pecked to access egg contents. Instead, breakage may facilitate grasping and removal of the egg, or simply render the egg inviable. Consumption of egg contents demands time, and the benefits of leaving immediately may outweigh the nutritional gains. The resident male guards his territory against intruders (Johnson and Kermott 1989) and checks the nesting cavity during the female's absence (i.e., when the female is foraging; S. Pribil, personal observation). If the intruder is confronted inside the cavity, it may sustain injuries or may even be killed by the resident male, as was suspected in two cases reported by Belles-Isles and Picman (1987).

Nesting cavities suitable for House Wrens are limited in our area. This is evident from the fact that the introduction of nesting boxes was followed by a dramatic increase in size of the study population from less than 2–4 pairs to 38–45 pairs in different years (J. Picman and S. Pribil, unpublished data). The observations of egg destruction followed by a cavity takeover support the hypothesis that the egg destroying behavior in House Wrens may have been favored by intense competition for nesting cavities. We cannot, however, establish the plausibility of the remaining hypotheses because a) the test specifically examined the nest site competition hypothesis; b) results of the test are not inconsistent with the other hypotheses; and c) the four hypotheses are not mutually exclusive. On the other hand, the fact that House Wrens apparently failed to consume contents of the broken eggs (i.e., spent a minimum amount of time handling the broken eggs) argues against the egg consumption hypothesis. Tests of the remaining hypotheses will require more information on the degree of foraging similarity between House Wrens and sympatric passerines, the movement of birds whose nests are destroyed by House Wrens, and the chance that the intruding male has to mate with the female whose nest the male destroyed.

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MITOCHONDRIAL DNA VARIATION INDICATES GENE FLOW ACROSS A ZONE OF KNOWN SECONDARY CONTACT BETWEEN TWO SUBSPECIES OF THE BROWN-HEADED COWBIRD[1]

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Key words: mitochondrial DNA; Brown-headed Cowbird; gene flow; hybridization.

The dynamics of gene flow are often easily observed and analyzed where differentiated taxa meet and interbreed in hybrid zones (Endler 1977; Barton and Hewitt 1985, 1989). In most studies, the amount of introgression of various traits or alleles is directly measured within and around such zones. However, few studies have historical data on the timing of secondary contact such that actual *rates* of introgression can be calculated (Endler 1977, Rand and Harrison 1989). Studies with historical data often involve species with well-documented range extensions (e.g., Gill 1980, Cooke et al. 1988, Fleischer and Rothstein 1988, Echelle and Connor 1989). Our studies of geographic variation in morphometric and colorimetric characters in the brown-headed cowbird have indicated recent and extensive gene flow between the differentiated subspecies *Molothrus ater obscurus* and *M. a. artemisiae* in the

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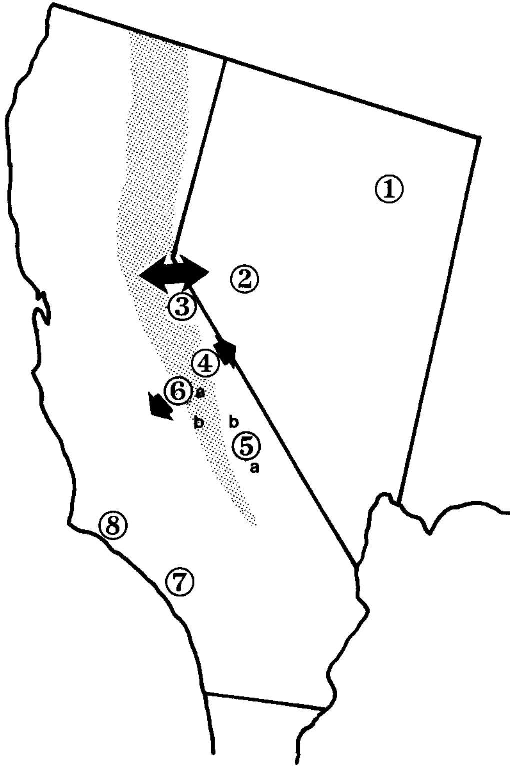

FIGURE 1. Map of California and Nevada showing the sites at which cowbirds were collected for this study. The sites, corresponding to the numerical and letter designations, are listed in Table 1. Note the position of the Sierra Nevada (stippled area) and two probable routes of intersubspecific gene flow at low passes (arrows) in the Lake Tahoe/Carson Valley drainage (north), and the Mammoth Lakes area (south).

