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# Genetic Analyses Through DNA Fingerprinting of Captive Populations of Hawaiian Geese

ELIZABETH H. RAVE

Department of Biology  
University of North Dakota  
Grand Forks, ND 58202, U.S.A.

ROBERT C. FLEISCHER

Molecular Genetics Lab  
National Zoological Park  
Smithsonian Institution  
Washington, DC 20008, U.S.A.

FERN DUVALL

Olinda Endangered Species Captive Propagation Facility  
535 Olinda Road  
Makawao, Maui, HI 96768, U.S.A.

JEFFREY M. BLACK

The Wildfowl and Wetlands Trust  
Slimbridge, Gloucester  
GL2 7BT England

**Abstract:** DNA fingerprinting was used to assess levels of genetic variation in 106 Hawaiian Geese, or Nene (*Branta sandvicensis*), from two captive colonies in Hawaii and Slimbridge, England. Mantel tests were used to determine differences in mean similarity coefficients obtained from DNA fingerprints between unrelated and related Nene within and between captive colonies and to determine whether pedigree-based estimates of relatedness correlated with DNA fingerprint-based estimates. Between colonies, mean similarity coefficients for unrelated and related Slimbridge Nene were higher than those for Hawaiian Nene. Within each colony, related Nene had higher mean similarity coefficients than did unrelated Nene. A positive relationship was found between coancestry coefficients and similarity coefficients. A greater number of founders for the Hawaiian colony con-

Análisis genético de poblaciones cautivas de gansos hawaianos usando "fingerprinting" de ADN

**Resumen:** Se uso "fingerprinting" de ADN para evaluar los niveles de variación genética en 106 gansos Hawaianos o Nene (*Branta sandvicensis*), de dos colonias cautivas en Hawaii, USA y en Slimbridge, Inglaterra. Se usaron tests de Mantel para determinar diferencias en los coeficientes de similitud promedio obtenidos a partir de "fingerprints" de ADN entre Nene emparentados y no emparentados dentro y entre colonias cautivas y para determinar si las estimaciones de parentesco basadas en pedigré se correlacionaban con estimaciones basadas en "fingerprint" de ADN. Entre colonias, los coeficientes de similitud promedio para los Nene emparentados y no emparentados de Slimbridge fueron mayores que para aquellos de Nene de Hawaii. Dentro de cada colonia, Nene emparentados tuvieron coeficientes de similitud promedio más altos que Nene no emparentados. Se encontró una relación positiva entre coeficientes de colinaje y coeficientes de similitud. Los coeficientes de simi-

Send correspondence to Robert C. Fleischer, Molecular Genetics Lab, National Zoological Park, Smithsonian Institution, Washington, D.C. 20008.

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tributed to the lower mean similarity coefficients. As genetic variation decreases, difficulty in distinguishing relatedness among individuals using DNA fingerprinting may increase. Lower genetic variation also may increase the error in estimating the relationship between coancestry and similarity coefficients. DNA fingerprinting of Nene identified unique alleles and can determine optimal pairings between individuals. The calibrated similarity coefficient distributions can help determine the relatedness of individuals in wild populations of Nene.

## Introduction

Severe reductions in a population's size can result in higher inbreeding and lower genetic variation (Wright 1931; Nei et al. 1975). Subsequently, a decrease in fitness may occur, as evidenced by lower rates of fecundity (Ralls & Ballou 1983) or survivability (Ralls & Ballou 1983; Templeton & Read 1983). Alternatively, retention of genetic variation may increase population vigor and the potential for future evolutionary adaptation (see Allendorf & Leary 1986) and may enhance the chances for successful reintroduction of captive animals into the wild (Hedrick & Miller 1992). For these reasons, population size reductions and genetic diversity are important issues for managers, especially those who must maintain or increase productivity in small captive populations (Frankel & Soulé 1981; Schonewald-Cox et al. 1983; Ralls & Ballou 1986; Soulé 1987). The effectiveness of DNA fingerprinting in the assessment of genetic diversity and relatedness of individuals within populations has prompted researchers to use this technique increasingly in population biology studies (Burke & Bruford 1987; Gilbert et al. 1990, 1991; Kuhnlein et al. 1990; Reeve et al. 1990; Packer et al. 1991; Piper & Rabenold 1992; Triggs et al. 1992).

