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Phylogeography and historical demography of Carib Grackle (*Quiscalus lugubris*)

Meghann B. Humphries^{1,2}, Maribel A. Gonzalez³, and Robert E. Ricklefs^{1,4}

Abstract We assessed the phylogeography of the Carib Grackle (*Quiscalus lugubris*), whose distribution includes eight subspecies in the Lesser Antilles and northern South America. We used the geographic distribution of variation in the mitochondrial genes ATPase 6 and ATPase 8 to assess the demographic history of the species and degree of concordance between phylogenetic relationships and subspecies assignments. We recovered a single haplotype in Guyana and French Guiana, which was shared by some samples from Trinidad, but Trinidad also hosts a second mitochondrial clade separated by 2.9% sequence divergence. Similarly, Venezuela is home to two sympatric clades separated by 3.6% divergence. Genetic relationships of island populations appeared largely discordant with currently described subspecies.

Keywords colonization, demography, introduced species, phylogeography, *Quiscalus lugubris*

Resumen Filogeografía y demografía histórica de *Quiscalus lugubris*—Evaluamos la filogeografía de *Quiscalus lugubris*, cuya distribución incluye ocho subespecies en las Antillas Menores y el norte de Sudamérica. Utilizamos la distribución geográfica de la variación en los genes mitocondriales ATPasa 6 y ATPasa 8 para evaluar la historia demográfica de la especie y el grado de concordancia entre las relaciones filogenéticas y las asignaciones de subespecies. Recuperamos un solo haplotipo en Guyana y Guyana Francesa, que fue compartido por algunas muestras de Trinidad. Trinidad también alberga un segundo clado mitocondrial separado por una divergencia secuencial del 2,9%. De manera similar, Venezuela alberga dos clados simpátricos separados por un 3,6% de divergencia. Las relaciones genéticas de las poblaciones de las islas fueron discordantes con las subespecies actualmente descritas.

Palabras clave colonización, demografía, especies introducidas, filogeografía, *Quiscalus lugubris*

Résumé Phylogéographie et démographie historique du Quiscale merle (*Quiscalus lugubris*)—Nous avons évalué la phylogéographie du Quiscale merle (*Quiscalus lugubris*), qui comprend huit sous-espèces dans les Petites Antilles et le nord de l'Amérique du Sud. Nous avons utilisé la répartition géographique de la variation des gènes mitochondriaux ATPase 6 et ATPase 8 pour évaluer l'histoire de la démographie de l'espèce et le degré de concordance entre les relations phylogénétiques et les affectations à des sous-espèces. Nous avons retrouvé un seul haplotype au Guyana et en Guyane française, partagé par quelques échantillons de Trinidad, mais Trinidad héberge également un deuxième clade mitochondrial séparé par une divergence de séquence de 2,9%. De même, le Venezuela abrite deux clades sympatriques séparés par une divergence de 3,6%. Les relations génétiques des populations insulaires semblaient en grande partie ne pas concorder avec les sous-espèces actuellement décrites.

Mots clés colonisation, démographie, espèces introduites, phylogéographie, *Quiscalus lugubris*

The New World blackbird genus *Quiscalus* (Icteridae), whose members are commonly known as grackles, exhibits a wide range of behaviors, morphologies, and ecological relationships across species (Griscom 1932, Ficken 1963, Yang and Selander 1968, Kok 1971, Howe 1978, Grabrucker and Grabrucker 2010). Populations of *Quiscalus* species have diverse demographic his-

stories involving natural colonization, local extinction, and rapid range expansions (Johnson and Lanyon 1999, Lanyon and Omland 1999, Christensen 2000, Wehtje 2003, DaCosta *et al.* 2008, Powell *et al.* 2008, Haemig 2011, 2012, 2014, Paulino *et al.* 2013), including a recent dramatic expansion into Colombia and Panama by Carib Grackle (*Q. lugubris*; Vallely and Dyer 2018). Land use changes have been considered the primary drivers of these population dynamics (Bond 1956, Miller *et al.* 2003) as grackles are generally tolerant of human activity and are considered commensal with human settlements, which provide many potential sources of food for the birds (Christensen 2000, Miller *et al.* 2003).