central Sierra Nevada of California (Fleischer and Rothstein 1988). Here we present data on mitochondrial DNA (mtDNA) variation that are in accord with our prior conclusions of extensive gene flow between *obscurus* and *artemisiae.*

The subspecies *artemisiae* was known historically from the Great Basin, occurring strictly *east* of the Sierra Nevada and Cascade mountain ranges (Rothstein et al. 1980). *Obscurus* was known historically from southern Arizona to Texas and south throughout most of Mexico (Rothstein et al. 1980). From records cited in the literature (summarized in Rothstein et al. 1980, Laymon 1987, Fleischer and Rothstein 1988) we know approximately when *obscurus* expanded its range into southern California and could have made contact with *artemisiae* in the central Sierra Nevada. Cowbirds invaded Southern California from the lower Colorado River about 1900 and colonized regions as far north as San Francisco by 1930. The records of movement show that secondary contact between the two subspecies in the Sierra Nevada range began sometime during the previous 20 to 50 years (Laymon 1987, Rothstein et al. 1980).

The subspecies differ in overall size (*artemisiae* is larger than *obscurus*; Grinnell 1909, Rothstein et al. 1986, Table 2 in Fleischer and Rothstein 1988), nestling rictal flange color (*artemisiae* has white flanges, *obscurus* yellow; Rothstein 1978), and vocalizations (Rothstein et al. 1986, Rothstein and Fleischer 1987). The population from the presumed contact zone near Mammoth Lakes, Mono County, California (see Fig. 1) is intermediate in average body size and flange color frequencies (Fleischer and Rothstein 1988). Here we show that each subspecies predominantly exhibits alternative mtDNA restriction fragment profiles for mtDNA digested with *Pst*I. Populations predicted to contain both of the subspecies and intersubspecific hybrids because of historical and morphological data (Fleischer and Rothstein 1988) exhibit intermediate frequencies of the mtDNA haplotypes.

METHODS

Collections. Cowbirds were collected from 10 sites in seven major regions during the breeding seasons of 1987–1989 (Table 1, Fig. 1). They were either captured with traps baited with seed or lured nearby with a recording of female vocalizations and shot. Specimens were weighed and measured (as described in Keys et al. 1986, Fleischer and Rothstein 1988). For nearly all specimens, liver, kidney and heart tissues were quickly removed, placed in MSB buffer (Lansman et al. 1981) in cryotubes, and then stored in liquid nitrogen until return to the laboratory. Tissues for five birds from site 7 (Fig. 1) were obtained from whole birds shipped to the laboratory on dry ice.

Mitochondrial DNA analyses. Tissue samples were stored in an ultracold freezer for up to 2.5 years prior to mtDNA isolation. MtDNA preparation followed the methods of Spolsky and Uzzell (1984 and pers. comm.). Briefly, tissues were homogenized in buffer and centrifuged slowly (1,000 × g) to pellet cellular debris and then rapidly (10,000 × g) to pellet mitochondria. These were resuspended and banded in a sucrose density gradient in an ultracentrifuge at 26,000 rpm. Mitochondria were lysed with SDS, the remaining proteins digested with proteinase K, high molecular weight DNA removed by potassium-acetate precipitation, and proteins and lipids extracted with phenol and chloroform. MtDNA was precipitated from this solution in sodium acetate and ice cold 95% ethanol. The mtDNA was pelleted, dried, and resuspended in 100–200 μl of 1X Tris-EDTA.

Between 10 and 20 ng of the purified mtDNA was digested with each restriction enzyme and RNAse A to eliminate contaminating RNA. The cohesive ends of the cleaved mtDNA were "fill-in" end-labeled with ^{32}P deoxynucleotides (dATP or dCTP depending on the enzyme used) and the Klenow fragment of DNA Polymerase I. The labelled fragments were separated in a 1.2% agarose gel in 1× TBE (Tris-borate-EDTA gel and running buffer; Perbal 1988), the gel was dried under vacuum and the fragments were visualized by autoradiography. DNA from bacteriophage lambda was digested with *Hind*III, end-labeled, and run on each gel as a size marker.

The preliminary survey of variation included six

TABLE 1. Collecting locations, number of birds that exhibited the *Pst*I-4 and *Pst*I-3 mtDNA haplotypes, and the year in which the sample was collected. See Figure 1 for locations.