The endangered Hawaiian Goose, or Nene, underwent a severe population bottleneck during the early part of this century. Habitat loss, introduced predators, and excessive hunting decimated Nene populations, declining from an estimated 25,000 birds during the late eighteenth century (Baldwin 1945) to 17 known individuals by 1950 (Elder & Woodside 1958). To save the species, captive propagation programs were established at the Pohakuloa Endangered Species Facility on Hawaii (currently Olinda on Maui) in 1949 and at The Wildfowl and Wetlands Trust in Slimbridge, England, in 1950 (Kear & Berger 1980). Both colonies began with only two geese and one gander. Five of these Nene were obtained from a privately owned inbred flock, and one was a wild female sent to Pohakuloa. Since the inception of these colonies, 21 additional founders have been

added to Pohakuloa (three of which were of known wild ancestry; Kear & Berger 1980; Duvall, unpublished data), whereas only four have been added at Slimbridge (Kear & Berger 1980). After a slow start, propagation efforts became successful, and in 1960 captive Nene were first released into the wild (Kear & Berger 1980). By 1992, 2147 Nene from both captive colonies had been released on Hawaii, Maui, and Kauai (Black et al. 1991; Duvall, unpublished data). Although the number of birds on Maui and Kauai have remained stable or have increased (Black et al. 1991), self-sustaining wild populations on Hawaii have yet to be established (Stone et al. 1983; Scott et al. 1985). Population numbers depend instead on the number of captive birds released (Black et al. 1991; Black & Banko 1994). A low level of genetic variation is a potential factor limiting Nene population growth (Kear & Berger 1980; Stone et al. 1983). Because few individuals founded both captive flocks, high levels of inbreeding and low genetic diversity would be expected. Although captive propagation has continued for over 40 years, genetic information on these birds is still unknown. Therefore, we assessed the genetic diversity and relatedness of individuals in two captive Nene populations using DNA fingerprinting. Our goals were (1) to determine differences in genetic variability between the two captive colonies, (2) to determine differences between related and unrelated individuals within each captive colony, and (3) to determine the relationship between pedigree-based estimates and DNA fingerprint-based estimates of relatedness.

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## Methods

### DNA Fingerprinting

One to two ml of whole blood were obtained from 29 Nene (88% of the flock from 1988 to 1992) at Pohakuloa, Hawaii, and at the Olinda Endangered Species Captive Propagation Facility, Maui, and from 77 Nene (48%

of the flock during 1990) at The Wildfowl and Wetlands Trust, Slimbridge, England. For comparisons with a related (Quinn et al. 1991) outbred species, whole blood also was obtained from 14 presumably unrelated wild Canada Geese (*Branta canadensis*) from Mandan, North Dakota.

Methods for obtaining DNA fingerprints were the same as those used by Rave (1994). Genomic DNA was isolated, digested with the restriction endonuclease *Hae*III, electrophoresed in an agarose gel, and vacuum-blotted to a nylon membrane. Molecular size markers were run on each gel, and internal standards were run within each lane. Every effort was made to equalize DNA concentrations between lanes. Membranes were prehybridized (Westneat et al. 1988) and then hybridized sequentially with radioactively labeled M13 bacteriophage DNA (Vassart et al. 1987), with Jeffreys' 33.15 and 33.6 human minisatellite DNA (Jeffreys et al. 1985), and with lambda *Hind*III to document occasional band shifts. The Canada Goose membrane was hybridized with Jeffreys' 33.15. Membranes were washed (Westneat et al. 1988) and exposed to X-ray film to produce autoradiographs of DNA fingerprints.

