Minisatellite DNA variability in two populations of Spotted Sandpipers *Actitis macularia* in Minnesota, U.S.A.

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The genetic structure within and between populations of shorebirds is poorly known. Here we present DNA fingerprint data from two populations of Spotted Sandpipers *Actitis macularia* from near the center of their geographic distribution, that are separated by 225 km. We found the amount of genetic variability within populations and the mutation rate to be similar to other avian species. In addition, each population has typical levels of minisatellite locus variability. Heterozygosity was 0.78 at one site and 0.77 at the other, and mean similarities within each site were almost identical (0.28 and 0.25, respectively), with S=0.18 between sites. Despite the sensitive assessments of genetic structuring allowed by DNA fingerprints, we observed little differentiation between populations (F_{ST}=0.10).

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INTRODUCTION

The genetic structure within and between populations of shorebirds is poorly known. Based on allozyme data, shorebird populations generally are genetically unstructured (Baker & Strauch 1988). Control-region sequences of mitochondrial DNA have provided greater resolution of global genetic structure in Dunlin *Calidris alpina* (Wenink *et al.* 1993), but regional structure is not resolved. Part of the lack of observed differentiation is probably a result of the coarse-grained methods used (*i.e.* assessment of loci with relatively low variability and rates of mutation). In addition, little is known about levels of genetic variability within populations of non-endangered shorebirds (*e.g.* Haig & Oring 1988). The hypervariable minisatellite loci assayed in DNA fingerprinting allow an assessment of genetic variability at a relatively fine-grained level (Jeffreys *et al.* 1985), and should give more-detailed information on microgeographic patterns of genetic differentiation. This method has been used to compare populations in only a few species of birds (Rabenold *et al.* 1991; Fleischer *et al.* in press), but has been used for other taxa (*e.g.* Gilbert *et al.* 1990; Parker & Whiteman 1993).

Here we present DNA fingerprint data from two populations of Spotted Sandpipers *Actitis macularia*, from near the center of their geographic distribution, that are separated by 225 km. We found the amount of genetic variability within populations and the mutation rate to be similar to other avian species. In addition, we show that each population has typical levels of minisatellite locus variability and that the two populations are only moderately differentiated in spite of the distance and low number of connecting populations between them.

STUDY SPECIES AND METHODS

Spotted Sandpipers breed across most of North America, they are migratory, and breeding habitat is widespread. Dispersal and breeding data show extensive breeder exchange between two sites separated by 7 km (see below; Oring & Lank 1986; Reed & Oring 1993). DNA fingerprint band-sharing of randomly sampled individuals at these sites (S=0.29, Oring *et al.* 1992) is not high relative to other bird species (*e.g.* Westneat 1990). Breeders on one of our study sites (LL, below) regularly are recruited from immigrants, and birds survey potential breeding sites for future breeding (Oring *et al.* 1983; Oring & Lank 1986; Oring 1988; Reed & Oring 1992, 1993). Because of these observations, and results from allozyme studies of other shorebird species (Baker & Strauch 1988), we expected little local genetic differentiation. Therefore, hypervariable minisatellite loci offer a good option for finding any underlying regional patterns that might exist.

Blood samples were collected from Spotted Sandpipers on Little Pelican Island and the adjacent mainland (7 km distant) at Leech Lake (LL), Minnesota (47°07'N, 94°21'W) (see Reed & Oring 1993 for a site description), and at Lake of the Woods (LOW), Minnesota (48°67'N, 94°78'W), 225 km away. Population size at LL varied

between 18 and 52 breeding birds (Oring *et al.* 1991); LOW is larger, with a minimum of 50 breeding birds annually (L.W.O. unpubl. data). No large concentrations of breeding birds occur between the sites, although scattered pairs exist (L.W.O. unpubl. data). Blood samples were taken at LL in 1990 and 1991 as part of a paternity study (Oring *et al.* 1992), and eight unrelated individuals were randomly selected for comparison to six individuals sampled in 1991 at LOW. All birds were run on a single gel to eliminate differences in fragment mobility that typically occur between gels. Laboratory methods of DNA isolation, digestion, transfer, and minisatellite probing are described in Oring *et al.* (1992) and Fleischer *et al.* (1994).