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Two described species of grackle occur in the West Indies, Greater Antillean Grackle (*Quiscalus niger*) and Carib Grackle (Fraga 2019). These species were formerly classified together in the genus *Holoquiscalus*, but molecular assessments have revealed a closer affinity of Carib Grackle to Nicaraguan Grackle (*Quiscalus nicaraguensis*) than to Greater Antillean Grackle, which is sister to a clade formed by Great-tailed Grackle (*Q. mexicanus*) and Boat-tailed Grackle (*Q. major*) (Johnson and Lanyon 1999, Lanyon and Omland 1999, DaCosta *et al.* 2008, Powell *et al.* 2008). These evolutionary relationships suggest that the West Indian archipelago may have been colonized from the north by Greater Antillean Grackle and from the south by Carib Grackle.

Carib Grackle has eight recognized subspecies divided into the Fortirostris and Lugubris groups (Fraga 2019). The Fortirostris group includes the subspecies *Q. l. fortirostris* on Barbados and *Q. l. contrusus* on Saint Vincent and is typified by blackish-brown females. The Lugubris group includes the remaining six races and is typified by paler brown females, although both male and female plumage coloration, body and bill size, and vocalizations vary among island populations of both groups (Fraga 2019).

Human facilitation appears to be a repeated feature of this group's geographic history. James Bond (1956) indicated frequent introductions throughout the West Indies as birds (of many species) often boarded ships and then dispersed at destination ports. Bond mentioned that Carib Grackle had been introduced to several of the smaller islands of the Lesser Antilles, including the population of Carib Grackle on Antigua, which he asserted was introduced between 1912 and 1914 (Bond 1956). No additional information about the Antigua introduction is known, except that earlier information provided by Ridgway (1902) supports the assertion that Carib Grackle (at that time, *Q. fortirostris*) was not present on Antigua before 1902. In another case, assessment of mitochondrial genetic diversity revealed that the Carib Grackle population on Barbados was introduced from a Trinidadian source, and that their sequences differed from those of populations on nearby Saint Vincent and Saint Lucia by 3.4% (Lovette *et al.* 1999). Finally, the population on

Aruba is purported to be the result of an introduction (Ridgely *et al.* 2005), though details concerning the origin of this population and the time of colonization are unknown.

In the present study, we used mtDNA sequences to assess the colonization history and genetic structure of Carib Grackle populations across most of its distribution in the Lesser Antilles and continental South America. We inferred evolutionary relationships and used the geographic distribution of genetic variation across the species' range to assess previously recognized subspecies limits.

Methods

Sample Collection

Birds were captured with mist nets and c. 10 µL of blood were obtained via sub-brachial venipuncture (field techniques described in Latta and Ricklefs [2010]). Populations at 14 locations were sampled in the West Indies and continental South America, with sample sizes ranging from 1 to 16 individuals per location (Table 1). DNA was extracted using the phenol-chloroform protocol described in Seutin *et al.* (1991), except that the final suspension was purified by dialysis rather than by ethanol precipitation. Mitochondrial ATP-synthase 8 (168 bp) and ATP-synthase 6 (684 bp) genes, which overlap by 10 base pairs, were amplified and sequenced using primers and protocols described in Hunt *et al.* (2001). Sequences were generated using an Applied Biosystems model 377 automated DNA sequencer and an ABI 3130xl capillary DNA sequencer (Thermo Fisher Scientific, Waltham, MA, USA). Sequenced gene fragments were aligned and proofread using Sequencher 4.5 (Gene Codes Corporation, Ann Arbor, MI, USA). Unique sequences were deposited in GenBank (accession numbers MG460151–MG460165 and MK524532–MK524575, pending annotation, and AD132426.1 and AF132425.1 previously reported by Lovette *et al.* [1999]).

