Preliminary results of population genetics of the Pacific Golden-Plover (*Pluvialis fulva*)

Stacey Philipoom-Lynn
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1 Department of Biology, Brigham Young University-Hawaii, 55-220 Kulanui Street, Laie, HI 96762, USA
Abstract. The Pacific Golden-Plover (*Pluvialis fulva*) has a wide distribution, but little is known about migration patterns and population connectivity. To assess genetic structure and determine population trends, 57 specimens from Hawaii and Saipan/Tinian populations were examined at eight highly variable microsatellite DNA loci. Mean gene diversity ($H_E$) was higher in Hawaii than in Saipan/Tinian (0.53 and 0.47; $P < 0.007$). Low heterozygote deficit ($F_{IS}$) suggested no sub-structuring in either population. Genetic differentiation ($F_{ST} = 0.011$, $P = 0.223$) using the current sampling conditions was unable to support the results of recent radio-tagging and leg banding studies that suggested the maintenance of distinct breeding populations. No evidence of a recent bottleneck was found. The seven genetic markers in Hardy Weinberg Equilibrium were adequate to distinguish one among 7,153 individuals in Saipan/Tinian, but due to a small sample set were unable to assign probability of identity values for the Hawaii population.

Introduction

The Pacific Golden-Plover (*Pluvialis fulva*) makes some of the longest migrations in the world. It nests on tundra in Siberia and western Alaska, and winters in locations ranging from coastal California, to the Pacific Islands, Australia, Asia, and northeast Africa (Johnson and Connors 1996). At present, there are only gross estimates as to how many *P. fulva* exist, and little information on population trends (Johnson 2003). Most recent estimates place world populations between 166,000-216,000 with 16,000 nesting in western Alaska (Delany and Scott 2002).

For the conservation and management of any migratory species, it is critical to know the range and movements of the species throughout its annual cycle. For the Pacific Golden-Plover, this migration extends over an extremely large geographical area. Assessing migration patterns and connectivity of plovers over such a vast range is
challenging. Radiotelemetry and leg banding studies have only been successful in showing direct interaction between plovers wintering in Hawaii and plovers nesting in Alaska (Johnson et al. 1997, Johnson et al. 2001, Johnson et al. 2004).

The Northern Mariana Islands, located 1700 km south of Japan, form an archipelago of 14 islands. They serve as wintering grounds for plovers. According to regular surveys conducted on Saipan from 1987-1993, the Pacific Golden-Plover is the most abundant shorebird in the Mariana Islands (Stinson et al. 1997). Radar studies showed that a significant number of the shorebirds that winter on small islands and atolls in the western and southern Pacific fly northward to distant breeding grounds (Williams and Williams 1988). These observations suggest that the Saipan, Mariana Islands population of plovers migrate northward to breed on tundra in Siberia or eastern Asia. Whether Saipan plovers interact with the Alaska/Hawaii populations or whether they are distinct breeding populations is unknown.

Population genetics tools, such as microsatellite DNA markers, have been useful in providing insights into population connectivity and demographics that are difficult to assess through field studies (Clegg et al. 2003, Talbot et al. 2003, Weckworth et al. 2005). Microsatellite markers were used to describe the levels of variability within plover populations in an attempt to understand population trends. This report details the results of a preliminary analysis of bi-parentally inherited microsatellite loci collected from plover populations in Hawaii and Saipan/Tinian.

Materials and methods

Sampling

Blood, heart, muscle tissue, and contour feathers were collected from plovers wintering in Hawaii (H, n = 10) and from populations on Saipan and Tinian (S and T, n = 47). Blood samples were stored in blood lysis buffer [100 mM Tris-HCl at pH 8.0, 100mM EDTA, 10 mM NaCl, 0.5% SDS] (Longmire et al. 1988), tissue samples were stored in tissue preservation buffer [4 M urea, 0.2 M NaCl, 100 mM Tris-HCl at pH 8.0, 0.5% N-lauroylsarcosine, 10 mM EDTA] (Pearce et. al 2000), and feathers were stored dry on silica gel.
Laboratory analysis

Genomic DNA was extracted from each sample using the “salting out” procedure described previously (Medrano et al. 1990), quantified using a Hoefer DyNA Quant 200 fluorometer (Hoefer, San Francisco, California, USA), and diluted to 50 ng/µL working solutions.

