coralville, IA). Polymerase chain reactions were carried out as follows: 95 C for 3 min; 35 cycles of 90 C for 30 s, 45—54 C (depending on locus) for 20 s, 72 C for 20 s; and final extension at 72 C for 5 min. Annealing temperatures (T_A) for each primer pair were as follows: 50 C for AV13 and MSMM5, 48 C for MSMM2, 54 C for MSMM3 and MOE2, and 45 C for MSMM6. For samples ($n = 54$) that failed to amplify under these PCR conditions, annealing time was increased up to 1.5 min and the number of cycles was increased up to 45. In addition, for some of the oldest samples ($n = 11$), we used a high fidelity DNA polymerase with the following reaction conditions: 10× PCR buffer, dNTPs (0.2 mM), $MgCl_2$ (1.5 mM), forward and reverse primers (0.4 μM), Platinum Taq (0.3 units; Invitrogen, Carlsbad, CA), DNA (15—150 ng), and water in a total reaction volume of 15 μL. Polymerase chain reactions using high fidelity DNA polymerase were carried out as follows: 94 C for 2 min; 35—40 cycles for 94 C for 30 s, T_A for 0.5—1.5 min, 72 C for 25 s; 72 C for 10 min. Analyses of fragment size (basepairs, bp) were performed with an ABI Genetic Analyzer using LIZ500 size standard (Applied Biosystems, Inc., Foster City, CA). Genotypes were scored using Gene Mapper v3.7 (Applied Biosystems, Inc., Foster City, CA), and TANDEM v1.07 (Matschiner and Salzburger, 2009) was used to bin DNA fragment sizes.

To detect null alleles (false homozygotes), we used Micro-Checker v2.2.3 (van Oosterhout *et al.*, 2004). To test for linkage disequilibrium among microsatellite loci, we used GENEPOP v4.0.10 (Rousset, 2008). A test of Hardy-Weinberg equilibrium (HWE) was conducted in GenAlEx v6.5 (Peakall and Smouse, 2012) with Bonferroni correction. We evaluated the genetic variation within putative subspecies by calculating the number of private alleles (N_P) and observed and expected heterozygosities (H_O and H_E, respectively) for each putative subspecies in GenAlEx. We calculated the rarefied allelic richness in ADZE (Szpiech *et al.*, 2008) to correct for differences in sample size. To compare genetic variation among putative subspecies, we conducted a one-way analysis of variance (ANOVA) on the rarefied allelic richness and arcsine square root transformed observed and expected heterozygosities using R (R Development Core Team, 2013). To evaluate genetic differentiation among putative subspecies, we calculated pairwise F_{ST} in GenAlEx. We explicitly tested our prediction that the inter-subspecific variation will be greater than intra-subspecific variation by conducting an analysis of molecular variance (AMOVA) in GenAlEx in which we compared the within-population, among-populations-but-within-subspecies, and among-subspecies genetic variation.

Additionally, we conducted a Bayesian cluster analysis using STRUCTURE (Pritchard *et al.*, 2000) to detect distinct genetic groups. This program uses only genotype data without assigning *a priori* subspecies or geographic locations to the samples. STRUCTURE analysis was conducted without the *M. o. ludovicianus* samples. We used an admixture model with correlated allele frequencies. The burn-in was set to 100,000 followed by 200,000 Markov

TABLE 1.—Descriptive statistics for six microsatellite loci for each of the putative subspecies of prairie vole (*Microtus ochrogaster*). Standard errors in parentheses

Subspecies	n	n_c	N_A	N_P	H_O	H_E
M. o. haydenii	52	9	4.459 (0.403)	8	0.728 (0.058)	0.828 (0.071)
M. o. ludovicianus	2	1	—	0	0.250 (0.171)	0.188 (0.120)
M. o. minor	30	6	4.489 (0.325)	4	0.792 (0.057)	0.840 (0.047)
M. o. ochrogaster	42	10	4.646 (0.406)	16	0.769 (0.071)	0.841 (0.068)
M. o. ohionensis	16	4	3.882 (0.486)	3	0.626 (0.118)	0.717 (0.113)
M. o. similis	15	4	3.905 (0.357)	2	0.649 (0.083)	0.730 (0.058)
M. o. taylori	13	2	4.219 (0.395)	4	0.643 (0.151)	0.782 (0.053)
Total	170	36	—	37	0.637 (0.047)	0.704 (0.044)

Note: n = number of samples, n_c = number of counties sampled, N_A = rarefied allelic richness, N_P = number of private alleles, H_O = mean observed heterozygosity, H_E = mean expected heterozygosity

Chain Monte Carlo (MCMC) iterations. The procedure was performed for possible genetic clusters of $k = 1$ through 6, with 20 replicates for each k. To determine the optimal number of clusters, we estimated delta K using STRUCTURE HARVESTER (Earl and vonHoldt, 2012), which employs the Evanno method (Evanno *et al.*, 2005). We then averaged the probability that each individual would be assigned to each distinct group over the 20 replicates for the optimal k using CLUMPP (Jakobsson and Rosenberg, 2007). The results were visually displayed using DISTRUCT v1.1 (Rosenberg, 2004). Finally, we performed a Mantel test to evaluate isolation-by-distance in GenAlEx using pairwise F_{ST} values by population and pairwise geographic distances among populations. Under this model, we expected as geographic distance increased, genetic distance should increase as well.

