

MITOCHONDRIAL DNA VARIATION, POPULATION STRUCTURE, AND EVOLUTION OF THE COMMON GRACKLE (*QUISCALUS QUISCALA*)¹

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Abstract. The Common Grackle (*Quiscalus quiscula*) includes two phenotypically differentiated forms, the Purple Grackle (*Q. q. quiscula*), which breeds along the Atlantic and Gulf coasts of the United States, and the Bronzed Grackle (*Q. q. versicolor*), which occurs over the rest of eastern North America. These grackles meet and hybridize in a zone that stretches from northeastern to the extreme south central United States. We used 20 restriction endonucleases to survey the level and pattern of mitochondrial DNA (mtDNA) variation within and among 35 individuals taken from eight sites. To establish the phylogenetic position of *Q. quiscula*, we also studied the Greater Antillean Grackle (*Q. niger*), the supposed sister taxon of *Q. quiscula*, the Boat-tailed Grackle (*Q. major*), and the Great-tailed Grackle (*Q. mexicanus*). A relatively high number (29) of clones was detected in *Q. quiscula*, of which 23 occurred in single individuals. Using results of simulations by Avise et al. (1988), we found times to common ancestry of the 31 clones that are much lower than predicted by neutral theory; however, it is likely that long-term effective population sizes of grackles are much lower than current population size, which potentially explains the discrepancy. Sixty-six percent of the birds were genetically most similar to an individual in another population sample, suggesting that gene flow is high. Inspection of the distribution of individual fragment profiles, composite haplotypes (clones) and a maximum parsimony phylogenetic tree of clones reveals no evidence of geographic variation. Thus, the mtDNA gene tree is paraphyletic with respect to the "plumage" tree of *Q. quiscula*. The mtDNA data do not reflect the historical event that lead to the origin of either Purple or Bronzed grackles. The low diversity among clones ($P = 0.28\%$) suggests a recent common ancestry of clones and recent colonization of North America, which we suggest explains the lack of geographic variation; plumage evolution must therefore have occurred rapidly, likely enhanced by sexual selection. The separation of *quiscula* and *niger* occurred 1-2 MY ago, but it is not clear that these species are sister taxa, whereas it is likely that *major* and *mexicanus* are.

Key words: Common Grackle; *Quiscalus quiscula*; mitochondrial DNA; population structure; gene flow.

INTRODUCTION

The advent and widespread application of molecular methods of detecting genetic variation have greatly facilitated empirical estimates of the degree of population subdivision and its relation (if any) to geographic barriers, and the nature and extent of gene flow (Avise et al. 1987). New methods of data analysis further encourage the molecular approach to genetic analysis of population structure and geographic variation (e.g., Slatkin and Maddison 1989). Geographic variation, in effect a measure of population structure, was previously studied by comparisons of external morphology. Studies of geographic variation in birds, especially, figured prominently in the

development of ideas concerning the evolution of populations (Mayr 1963). However, the degree to which morphological patterns of avian geographic variation are genetically based is rarely tested. It is appropriate therefore, that modern molecular approaches be used to describe genetic variation within and among avian populations to provide a new perspective on geographic variation.

Although ornithologists lagged behind workers in other fields in applying techniques of protein (allozyme) electrophoresis to problems in geographic variation, there is a moderate data base now available for birds from the New World temperate zone (Evans 1987, Zink 1988). In general, most allozyme studies have revealed little or no intraspecific genetic differentiation (Barrowclough 1983). In contrast, analysis of the geography of mitochondrial DNA (mtDNA) differentiation has yielded mixed results in avian

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species. Ball et al. (1988) found little geographic structure in the Red-winged Blackbird (*Agelaius phoeniceus*), whereas Avise and Nelson (1989) found considerable differentiation between populations of the Seaside Sparrow (*Ammodramus maritimus*). In samples taken from nine geographic sites in western North America, Zink (in press) found geographically segregating mtDNA differences in Fox Sparrows (*Passerella iliaca*), whereas in samples of Song Sparrows (*Melospiza melodia*) taken at the same sites there was some mtDNA differentiation, but it occurred in a geographically mosaic pattern. These studies reveal that temperate-breeding avian species might be more genetically substructured than predicted by the bulk of allozyme studies (Zink, in press), which necessitates additional mtDNA surveys.

Another aspect of population structure that can be addressed with mtDNA concerns neutral, or mutation-genetic drift, theory. Specifically, Avise et al. (1988) concluded that under the assumption of selective neutrality (i.e., mutation and genetic drift), a rate of evolution of 2% per million years (MY), and a long-term effective population size equal to current population size, several vertebrate species with seemingly high gene flow exhibit far shorter times to common ancestry (i.e., less inter-clone diversity) than expected. They concluded that long-term effective population sizes were much lower than current population sizes, a conclusion independently reached by Barrowclough and Shields (1984) for birds. Avise et al.'s methods and analysis deserve extension to other species to assess the generality of their conclusions. This is especially true because the distributions of alleles detected by protein electrophoresis within and among avian populations are consistent with neutral theory (Barrowclough et al. 1985).