Data Analysis

To visualize genetic relationships among sampled individuals, we generated a median-joining haplotype network (Bandelt *et*

Table 1. Carib Grackle group assignment, subspecies assignment, location, and sample size. One Brewer's Blackbird sampled in Siskiyou County, California, USA, served as the outgroup.

Region	Group	Subspecies	Sampling Location	N
Antigua	unknown	unknown	Willocks (17°04'52.8"N, 61°46'58.6"W)	2
Barbados	Fortirostris	<i>fortirostris</i>	Barclay's Park (13°11'37.9"N, 59°32'35.5"W)	2
Dominica	Lugubris	<i>gadeloupenis</i>	20 km N of Rouseau (15°24'54.0"N, 61°22'15.5"W)	1
French Guiana	Lugubris	<i>lugubris</i>	Cayenne (4°56'27.6"N, 52°20'08.0"W)	3
Grenada	Lugubris	<i>luminosus</i>	Mt. Hartman Estate (12°00'26.3"N, 61°45'19.4"W)	10
Guadeloupe	Lugubris	<i>gadeloupenis</i>	Duquerry (16°15'54.0"N, 61°33'03.6"W)	2
Guyana	Lugubris	<i>lugubris</i>	Ceiba Biological Centre (6°29'57.7"N, 58°13'07.2"W)	6
Martinique	Lugubris	<i>gadeloupenis</i>	Pointe Rouge (14°38'29.5"N, 61°01'27.0"W)	2
Montserrat	Lugubris	<i>gadeloupenis</i>	Plymouth Cemetery (16°44'32.9"N, 62°11'14.5"W)	2
Saint Lucia	Lugubris	<i>inflexirostris</i>	Anse la Sorciere (13°54'21.0"N, 60°59'25.6"W)	2
Saint Vincent	Fortirostris	<i>contrusus</i>	St. George Parish (13°13'21.5"N, 61°12'40.5"W)	1
Trinidad	Lugubris	<i>lugubris</i>	Caroni Swamp (10°35'17.2"N, 61°27'19.1"W)	16
Venezuela	Lugubris	<i>lugubris</i>	Power Line Rd (10°34'07.6"N, 63°46'31.9"W)	5
Venezuela	Lugubris	<i>lugubris</i>	Falcon Bocas del Tocuyo (11°03'35.7"N, 68°20'20.2"W)	6

al. 1999) using PopART (Leigh and Bryant 2015). We performed a partitioned Maximum Likelihood Analysis using RAXML HPC 8 (Stamatakis 2014) on the XSEDE computing cluster on the CIPRES Science Gateway (Miller *et al.* 2010). Nodal support was evaluated with 100 nonparametric bootstrap pseudoreplications. We visualized the final tree using FigTree 1.4.2 (tree.bio.ed.ac.uk/software/figtree/). We used the Brewer’s Blackbird (*Euphagus cyanocephalus*) as the outgroup, as previous work (Johnson and Lanyon 1999, Lanyon and Omland 1999) had shown *Euphagus* to be sister to the monophyletic clade of *Quiscalus* species.

Results

Assessment of phylogenetic relationships among the sampled Carib Grackle individuals revealed three mitochondrial clades (Figs. 1 and 2), the first of which had a disjunct distribution including Venezuela, Trinidad, Barbados, and Antigua (Fig. 3). The individuals from Antigua, Barbados, and four (of five) individuals from Trinidad in this clade had identical haplotypes. The fifth Trinidadian haplotype differed from these by nine nucleotide substitutions, some of which are shared with the Venezuelan individuals belonging to Clade 1. Clade 2 is continuously distributed through the Lesser Antilles, but also represented by two individuals captured in Venezuela; the clade is absent from our Trinidad sample (Figs. 1 and 2). Clade 3 occurred only within

continental South America. Our sampling locations in Venezuela produced individuals belonging to Clades 1 and 2, which were separated by 3.6% sequence divergence, while locations on Trinidad included individuals of Clades 1 and 3, which were separated by 2.9% sequence divergence. All other sampling locations included individuals of only one clade.