RESULTS

Approximately 56% of all potential locus-by-individual combinations were able to be amplified across the 170 individuals (a total of 571 locus-by-individual genotypes), which may be attributed to the difficulties associated with extracting and amplifying ancient DNA from museum study skins (Rowe *et al.*, 2011). There was no evidence for linkage disequilibrium between any of the six loci. Null alleles were detected at three loci (AV13, MSMM2, and MSMM6), but because they were not consistently detected across all subspecies (Appendix II) we used all loci in subsequent analyses. The genotyping error rate at the loci used in this study was previously estimated to be approximately 0.02 (errors per locus per generation) in *M. ochrogaster* due to mutation and mis-scoring (Solomon *et al.*, 2004). Significant deviations from HWE due to heterozygote deficiencies occurred at two loci (AV13 and MSMM6) in three of the seven subspecies (*M. o. haydenii*, *M. o. ochrogaster*, and *M. o. ohionensis*) after Bonferroni correction. Since the deviations were not consistent across subspecies, we kept all loci for all further analyses.

Microtus ochrogaster ludovicianus had the lowest genetic diversity, likely an artifact of small sample size (n = 2); therefore, we do not include it when summarizing variation among subspecies. For all other subspecies, the mean rarefied allelic richness ranged from 3.88 (SE = 0.486) in *M. o. ohionensis* to 4.65 (SE = 0.406) in *M. o. ochrogaster* (Table 1). An analysis of variance (ANOVA) was not statistically significant for the rarefied allelic richness among putative subspecies ($F_{5,30} = 0.64$, $P = 0.668$). The mean observed heterozygosity ranged from 0.626 (SE = 0.118) in *M. o. ohionensis* to 0.792 (SE = 0.057) in *M. o. minor* but was not significantly different among putative subspecies ($F_{5,30} = 0.48$, $P = 0.788$). Mean expected

TABLE 2.—Percentage of molecular variance and corresponding statistics calculated from the analysis of molecular variance (AMOVA) using populations with greater than one sample from the six well-sampled putative subspecies of prairie vole (*M. ochrogaster*)

Source	DF	SS	MS	EV	%
Within populations	298	653.881	2.194	2.194	88%
Among populations	26	121.930	4.690	0.256	10%
Among subspecies	5	39.228	7.846	0.047	2%
Total	329	815.039	—	2.497	100%

Note: DF = degrees of freedom, SS = sum of squares, MS = mean square, EV = estimated variance, % = percent molecular variance

heterozygosity ranged from 0.717 (SE = 0.113) in *M. o. ohionensis* to 0.841 (SE = 0.068) in *M. o. ochrogaster* but was also not significantly different among putative subspecies ($F_{5,30} = 0.791$, $P = 0.564$). The AMOVA for the six well-sampled putative subspecies revealed 88% of the molecular variance was within populations, 10% was among populations but within the same putative subspecies, and only 2% was found among the six putative subspecies (Table 2). Due to the relatively large amount of missing data, tests were repeated with samples that had one missing locus or fewer (n = 95). This alternative sampling in the analysis did not change the outcome described above (data not shown).

The overall F_{ST} value was 0.045 ($P = 0.001$), and the highest pairwise F_{ST} values were between *M. o. ludovicianus* and the other putative subspecies (Table 3). However, the very small sample size of *M. o. ludovicianus* (n = 2) may have disproportionately influenced the F_{ST} estimations. When samples of *M. o. ludovicianus* were excluded, the overall F_{ST} fell slightly to 0.040 ($P < 0.001$). The highest F_{ST} values were between *M. o. similis* and *M. o. ohionensis* ($F_{ST} = 0.097$, $P < 0.001$), followed by *M. o. taylori* and *M. o. ohionensis* ($F_{ST} = 0.072$, $P < 0.001$; Table 3). Repeated tests on only the near complete samples resulted in similar F_{ST} values (data not shown). We found a significant relationship between population-pairwise-F_{ST} and geographic distance with the *M. o. ludovicianus* samples included (Mantel's $r = 0.142$, $P = 0.030$; Fig. 2) and excluded (Mantel's $r = 0.167$, $P = 0.020$), indicating a pattern of isolation-by-distance. We excluded the two *M. o. ludovicianus* samples to be certain that this isolated population was not driving the geographic pattern. Using only samples that

TABLE 3.—Pairwise F_{ST} values for the seven putative subspecies of prairie vole (*Microtus ochrogaster*) are displayed below the diagonal

	haydenii	*ludovicianus*	*minor*	*ochrogaster*	*ohionensis*	*similis*	*taylori*
M. o. haydenii	—	0.000	0.000	0.000	0.000	0.000	0.000
M. o. ludovicianus	0.204	—	0.000	0.003	0.000	0.026	0.000
M. o. minor	0.039	0.171	—	0.000	0.000	0.000	0.000
M. o. ochrogaster	0.021	0.109	0.024	—	0.000	0.012	0.000
M. o. ohionensis	0.056	0.236	0.052	0.055	—	0.000	0.000
M. o. similis	0.043	0.102	0.062	0.016	0.097	—	0.000
M. o. taylori	0.032	0.246	0.058	0.052	0.072	0.059	—