We studied mtDNA variation in samples of the Common Grackle (*Quiscalus quiscula*). This abundant species consists of two forms, formerly considered separate species, the Bronzed Grackle (*Q. q. versicolor*), and Purple Grackle (*Q. q. quiscula*). The forms differ ecologically, in plumage color, and morphologically (Yang and Selander 1968); the Purple Grackle has a relatively stouter bill and forages nearer the ground and on more aquatic prey. Purple Grackles occur in a fairly narrow zone along the east coast from the northern United States to Louisiana, except that they occur throughout Florida. Bronzed Grackles range throughout eastern Canada to Saskatche-

wan, south to New Mexico and the eastern United States. A hybrid zone stretches from the northeastern United States, southward to Virginia, where it is 200–400 km in width, and westward to Louisiana, where it is very narrow (Huntington 1952, Yang and Selander 1968). Yang and Selander (1968) noted that the hybrid zone in Louisiana seemed to have shifted northward between 1930 and 1965, but remained narrow, and that introgression of genes of the Purple Grackle into the Bronzed Grackle was much less than the reverse. Studies of the hybrid zone revealed non-assortative mating, which suggested that the forms were one biological species (Yang and Selander 1968). Moore and Dolbeer (1989) studied dispersal characteristics of *Q. quisculus* and concluded that gene flow is high. Selander (in Yang and Selander 1968) suggested that the Purple Grackle was closely related to grackle species in the Caribbean, in particular the Greater Antillean Grackle (*Q. niger*), and postulated that a *niger*-type grackle invaded Florida and subsequently spread into North America. The Bronzed Grackle was therefore derived and the Purple Grackle "ancestral." Selander did not specify where the bronzed phenotype arose.

Questions we addressed included: what is the geographic nature of mtDNA variation among populations, is there evidence of high gene flow as postulated by Moore and Dolbeer (1989), do mtDNAs of Bronzed and Purple grackles differ, does the distribution of times to common ancestry of clones conform to neutral predictions, and is *Q. niger* closer to the purple than to the Bronzed Grackle? If Selander's hypothesis is correct, clonal diversity and differences among clones should be greatest in the Purple Grackle because it has been in place longer, and these measures should decrease in the Bronzed Grackle to the northwest, the presumed path of colonization following glacial retreats. To test Selander's hypothesis further, we determined the phylogenetic relationships of *Q. quiscula*, *Q. niger*, and two other congeners, the Boat-tailed Grackle (*Q. major*), and the Great-tailed Grackle (*Q. mexicanus*).

METHODS

The following 35 specimens of *Q. quiscula* were used (general locality, *n*): New York (3), Wisconsin (4), Minnesota (5), Saskatchewan (4), Missouri (5), northern Louisiana (3), southern Louisiana (5), and North Carolina (6) (see Fig.

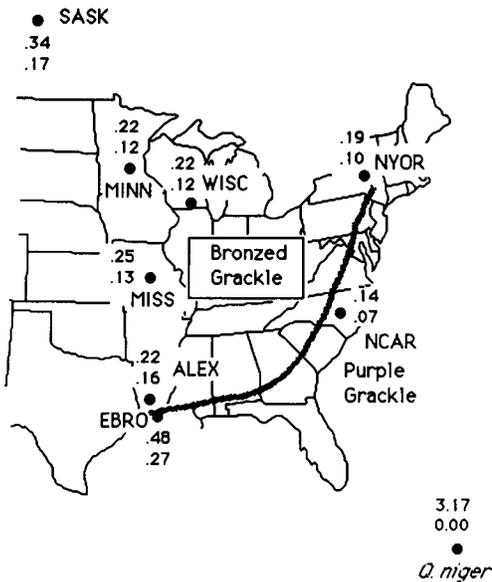


FIGURE 1. Map of collecting sites and their identifying acronyms, percentage nucleotide difference among clones within localities (bottom number), and percentage nucleotide difference from the all-A clone (clone 1; top number). Approximate center of hybrid zone derived from literature.

1); the only sample of "pure" Purple Grackles was that from North Carolina. In addition, four *Q. niger*, three *Q. major*, and three *Q. mexicanus* were analyzed. MtDNA was isolated from frozen tissue and purified in cesium chloride density equilibrium gradients following established protocols (Lansman et al. 1981, Avise and Zink 1988). MtDNA from each individual was digested with 20 restriction endonucleases and fragments were end-labeled with ^{32}P and ^{35}S , separated in agarose gels of 0.8% to 1.5%, and visualized with autoradiography. A molecular size standard, a 1 kb ladder purchased from Bethesda Research Laboratories, was used to determine the sizes of mtDNA fragments. For each individual the fragment pattern for each enzyme was scored, and the scores tallied into a multi-endonuclease haplotype. In addition, each individual was scored for the presence or absence of each fragment. Although restriction site maps are more informative than fragment analyses, restriction fragments do qualify as characters for phylogenetic analysis (Zink and Avise 1990). Using estimates of fragment sizes, restriction sites were inferred from the fragment profiles, and individuals were also scored for the presence and

absence of sites. Some fragment profiles, those derived from endonucleases with four-base recognition sites, were too complicated for inference of sites, and thus both fragments and sites were analyzed separately. We computed genotypic diversity, G (Nei 1987) as $(n/n - 1) (1 - \sum f_i^2)$, where f_i is the frequency of the i^{th} mtDNA haplotype in a sample of n individuals. This value, similar to single-locus heterozygosity, gives the probability that random pairs of individuals surveyed have different mtDNA haplotypes. The presence/absence matrix of fragments was used to compute the percent nucleotide divergence (p) following Nei and Li (1979). We used the simulation results of Avise et al. (1988) to investigate the times to common ancestry in *Q. quiscula* expected under neutral theory; we converted our estimates of p into generations using the formula $t = [0.5 \times 10^8(p)]/3$, which assumes that mtDNA evolves at a rate of 2%/MY (Shields and Wilson 1987) and that three years is a reasonable estimate of generation length in grackles. For phylogenetic analysis, we analyzed the data in two parts. First, we entered the fragment and site data into HENNIG86, written by J. S. Farris, to approximate the most parsimonious tree using Wagner parsimony for each clone of *Q. quiscula*, with *Q. niger* and *Q. mexicanus* designated as outgroups. To estimate phylogenetic relationships at the species level, we eliminated all but three *quiscula* (chosen to represent diversity within the species) from the foregoing analysis of fragments and added *major*.

