

Conservation genetics of American Dipper (*Cinclus mexicanus*): the genetic status of a population in severe decline

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Abstract American Dippers (*Cinclus mexicanus*) were once known to occur in streams throughout the Black Hills of South Dakota and Wyoming, but now dippers number about 50–75 individuals and reside almost exclusively in a single stream. The recent decline of the American Dipper in the Black Hills of South Dakota is thought to be due to local stream degradation. As a result of the decline of *C. mexicanus* in the Black Hills of South Dakota and Wyoming, the Black Hills population of American Dippers is a candidate for designation as a distinct population segment (DPS) and might warrant protection and special management. One criterion for DPS designation is genetic uniqueness. Here we present the results of a genetic assessment of the Black Hills population of *C. mexicanus*. Data presented here indicate that the dipper population in the Black Hills is genetically distinct from other sampled populations. Further population sampling will be needed to understand the genetic population structure of *C. mexicanus* throughout its range. Furthermore, the recent decline in the Black Hills dipper population should be a warning that other populations (and other species) may be experiencing similar declines and that such montane habitats are worthy of special management.

Keywords American Dipper · Microsatellites · Conservation genetics · Distinct population segment · Endangered

Introduction

The American Dipper (*Cinclus mexicanus*) is an aquatic songbird that inhabits mountainous regions of western North America. It is non-migratory and lives year-round near fast moving, clear, rocky streams with the highest abundance of aquatic invertebrates (Pennak and van Gerpaen 1947). Dippers are rarely seen away from water and do not make long distance flights over land (Price and Brock 1983). Because dippers require invertebrates found in clear, fast-moving streams, stream degradation can lead to a decline in the availability of this major food source. Therefore, dippers are sensitive to habitat disturbance and water quality and have been suggested as a bioassessment indicator for mountain streams (Feck and Hall 2004; Morrissey et al. 2004).

The Black Hills of South Dakota and Wyoming are at the eastern edge of the range of *C. mexicanus*. The Black Hills are surrounded by the Great Plains and the nearest mountain range is the Big Horn Mountains, which are approximately 200 miles to the west and not connected by dipper habitat. American Dippers were once known to occur in streams throughout the Black Hills, but now dippers number about 50–75 individuals and reside principally in a single stream. The recent decline of the American Dipper in the Black Hills of South Dakota is thought to be due to local stream degradation (Backlund 2007). A number of factors are thought to negatively impact dipper populations including: livestock (Osborn 1999), mining, industrial and agricultural pollution, silting (Feck and Hall 2004), deforestation

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(Sullivan 1973), dams, and acidification (Tyler and Ormerod 1994). The Black Hills have a history of mining that has dramatically impacted some streams. For example, Whitewood Creek was declared a dead stream due to heavy metal contamination from mining and municipal sewage until measures were taken to clear Whitewood Creek of pollution. Whitewood Creek still has periods of heavy metal pollution during high flow periods. The extinction of dippers on Rapid Creek is likely due to Pactola Dam, which has been responsible for no-flow periods below the dam (Backlund 2007). Such reduced flows are responsible for winter freezing of the stream, the proliferation of an invasive diatom (*Didymosphenia geminata*) and reduced invertebrate abundance (Backlund 2007). While comprehensive data are lacking, there is evidence of declining dipper populations in Colorado, Arizona and Wyoming (Anderson 2002; Backlund pers. comm.). Dippers in other parts of its range may also be in decline but not documented because they are not being monitored.

Concern over the recent decline in the Black Hills American Dipper population has led resource managers to consider whether protection and special management are warranted. Special management might be warranted if Black Hills dippers are distinct from other populations of dippers. The concept of the distinct population segment used by the US Fish and Wildlife Service and the National Marine Fisheries Service is appropriate. Designation as a DPS is based on two general criteria: (1) The discreteness of the population segment in relation to the remainder of the species or subspecies to which it belongs; and (2) the significance of the population segment to the species or subspecies to which it belongs. The present study was undertaken to determine if the Black Hills American Dipper population represents a genetically discrete population of *C. mexicanus*.

