Supplemental Material

Sequence reliability: The 13 variable sites (12 transitions, one transversion; Fig. 1B) in our sequences appear to represent *in vivo* substitutions, and not artifacts because: 1) all specimens were extracted at least twice, and no sequences differed among extractions or PCRs; 2) bone PAHJ was independently extracted and amplified at the Smithsonian and UCLA, and identical sequences were obtained; 3) 12 of the variable sites also varied within or among other species of geese (ref. 5); 4) the conserved right domain sequences showed no variation, as expected from comparisons of modern sequences (e.g., in *Branta canadensis*, 11.5% of 217 left domain sites varied, while only 1.1% of 92 right domain sites varied; ref. *5*); and 5) in the paleontological sample there was no correlation between age of a bone and number of sites differing from RH (r^2=0.06, p=0.50, n=10). Finally, four subfossil bones from four other islands contained RH while four contained additional unique haplotypes.

Confidence intervals and simulation model: In addition to multinomial 95% confidence intervals for our heterozygosity estimate, we calculated bootstrap resampling confidence limits of 0.19 upper (for H=0.067), and 0.57 lower (for H=0.802). Both methods indicate a major and significant decline in heterozygosity.

To determine the severity of a population decline that could have caused such a loss of mtDNA gene diversity, we conducted a Monte Carlo simulation. We considered Baldwin's estimate of 25,000 nene living on Hawaii Island at the time of Cook's arrival as an upper limit. Accordingly, our simulations used female effective population sizes (N_e) starting at 500, 1000 and 10,000. We reduced these initial population sizes at different rates to simulate different

degrees of bottlenecks. Our estimated maximal rate of population decline from Cook's time (N=25,000) until the 1930s (N=30) is r = -0.16. To be conservative, we used rates of decline in the model of 0, -0.01, -0.05 and -0.10. The simulation ran for a period of 150 generations (600 years given an estimated generation time of 4 years) and estimated the average H for each generation. Initial H was simulated by randomly drawing individuals from the observed frequency distribution of the paleontological sample with a mean H of 0.80. The simulation continued until: 1) gene diversity dropped to 0.26 (the upper 95% bound of the observed H) or 2) the number of generations exceeded 150. This was repeated 1000 times for each r and starting female N combination.

In no case did the simulation reach 0.26 when r was 0, indicating that some level of decline was necessary to achieve the observed loss. In addition, our simulations show that even at the slowest rate of population decline (r = -0.01), the population needed to fall below 270 females to reach 0.26. At more likely rates of decline (r = -0.05, -0.10) the population must fall to under 20 females on average. Thus, even with a single mtDNA marker, the observed loss of genetic variation is best explained by a major population decline, most likely to below 20 females.

Acknowledgments: We thank C. Kishinami, R. Prys-Jones, A. Baker, G. Barrowclough, R. Dekker, C. Fisher, C. Voisin, K. Garrett, J. Fjeldsa, R. Payne, N. Johnson, J. Black, K. Bauer, E. Rave, F. Duvall, C. Terry, J. Black, A. Cooper, B. Schaefer, M. Severns, J. Giffin, B. Covington, and D. Taylor for access to nene specimens, samples or logistic assistance, and P. Banko, L. Hartzell, T.&L. Pratt, C. Tarr and B. Slikas for help with analyses or discussion. Funding was provided by the Smithsonian Institution, NSF (DEB-9100057 to R. C. Fleischer

and OPP-9817937 to R. Wayne), FONZ, Hawaii DLNR, Wildfowl and Wetland Trust, and National Geographic Society.