RESULTS

Q. quiscula. On average, 96 restriction fragments were scored for each individual. Based on 10 endonucleases that generated mtDNA fragments between 500 and 9,500 base pairs, the size of the mtDNA molecule was estimated at 16,650 (± 298 , SD), typical for passerine birds (Shields and Helm-Bychowski 1987). Nine endonucleases (*Sst* II, *EcoR* I, *Nco* I, *Ava* I, *Bcl* I, *Bgl* I, *Bgl* II, *Pvu* II, *BamH* I) failed to produce more than one pattern (Table 1). The other 11 endonucleases each yielded from two to nine (*Rsa* I) patterns; in total, 29 clones were observed in the 35 birds. Two birds possessed clone 10 (NCAR 86, MINN 111) and six birds from four sites possessed clone 1 (ALEX 34, MISS 113, MISS 114, NYOR 87, NCAR 84, NCAR 101). Thus, for the single mtDNA genome, there were 29 clones, with most individuals possessing a unique clone; the most

TABLE 1. MtDNA haplotypes for grackles. Letters refer to restriction fragment profiles; adjacency in alphabet does not imply genetic relatedness. An asterisk marks the six occurrences of clone 1, and two asterisks mark the two occurrences of clone 10. Sequence of restriction enzymes is: *Ava* I, *Ava* II, *Bam*H I, *Bcl* I, *Bgl* I, *Bgl* II, *Hinc* II, *Kpn* I, *Nci* I, *Nde* I, *Pst* I, *Pvu* II, *Stu* I, *Xba* I, *Hinf* I, *Rsa* I, *Cfo* I, *Sst* II, *Eco*R I, and *Nco* I. Note that all individuals of *Q. niger* share clone 30, all *Q. major* share clone 33, and two *Q. mexicanus* share clone 32.

Clone	Specimen ID*	Composite haplotype																	
<i>Quiscalus quiscula</i>																			
1*	ALEX 34	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2	ALEX 35	B	.	C	C	.	.
3	ALEX 37	C	C	.
4	EBRO 102	.	B	B	.	.	B	.	.
5	EBRO 103	B
6	EBRO 104	B	.	.	C	.	.
7	EBRO 98	C	.	.
8	EBRO 105	C	D	E	.
9	NCAR 85	.	C
10**	NCAR 86	B
11	NCAR 99	J	.	.
12	NCAR 100	C	C
1*	NCAR 101
1*	NCAR 84
13	SASK 121	E	.	.	.
14	SASK 122	F	.	E	.
15	SASK 123	C	H	.	.
16	SASK 124	E	.	H	C	.	.	.
17	NYOR 90	.	E	B
1*	NYOR 87
18	NYOR 89	C
1*	MISS 113
1*	MISS 114
19	MISS 115	C	D	E	.	.
20	MISS 118	B	I	L	.	.
21	MISS 119	F	.	.
22	MINN 72	B	B	.	.
23	MINN 106	B
24	MINN 107	B
25	MINN 110	B	.	K	.	.	.
10**	MINN 111	B
26	WISC 91	B
27	WISC 92	C
28	WISC 96	B	B
29	WISC 95	.	G
<i>Quiscalus niger</i>																			
30	GAGR 1-4	B	F	B	B	C	B	D	B	D	B	C	.	F	B	G	I	F	.
<i>Quiscalus mexicanus</i>																			
31	GTGR 1	B	D	.	.	B	.	E	B	E	C	C	B	G	B	C	E	D	.
32	GTGR 2-3	B	D	.	.	B	.	E	B	E	C	C	B	D	B	C	E	D	.
<i>Quiscalus major</i>																			
33	BTGR 1-3	B	H	.	.	D	.	E	B	F	.	C	B	H	B	H	M	D	.

* For specimen location codes see Fig. 1.

common "allele," clone 1, occurred in an overall frequency of 15%. The large number of rare alleles and our small sample sizes do not warrant calculation of F_{st} . Genotypic diversity, G , was 0.97. Percentage nucleotide difference among clones averaged 0.28, and the range was 0 (sev-

eral comparisons) to 0.98% (EBRO 102 vs. EBRO 105); the matrix of p -values is available from the senior author or can be computed from the data in Appendix 1. There were too many clones to permit exhaustive searches for the most parsimonious tree(s); using a heuristic search (options