We found that the individual sampled on Saint Vincent (Fortirostris group, *Q. l. contrusus*) possessed a haplotype also detected on Grenada (Lugubris group, *Q. l. luminosus*) and closely allied with samples from the north-central Lesser Antilles (Lugubris group, *Q. l. guadeloupensis*). Samples from Saint Lucia (Lugubris group, *Q. l. inflexirostris*) were separated by only two mutations from two haplotypes found on Grenada (Lugubris group, *Q. l. luminosus*), Martinique (Lugubris group, *Q. l. guadeloupensis*), and Saint Vincent (Fortirostris group, *Q. l. contrusus*). Finally, we recovered shared haplotypes in Guyana, French Guiana, and Trinidad (spanning more than 1,100 km) and the absence of this haplotype in neighboring Venezuelan populations, which were more than 2% divergent.

Discussion

Utilizing the geographic distribution of contemporary genetic diversity, we inferred the phylogenetic relationships of representatives of six of the eight recognized subspecies of Carib

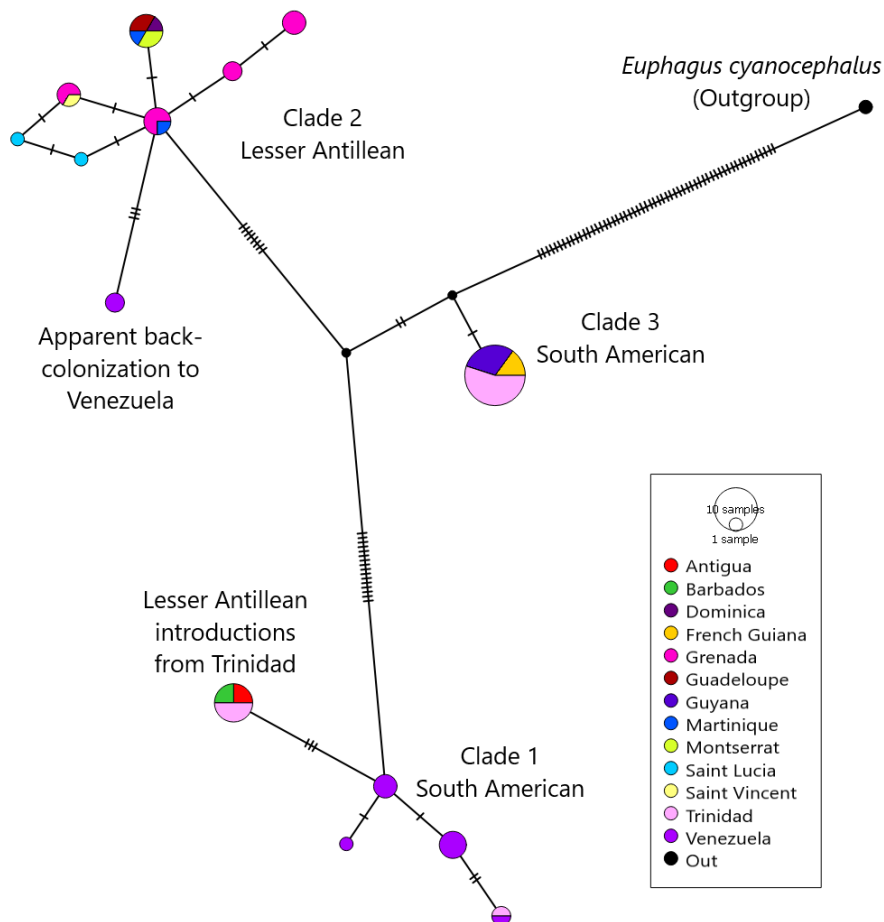


Fig. 1. Median-Joining Haplotype Network. Circle size indicates number of individuals with a given haplotype, circle color indicates sampling location, and mutations are indicated by hash marks.