#### DNA Fingerprint Analyses

For each autoradiograph, similarity coefficients ( $S$ ; Lynch 1990) between all paired combinations of birds were calculated using the following formula:

$$S = 2N_{AB}/(N_A + N_B),$$

where  $N_{AB}$  is the number of fragments shared by individuals  $A$  and  $B$ , and  $N_A$  and  $N_B$  are the total number of fragments present for individuals  $A$  and  $B$ , respectively. All bands greater than 1.9 kilobase pairs were scored and assumed to be unlinked. Although a detailed pedigree of the Nene was available at Olinda, only immediate familial relationships were known at Slimbridge. Therefore, only parents and offspring and siblings at both sites were classified as related, with a coefficient of relatedness ( $r$ ; Wright 1922) equal to 0.5. All other individuals were "unrelated" ( $r < 0.5$ ).  $R$  did not reflect past history of inbreeding within the colonies. Mean similarity coefficients for unrelated and related Nene at both colonies were obtained for each probe and for all probes combined. A mean similarity coefficient for Canada Geese was calculated only for Jeffreys' 33.15.

Because similarity coefficients were calculated for pair-wise combinations of birds, nonindependent observations resulted. To account for these interdependencies, Mantel tests (NTSYS; Rohlf 1990) were used to determine differences in mean similarity coefficients between unrelated and related Nene within and between captive colonies for each probe and for all probes combined. Two symmetrical similarity matrices consisting

of similarity coefficients between all pair-wise combinations of birds for the four probe categories and their corresponding relatedness or site were compared using the Mantel test statistic  $Z$  (NTSYS; Rohlf 1990).  $T$ -values then were calculated and compared with the standard  $t$ -distribution with infinite degrees of freedom ( $t = \pm 1.96$  at the 0.05 probability level) to determine the presence of a statistical association between the two variables (Schnell et al. 1985). Mantel tests also were used to determine differences between mean similarity coefficients of Canada Geese and unrelated Nene at Olinda and Slimbridge using data from Jeffreys' 33.15.

Differentiation between the two colonies was assessed using an estimate of  $S_{ij}$  (equation 11; Lynch 1990) and  $F'$  (equation 14; Lynch 1990). Because  $S$  is potentially biased (Lynch 1990), we also calculated band-sharing probabilities ( $x$ ; Jeffreys et al. 1985), or the probability that a band present in one individual is found in another, using the following formula:

$$x = [(N_{AB}/N_A) + (N_{AB}/N_B)]/2.$$

$X$  was used to calculate average allele frequencies ( $q$ ) and the average number of alleles per locus ( $1/q$ , assuming little variance in  $q$  between alleles; Jeffreys et al. 1985) for unrelated pairs of Nene in each captive colony for all probes combined.

#### Pedigree Analysis

From the detailed pedigree of Nene at Pohakuloa/Olinda, we used a pedigree analysis program (Genetics; Ballou 1989) to calculate coancestry coefficients ( $F_{ij}$ ), or inbreeding coefficients of hypothetical offspring resulting from matings between all possible pair-wise combinations of birds. This program uses the additive relationship matrix method to calculate inbreeding coefficients, with  $F_{ij} = 1/2r_{ij}$  (Ballou 1983). Unlike  $r$  (see above),  $F$  reflects past history of inbreeding within the Pohakuloa/Olinda colony. We used a Mantel test (NTSYS; Rohlf 1990) to determine whether pedigree-based estimates of relatedness (coancestry coefficients) correlated with DNA fingerprint-based estimates (similarity coefficients).

Five of the 29 Nene in the current colony at Olinda were captured from the wild. Because these birds may be descendants of captive-released birds (see discussion), their inbreeding coefficients could not be accurately estimated. To alleviate the potential bias due to unknown ancestry, these five birds were eliminated, and the remaining 24 birds were used in the analysis.