Thirty-five microsatellite DNA loci known to be polymorphic in shorebirds were screened (Van Treuren et al. 1999, Thuman et al. 2002); eight were found to be polymorphic in the Pacific Golden-Plover and were used in subsequent analyses (4A11, 18F9, 54F2, Hbau4, Hbau7, K31, 20H7, and Ruff6). These loci were analysed by PCR with end-labelled primers (IRDye 700 and 800 fluorescent tags LI-COR). PCR amplifications were prepared to contain approximately 50 ng of genomic DNA, 2 mM dNTPs, 10 mM Tris-HCl at pH 8.3, 1.5 mM MgCl₂, 50 mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton-X 100, 1 µg of BSA, 1.5 pmoles of IRD-labelled primer, 5.0 pmoles of unlabelled forward primer, 5.0 pmoles of unlabelled reverse primer, and 0.1 unit of Taq polymerase (Promega, Madison, Wisconsin, USA) in a total volume of 10 µL. Typical reactions included an initial denaturation at 94 °C for two minutes and continued with 40 cycles each of 94 °C for 15 seconds, 50 °C for 15 seconds, and 72 °C for 30 seconds. A 30 minute extension at 72 °C concluded each reaction. The fluorescently-labelled PCR products were electrophoresed on a 48-well 6% polyacrylamide gel using a LI-COR 4200 L-2 LR automated sequencer. Amplified microsatellite fragments were compared with a fluorescently labeled M13 DNA sequence ladder (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA). Microsatellite fragment data were captured using Gene ImagIR data analysis software. For quality control purposes, a minimum of 10% of samples at each locus were randomly selected and re-analysed.

Statistical analysis

Excel Microsatellite Toolkit (version 2.1, Park 2001) was used to prepare data for analysis. GENEPOP (version 3.3, Raymond and Rousset 1995) was used to determine allelic frequencies, observed heterozygositites (H₀), and expected heterozygositites (Hₑ). The Markov chain parameters provided were used to estimate exact P-values for deviation from Hardy-Weinberg equilibrium (HWE) and to test for linkage
disequilibrium (LD). P-values for tests were corrected using a strict Bonferroni adjustment (initial \( \alpha = 0.05 \)) for multiple comparisons. Allelic richness (\( AR \)) per locus and per population was calculated using FSTAT (version 2.9.3, Goudet 2001).

Significance of spatial variation in allele frequency was assessed using F-statistics (Weir and Cockerham 1984). These measures describe the apportionment of allelic variance among individuals within (\( F_{IS} \)) and between (\( F_{ST} \)) populations. Pairwise \( F_{ST} \) values, which estimate interpopulational variance, were calculated using ARLEQUIN (version 2.0, Schneider et al. 2000). Significance of pairwise \( F_{ST} \) values were based on random permutation tests (\( n = 1,000 \)).

The statistical program GIMLET (version 1.3.2, Valieré 2002) was used to generate \( P_{ID} \) and \( P_{IDsib} \) values to establish whether the microsatellite markers were sufficiently variable for use in studies requiring individual identification.

Populations were assessed for evidence of a recent reduction in population size using the program BOTTLENECK (Piry et al. 1999). The Wilcoxon test was used to detect excess heterozygosity for polymorphic microsatellite loci and was performed under three models thought to represent the range of possible mutation modes generating polymorphism at microsatellite loci (Chakraborty and Jin 1992). These include the step-wise mutation model (SMM, Ohta and Kimura 1973), the infinite-alleles model (IAM, Kimura and Crow 1964), and the two-phase model (TPM, Di Rienzo et al. 1994) of microsatellite mutation. One thousand simulations were performed for each population.

**Results**

**Population structure**

Multilocus genotypes were obtained for all 57 individual plovers across the eight polymorphic loci. One of the loci (18F9) deviated from HWE proportions due to heterozygote deficit, and was excluded from further analyses. The remaining seven loci showed no significant deviations from HWE and appeared to be useful for detecting potential population genetic differentiation. The number of alleles per locus for the seven-locus genotype ranged from two (20H7) to 15 (Hbau4) and the mean number of alleles per locus per population (observed allelic diversity) was 4.4 for the Hawaii plovers and 6.0 for Saipan/Tinian (Table 1). Allelic richness (\( AR \)) was lower in the Saipan/Tinian
population than Hawaii plovers. Expected heterozygosities ($H_E$) for each population ranged from 0.47 to 0.53 and observed heterozygosities ($H_O$) ranged from 0.44 to 0.49. Heterozygosity (both observed and expected) was lower in the Saipan/Tinian population (Table 1). $F_{IS}$ (heterozygote deficit) values were not significant in either population and overall (Table 1). The $F_{ST}$ value for both populations was not significant ($0.011$) ($P = 0.223$, Bonferroni corrected $\alpha = 0.007$, data not shown), indicating no significant genetic difference between populations.