Note: Values above the diagonal are probabilities of $F_{ST} > 0$ based on 9999 nonparametric permutations of the data

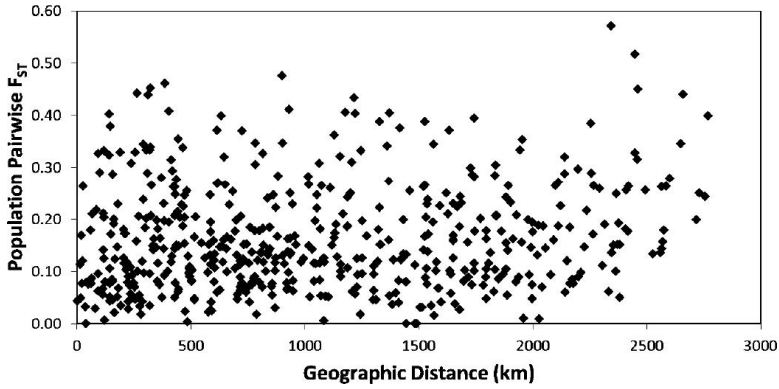

FIG. 2.—Plot of pairwise F_{ST} values versus pairwise geographic distances (km) between populations from all seven putative subspecies of prairie vole, *Microtus ochrogaster,* showing a significant relationship between population pairwise F_{ST} and geographic distance. This relationship held whether or not *M. o. ludovicianus* samples were included

have data for five out of six loci, the correlation between geographic and genetic distance strengthens (Mantel's $r = 0.323$, $P = 0.020$).

The Bayesian cluster analysis indicated the optimal number of genetic clusters was $k = 3$ (Fig. 3) for the putative subspecies, excluding *M. o. ludovicianus* (this result did not change when only samples with data for five out of six loci were used). We chose $k = 3$ after running our data through STRUCTURE HARVESTER because it had the highest delta K (20.74) and had a mean likelihood of -3748.61 ± 14.63. Most individuals were admixed for the three genetic clusters, and only individuals of *M. o. ohionensis* were distinctive in being essentially represented by a single cluster.

DISCUSSION

Almost 90% of the genetic variation in *M. ochrogaster* was found within populations, meaning that there is little genetic differentiation among populations and the likelihood of gene flow is high. Nonetheless, we did detect an isolation-by-distance pattern across the geographical range of *M. ochrogaster* even excluding the geographically isolated *M. o. ludovicianus* samples ($n = 2$).

The "abundant centre" model of a species' distribution states a species is expected to be most abundant at the geographic center of its range with populations on the periphery of the range smaller and more geographically isolated (Vucetich and Waite, 2003). As a result, peripheral populations should have lower genetic diversity due to high genetic drift and low gene flow. Within the six contiguous subspecies, we found no evidence that genetic diversity (measured as allelic richness and heterozygosity) was significantly lower towards the periphery of the range, in contrast to results from many other animal species (Eckert *et al.*, 2008).

We do not find any congruence between subspecies designation and membership in one of the three genetic groups identified by STRUCTURE. Individuals of the six subspecies were admixed for the three genetic clusters, and only individuals of *M. o. ohionensis* were in essentially a single cluster. This is further evidence of relatively high gene flow and little geographic differentiation throughout the range of the six contiguous subspecies.