## Results

### DNA Fingerprints

DNA fingerprints and similarity coefficients revealed low levels of genetic diversity within the two captive

populations (Figs. 1 and 2). The means and overall ranges of similarity coefficients were higher for Slimbridge than for Olinda (Table 1 and Fig. 2). Because of common ancestry between the groups, many bands were shared by both populations (Fig. 1). All DNA fingerprint bands from Slimbridge Nene also were represented in Olinda birds, but not all bands present in Olinda Nene were found in Slimbridge birds. The degree of differentiation between Olinda and Slimbridge ( $F'$ ; Lynch 1990) was 0.203.

Mean similarity coefficients for unrelated and related Nene at Slimbridge were greater than for those at Olinda for all four probe categories ( $t = 5.52-33.57$ ,  $p < 0.05$ ; Table 1, Fig. 2). Mean similarity coefficients for related Nene were greater than those for unrelated Nene at both Olinda and Slimbridge for all four probe categories ( $t = 4.29-17.07$ ,  $p < 0.05$ ; Table 1, Fig. 2). In comparison, the mean similarity coefficient for a sample of putatively unrelated Canada Geese was 0.470, which was lower than those for unrelated Nene at both Olinda and Slimbridge ( $t = 10.89$  and  $24.00$ , respectively,  $p < 0.05$ ).

Combining all probes, the total number of bands scored for each Nene ranged from 55 to 78 at Olinda and from 48 to 81 at Slimbridge. Mean number of bands scored did not differ between sites ( $t = 1.86$ ,  $p > 0.05$ ; Table 2). Mean band-sharing probability and allele frequency were lower at Olinda, whereas number of alleles per locus was lower at Slimbridge (Table 2).

## Pedigrees

A positive relationship was found between coancestry coefficients and similarity coefficients for the 24 Nene from Pohakuloa/Olinda ( $t = 4.27$ ,  $p < 0.05$ ; Fig. 3). The product-moment correlation coefficient ( $r$ ) was 0.269, a low number reflected by the correspondingly high scatter in Fig. 3. Consequently, only 7% of the variation in similarity coefficients can be explained by the coancestry coefficients.

## Discussion

### DNA Fingerprints

DNA fingerprints revealed that Nene at Olinda were more genetically diverse than those at Slimbridge. Throughout the colonies' histories, Olinda had more founders than did Slimbridge (24 versus 7 Nene; Kear & Berger 1980; Duvall, unpublished data). This explains the lower mean similarity coefficient at Olinda. Prior to the first captive releases in 1960, three wild Nene were added to the Pohakuloa/Olinda flock (Kear & Berger 1980). These birds were individuals from, or descendants of, the estimated 30 wild Nene that remained by 1951 (Smith 1952). These truly wild Nene undoubtedly contributed new genetic diversity to the highly inbred flock. Also, an additional 18 Nene were added to the flock after reintroductions began. Although these birds may have been offspring of released captives, lower sim-