Methods

DNA was isolated from either blood or feathers using standard phenol-chloroform extractions. Sampling locations include: Black Hills, SD ($N = 26$), Big Horn mountains, WY ($N = 18$), Lochsa River, ID ($N = 26$), Clearwater River, ID ($N = 13$), Selway River, ID ($N = 11$) and Bitterroot River, MT ($N = 14$). Primers developed for *Cinclus cinclus* were screened (Lukas Keller, Zoological Museum, University of Zurich, unpublished data along with other previously published primers (Primmer et al. 1996)). Each 20 μ l polymerase chain reaction (PCR) was done using Promega PCR Master Mix with approximately 50 ng genomic DNA and 0.5 μ M primer. All presumptive microsatellite loci were cloned and sequenced to assure homology with published loci. All genotyping and

sequencing was done at the Western South Dakota DNA Core Facility (WestCore) on an ABI 3130 genetic analyzer. Allele sizes were determined using the software *Genemapper* (Applied Biosystems).

Data were tested for deviations from Hardy-Weinberg equilibrium, and the observed and expected heterozygosities were estimated using GENEPOP web version (Raymond and Rousset 1995). ARLEQUIN version 3.01 (Excoffier et al. 2005) was used to compute pair-wise comparisons of F_{st} (corrected for multiple comparisons). A Mantel test was used to test for a positive correlation between genetic distance and geographic distance with 10,000 permutations using ARLEQUIN. To visualize patterns of genetic diversity across the geographic landscape, we implemented a landscape shape interpolation analysis (Miller 2005), where genetic distances between sampling locations are placed at geographic midpoints and peaks represent areas with large genetic distances. Bayesian clustering with STRUCTURE software (Pritchard et al. 2000) was used to assign individuals to populations (K) based on posterior probabilities where K is unknown. The number of groups was set to 1–7 with 3 runs per K . Posterior probabilities were calculated for all K hypothetical populations. All analyses were based on 1,000,000 Markov Chain Monte Carlo iterations following a burn in of 50,000 iterations.

Results

The number of alleles per locus ranged from 3–16 and some significant departures from Hardy-Weinberg equilibrium were observed (especially Cici02 and Cici12) due to deficiencies of heterozygous genotypes (Table 1). The cause of these departures from Hardy-Weinberg is not known, but the presence of null alleles appears to be a possible cause. Departures from Hardy-Weinberg expectations caused by a deficiency of heterozygous genotypes are a common feature of microsatellites and this is most often attributed to null alleles (Garner 2002). Pair-wise comparisons of F_{st} indicate significant differences between the Black Hills and Bighorn populations of *C. mexicanus* and all other populations. All other comparisons were not significant (Table 2). Mantel tests provided no support for a correlation between F_{st} and geographic distance ($r = 0.4454$, $P = 0.0908$). There is no significant difference in allelic diversity between the Black Hills population and other populations. A 3-dimensional surface plot of geographic locations and genetic distance indicates that the greatest difference in genetic distance occurs between the Big Horns, Black Hills and all other populations (Fig. 1b). Bayesian cluster analyses indicate that 2 populations ($K = 2$) had the highest probability rather than $K = 1, 3, 4, 5, 6, 7$. The majority of Black Hills individuals were

Table 1 Microsatellite loci used in this study. Observed sizes are based on the obtained genotypes