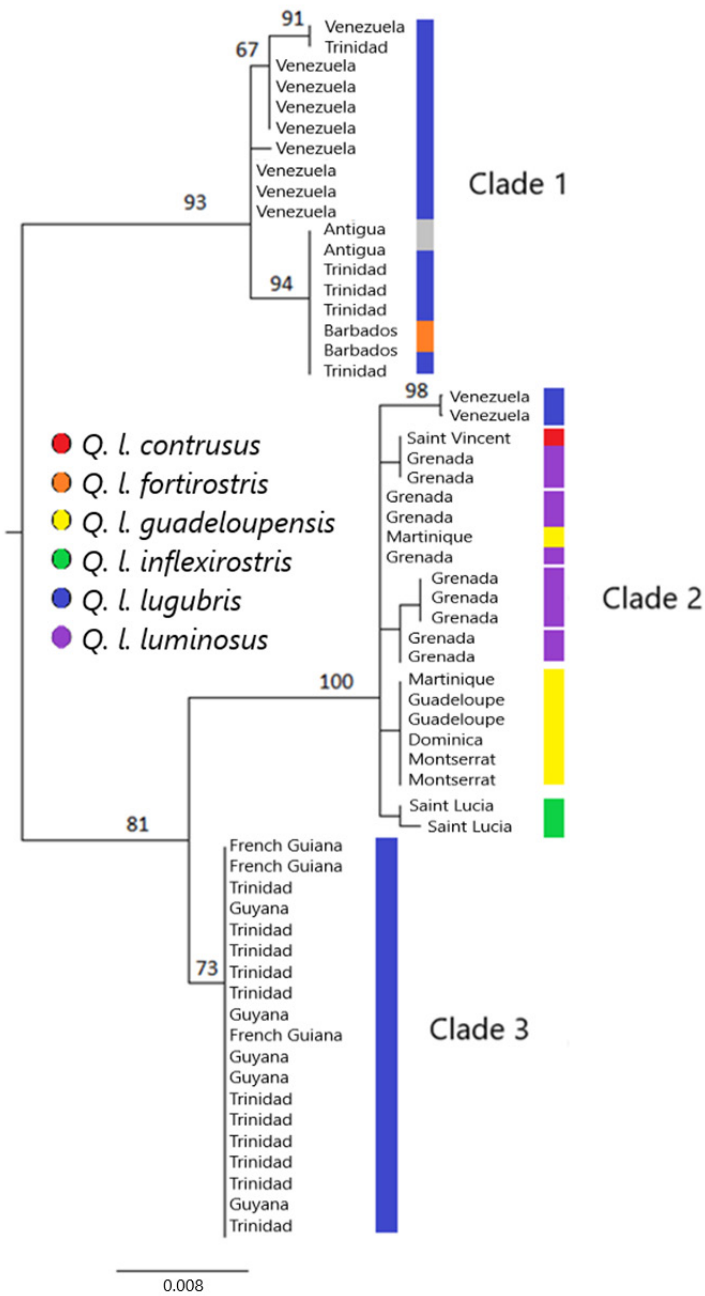


Fig. 2. Maximum Likelihood Tree. Branch labels are bootstrap values > 70 (outgroup removed). Colored boxes indicate subspecific assignment based on sampling locality.

Grackle across most of the range of the species. Our assessment revealed that subspecies names are not consistent with mitochondrial genetic relationships among locations. We found five subspecies nested within a primarily *Q. l. lugubris* clade and that *Q. l. guadeloupensis* and *Q. l. luminosus* are polyphyletic. We further determined that samples of *Q. l. fortirostris* on Barbados were genetically indistinct from samples on Trinidad and Antigua, which suggests that Trinidad was the source of the birds introduced to Antigua as well as Barbados (the latter previously reported by Lovette et al. [1999]).