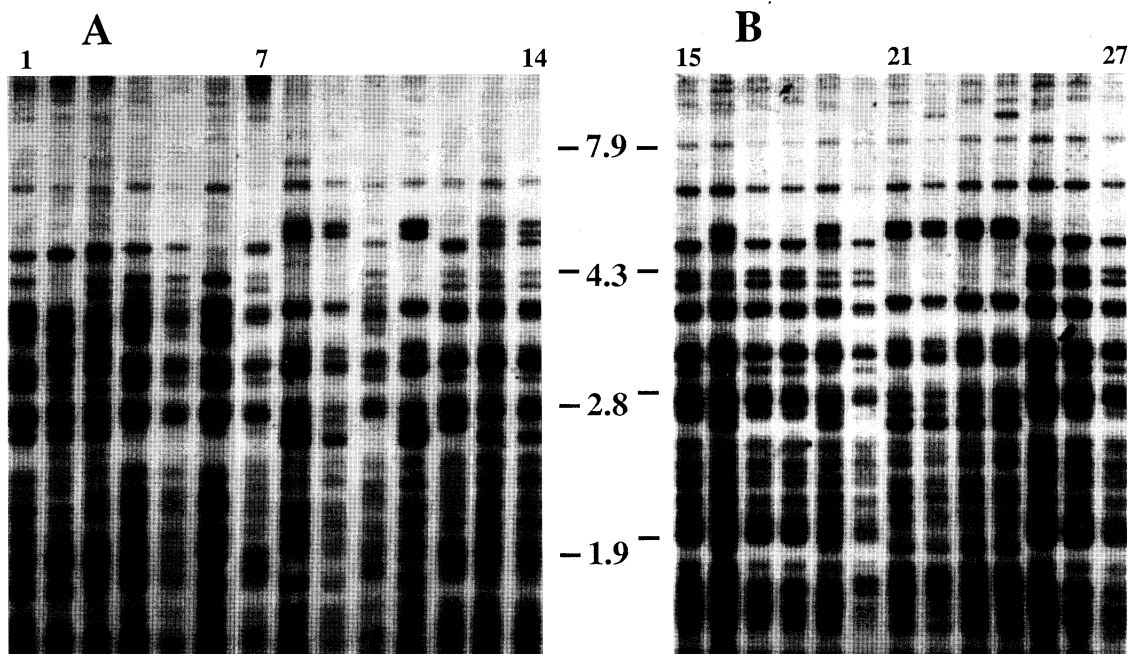


Figure 1. DNA fingerprints of unrelated and related Nene digested with *HaeIII* and probed with M13. Marker is in kilobase pairs. (a) Nene from Pohakuloa/Olinda, Hawaii (lanes 1-8 and 10-12), and Slimbridge, England (lanes 9, 13, and 14); lanes 4 and 5 are siblings, and lanes 3-4 and 13-14 are separate parent/offspring groups; lanes 10 (parent), 11 (offspring), and 12 (parent) comprise a misidentified family group (see text for details). (b) Nene from Slimbridge, England; lanes 15-19, 21-24, and 25-27 are separate family groups.

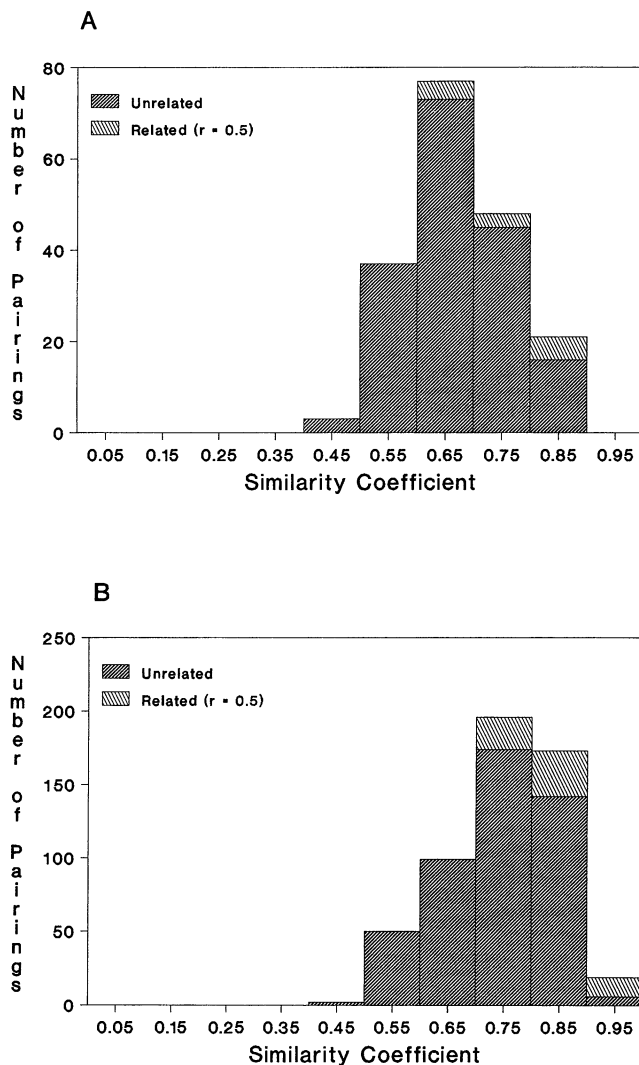


Figure 2. Distribution of similarity coefficients for unrelated and related Nene at Olinda, Hawaii (a), and Slimbridge, England (b).  $r$  = coefficient of relatedness.