Locus	Species from which microsatellite was isolated		Population					
			Black Hills	Lochsa	Bitterroot	Clearwater	Selway	Bighorns
^a Cuu05	<i>Catharus ustulatus</i>	Observed size range (bp)	156–177	156–169	151–171	156–171	158–171	158–177
		Ho	0.7143	0.5200	0.5714	0.8462	0.5455	0.4118
		He	0.6852	0.5861	0.7143	0.7108	0.7186	0.4100
		HWE (<i>P</i> -val)	0.8787	0.0160	0.1644	0.6432	0.2871	0.4972
^a Cuu10	<i>Catharus ustulatus</i>	Observed size range (bp)	158–168	152–168	158–168	152–166	152–168	152–168
		Ho	0.0769	0.2609	0.6923	0.4167	0.2222	0.1667
		He	0.2123	0.3159	0.5446	0.5797	0.7386	0.6341
		HWE (<i>P</i> -val)	0.1195	0.1334	0.8078	0.2601	0.0029	0.0000
^b Ase64	<i>Acrocephalus sechellensis</i>	Observed size range (bp)	424–458	409–458	424–454	412–445	424–461	428–451
		Ho	0.7826	0.7917	0.7857	0.9231	0.9091	0.7059
		He	0.7816	0.8679	0.8915	0.8585	0.8831	0.8360
		HWE (<i>P</i> -val)	0.3598	0.2284	0.2014	0.2089	0.2609	0.0234
^c Cici02	<i>Cinclus cinclus</i>	Observed size range (bp)	115–119	109–119	109–119	107–119	107–119	102–119
		Ho	0.4000	0.4762	0.4286	0.5385	0.2727	0.7222
		He	0.6577	0.7619	0.7858	0.7815	0.7835	0.7889
		HWE (<i>P</i> -val)	0.0402	0.0004	0.0111	0.0770	0.0000	0.0560
^c Cici04	<i>Cinclus cinclus</i>	Observed size range (bp)	164–202	164–206	164–202	164–198	164–202	164–202
		Ho	0.8182	0.7200	1.0000	0.6923	0.9091	0.8421
		He	0.5930	0.7616	0.8307	0.6185	0.8268	0.7937
		HWE (<i>P</i> -val)	0.2553	0.3149	0.7091	0.9273	0.4352	0.5918
^c Cici05	<i>Cinclus cinclus</i>	Observed size range (bp)	250–259	246–259	250–257	246–259	246–257	250–257
		Ho	0.4000	0.7500	0.6429	0.4615	0.6364	0.3158
		He	0.4692	0.7115	0.7169	0.5600	0.5844	0.5306
		HWE (<i>P</i> -val)	0.1392	0.5914	0.5851	0.4385	0.4638	0.0236
^c Cici08	<i>Cinclus cinclus</i>	Observed size range (bp)	221–234	221–237	221–237	221–234	225–234	221–234
		Ho	0.5500	0.7619	0.8571	0.6923	0.9091	0.6111
		He	0.6000	0.7933	0.7143	0.7508	0.7749	0.7079
		HWE (<i>P</i> -val)	0.6132	0.7911	0.5116	0.4343	0.9813	0.1270
^c Cici10	<i>Cinclus cinclus</i>	Observed size range (bp)	125–142	129–151	117–147	129–147	129–142	125–142
		Ho	0.4211	0.2381	0.4615	0.3333	0.5000	0.3125
		He	0.7866	0.8165	0.8862	0.7065	0.7684	0.8206
		HWE (<i>P</i> -val)	0.0012	0.0000	0.0000	0.0074	0.3119	0.0000
^c Cici11	<i>Cinclus cinclus</i>	Observed size range (bp)	173–200	173–202	173–198	173–202	177–202	173–200
		Ho	0.9048	0.7308	0.7857	1.0000	1.0000	0.6842
		He	0.8595	0.9178	0.8862	0.9110	0.8095	0.8848
		HWE (<i>P</i> -val)	0.9444	0.0000	0.4084	0.9216	0.6693	0.2965
^c Cici12	<i>Cinclus cinclus</i>	Observed size range (bp)	263–294	240–297	271–322	248–294	263–294	260–294
		Ho	0.6364	0.6000	0.5714	0.5385	0.5455	0.4118
		He	0.7727	0.8897	0.8413	0.8594	0.8918	0.8610
		HWE (<i>P</i> -val)	0.0054	0.0027	0.0428	0.0019	0.0021	0.0000
^c Cici13	<i>Cinclus cinclus</i>	Observed size range (bp)	160–180	160–176	160–176	160–180	160–172	160–184
		Ho	0.7500	0.6957	0.7143	0.8462	0.7273	0.7368
		He	0.7513	0.7556	0.6032	0.8246	0.6797	0.7454
		HWE (<i>P</i> -val)	0.7293	0.1429	0.6842	0.8189	0.0385	0.8870

