Genetic relationships within a population of Blue Duck *Hymenolaimus malacorhynchos*

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DNA fingerprinting revealed a very high degree of genetic similarity within a small population of Blue Ducks, supporting observations of limited dispersal. The three major family groups studied proved to be interrelated. One example of close inbreeding was confirmed and another suggested. All genetic relationships indicated by observational studies were confirmed by genetic data, including correct parentage of ten broods. Thus, no evidence was found of multiple paternity or maternity in this territorial riverine species.

One difficulty with observational studies of long-lived species is that genetic relationships within a population and between neighbouring populations can be assigned only after many years of detailed study. Even then, assignment of offspring to particular breeding birds based on observed parental care may be confounded by successful (but unobserved) extra-pair copulations (Burns *et al.* 1980, Gavin & Bollinger 1985, Birkhead *et al.* 1988), nest parasitism (Yom Tov 1980), creching of young or brood amalgamation (Kear 1970, Gorman & Milne 1972, Williams 1974).

Recent advances in molecular genetic techniques and in particular the development of DNA fingerprinting (Jeffreys *et al.* 1985a) now allow parentage to be assigned with a very high degree of certainty, as well as allowing estimates of other degrees of genetic relatedness (Burke & Bruford 1987, Wetton *et al.* 1987, Quinn *et al.* 1987, 1989, Burke *et al.* 1989).

In waterfowl, forced extra-pair copulations appear as a secondary male reproductive strategy in many species (McKinney *et al.* 1983). From studies of sperm competition (Burns *et al.* 1980, Cheng *et al.* 1983) and the widespread occurrence of forced copulations in *Anas* ducks, multiple paternity is predicted to be a common feature of dabbling duck broods. First evidence of this has come from Evart & Williams (1987).

One species of Anas in which forced copulations have not been observed is African Black Duck *Anas sparsa* (McKinney *et al.* 1978). This and the other riverine *Anas* (*A. waiguensis*; Kear 1975), in contrast to almost all of their congeners, retain pair associations year round and defend territories, as pairs, on rivers at all times of the year. In this social system extra-pair liaisons or inseminations seem incompatible with year-round resource defence, and multiple paternity within broods is not expected.

The Blue Duck *Hymenolaimus malacorhynchos* of New Zealand is another riverine anatid that maintains year round pair associations and territorial defence. The male Blue Duck (unlike the male Black Duck) plays a full role in raising the brood and forced copulations have not been observed (M. Williams pers. obs.).

A long-term demographic study of Blue Ducks on the Manganuiateao River in the central North Island of New Zealand revealed that some neighbouring birds were relatives, and that sibling pairings had formed (Williams 1991). These close genetic relationships appear to be a consequence of the limited dispersal shown by this species and the tendency of both sexes to attempt settlement close to their natal range. In this study, we have used DNA fingerprinting to test the prediction that all young within a brood are the progeny of the guardian adults and to determine genetic relationships between neighbouring territorial adults. We have sought to quantify the genetic relationships of seven contiguous territorial pairs and their offspring of 1987 and 1988 on the Manganuiateao River and to look for relationships which pre-date the observational study.

Table 1. Genetic similarity D between adult Blue Ducks of no known relationship within the Manganuiateao population (3'HVR below and 33.15 above diagonal).

	ML1	ML2	ML3	ML9	ML10	ML11	ML12	MM1	ML13	ML14	ML17	ML20
ML1	——	0.38	*	-	0.35 0.	43	0.43	0.43	0.40	0.50	0.59	0.31
ML2	0.33	——	*	-	0.56	0.56	0.29	0.54	0.53	0.26	0.67	0.43
ML3	*	*	——	-	0.68	0.78	0.51	-	0.48	-	0.45	0.57
ML9	-	-	-	——	0.38	*	0.63	*	*	*	0.58	-
ML10	0.35	0.50	0.49	-	—— *	0.38	*	*	*	0.89	0.17	
ML11	0.41	0.45	-	*	*	——	0.38	*	*	*	0.51	0.37
ML12	-	0.27	-	-	0.41	0.42	——	0.58	0.34	*	0.43	0.25
MM1	-	-	-	-	-	-	-	——	-	*	0.65	-
ML13	-	-	-	*	*	*	0.48	-	——	*	0.56	0.25
ML14	-	0.38	-	*	*	*	*	-	-	——	0.35	0.16
ML17	-	-	-	-	0.69	0.44	0.27	-	-	-	——	0.36
ML20	-	0.31	-	-	0.22	0.38	0.26	0.33	0.36	-	0.32	——

- No data.
* Known relationship (see Table 2).