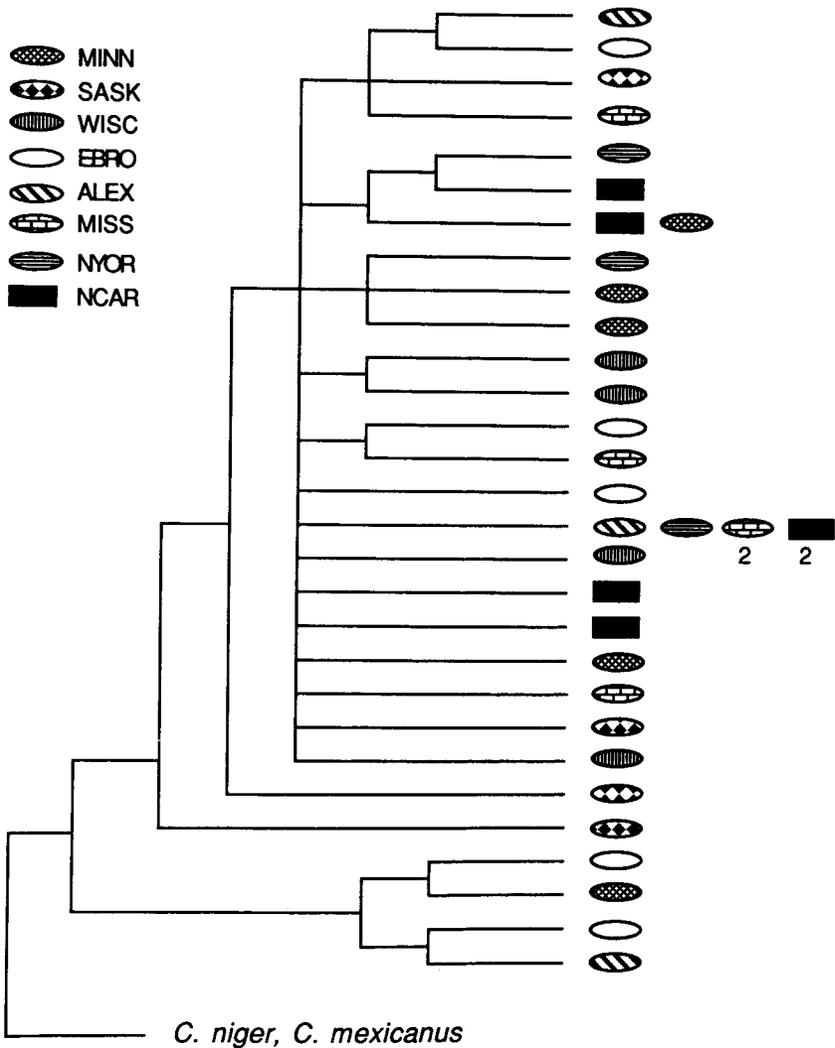


FIGURE 2. Tree relating mtDNA clones derived using maximum parsimony and rooted at *Q. niger* and *Q. mexicanus*. The ci = 0.78 and ri = 0.64.

m* and bb) in HENNIG86, 100+ equally parsimonious trees of 193 steps (consistency index = 0.78, retention index = 0.64) were found for the fragment data; an example of one is shown in Figure 2. This tree, like the others and those derived from the distribution of restriction sites (not shown), reveals little geographic structure; clones from the same site were often not grouped together. Plotting the average inter-clone difference reveals that the Saskatchewan and Baton Rouge samples are the most differentiated *intra se*, with values generally decreasing from Saskatchewan to North Carolina (Fig. 1). Time in generations to common ancestry of the 29 clones

(Fig. 3) resembles that observed by Avise et al. (1988) for *Agelaius phoeniceus*, but not that predicted from neutral expectation based either on a long-term population size of 20,000,000 (not shown, see Fig. 1 of Avise et al. [1988]) or 36,700 (Fig. 3).

Other species. The four specimens of *Q. niger* each possessed clone 30, the three individuals of *Q. major* each possessed clone 33, whereas there were two clones (31, 32) represented in the three *Q. mexicanus* (Table 1). Interspecific *p*-values ranged from 1.5% (*mexicanus* vs. *major*) to 4.3% (*niger* vs. *major*; Table 2). The sample of *Q. niger* differed from *Q. quiscula* at an average *p* of 3.47%,

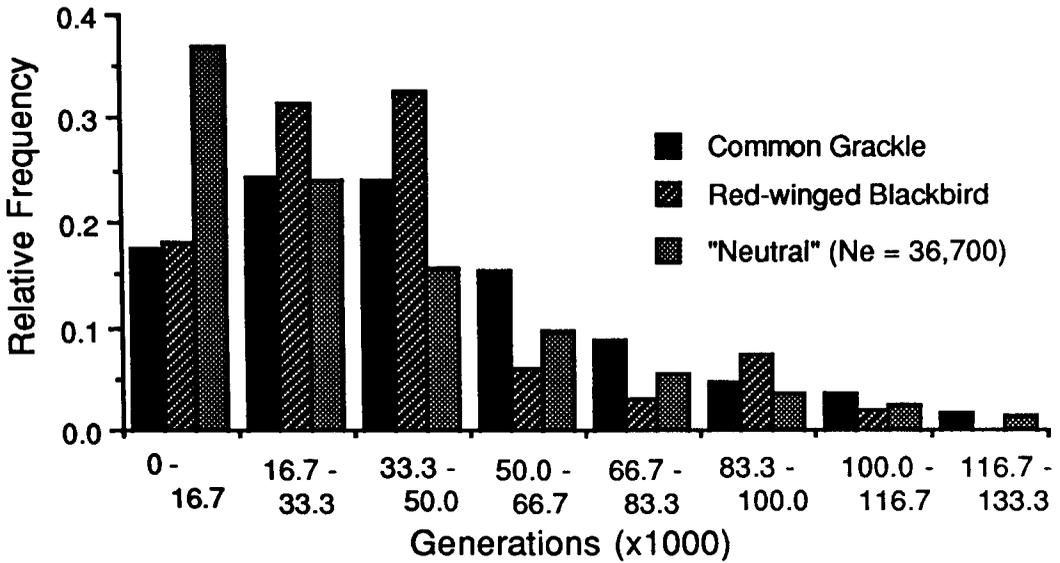


FIGURE 3. Distribution of times in generations to common ancestry for Red-winged Blackbird (data from Avise et al. [1988]), Common Grackle (this study), and the neutral prediction for $N_e = 36,700$ (from Avise et al. [1988]). One Common Grackle is not shown at a separation of 165,000 generations.