Table 1 continued

Locus	Species from which microsatellite was isolated		Population					
			Black Hills	Lochsa	Bitterroot	Clearwater	Selway	Bighorns
°Cici15	<i>Cinclus cinclus</i>	Observed size range (bp)	209–229	209–223	209–223	209–223	209–223	209–223
		Ho	0.4348	0.5600	0.6429	0.7692	0.5455	0.6316
		He	0.4783	0.5037	0.6640	0.7015	0.7056	0.6501
		HWE (<i>P</i> -val)	0.2188	0.3149	0.4827	0.7264	0.0169	0.4691
°Cici16	<i>Cinclus cinclus</i>	Observed size range (bp)	268–270	268–270	268–272	268–272	268–270	268–272
		Ho	0.2857	0.2857	0.2857	0.4615	0.0909	0.3158
		He	0.2509	0.3159	0.2646	0.5323	0.0909	0.3997
		HWE (<i>P</i> -val)	1.0000	1.0000	1.0000	0.7632	NA	0.2399

Expected (HE) and observed (HO) levels of heterozygosity and HWE were calculated using Genepop on the Web, <http://genepop.curtin.edu.au/history.html> (Raymond and Roussett, 1995). Primer sequences and PCR conditions were as published (°Gibbs et al., 1999; °Richardson et al., 2000), or as provided by °Keller pers. comm., 2005

assigned to group 1 and all other samples were mixed for group 1 and group 2 (Fig. 1a).

Discussion

Pair-wise F_{st} comparisons indicate significant differences among populations of American Dippers and Bayesian cluster analysis suggests an overall population structure for the populations included in the study of $K = 2$, with the Black Hills population separate from all other populations. The Black Hills population of dippers is likely isolated due to stream piracy and climatic trends at the eastern most periphery of its range separated from other populations by vast expanses of unsuitable habitat (Kingery 1996). Lack of gene flow and subsequent genetic divergence is supported by evidence suggesting little long-range movements of dippers (Price and Brock 1983). Interestingly, F_{st} comparisons show the greatest differences between the Black Hills and the most geographically proximate population included in our study, the Big Horn Mountains in Wyoming. This may seem counterintuitive, but declining population size in Black Hills Dippers has increased the likelihood of genetic change due to genetic drift possibly

creating an island pattern of population structure rather than an isolation by distance pattern. It is also possible that the Black Hills population has affinities to populations not included in the present study (e.g. Medicine Bow Mountains, Wyoming). This appears somewhat unlikely given the present data, the current status of Black Hills dippers and the biogeographic history of the Black Hills, but future sampling of other populations is certainly warranted given the results of the present study.

To be considered a DPS, a population must first be demonstrated to be discrete genetically or morphologically. If a population is deemed discrete, it must also be demonstrated that the population persists in a unique geographical and ecological setting, the loss of which would represent a gap in the distribution of the taxon. Here we provide evidence that the Black Hills population is genetically distinct but there is a general lack of other biologically relevant information available for American Dippers. Estimates of local abundance, total population size and geographic trends are lacking (Anderson 2002; Price et al. 1995). It is not known if there is geographic variation in ecology, behavior or population biology among dipper populations. Five subspecies of *C. mexicanus* are recognized based on variation in color, but their exact

Table 2 Matrix of F_{st} comparisons

	Black Hills	Lochsa	Clearwater	Bitterroot	Selway	Big Horns
Black Hills, SD ($N = 23$)	0					
Lochsa, ID ($N = 26$)	0.02468**	0				
Clearwater, ID ($N = 13$)	0.04848***	0.0073	0			
Bitterroot, MT ($N = 13$)	0.0426***	0.01149	0.01846	0		
Selway, ID ($N = 11$)	0.0593***	0.01924	0.01542	0.01846	0	
Big Horns, MT ($N = 19$)	0.09541***	0.03998***	0.04309**	0.06594***	0.05379**	0

** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$

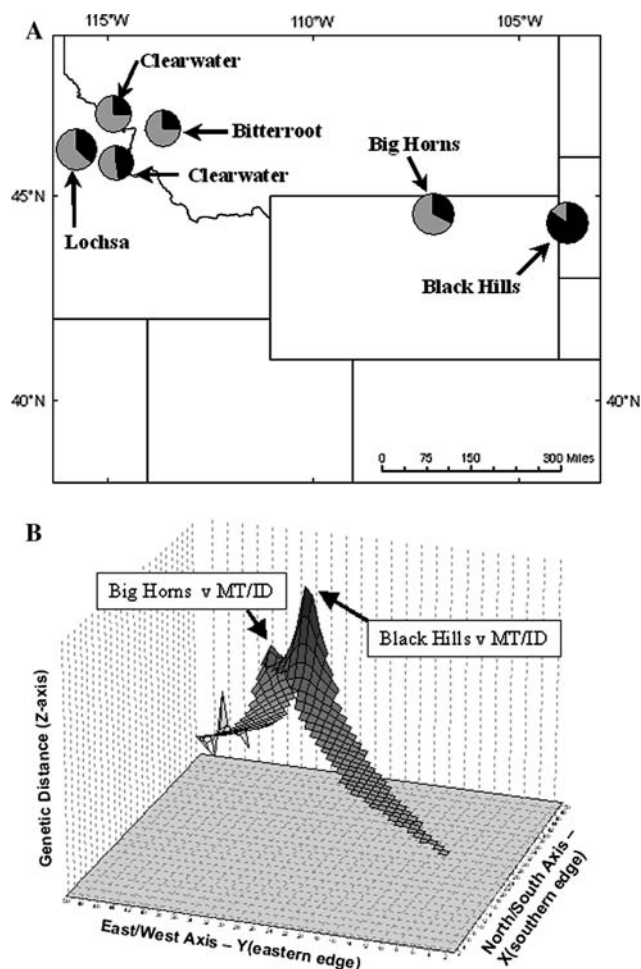


Fig. 1 (A) Map showing the results of the Bayesian cluster analyses ($K = 2$). Each pie chart represents a sampled population. The shading in the pie charts shows the overall proportion of individuals assigned to the two groups (Group 1 = Black, Group 2 = Gray). (B) Results of the landscape shape interpolation analysis. X and Y axes correspond to geographic locations within the sampled landscape converted to a 50×50 grid and a distance weighting parameter (a) = 1. The surface plot reflects genetic distances placed at the geographic midpoints between sample sites. High points of genetic distance between the Black Hills and Big Horn mountain ranges and all other sample sites are marked

boundaries and regions of contact are not well understood (Phillips 1986). Genetic studies have not been done to assess the taxonomic status of these putative sub-species. In addition to the lack of biological information for dippers, management of *C. mexicanus* is confused because state and federal agencies have different criteria for listing dippers.

While the present study showed that the Black Hills were genetically distinct from other populations in the study, designation of the Black Hills population as a DPS would likely require additional sampling of populations not included in the present study. Given the precarious status of the Black Hills population (only 50–75 birds) managers

may be required to make critical decisions before a more extensive genetic study can be done. Supplementation of the Black Hills population with dippers from other populations might be seen as a way to preserve or enhance the Black Hills population. Translocating birds from the most geographically proximate population in the Big Horn Mountains in Wyoming would seem like a likely source population, but the genetic data indicate that the Black Hills population is genetically distinct from the population in the Big Horn Mountains (as well as all other populations included in the study). Such a translocation would alter the genetic integrity of the Black Hills population, interfere with the evolutionary process that created the existing pattern of population structure and possibly reduce the fitness of the population if they are ecologically adapted to local environmental conditions. While the addition of samples from other populations would clarify the overall genetic population structure, protection of the existing population of dippers in the Black Hills and the habitat that supports this population is clearly justified.

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