	No. *Pst*I-4	No. *Pst*I-3	Year collected
Eastern Nevada:			
1. Lamoille area, Elko County	1	12	1988
Western Nevada:			
2. Fallon area, Churchill County	6	6	1988
Eastern Sierra Nevada, California:			
3. Bridgeport, Mono County	2	3	1988
4. Mammoth Lakes, Mono County	3	5	1988
Owens Valley, California:			
5a. Bishop, Inyo County	0	1	1988
5b. Lone Pine, Inyo County	1	5	1988
Western Sierra Nevada, California:			
6a. Lake Edison, Fresno County	2	2	1987
6b. Dinky Creek, Fresno County	3	3	1987
Southern California:			
7. Corona, Riverside County	5	0	1988
8. Santa Barbara, Santa Barbara County	6	3	1989

"pure" *artemisiae* from eastern Nevada (site 1, Fig. 1), six "pure" *obscurus* from southern California (sites 7 and 8, Fig. 1), and seven individuals from the region of purported hybridization (site 4, Fig. 1). These 19 samples were digested with 10 hexanucleotide-recognizing restriction enzymes: *Apa*I, *Bam*HI, *Bgl*II, *EcorR*I, *Hin*dIII, *Pst*I, *Sac*I, *Sal*I, *Xba*I, and *Xho*I. MtDNA samples from all individuals listed in Table 1 were digested with *Pst*I, because this enzyme produced fragments that varied geographically (see below).

RESULTS

MtDNA variability. The preliminary survey of 10 restriction enzymes produced a total of 36 fragments that could be resolved on the agarose gels (fragments smaller than 0.5 kilobase pairs [kb] were not usually resolved). The total size of cowbird mtDNA was estimated to be 17.7 kb, well within the normal range of size variation of vertebrate and avian mtDNA (Brown 1985, Moritz et al. 1987, Gray 1989, Shields and Helm-Bychowski 1988). Only restriction fragment profiles for *Pst*I exhibited variation. All 20 individuals had identical haplotypes for each of the other nine restriction enzymes.

*Pst*I-digested mtDNA exhibited three-banded (consisting of approximately 9.3 kb, 7.1 kb, and 1.2 kb fragments) or four-banded haplotypes (consisting of 7.1 kb, 5.5 kb, 3.8 kb and 1.2 kb fragments). The three-banded haplotype (*Pst*I-3) differed from the four-banded (*Pst*I-4) by a single restriction site, cutting the 9.3 kb fragment into the 5.5 and 3.8 kb fragments. Geographic variation in the *Pst*I haplotype was apparent and significant ($G = 18.7$, 5 df, $P < 0.005$; Table 1). To increase sample sizes for the above statistical test we combined the closely-located samples from Southern California (sites 7 and 8), the Sierran west slope (sites 6a and 6b) and the Owens Valley (sites 5a and 5b). *Pst*I-3 was nearly fixed in the eastern Nevada *artemisiae* sample (12 of 13), whereas *Pst*I-4 was in high frequency in the southern California *obscurus* sample (11 of 14). This indicates that *Pst*I-3 is predominant in *artemisiae* and *Pst*I-4 is predominant in *obscurus*. Additional sampling from the ranges of each subspecies is necessary to confirm this.

The west slope and east slope Sierra Nevada samples and the western Nevada sample (Fallon) are all intermediate in frequency to the two "pure" populations (Table 1). The Lone Pine sample, from the Owens Valley, is mostly *Pst*I-3 (6 of 7). This was predictable because of the morphological similarity of Owen's Valley cowbirds to pure *artemisiae* (Fleischer and Rothstein 1988) and the probable isolation caused by the Mojave Desert of Owen's Valley populations from much gene flow from *obscurus*.

DISCUSSION

The mtDNA of brown-headed cowbirds exhibits a *Pst*I polymorphism which appears nearly diagnostic of subspecies (*Pst*I-3 in eastern Nevadan *artemisiae* and *Pst*I-4 in southern California *obscurus*). Cowbirds from eastern Nevada have a *Pst*I-3 frequency of 92%, whereas those from southern California have a *Psti*I-3 frequency of 21%. Cowbirds in the putative area of contact in the central Sierra Nevada exhibit *Pst*I-3 frequencies of 50–60%, exactly intermediate to the two "pure" populations. These distributions are thus concordant with our previous assessment based on morphology (both body size and juvenile flange colors; Fleischer and Rothstein 1988, Rothstein 1978), and further indicate to us that gene flow is occurring across a zone of secondary contact between the two subspecies along the Sierran crest. The historically-documented colonization of the western seaboard by cowbirds in the past 90 years rules out the possibility that the clinal change in haplotype frequencies from southern California to eastern Nevada is primary in origin.