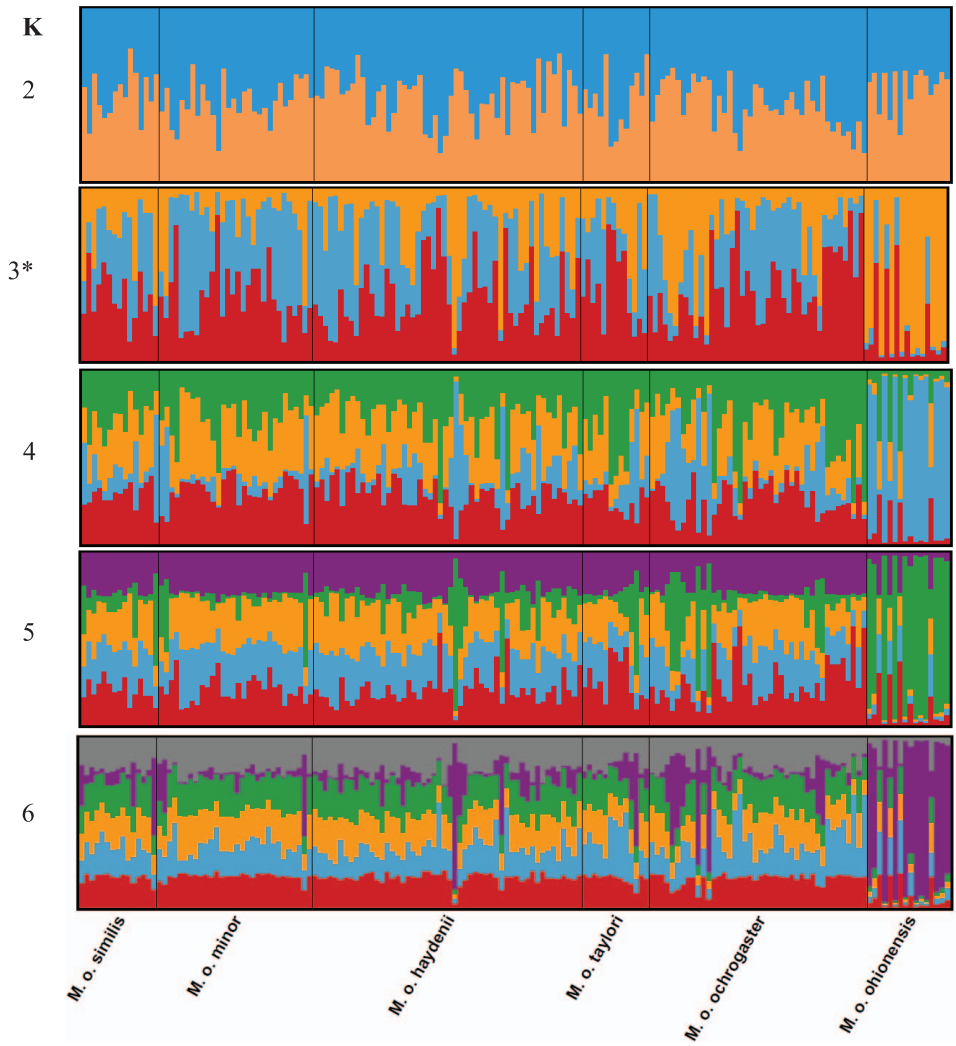

FIG. 3.—Bar plot result from STRUCTURE analysis, assigning individuals into groups (k = 2–6). Samples were grouped together by putative subspecies. The optimal number of genetic clusters, indicated by an asterisk (*), was $k = 3$

Our results did not support the hypothesis that genetic structure was congruent with the current classification of subspecies based on the geographic boundaries of subspecies; populations within the geographic range of one subspecies were often not genetically distinguishable from populations within the geographic range of the other subspecies. However, if microsatellite similarities were due to homoplasy instead of true identity-by-descent, we would not be able to detect evolutionary differences using these methods, which could possibly result in putative subspecies that are less-similar than reported here (Garza

and Freimer, 1996). Furthermore, the allele size range in microsatellites is limited, reducing the efficacy of detecting genetic structure (Nauta and Weissing, 1996).

The lack of congruence between the genetic data and subspecies designations could be due to recent isolation. Speciation is a continuous process, therefore the subspecies of *M. ochrogaster* may not have been isolated long enough to detect a genetic difference. One study attributed a lack of genetic differentiation within *M. ochrogaster* to recent range expansion and ongoing divergence (Fink *et al.*, 2010). Similarly, another study suggested Nearctic and Palearctic continental subspecies tend to have low phylogenetic differentiation due to insufficient time since post-glacial recolonization (Phillimore and Owens, 2006). Investigators of *M. californicus* attributed the high level of gene flow to large effective population sizes and recent or incomplete isolation of populations (Adams and Hadly, 2010). High gene flow has also been recorded across fragmented habitats (Aars *et al.*, 2006), which could contribute to panmixia across the range of *M. ochrogaster*.

Additionally, the lack of congruence between the genetic data and subspecies designations could be due to hybridization. The three genetic groups, identified by STRUCTURE, within *M. ochrogaster* could be historical lineages that have introgressed. Typically, introgression happens in a unidirectional manner where one geographic lineage invades the range of another (Bastos-Silveira *et al.*, 2012; Beysard *et al.*, 2012). However, our results did not suggest the three lineages were correlated with geography, which may indicate an older introgression event as seen in the European pine voles, *M. duodecimcostatus*, and *M. lusitanicus*, (Bastos-Silverira *et al.*, 2012). *Microtus ochrogaster* has a short generation time due to year-round breeding in some areas, a 21 d gestation and lactation period, and age of reproduction of 31 d (Solomon, 1991). Therefore, introgression and maintenance of a hybrid lineage is plausible because of rapid reproduction. Introgression also has been documented in other North American small mammals including red-backed voles (*Myodes gapperi* and *M. rutilus*; Runck *et al.*, 2009) and hispid cotton rats (*Sigmodon hispidus*; Phillips *et al.*, 2007), although on smaller geographic scales.

Another reason the genetic data may not correspond to the subspecies designations may be because the described subspecies do not represent genetically distinct subgroups. A previous study that used a 160 bp tandem satellite array (MSAT-160) found identical results for *M. o. ochrogaster* and *M. o. similis* using a Southern blot analysis, suggesting no genetic differentiation between these two putative subspecies (Modi, 1993). Morphological studies by Choate and Williams (1978) and Stangl *et al.* (2004) found *M. o. ochrogaster*, *M. o. similis*, and *M. o. taylori* were not morphologically distinct based on external and cranial morphometric measurements, suggesting the morphological diversity reported for putative *M. ochrogaster* subspecies is likely due to phenotypic plasticity and/or local adaptation rather than subspecific differences. Similarly, a morphological study of southeastern populations of *M. ochrogaster* concluded clinal morphological differences among populations were most likely due to local phenotypic adaptation and not genetic divergence (Huggins and McDaniel, 1984). Therefore, the majority of the existing data (morphological and genetic) do not support the classification of *M. ochrogaster* into seven subspecies and suggest the classification of *M. ochrogaster* may need to be re-evaluated using integrative taxonomic approaches. Because our current study is based on a limited number of microsatellites ($n = 6$), the results should be viewed with caution. Future use of mitochondrial and additional nuclear loci may help to further elucidate the evolutionary histories of these three *M. ochrogaster* lineages.