RESULTS

From the 14 individuals sampled, we found 78 fragments greater than about 1 kb in size. The mean number of fragments per individual was 15.3 (s.e.m 3.3, range 9-20). Results are summarized in Table 1. There were no monomorphic bands at either site. Heterozygosity (Gilbert *et al.* 1991) was 0.78 at LL and 0.77 at LOW, and the estimate is unbiased because there were no monomorphic bands (Jin & Chakroborty 1993). Fisher's exact tests of band-frequency distributions between sites showed significant differences ($P<0.05$) for only three bands (3.9 were expected by chance). Mean similarities within each site were almost identical (LL=0.28, LOW=0.25), with S=0.18 between sites. The LL estimate was essentially the same as found for the overall study at the LL site (Oring *et al.* 1992). Between-site similarity corrected by within-site similarity was 0.92 (Table 1). If we assume each band site represents an independent allele, F_{ST}=0.10 (Table 1).

Table 1. Summary of DNA fingerprint data, using Jeffreys' 33.15 probe, of Spotted Sandpipers from two study sites (LL and LOW) that are separated by 225 km ([a]Heterozygosity (Jin & Chakroborty 1993), [b]Lynch (1990), [c]Li *et al.* (1993), [d]Corrected for within-population similarity (Lynch 1991), [e]Lynch (1991, eqs 10 and 5); using a mutation rate of 0.018).

Statistic	LL	LOW	Combined/ Between
number of birds	8	6	14
fragments/bird	14.9	16.5	15.3
mean			
s.e.m.	3.7	2.5	3.3
unique bands	24	17	
unbiased H[a]	0.78	0.77	0.85
similarity (S)[b]	0.27	0.25	0.18
var (S)	0.002	0.001	
unbiased S[c]	0.28	0.25	0.18
corrected S[d]			0.92
F_{ST}[d]			0.10
effective size[e]	35.8-78.4	41.7-90.3	

Mutation rate can be estimated from DNA fingerprints (Jeffreys *et al.* 1988; Westneat 1990) and used to calculate long-term effective population size, assuming populations are at genetic equilibrium and fragments are independent (Lynch 1991). We used offspring of unexcluded parents (Oring *et al.* 1992) to approximate a mutation rate of 0.018 mutations / fragment / meiotic event. We used equation 10 of Lynch (1991) to estimate downwardly-biased effective sizes for the populations and equation 5 for upwardly-biased estimates. These values ranged from 35.8 to 78.4 at LL and 41.7 to 90.3 at LOW.

DISCUSSION

Genetic variability, as expressed by minisatellite DNA, was similar for the two Spotted Sandpiper populations. The mutation rate (mutation/artifact rate) was fairly high (0.018), but not very different from that reported for other species (0.011, Westneat 1990; 0.024, Delehanty *et al.* in review).

We found little substructuring between the populations. Allozyme studies have shown little substructuring among bird populations. Barrowclough (1983), Evans (1987), and Baker & Strauch (1988) reviewed avian studies and found 38/41 species had $F_{ST} < 0.10$. When genetic differentiation has been found, it is usually associated with island (or island-like) distributions, or behavioral barriers to mating or dispersal (*e.g.* Barrowclough 1983; Evans 1987, Caparella 1988; Fleischer & Rothstein 1988; Fleischer *et al.* 1991). F_{ST} from allozyme allele frequencies for nine species of shorebirds were all below 0.06) (Baker & Strauch 1988; Haig & Oring 1988). DNA fingerprints allow more sensitive assessments of genetic structuring. Despite this, we observed little more differentiation (F_{ST}=0.10) in Spotted Sandpipers. This degree of differentiation is consistent with regular movement between sampled populations. However, despite many banded birds over the last 20 years at LL, and regular surveys at LOW, no marked bird from LL has been seen at LOW.

The large calculated population growth rate (R_0=1.4 for LL) implies the LL population ought to be growing rapidly, but the population has fluctuated annually with no net growth for at least ten years. This is because adult return rates following years with unsuccessful breeding is less than half of that following successful breeding, assuming no difference in survival related to reproductive success (Reed & Oring 1993). This causes LL to act as a source of immigrants for other populations. If this is a general pattern for Spotted Sandpipers, it would result in dispersal among populations, thus decreasing regional genetic structuring.

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