and *niger* was most similar ($p = 3.07\%$) to *mexicanus*. The phylogenetic network (Fig. 4) of species, rooted arbitrarily at *Q. niger*, shows *mexicanus* and *major* as sister taxa, and this clade is a sister taxon of *quiscula*.

DISCUSSION

LEVELS OF VARIATION

The number of clones in grackles is high, relative to other surveys that used a similar array of restriction endonucleases. For example, Ball et al. (1988) observed 34 clones in 124 Red-winged Blackbirds taken from throughout the range. Zink (in press) found five clones in 27 Song Sparrows (*Melospiza melodia*) and 5 clones in 46 Fox Sparrows (*Passerella iliaca*) taken in the western United States. The relatively high number of rare clones (alleles) is perhaps indicative of a non-equilibrium population structure (Maruyama and Fuerst 1984). Genotypic diversity in *Q. quiscula*, 0.97, is high, although most birds exhibit values in excess of 0.70 (Avise, unpubl. ms.). Thus, mtDNA polymorphism is a frequent phenomenon in avian species.

TIMES TO COMMON ANCESTRY

Avise et al. (1988) found that times to common ancestry of mtDNA clones in several high-gene-flow vertebrate species were far lower than those expected from neutral theory; as caveats they

noted that their tests used estimates of rate of evolution and long-term effective population sizes that were best guesses. They concluded that either the rate of mtDNA evolution commonly cited, 2% per million years, or a long-term effective population size of 20,000,000, are off by two to three orders of magnitude. They suggested, for example, that to bring observed and expected times to common ancestry in line, an effective population size of 36,700 would be needed for *A. phoeniceus* (Fig. 3). *Quiscalus quiscula* and *A. phoeniceus* have very similar mtDNA

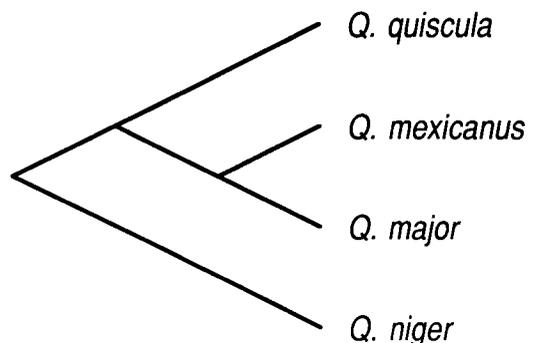


FIGURE 4. Single most parsimonious tree (151 steps, $ci = 93$, $ri = 87$) for species of grackles including three *quiscula* (EBRO 102, EBRO 105, ALEX 34 from Appendix), the latter of which are not distinguished on the phylogeny (they are nearest relatives).

population structures, and we hypothesize that the current effective population sizes of breeding females are similar. The distributions of times to common ancestry of clones detected in *Q. quiscula* and *A. phoeniceus* (Fig. 3) are very similar. *Quiscalus quiscula*, like *A. phoeniceus*, exhibits a distribution of times to common ancestry that conflicts markedly with neutral expectation if the long-term effective population size is 20,000,000 (Fig. 1 of Avise et al. [1988]). Both species show a closer fit to expectation if long-term effective population size is 36,700 (Fig. 3). However, in both species the shape of the distribution differs from expectation, especially in the relatively low number of clones observed with ancestry tracing to within 16,700 generations. Avise et al. (1988) suggested that this distribution pattern of times to common ancestry was due to slight geographic structure in *A. phoeniceus*. In grackles, however, geographic variation in mtDNA characters appears lacking. However, Ball et al. (1990) reported that because of non-independence of mtDNA lineages, the expected distribution of times to common ancestry might differ from that shown; they could find no consistent bias that would alter the nature of the expected distribution in a predictable fashion. Thus, the similar distributions of times to common ancestry in grackles and blackbirds suggest common processes affecting mtDNA lineage sorting, but it is not possible to discern the effects, if any, of natural selection.

We think it likely that *Q. quiscula* populations have expanded greatly in recent years owing to human-induced alterations in habitat (Yang and Selander 1968). We agree with Avise et al. (1988) that the long-term effective population size is orders of magnitude smaller than current N_e . Barrowclough and Shields (1984) suggested that avian effective population sizes were on the order of hundreds, much lower than that suggested by Avise et al. (1988). Clearly a consensus on effective size of avian populations is lacking. Because avian allozymic variation seems consistent with neutral theory (Barrowclough et al. 1985), it is relevant to continue to explore the behavior of the mtDNA "gene," especially because many assume that mtDNA evolves at a uniform rate, whereas others do not (Moritz et al. 1987).