In addition to discordance among subspecies identity and genetic relationships, we recovered sympatric clades in Venezuela

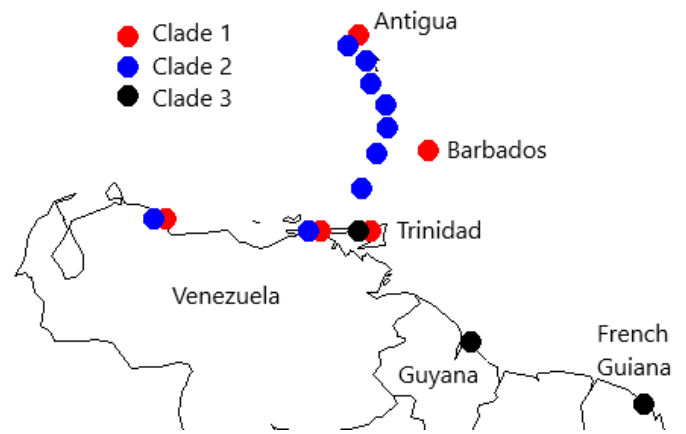


Fig. 3. Map of sampling localities and clade distributions (overlapping points displaced for clarity).

and Trinidad. A possible explanation for this could be back-colonization from the Lesser Antilles to South America, since one of the five haplotypes (represented by two individuals) detected in Venezuela belonged to the clade of otherwise Lesser Antillean samples. This haplotype was distinct from other Lesser Antillean sequences by 4 nucleotide substitutions and was more than 20 nucleotide substitutions distant from the other four Venezuelan haplotypes, suggesting that its presence in Venezuela is likely from an island source. Back-colonization, i.e., colonizing from the island chain to the mainland, has been reported for several other avian species as well as reptiles, amphibians, mammals, and invertebrates (Bellemain and Ricklefs 2008), though more thorough sampling within localities will be required to confirm whether this is the case here.

Differentiation of Venezuelan samples from the more easterly South American samples, which were themselves undifferentiated, and the apparent sympatry of these phylogenetically isolated groups on Trinidad, are intriguing. Further sampling of intracontinental localities will be necessary to determine whether the lack of diversity in the east and the increased diversity detected in Venezuela is due to limited sampling.

Regarding the origin of the Antiguan population, our findings are consistent with Bond's (1956) assertion that Carib Grackle was introduced by humans to Antigua, perhaps unintentionally, in the early part of the 20th century. The source of the Antiguan and Barbadian populations appears to be Trinidad (Lovette et al. 1999). We also note that a population of the Lesser Antillean clade (Clade 2), which is genetically identical throughout Martinique, Dominica, and Guadeloupe, is also present on Montserrat, but not on the intervening islands of Antigua and Barbuda, again suggesting the possibility of introduction. Interestingly, though we lack sufficient samples to determine whether Clade 2 populations are differentiated genetically among islands within the Lesser Antilles, differences in songs among island populations appear to be related to the degree of plumage variation of females, where the songs in populations with pale females are distinct from those with darker females (A. Jaramillo pers. comm.). Songs of birds in South America, Trinidad, and Tobago also differ from those in the Lesser Antilles, with Lesser Antillean songs being less complex with fewer trills (A. Jaramillo pers.

comm.). The degree to which these behavioral differences are consistent with the genetic patterns we identified will require additional investigation including increased genetic and behavioral sampling within islands and assessment of nuclear genetic variation.

Conclusion

In the present study, we utilized the distribution of contemporary mitochondrial genetic diversity to assess the phylogeographic history of Carib Grackle throughout most of its range. Our assessment revealed genetic relationships inconsistent with current subspecies designations of both island and mainland populations. We recovered three divergent mitochondrial clades across the species' range that are inconsistent with current subspecies groups, and with subspecific assignments of *Q. l. lugubris*, *Q. l. guadeloupensis*, *Q. l. luminosus*, and *Q. l. fortirostris*. We report previously unidentified sympatric populations in Venezuela and Trinidad, and differentiation between the eastern and western populations within northern South America. Finally, we found support for the assertion that the Antiguan population was introduced and determined that it, like the population on Barbados, was from a Trinidadian source.

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Authors declare that all field sampling and laboratory analyses complied with the current laws of the country in which they were performed.

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