Other studies have found discrepancies between results using genetic markers and existing taxonomic classifications based on morphology, including species within the

genus *Microtus* (Jaarola and Searle, 2002; Haring *et al.*, 2011). Specifically, researchers found little phylogenetic support for the current subspecies of *M. agrestis* (field vole; Jaarola and Searle, 2002). Another study of a Palearctic *Microtus* species, the reed vole (*M. fortis*), showed that the existence of two subspecies (*M. f. pelliceus* and *M. f. michnoi*) was not supported based on mitochondrial DNA sequences (Haring *et al.*, 2011). The same study showed the genetic data for two subspecies of Maximowicz's vole (*M. maximowiczii maximowiczii* and *M. m. ungurensis*) also did not correspond to established taxonomy (Haring *et al.*, 2011).

Subspecific taxonomy of other Nearctic mammals has also been re-evaluated in light of emerging molecular data. For example, subspecies of the common raccoon (*Procyon lotor*) described by morphology were not supported by mitochondrial data (Cullingham *et al.*, 2008). Additionally, a study of four muskrat (*Ondatra zibethicus*) subspecies showed evidence for the existence of two subspecies (*O. z. obscurus* and *O. z. zibethicus*) but could not differentiate between the remaining two subspecies (*O. z. albus* and *O. z. spatulus*; Laurence *et al.*, 2011).

An improved understanding of the phylogeography of a species can have implications beyond simply improving our understanding of the evolutionary history of the species. For example, *M. ochrogaster* has become a model organism for studying monogamy in mammals (Young *et al.*, 1998), a rare lifestyle with less than 3–5 % of mammalian species known to be monogamous (Kleiman, 1977). A previous study demonstrated geographic variation in monogamous behavior: a greater percentage of *M. ochrogaster* in Kansas were classified as socially monogamous compared to *M. ochrogaster* in Indiana (Streatfeild *et al.*, 2011). Genetic monogamy also was more frequent in Kansas than in Indiana (Streatfeild *et al.*, 2011). Therefore, an important question for future research is: how much behavioral variation in these populations is due to ecological versus genetic factors associated with phylogenetic differences and to what extent do existing behavioral differences affect speciation in *M. ochrogaster*?

Although our results suggest *M. ochrogaster* is panmictic throughout large stretches of its range, the prairie and grasslands of North America (the main habitats of *M. ochrogaster*) are experiencing significant anthropogenic alterations that are resulting in habitat fragmentation (Samson and Knopf, 1994; Samson *et al.*, 2004). As the prairie becomes more fragmented, gene flow among populations of *M. ochrogaster* may be reduced. Such loss could lead to small isolated populations that become differentiated due to genetic drift and potentially extirpated. Development and conversion to agricultural land also threaten the coastal tall grass prairie and have been implicated in the likely extirpation of the isolated *M. o. ludovicianus* (Lowery, 1974; Allain *et al.*, 1999). The use of subspecies in taxonomy can greatly influence conservation efforts. Correct delineation of subspecies will hopefully lead to more efficient resource use, which is gravely important in the crisis discipline that is conservation.

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TABLE A1.—*Microtus ochrogaster* sample details. Abbreviations for the samples sources are as follows: CMM = University of Colorado Museum of Natural History; CMNH = Museum of Natural History and Science, Cincinnati Museum Center; FHSM = Sternberg Museum of Natural History, Fort Hays State University; INHS = Illinois Natural History Survey; NMNH = National Museum of Natural History; OSU = Museum of Biological Diversity, The Ohio State University; UIMNH = University of Illinois Museum of Natural History; UKMNH = University of Kansas Biodiversity Institute; UNSMZM = University of Nebraska State Museum; UWSP = University of Wisconsin Stevens Point Museum of Natural History. Tissues are categorized as being from a museum study skin or from organ tissue preserved in ethanol

Source ID	Sample ID	Putative subspecies	County	State/province	Year collected	Tissue type
CMM	483	*haydenii*	Boulder	Colo	2010	preserved
CMM	484	*haydenii*	Boulder	Colo	2010	preserved
CMM	694	*haydenii*	Larimer	Colo	2010	preserved
CMM	714	*haydenii*	Larimer	Colo	2010	preserved
CMM	756	*haydenii*	Boulder	Colo	2010	preserved
CMM	765	*haydenii*	Larimer	Colo	2010	preserved
CMNH	M63	*ohionensis*	Brown	Ohio	1971	study skin
CMNH	M789	*ohionensis*	Gallia	Ohio	1984	study skin
CMNH	M790	*ohionensis*	Gallia	Ohio	1984	study skin
CMNH	M791	*ohionensis*	Gallia	Ohio	1984	study skin
CMNH	M1080	*ochrogaster*	Jefferson	Ind	1954	study skin
CMNH	M1810	*ochrogaster*	Ripley	Ind	1953	study skin
CMNH	M1820	*ochrogaster*	Jefferson	Ind	1954	study skin
CMNH	M1826	*minor*	Winona	Minn	1960	study skin
CMNH	M1827	*minor*	Winona	Minn	1960	study skin
CMNH	M1828	*minor*	Winona	Minn	1960	study skin
CMNH	M1829	*minor*	Winona	Minn	1960	study skin
CMNH	M1834	*minor*	Winona	Minn	1960	study skin
CMNH	M1900	*ochrogaster*	Ripley	Ind	1952	study skin
FHSM	3398	*taylori*	Hamilton	Kan	1964	study skin
FHSM	3399	*taylori*	Hamilton	Kan	1964	study skin
FHSM	3400	*taylori*	Hamilton	Kan	1964	study skin
FHSM	3401	*taylori*	Hamilton	Kan	1964	study skin
FHSM	5681	*ochrogaster*	Dickinson	Kan	1965	study skin
FHSM	5682	*ochrogaster*	Dickinson	Kan	1965	study skin
FHSM	5683	*ochrogaster*	Dickinson	Kan	1965	study skin
FHSM	5797	*haydenii*	Ellis	Kan	1965	study skin