ilarity coefficients would result if any contained unique alleles. At Slimbridge, two siblings that were descendants of one truly wild bird and one Pohakuloa bird were obtained in 1962. Two more Nene from Hawaii were obtained in 1967 (Kear & Berger 1980). Because these were the only birds added, with no full-blooded wild bird among them, higher similarity coefficients among Slimbridge Nene would be expected.

Different rearing techniques also may have contributed to lower similarity coefficients at Olinda. From the inception of this flock, all birds were manually paired to minimize inbreeding and maximize productivity (Kear & Berger 1980). Pairings were changed as new birds were added, as old or low-producing birds were culled, or as a pair's offspring were sufficiently represented among captive-released birds. At Slimbridge, Nene were manually paired only initially (Kear & Berger 1980).

Table 1. Mean similarity coefficients for each probe and for all probes combined of unrelated and related Nene in captive colonies at Olinda, Hawaii, and Slimbridge, England.\*

	Probe	Olinda	Slimbridge
Unrelated	M13	0.671 <sup>A1</sup> (174)	0.771 <sup>B1</sup> (473)
	33.15	0.704 <sup>A1</sup> (100)	0.737 <sup>B1</sup> (462)
	33.6	0.610 <sup>A1</sup> (100)	0.700 <sup>B1</sup> (437)
	Combined	0.666 <sup>A1</sup> (174)	0.739 <sup>B1</sup> (473)
Related	M13	0.758 <sup>A2</sup> (12)	0.876 <sup>B2</sup> (66)
	33.15	0.798 <sup>A2</sup> (8)	0.817 <sup>B2</sup> (62)
	33.6	0.774 <sup>A2</sup> (8)	0.805 <sup>B2</sup> (62)
	Combined	0.761 <sup>A2</sup> (12)	0.837 <sup>B2</sup> (66)

\* Means within a row sharing letters are not different, and means within a column sharing numbers between each unrelated and related probe category are not different ( $p > 0.05$ ). (Number of pairings.)

Beginning in the early 1970s, Nene were permitted to pair freely, which may have resulted in the mating of related birds. If inbreeding increased, DNA fingerprint profiles would show a higher degree of similarity between individuals (Kuhnlein et al. 1990).

Similarity coefficients at both Olinda and Slimbridge were high when compared with values obtained from outbred avian species, which typically average less than 0.30 (Burke & Bruford 1987; Wetton et al. 1987; Meng et al. 1990; Westneat 1990). Although the mean similarity coefficient for Canada Geese in this study—(0.470)—was higher than 0.30, it was still significantly lower than Nene values. It could be argued, however, that the high similarity coefficients for unrelated individuals at Olinda were upwardly biased. Related birds included only parent/offspring and siblings, with half-siblings, cousins, grandparents/grandchildren, and aunts and uncles/nieces and nephews defined as “unrelated.” If these more distantly related individuals were removed from the unrelated category, the mean similarity coefficient for unrelated Nene still would be high: 0.667 for all probes combined.