GEOGRAPHY OF MTDNA VARIATION IN *Q. QUISCU*

Our mtDNA comparisons of *Q. quiscula* revealed a relatively high number of closely related

clones, which implies recency of common clonal ancestry. The level of similarity among clones, $\bar{p} = 0.28\%$, is consistent with that observed in some avian intraspecific studies (Avise and Zink 1988) but not all (Zink, in press). The degree of mtDNA differentiation over 2,000 km (Saskatchewan vs. North Carolina) is low relative to some other vertebrates (Kessler and Avise 1985), but consistent with that found in surveys of allozyme variation in birds (Barrowclough 1983). Although our sample sizes are small, the nature of variation uncovered by our analysis makes it unlikely that additional samples would change our results qualitatively.

The distributions of mtDNA haplotypes (Table 1) and fragments (Appendix 1) reveal no evidence of geographic structure. Because most individuals exhibited the same pattern (all "A") for most restriction enzymes, we examined the geographic distribution of intra-sample mean differences from clone 1 (that was found in six birds; Table 1). There is a trend for difference from the A clone to decrease from Saskatchewan to North Carolina and northern but not southern (Baton Rouge) Louisiana (Fig. 1). Therefore, one might predict that populations in Saskatchewan and southern Louisiana are relatively old. The increased variation in the Baton Rouge sample might reflect its position in the center of the hybrid zone (Fig. 1). Saskatchewan grackles might differ relatively more from the composite A pattern because they immigrated historically from several directions. However, such conclusions are justified only if clones in a sample are descended from an ancestral clone in that population, an assumption we cannot make. Phylogenetic analysis of mtDNA data failed to produce a tree (e.g., Fig. 2) that grouped samples consistent with their geographic locations. To test this relationship further, and because we could not find the shortest tree, we input a tree into HENNIG86 that grouped all individuals by sampling locality. This tree required 267 steps to explain the mtDNA fragment data, relative to the 100+ trees that were 193 steps in length. We interpret this as strong evidence that there is not a geographically organized pattern to mtDNA variation; instead, it exhibits a mosaic pattern. There is no evidence of historical isolation of sufficient duration for a geographically ordered pattern of mtDNA variation to emerge.

Lack of a geographically structured phylogeny of alleles (clones) and the occurrence of clone 1 at four sites prevented use of Slatkin and Mad-

dison's (1989) approach for estimating gene flow (Slatkin, pers. comm.). They noted that the number of coalescent events (the occurrence of "sister" clones in different geographic samples) was positively associated with levels of gene flow (Nm). Another way of assessing gene flow, related to Slatkin and Maddison's (1989) approach, is to observe that 12 of 35 birds (34%) were genetically (p) most similar to a specimen from the same sample, 13 (37%) were most similar to a specimen in an adjacent sample (which were often hundreds of km apart; Fig. 1), and the remainder (10, 29%) were genetically most similar to a specimen in a sample at least two geographic sites removed. Thus, 66% of individuals surveyed seemed to have an ancestor that came from a different location, which is consistent with Moore and Dolbeer's (1989) conclusion that gene flow is high in *Q. quiscula*. This result would contrast markedly with a gene frequency approach, in which one could note that in the sample from Saskatchewan, for example, there are four unique alleles each in a frequency of 0.25. This could be interpreted as evidence of reduced gene flow (Slatkin 1985), were it not for the information on phylogenetic and geographic relations of clones (Slatkin and Maddison 1989).

The observation that there are broad regions of phenotypic uniformity corresponding to bronzed and purple phenotypes conflicts with the interpretation of high gene flow. Specimens of grackles from the hybrid zone, such as those breeding in Baton Rouge, exhibit obviously intermediate plumages, and therefore, high levels of gene flow would be detectable in specimens far from the hybrid zone. Because plumage variation is geographically organized, we suggest that the mtDNA gene genealogy is paraphyletic with respect to the "plumage" tree; specimens of Purple Grackles (the six NCAR specimens; Table 1) are interspersed among the bronzed individuals (Fig. 2). Several explanations exist. One might postulate strong natural selection for plumages, which would counteract high gene flow. This seems unlikely in that the selective load would be enormous. Sex-biased dispersal can yield discordant nuclear (e.g., plumage) and single-gene organellar genealogies (Neigel and Avise 1986). However, dispersal distances in male and female grackles are equivalent because young female grackles disperse farther than males, but the situation is reversed for adults (Moore and Dolbeer 1989). Because mtDNA is inherited as a single linkage group (i.e., a single gene), one might ex-

pect discordance with nuclear gene genealogies owing to stochastic lineage sorting (Neigel and Avise 1986, Pamilo and Nei 1988).

We favor the explanation that the seemingly high current gene flow is an artifact of recent and extensive range expansion, and that stochastic lineage sorting explains the mtDNA-plumage discordance. The degree of differentiation among individuals is low, indicating that continental clones share a recent common ancestry. Extensive range expansion is consistent with the geographically chaotic distribution of haplotype ancestry (Fig. 2). Thus, the lack of geographically ordered mtDNA variation is a result of recent range expansion, and not necessarily a reflection of current gene flow. Evolution of the bronzed phenotype, a polygenic trait almost certainly influenced by sexual selection, must have occurred more rapidly than the time required for mtDNA clones within a sample to trace their ancestry to a common clone at that site. Because grackles do not breed in continuous forest, and much of eastern North America was forested until a few hundred years ago, it seems likely geographic isolation was a factor in the evolution of bronzed (or purple, see below) grackles. Alternatively, sexual selection could drive plumage evolution rapidly without geographic isolation. The minimal conditions of our model of evolution in *Q. quiscula* include 1) recent isolation of a group of grackles in which the bronzed phenotype evolved rapidly, and 2) extensive and rapid range expansion throughout eastern North America, including the formation of the hybrid zone; note that the two steps need not be in this order. Although gene flow is likely high at least within the two major forms of grackle (Moore and Dolbeer 1989), we doubt that there is a continent-wide gene exchange between the two taxa—rather, this is an illusion owing to nonequilibrium, or retained ancestral polymorphism.