TABLE A1.—Continued

Source ID	Sample ID	Putative subspecies	County	State/province	Year collected	Tissue type
FHSM	5798	*haydenii*	Ellis	Kan	1965	study skin
FHSM	5801	*haydenii*	Ellis	Kan	1965	study skin
FHSM	5820	*haydenii*	Ellis	Kan	1965	study skin
FHSM	24591	*taylori*	Hamilton	Kan	1986	study skin
FHSM	24593	*taylori*	Hamilton	Kan	1986	study skin
FHSM	24594	*taylori*	Hamilton	Kan	1986	study skin
FHSM	29506	*taylori*	Meade	Kan	1993	study skin
FHSM	29514	*taylori*	Meade	Kan	1993	study skin
FHSM	29515	*taylori*	Meade	Kan	1993	study skin
FHSM	29517	*taylori*	Meade	Kan	1993	study skin
FHSM	29518	*taylori*	Meade	Kan	1993	study skin
FHSM	29520	*taylori*	Meade	Kan	1993	study skin
FHSM	37950	*haydenii*	Chautauqua	Kan	2007	preserved
FHSM	38011	*ochrogaster*	Dickinson	Kan	2007	preserved
FHSM	38012	*ochrogaster*	Dickinson	Kan	2007	preserved
FHSM	38013	*ochrogaster*	Dickinson	Kan	2007	preserved
FHSM	38014	*ochrogaster*	Dickinson	Kan	2007	preserved
FHSM	38109	*ochrogaster*	Dickinson	Kan	2007	preserved
FHSM	38425	*ochrogaster*	Washington	Kan	2007	preserved
FHSM	38623	*ochrogaster*	Greenwood	Kan	2008	preserved
FHSM	38624	*ochrogaster*	Greenwood	Kan	2008	preserved
FHSM	38625	*ochrogaster*	Greenwood	Kan	2008	preserved
FHSM	38986	*haydenii*	Ellis	Kan	2008	preserved
FHSM	39305	*ochrogaster*	Dickinson	Kan	2007	preserved
FHSM	39306	*haydenii*	Ellis	Kan	2008	preserved
FHSM	39307	*ochrogaster*	Washington	Kan	2007	preserved
INHS	47932	*haydenii*	Cherry	Neb	1973	study skin
INHS	47933	*haydenii*	Cherry	Neb	1973	study skin
INHS	47934	*haydenii*	Cherry	Neb	1973	study skin
INHS	47935	*haydenii*	Cherry	Neb	1973	study skin
INHS	47936	*haydenii*	Cherry	Neb	1973	study skin
INHS	47937	*haydenii*	Cherry	Neb	1973	study skin
INHS	47938	*haydenii*	Cherry	Neb	1973	study skin
INHS	47940	*haydenii*	Cherry	Neb	1973	study skin
INHS	47941	*haydenii*	Cherry	Neb	1973	study skin
INHS	47942	*haydenii*	Cherry	Neb	1973	study skin
INHS	47943	*haydenii*	Cherry	Neb	1973	study skin
INHS	47986	*haydenii*	Scottsbluff	Neb	1973	study skin
INHS	47987	*haydenii*	Scottsbluff	Neb	1973	study skin
INHS	47988	*haydenii*	Scottsbluff	Neb	1973	study skin
INHS	47989	*haydenii*	Scottsbluff	Neb	1973	study skin
INHS	47990	*haydenii*	Scottsbluff	Neb	1973	study skin
INHS	47991	*haydenii*	Scottsbluff	Neb	1973	study skin
INHS	47992	*haydenii*	Scottsbluff	Neb	1973	study skin
INHS	47993	*haydenii*	Scottsbluff	Neb	1973	study skin
INHS	47994	*haydenii*	Scottsbluff	Neb	1973	study skin
INHS	47995	*haydenii*	Scottsbluff	Neb	1973	study skin
INHS	47997	*haydenii*	Scottsbluff	Neb	1973	study skin
NMNH	75428	*minor*	Wingard	Sask	1895	study skin