Within each captive flock, related birds had higher mean similarity coefficients than did unrelated birds. Several studies show similar results, with little or no overlap between related and unrelated individuals (Wetton et al. 1987; Westneat 1990; Oring et al. 1992; Piper & Rabenold 1992). Even less genetically variable species show distinctions between the two categories (Gilbert et al. 1991; Packer et al. 1991). With Nene, however, considerable overlap existed, although related Nene were clustered toward the upper end of the histograms, whereas unrelated Nene ranged down to 0.450 at Olinda and 0.433 at Slimbridge. Because of these ten-

**Table 2.** Mean number of total bands scored for all individuals  $\pm$  sd and comparison of DNA fingerprint analyses for all probes combined of unrelated Nene in captive colonies at Olinda, Hawaii, and Slimbridge, England.

	Mean Number of Bands per Individual $\pm$ sd	Mean Band Sharing Probability ( $\bar{x}$ )	Mean Allele Frequency ( $\bar{q}$ )	Number of Alleles per Locus (1/q)
Olinda	65.6 $\pm$ 6.31 (26)*	0.670 (174)**	0.426	2.3
Slimbridge	62.4 $\pm$ 7.97 (77)*	0.743 (473)**	0.493	2.0

\* Number of fingerprint profiles.

\*\* Number of pairings.

dencies, we have greater confidence that Nene with low band-sharing similarity are unrelated than we do that those with high band-sharing similarity are related. Our data indicate that the more inbred and less genetically variable a population, the greater the difficulty in distinguishing between related and unrelated individuals based only on DNA fingerprints.

DNA fingerprinting in conjunction with pedigree information is more appropriate for determining relatedness in Nene. Even with available pedigrees, however, inaccurate records can affect similarity coefficients. Based on DNA fingerprint data, two mistakes in the Pohakuloa/Olinda pedigree were found. In two families, an

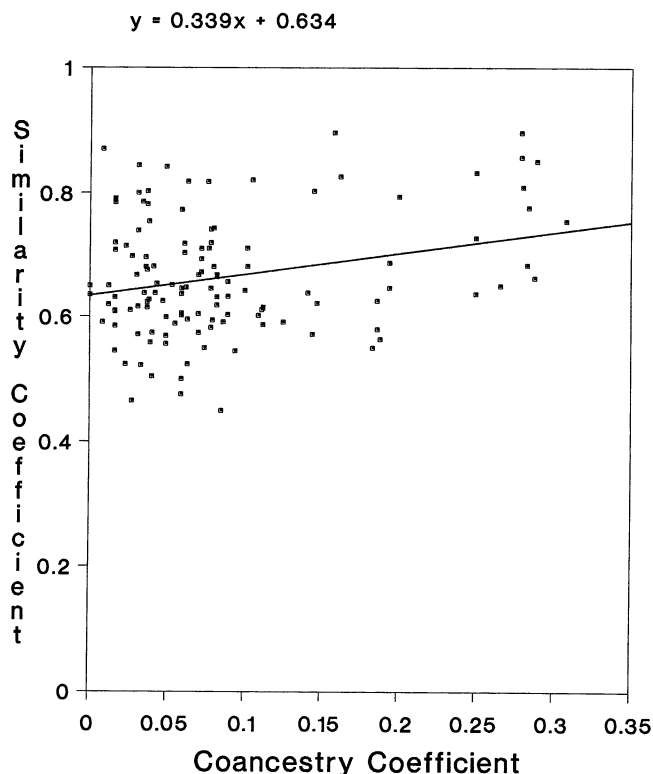
offspring's banding pattern differed from its parents. Many bands in both offspring were not found in either of their alleged parents, and some bands found in both parents were not found in their alleged offspring (see Fig. 1a). Because fingerprint bands match expectations of Mendelian inheritance (Jeffreys et al. 1985), these were not true family groups. If other mistakes such as these went undetected, distributions for "related" individuals would be skewed downward. Conversely, if "unrelated" individuals were in fact related, distributions would be skewed upward.