EVOLUTION OF GRACKLE SPECIES

The proper context in which to evaluate Selander's hypothesis is a phylogenetic one. However, because we cannot root our tree (Fig. 4) without an additional taxon, interpretation of the direction of plumage evolution is not possible—if the root is indicated between *mexicanus/major* and the other taxa, then *quiscula* and *niger* would be sister taxa and Selander's hypothesis would be consistent with phylogeny. If mtDNA evolution is clocklike, which is controversial, then Selander's hypothesis is falsified by the observation

TABLE 2. Matrix of *p*-values among species of grackles. The first three individuals are *Q. quiscula* and are identified in Table 1.

EBRO 102	0.0000					
EBRO 105	0.0098	0.0000				
ALEX 34	0.0056	0.0044	0.0000			
<i>Q. mexicanus</i>	0.0317	0.0348	0.0288	0.0000		
<i>Q. niger</i>	0.0354	0.0370	0.0317	0.0307	0.0000	
<i>Q. major</i>	0.0367	0.0389	0.0323	0.0153	0.0426	0.0000

that *Q. niger* is genetically more similar to *Q. mexicanus* than to *Q. quiscula* (Table 2). If bronzed *quiscula* was the basal taxon, the purple phenotype would be derived, and Caribbean grackles could have been derived from an ancestor of the Purple Grackle, which is consistent with the lack of clonal variation in our four *niger* if a founder event occurred.

Neigel and Avise (1986) noted that, with respect to nuclear genome evolution, mtDNA lineages exhibit a transition from polyphyly to paraphyly to monophyly, with the last stage occurring approximately 4N generations after cessation of gene flow, where N is the population size. In our study, times to common ancestry of the oldest clones within *quiscula* do not predate the age of the lineage. That is, *quiscula* differs by an average *p*-value of 3.17% from its nearest relative in our survey (Table 2), which corresponds to 500,000 generations, whereas the most different clones currently existing within the species (Fig. 3) trace their common ancestry to a clone that existed ca. 165,000 generations ago. Therefore, with respect to the species-level comparison, mtDNA has reached a stage of monophyly (Neigel and Avise 1986), whereas this is not apparently true in comparison of mtDNA and plumage evolution in the bronzed and purple forms of *Q. quiscula* (Fig. 2).

Selander's hypothesis is not consistent with either the average difference between clones (Fig. 1) or the phylogenetic array (Fig. 2), because neither supports an invasion of southeastern North America followed by range expansion, colonization, and differentiation, as Barrowclough and Johnson (1988) suggested for the Swamp Sparrow (*Melospiza georgiana*). In fact, the reverse seems true for the intrasample differences between grackle clones (Fig. 1). It is possible, given the clonal variation in the Saskatchewan sample, that a refugium existed in the north allowing the evolution of the bronzed phenotype.

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APPENDIX. Presence and absence of mtDNA restriction fragments in grackles. Omitted are NCAR 101, NCAR 84, NYOR 87, MISS 113, and MISS 114, which have the same fragment profile ("1") as ALEX 34, MINN 111, which has the same fragment profile ("10") as NCAR 86, and one *Q. mexicanus* which differs by two unique fragments from conspecifics. All *Q. niger* and *Q. major* possessed identical fragment profiles for all endonucleases. See footnote ^a.

Q. quiscula

SASK 121

11110111111110000000001001111010011110-
000011101111110000000000111011111010-
00000111111100000001111001111100001111-
11000111000111000111111111000000000111-
1110000111111000000000

SASK 122

11110111111110000000001001111010011110-
0000111010111011000000000111011111001-
0000011111110000000111001111100001111-
11000111000111000111111111000000000111-
1110000111111000000000

SASK 123

11110111111110000000001001111010011110-
00001110111111000000000111111111000-
000001101111100001101111001111100001111-
11000111000111000111111111000000000111-
1110000101111000010000

SASK 124

11110111111110000000001001111010011110-
00001110111111000000000111111111000-
1000011111110000000111001111100001111-
11000111000111000111111010000001000111-
11100001101111010000000

MISS 115

11110111111110000000001001111010011110-
00001110111111000000000111001111100-
00000111111100000001001101111100001111-
11000111000111000111111111000000000111-
1110000110111000100000

MISS 118

11110111111110000000001001111010011110-
00001110111111000000000111101111000-
00000111111100000001100011111100001111-
11000111000111000111111111000000000111-
11100001101101010000000

MISS 119

11110111111110000000001001111010011110-
00001110111111000000000111111111000-
0000011111110000000111001111100001111-
11000111000111000111111111000000000111-
111000011101100001000

MINN 72

11110111111110000000001001111010011110-
00001110111111000000000111111111000-
0000011111110000000111001111100001101-
1000111000111000111111111000000000111-
1110000110111100000000

APPENDIX. Continued.