TABLE A1.—Continued

Source ID	Sample ID	Putative subspecies	County	State/province	Year collected	Tissue type
NMNH	75429	*minor*	Wingard	Sask	1895	study skin
NMNH	92856	*ochrogaster*	Racine	Wis	1898	study skin
NMNH	92857	*ochrogaster*	Racine	Wis	1898	study skin
NMNH	96628	*ludovicianus*	Calcasieu	La	1899	study skin
NMNH	96631	*ludovicianus*	Calcasieu	La	1899	study skin
NMNH	214417	*similis*	Big Horn	Mont	1916	study skin
NMNH	222873	*similis*	Big Horn	Mont	1916	study skin
NMNH	248632	*minor*	Clark	Wis	1927	study skin
NMNH	248633	*minor*	Clark	Wis	1927	study skin
NMNH	248634	*minor*	Clark	Wis	1927	study skin
OSU	1294	*ohionensis*	Clermont	Ohio	1942	study skin
OSU	1519	*ohionensis*	Clermont	Ohio	1947	study skin
OSU	2867	*ohionensis*	Licking	Ohio	1970	study skin
OSU	3006	*ohionensis*	Brown	Ohio	1971	study skin
OSU	3010	*ohionensis*	Brown	Ohio	1971	study skin
OSU	3742	*ohionensis*	Licking	Ohio	1972	study skin
OSU	3743	*ohionensis*	Licking	Ohio	1972	study skin
OSU	3760	*ohionensis*	Licking	Ohio	1972	study skin
OSU	3766	*ohionensis*	Brown	Ohio	1971	study skin
OSU	3767	*ohionensis*	Licking	Ohio	1972	study skin
OSU	5609	*ohionensis*	Licking	Ohio	1974	study skin
OSU	6182	*ohionensis*	Licking	Ohio	1968	study skin
UIMNH	10181	*ochrogaster*	Madison	Ala	1955	study skin
UIMNH	10182	*ochrogaster*	Madison	Ala	1955	study skin
UIMNH	48163	*minor*	Clay	Minn	1973	study skin
UIMNH	59059	*ochrogaster*	Alexander	Ill	1981	study skin
UIMNH	59060	*ochrogaster*	Alexander	Ill	1981	study skin
UIMNH	59061	*ochrogaster*	Alexander	Ill	1981	study skin
UIMNH	59065	*ochrogaster*	Alexander	Ill	1981	study skin
UIMNH	59066	*ochrogaster*	Alexander	Ill	1981	study skin
UIMNH	59479	*haydenii*	Gregory	SD	1981	study skin
UIMNH	59480	*haydenii*	Gregory	SD	1981	study skin
UIMNH	59481	*haydenii*	Gregory	SD	1981	study skin
UIMNH	59483	*haydenii*	Gregory	SD	1981	study skin
UIMNH	59484	*haydenii*	Gregory	SD	1981	study skin
UIMNH	59485	*haydenii*	Gregory	SD	1981	study skin
UIMNH	59486	*haydenii*	Gregory	SD	1981	study skin
UKMNH	20745	*similis*	Niobrara	Wyo	1947	study skin
UKMNH	20747	*similis*	Niobrara	Wyo	1947	study skin
UKMNH	27652	*similis*	Natrona	Wyo	1948	study skin
UKMNH	27653	*similis*	Natrona	Wyo	1948	study skin
UKMNH	27654	*similis*	Natrona	Wyo	1948	study skin
UKMNH	42360	*similis*	Niobrara	Wyo	1951	study skin
UKMNH	113514	*similis*	Fall River	SD	1967	study skin
UKMNH	113515	*similis*	Fall River	SD	1967	study skin
UKMNH	113516	*similis*	Fall River	SD	1967	study skin
UKMNH	113517	*similis*	Fall River	SD	1967	study skin
UKMNH	113518	*similis*	Fall River	SD	1967	study skin
UKMNH	113519	*similis*	Fall River	SD	1967	study skin