As we expected from our previous work (Fleischer and Rothstein 1988), there is evidence of gene flow between the east and west slopes in the Mammoth Lakes area (sites 4, 6a and 6b in Fig. 1). The Bridgeport (site 3) sample's heterogeneity probably reflects gene flow from the north (Lake Tahoe), rather than from Mammoth or from directly across the crest on the west slope. There is probably west to east slope gene flow in the Lake Tahoe/Carson River drainage: we have found a west slope *obscurus* song type (the "coastal flight whistle") to be prevalent in much of the Lake Tahoe area (Rothstein and Fleischer, unpublished data). The intermediate *Pst*I-3 frequency at site 2 (Fig. 1) also suggests extensive gene flow.

Cowbirds from Lone Pine and Bishop in the Owen's Valley (sites 5a and 5b) are more like *artemisiae* in both morphology and mtDNA. In spite of a previous suggestion (Grinnell and Miller 1944), cowbird populations in the Owens Valley are likely not to be subject to gene flow from *obscurus*. This is because the populations of cowbirds in the Mojave Desert south of the Owens Valley are extremely sparse, and because the Sierran crest is higher and more massive at Lone Pine than it is further to the north from Mammoth to Bridgeport.

Our study is one of few to show statistically significant geographic structuring in mtDNA within a passerine species (see Avise and Nelson 1989). Variation in only one restriction site (*Pst*I) might not by itself indicate a biologically significant pattern, but this variation along with our extensive data from other sources (variation in body size, rictal flange color, changes in body size over time [Fleischer and Rothstein 1988]) all but confirms the existence of extensive gene flow between *artemisiae* and *obscurus* in the central Sierra Nevada.

In another published study that assesses widespread intraspecific geographic variation in mtDNA in a passerine, the red-winged blackbird (*Agelaius phoeniceus*; Ball et al. 1988), greater intrapopulational and overall variability was found, but geographic structuring was not apparent. On the other hand, Avise and Zink (1988) found considerable intertaxa heterogeneity in an examination of a few individuals from each of four pairs of closely-related taxa, including one pair of currently-recognized subspecies (*Parus bicolor bicolor* and *P. b. atricristatus*). Thus, it may not be too surprising that a species with such morphologically-differentiated subspecies as the brown-headed cowbird would have diagnostic mtDNA variation. Studies of other organisms have shown similar concordance between mitochondrial and nuclear genes or their products (e.g., Avise et al. 1984, Szymura et al. 1985, Baker et al. 1989).

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PHILOPATRY IN MALE AND FEMALE AMERICAN BLACK DUCKS[1]

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Key words; American Black Ducks; Anas rubripes; *male/female philopatry; Nova Scotia.*

Breeding philopatry in waterfowl is female-biased (Greenwood 1980, Lessells 1985) and female philopatry in North American dabbling ducks (*Anas* spp.) has been documented for several species (Lincoln 1934, Lokemoen et al. 1990, Sowls 1955). Male philopatry is less common and low return rates have been reported by Dwyer et al. (1973), Titman (1983), Evrard (1990) for Mallards (*A. platyrhynchos*), by Sowls (1955) for Northern Pintails (*A. acuta*), by Poston (1974) for Northern Shovelers (*A. clypeata*), and by Blohm (1978) for Gadwalls (*A. strepera*). Homing by American Black Ducks (*A. rubripes*) to nest sites (Coulter and Miller 1968, Reed 1970) and to rearing areas (Reed 1970) has been documented and Ringelman et al. (1982) report fidelity to home range and use of the same wetlands by an adult female, but there are few reports of homing by juvenile and male Black Ducks. This note documents adult and juvenile male and female philopatry by Black Ducks.

From 1973 through 1989, 591 adult males and 393 adult females were banded and another 63 adult males and 47 adult females were individually marked with color-coded nasal discs (Bartonek and Dane 1964). In addition, 65 male and 50 female ducklings were captured on their natal wetlands in 1987–1989 and banded and marked with nasal-discs. The study area was a 750 km^2 drainage basin but most data were collected in a 1.5 km^2 *Spartina* marsh which has formed where the three main rivers of the basin enter a 10 × 2 km tidal estuary in northeastern Nova Scotia.

Some adult and juvenile (10–11 months old and captured as a duckling) males and females remain on the

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