MINN 106

11110111111110000000001001111010011110-
0000111011111110000000000111111111000-
0000011111110000000111001111100001101-
10001111000111000111111111000000000111-
1110000111111000000000

MINN 107

11110111111110000000001001111010011110-
0000111011111110000000000111111111000-
0000011111110000000111001111100001111-
1100001101111000111111111000000000111-
1110000111111000000000

MINN 110

11110111111110000000001001111010011110-
0000111011111110000000000111111111000-
00000111111100000001110011111100001111-
11000111000111000111111111000000000110-
11010001110111000000000

NYOR 89

11110111111110000000001001111010011110-
0000111011111110000000000111111111000-
00000111111100000001110001011100101111-
11000111000111000111111111000000000111-
1110000111111000000000

NYOR 90

1111011011111100000000010001111010011110-
0000111011111110000000000111111111000-
00000111111100000001110011111100001101-
10001111000111000111111111000000000111-
1110000111111000000000

NCAR 85

1111001111111100100010001001111010011110-
0000111011111110000000000111111111000-
00000111111100000001110011111100001111-
11000111000111000111111111000000000111-
1110000111111000000000

NCAR 86

111101111111110000000001001111010011110-
0000111011111110000000000111111111000-
00000111111100000001110011010110001111-
11000111000111000111111111000000000111-
1110000111111000000000

NCAR 99

111101111111110000000001001111010011110-
0000111011111110000000000111111111000-
00000111111100000001110011111100001111-
11000111000111000111111111000000000111-
1110000111111000000001

NCAR 100

111101111111110000000001001111010011110-
0000111011111110000000000111111111000-
00000111111100000001110001011100101111-
11000111000111000111111111000000000110-
11101101111111000000000

APPENDIX. Continued.

WISC 91

11110111111110000000001001111010011110-
 0000111011111110000000000111111111000-
 0000011111110000000111001111100001111-
 11000111000111000011111110010000000111-
 1110000111111000000000

WISC 92

11110111111110000000001001111010011110-
 0000111011111110000000000111111111000-
 00000111111100000001110011111100001111-
 11000111000111000101111101100000000111-
 1110000111111000000000

WISC 95

11110111111110000000001010011010011110-
 0000111011111110000000000111111111000-
 00000111111100000001110011111100001111-
 1100011100011100011111111000000000111-
 1110000111111000000000

WISC 96

11110111111110000000001001111010011110-
 0000111011111110000000000111111111000-
 00000111111100000001110011111100001111-
 11000111000111000011111110010000000111-
 1110000111111000000000

EBRO 98

11110111111110000000001001111010011110-
 00001110101111110110000000111111111000-
 00000111111100000001110011111100001111-
 1100011100011100011111111000000000111-
 1110000110111010000000

EBRO 102

11110101111111000000001001111010011110-
 0000111010101111011100000111111111100-
 00000111111100000001110011111100001111-
 1100011100011100011111111000000000110-
 1101000111111000000000

EBRO 103

11110111111110000000001001111010011110-
 0000111011111110000000000111111111000-
 00000111111100000001110011111100001111-
 1100011100011100011111111000000000111-
 1110000111111000000000

EBRO 104

11110111111110000000001001111010011110-
 0000111011111110000000000111111111000-
 00000111111100000001110011111100001111-
 1100011100011100011111111000000000110-
 1101000111111000000000

APPENDIX. Continued.

EBRO 105

11110111111110000000001001111010011110-
 00001110101111101100000000011111111000-
 00000111111100000001001011111100001111-
 1100011100011100011111111000000000111-
 11100001010111000000110

ALEX 34

11110111111110000000001001111010011110-
 0000111011111110000000000111111111000-
 00000111111100000001110011111100001111-
 1100011100011100011111111000000000111-
 1110000111111000000000

ALEX 35

11110111111110000000001001111010011110-
 000011101011111101100000000111111111000-
 00000111111100000001110011111100001111-
 1100011100011100011111111000000000110-
 11010001101111010000000

ALEX 37

11110111111110000000001001111010011110-
 000011101011111101100000000111111111000-
 00000111111100000001110011111100001111-
 11000111000111000111111110000000000111-
 1110000111111000000000

Q. niger

1010111001011100000001101101100101100111-
 1111000100011111000001110001111000111000-
 000111110100011000010100011100000011101-
 10001000100011100001111101111000000011110-
 11010010111011000000000

Q. mexicanus

1010110101011100011100000001111010000011-
 1101111000010111000001111101111011111000-
 0110011100111001100011000111010001001111-
 1011000010000011101101010110011110000110-
 110100011110010010000000

Q. major^a

101010100100100000000000000011010010110-
 0000111000010111000001111101111011111000-
 0110011100111001100011000111011000001111-
 1100000010000011100100010110010110000110-
 11010001111000000000000

^a Order of restriction enzymes (# fragments) = *Ava* I (5), *Ava* II (24), *Bam*HI (3), *Bcl* I (3), *Bgl* I (9), *Bgl* II (4), *Cfo* I (19), *Eco*R I (3), *Hinf* I (15), *Hinc* II (16), *Kpn* I (5), *Nci* I (10), *Nco* I (1), *Nde* I (8), *Pst* I (6), *Pvu* II (6), *Stu* I (20), *Sst* II (2), *Xba* I (7), *Rsa* I (17).

^b 14 autapomorphous fragments were added to *major* in the interspecific analysis, with corresponding zeros for other species.