TABLE A1.—Continued

Source ID	Sample ID	Putative subspecies	County	State/province	Year collected	Tissue type
UKMNH	113520	*similis*	Fall River	SD	1967	study skin
UNSMZM	13236	*haydenii*	Cherry	Neb	1969	study skin
UNSMZM	15110	*ochrogaster*	Lancaster	Neb	1981	study skin
UNSMZM	15417	*ochrogaster*	Lancaster	Neb	1983	study skin
UNSMZM	16861	*ochrogaster*	Lancaster	Neb	1987	study skin
UNSMZM	16886	*ochrogaster*	Lancaster	Neb	1987	study skin
UNSMZM	17484	*haydenii*	Antelope	Neb	1989	study skin
UNSMZM	19950	*ochrogaster*	Lancaster	Neb	1993	study skin
UNSMZM	23153	*haydenii*	Sioux	Neb	1973	study skin
UNSMZM	23154	*haydenii*	Sioux	Neb	1973	study skin
UNSMZM	23157	*haydenii*	Sioux	Neb	1973	study skin
UNSMZM	23158	*haydenii*	Sioux	Neb	1989	study skin
UNSMZM	23159	*haydenii*	Sioux	Neb	1989	study skin
UNSMZM	23160	*haydenii*	Sioux	Neb	1989	study skin
UNSMZM	23161	*haydenii*	Sioux	Neb	1989	study skin
UNSMZM	23162	*haydenii*	Sioux	Neb	1989	study skin
UNSMZM	29065	*ochrogaster*	Lancaster	Neb	2002	study skin
UWSP	941	*minor*	Portage	Wis	1968	study skin
UWSP	1410	*minor*	Portage	Wis	1969	study skin
UWSP	1717	*minor*	Portage	Wis	1969	study skin
UWSP	2134	*minor*	Portage	Wis	1970	study skin
UWSP	2135	*minor*	Portage	Wis	1970	study skin
UWSP	2136	*minor*	Portage	Wis	1970	study skin
UWSP	2142	*minor*	Portage	Wis	1970	study skin
UWSP	2145	*minor*	Portage	Wis	1970	study skin
UWSP	2757	*ochrogaster*	Sauk	Wis	1971	study skin
UWSP	2839	*ochrogaster*	Sauk	Wis	1971	study skin
UWSP	2840	*ochrogaster*	Sauk	Wis	1971	study skin
UWSP	2939	*minor*	Portage	Wis	1970	study skin
UWSP	3277	*ochrogaster*	Sauk	Wis	1971	study skin
UWSP	3691	*ochrogaster*	Sauk	Wis	1972	study skin
UWSP	3694	*ochrogaster*	Sauk	Wis	1972	study skin
UWSP	3696	*ochrogaster*	Sauk	Wis	1972	study skin
UWSP	3697	*ochrogaster*	Sauk	Wis	1972	study skin
UWSP	3962	*ochrogaster*	Sauk	Wis		study skin
UWSP	4645	*minor*	Portage	Wis	1972	study skin
UWSP	9611	*minor*	Monroe	Wis	2009	preserved
UWSP	9612	*minor*	Monroe	Wis	2009	preserved
UWSP	9613	*minor*	Monroe	Wis	2009	preserved
UWSP	9616	*minor*	Monroe	Wis	2009	preserved
UWSP	9617	*minor*	Monroe	Wis	2009	preserved
UWSP	9620	*minor*	Monroe	Wis	2009	preserved
UWSP	9623	*minor*	Monroe	Wis	2009	preserved
UWSP	9657	*minor*	Monroe	Wis	2009	preserved
UWSP	10204	*minor*	Monroe	Wis	2009	preserved

TABLE A2.—Allele statistics by locus and subspecies of *Microtus ochrogaster*. Bold values indicate statistically significant deviations from HWE. The frequency of null alleles was calculated in MicroChecker and the Oosterhout estimate is reported. Italicized values indicate the presence of null alleles. Two subspecies (*M. o. ludovicianus* and *M. o. taylori*) did not have enough data to calculate null allele frequencies

Subspecies	Locus	n	n_{amp}	n_{al}	H_O	H_E	n_f
M. o. haydenii	MSMM2	52	45	18	0.756	0.909	*0.080*
	MSMM3	52	22	11	0.864	0.883	0.013
	MSMM5	52	37	23	0.838	0.911	0.043
	MSMM6	52	48	9	0.521	0.476	−0.054
	AV13	52	36	20	**0.583**	0.919	*0.183*
	MOE2	52	36	13	0.806	0.868	0.037
M. o. ludovicianus	MSMM2	2	0	0	0.000	0.000	
	MSMM3	2	2	3	0.500	0.625	
	MSMM5	2	0	0	0.000	0.000	
	MSMM6	2	1	1	0.000	0.000	
	AV13	2	1	2	1.000	0.500	
	MOE2	2	0	0	0.000	0.000	
M. o. minor	MSMM2	30	26	18	0.962	0.907	–0.030
	MSMM3	30	27	14	0.704	0.847	*0.086*
	MSMM5	30	22	15	0.909	0.917	0.005
	MSMM6	30	26	7	0.577	0.612	0.026
	AV13	30	25	15	0.800	0.880	0.044
	MOE2	30	25	13	0.800	0.874	0.039
M. o. ochrogaster	MSMM2	42	28	16	0.857	0.911	0.032
	MSMM3	42	30	14	0.833	0.883	0.031
	MSMM5	42	25	24	0.880	0.946	0.035
	MSMM6	42	36	11	**0.417**	0.504	0.089
	AV13	42	30	22	0.800	0.918	*0.066*
	MOE2	42	23	15	0.826	0.888	0.033
M. o. ohionensis	MSMM2	16	15	8	0.667	0.847	0.104
	MSMM3	16	12	8	0.833	0.823	–0.004
	MSMM5	16	12	10	0.833	0.872	0.015
	MSMM6	16	12	3	**0.083**	0.156	*0.204*
	AV13	16	13	9	**0.538**	0.793	*0.153*
	MOE2	16	15	8	0.800	0.811	0.019
M. o. similis	MSMM2	15	10	10	0.700	0.835	0.079
	MSMM3	15	10	8	0.800	0.855	0.029
	MSMM5	15	8	8	0.750	0.844	0.047
	MSMM6	15	11	4	0.273	0.492	*0.186*
	AV13	15	7	5	0.571	0.673	0.077
	MOE2	15	5	5	0.800	0.680	–0.095
M. o. taylori	MSMM2	13	13	10	0.846	0.867	
	MSMM3	13	7	8	1.000	0.857	
	MSMM5	13	9	12	0.778	0.870	
	MSMM6	13	12	7	0.417	0.573	
	AV13	13	3	3	0.000	0.667	
	MOE2	13	11	10	0.818	0.860	

n = number of samples, n_{amp} = number of samples amplified, n_{al} = number of alleles, H_O = mean observed heterozygosity, H_E = mean expected heterozygosity, n_f = Oosterhout's null allele frequency