**Table 1.** Measures of genetic variability at seven loci within two populations of Pacific Golden-Plovers (*Pluvialis fulva*).

<table>
<thead>
<tr>
<th>Population</th>
<th>$N$</th>
<th>$A$</th>
<th>$AR$</th>
<th>$H_E$</th>
<th>$H_O$</th>
<th>HWE($P$)</th>
<th>$F_{IS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hawaii</td>
<td>10</td>
<td>4.4</td>
<td>4.1</td>
<td>0.53</td>
<td>0.49</td>
<td>ns</td>
<td>0.084, ns</td>
</tr>
<tr>
<td>Saipan/Tinian</td>
<td>47</td>
<td>6.0</td>
<td>3.6</td>
<td>0.47</td>
<td>0.44</td>
<td>ns</td>
<td>0.052, ns</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.074, ns</td>
</tr>
</tbody>
</table>

$n$, sample size; $A$, mean number of alleles per locus; $AR$, allelic richness; $H_E$, expected heterozygosity; $H_O$, observed heterozygosity; HWE($P$), probability of departure from Hardy-Weinberg Equilibrium; $F_{IS}$, heterozygote deficit.

*Significance assessed at $P < 0.007$, Bonferroni correction applied ($\alpha = 0.05/7$ loci); ns = not significant.

The probability of observing identical multilocus genotypes between two individuals sampled from a population ($P_{ID}$) was $1/7,153$ in Saipan/Tinian. This value could not be determined in the Hawaii population due to the small sample size (Table 2). A seven-locus genotype appears adequate to distinguish $1/34$ first-order related individuals ($P_{ID_{sib}}$) in Saipan/Tinian and $1/51$ in Hawaii (Table 2).

**Table 2.** Probability that an individual Pacific Golden-Plover (*Pluvialis fulva*) is a member of either the Hawaii or Saipan/Tinian population based on a multilocus panel of seven microsatellite loci.

<table>
<thead>
<tr>
<th>Probability</th>
<th>Hawaii Population</th>
<th>Saipan/Tinian Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{ID_{multilocus}}$</td>
<td>n.d.</td>
<td>1.948e-02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.398e-04</td>
</tr>
</tbody>
</table>
No genetic signature of a recent demographic bottleneck was detected in either population (Table 3). The SMM model (Step-wise Mutation Model) did indicate a significant heterozygote deficit relative to the number of alleles in the Saipan/Tinian samples; however, this could be due to a population expansion or a recent influx of new alleles (Luikart and Cornuet 1998).

Table 3. Wilcoxon test for heterozygosity excess and deficit in seven microsatellite loci in two populations of Pacific Golden-Plovers (*Pluvialis fulva*).

<table>
<thead>
<tr>
<th>Population</th>
<th>IAM deficit</th>
<th>IAM excess</th>
<th>TPM Deficit</th>
<th>TPM excess</th>
<th>SMM deficit</th>
<th>SMM excess</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hawaii (31)</td>
<td>0.344</td>
<td>0.711</td>
<td>0.055</td>
<td>0.961</td>
<td>0.039</td>
<td>0.973</td>
</tr>
<tr>
<td>Saipan/Tinian (44)</td>
<td>0.055</td>
<td>0.961</td>
<td>0.008</td>
<td>0.996</td>
<td>0.003*</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*n*, number of alleles; heterozygote deficit or heterozygote excess; IAM, Infinite alleles model; TPM, Two-phase mutation model; SMM, Step-wise mutation model. Values shown are *P*-values; *significant value based on Bonferroni corrected α = 0.007.

Discussion

No significant genetic differentiation between Pacific Golden-Plover populations of Hawaii and Saipan/Tinian were found in this study, possibly due to a limited sample size which was unable to represent total genetic diversity across both populations. These data indicate that the two sampling populations are the same breeding population. These data do not support current radiotelemetry and leg-banding studies which suggest Saipan plovers breed in Siberia (Johnson et al., in preparation). The apparent absence of population differentiation might show that the species is comprised of populations that are not demographically distinct; however, analyses with a larger sample set are necessary to determine population connectivity.
A larger dataset is currently being analyzed with additional samples and loci. In the future, this population genetic work will be augmented with radiotelemetry and leg-banding data on plover populations in Hawaii and Saipan/Tinian.

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References