Band-sharing probabilities, allele frequencies, and number of alleles per locus also reflected low levels of diversity in Nene. Many bands in the fingerprint profiles were shared by all individuals, especially among Slimbridge birds (see Fig. 1). Correspondingly, band-sharing probabilities were high, and number of alleles per locus was low. In contrast, Meng et al. (1990) found lower band-sharing values (0.206–0.284) and a greater number of alleles per locus (6.5–9.1) in three species of swan (*Cygnus* sp.). If fingerprint fragments are chromosomally linked, allele number is likely biased. Some fragments of Jeffreys' 33.6 in swans and fragments of M13 in Nene appear to be linked (Meng et al. 1990; Fig. 1), so the exact number of alleles per locus may be incorrect. However, we believe that the large relative difference in variability between Nene and other species is real.

### Pedigrees

As the level of inbreeding increased, so did similarity coefficients. Kuhnlein et al. (1990) found similar results, with a nonlinear but positive relationship between inbreeding and similarity coefficients in defined genetic strains of chickens. Other studies also show higher similarity values associated with smaller, presumably more inbred, populations, and lower values associated with larger populations (Gilbert et al. 1990, 1991; Triggs et al. 1992).

The relationship between coancestry and similarity coefficients of Nene revealed a high degree of scatter, because the coancestry coefficients were biased downward. Two of the three original founders at Pohakuloa/Olinda were highly inbred (Kear & Berger 1980). No



**Figure 3.** Comparison of coancestry coefficients obtained from the pedigree of Nene at Pohakuloa/Olinda, Hawaii, and similarity coefficients obtained from DNA fingerprints for the same pairings. The best-fit line and its equation are shown.

pedigrees were available before the founding of the colony, so inbreeding coefficients for those birds, along with subsequent founders captured after reintroductions began, could not be identified accurately. Consequently, they were assigned an inbreeding coefficient of 0, even though this number was often too low. Although the five wild Nene currently in the colony were removed from the analysis, the bias caused by the compounding effect of Nene added earlier to the flock was not alleviated.

A relatively narrow range of similarity coefficients was revealed through DNA fingerprinting (see Fig. 2a). Perhaps as this range decreases (the more inbred the species), the greater the error in estimating the relationship between coancestry based on pedigrees and relatedness based on DNA fingerprints. Precise knowledge of inbreeding coefficients of individuals from the onset may result in better estimates, however, regardless of the range of similarity coefficients.

### Management Implications

DNA fingerprint profiles identified unique alleles that could prove useful in the management of Nene populations. When fingerprints were analyzed from the Pohakuloa/Olinda flock, one Nene had several bands that were not found in any other captive bird. In 1992, this bird was to be culled from the breeding population and released into the wild. But after its genetic value was confirmed through DNA fingerprinting, it was released only after a semen sample was obtained. Because the goal of many captive propagation programs is to maintain or increase genetic diversity among individuals (Schonewald-Cox et al. 1983; Ralls & Ballou 1986; Tomlinson et al. 1991), the usefulness of DNA fingerprinting in identifying genetic variation becomes apparent.

Similarity coefficients obtained from DNA fingerprints could be used to determine male/female pairings that would maximize genetic diversity or minimize inbreeding in the captive colonies. For example, Nene with low similarity coefficients should be mated more often than those with high similarity coefficients. This would increase the probability of pairing unrelated birds, because low similarity values generally indicated unrelated individuals (see Figs. 2a and 3). Without this genetic information, lower genetic diversity still could result between pairings of unrelated Nene if their similarity values were high. Similarity coefficients, therefore, may prove more useful than a pedigree in determining optimal pairings among captive Nene.

Lastly, the calibrated distributions of similarity coefficients for unrelated and related captive Nene can be used as a standard to determine relatedness among individuals in wild populations (Rave, unpublished data). Some populations of Nene are not yet self-sustaining (Stone et al. 1983; Scott et al. 1985), perhaps due to the

low genetic diversity (Kear & Berger 1980; Stone et al. 1983). Because most wild birds were descended from released captives, increasing genetic diversity of captive populations through DNA fingerprint analyses may ultimately enhance the success of reintroduced individuals and increase the chances of restoring self-sustaining populations to the wild.

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