Methods

Blood samples for genetic analysis were taken from a total of 22 Blue Ducks: 11 breeders/ independent juveniles and ten ducklings from a 9 km stretch of the Manganuiateao River (the study site of M. J. Williams), and one individual (MM1) known to have been hatched within this stretch of river and now resident approximately 10 km upriver. Blue Ducks were captured by gently herding them downstream into a mistnet erected across the river. Individuals were banded (or previous bands recorded and checked), sexed and weighed, and a blood sample of up to 1 ml was taken by venipuncture using a sterile, heparinized syringe and 26 ga needle. Blood samples were separated into serum and red cell fractions by field centrifuge (2000 rpm for 5 minutes), kept on ice, and frozen in liquid nitrogen as soon as possible (0-4 hours). Samples were stored at -70°C for the duration of the study.

DNA fingerprints were produced using two minisatellite DNA probes, 33.15 (Jeffreys 1985a) and 3'HVR. Two probes were used to give two independent estimates of genetic similarity values and also because variablity within a species (and hence discriminatory power) may depend on probe type (G.K. Chambers unpubl.). Red cell fractions (50 ul) were digested overnight at 37°C in a solution containing proteinase K (BRL-Bethesda Research Laboratories) and sodium dodecyl sulphate (BRL) according to the method of Maniatis *et al.* (1982). Red blood cell debris was removed by solvent extraction with phenol and chloroform/ isoamyl alcohol (Maniatis *et al.* 1982). DNA was precipitated with ethanol, air-dried, and redissolved in TE (10 mM Tris.Cl, 1 M EDTA, pH 8.0). DNA yields were estimated by gel

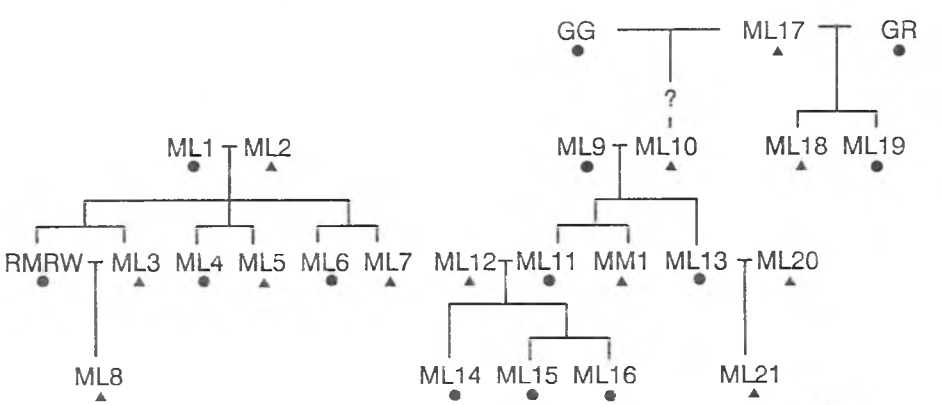

Figure 1 Genealogy of Blue Ducks sampled, as determined by field observations. ▲ = male, ● = female. (Cross-reference to Williams (1991: Fig. 7) is as follows: ML1 - Ruatiti female 1983-89; ML2 - Rain male 1980-88; ML3 - Fern male 1985-89; ML9 - Meyers female 1980-88; ML10 - Meyers male 1981-89; ML11 - Orautoha female 1985-89; ML12 - Orautoha male 1986-89; ML13 - Hoihenga female 1983-89; ML14 - Beeches female 1986-88; ML16 - Beeches female 1989; ML17 - Ruatiti male 1980-88; ML18 - Beeches male 1989; ML20 - Hoihenga male 1981-89.)

electrophoresis and comparison with bacteriophage lambda DNA standards. Samples of DNA (2 ug) were digested with HaeIII (BRL) as per supplier's instructions then electrophoresed under standard conditions (Maniatis *et al.* 1982) on 1% agarose gels in TBE buffer (0.089 M Tris, 0.089 M Boric acid, 0.002 M EDTA). DNA in the gels was depurinated and transferred by Southern blotting with 20X SSC (3 M NaCl, 0.3 M NaCitrate, pH 7.0) onto Amersham Hybond N nylon membranes (Southern 1975). Blots were baked for two hours at 80°C under vacuum to bind DNA to the membrane, then hybridized overnight at 55°C with ^{32}P-labelled 33.15 or 3'HVR probe, washed under conditions appropriate for the analysis of human DNA (Fowler *et al.* 1988, Jeffreys *et al.* 1985b), and autoradiographed at -70°C.

The degree of genetic similarity (D) was calculated from DNA fingerprints as the proportion of bands (DNA fragment) shared between each pair of individuals, i.e. $D = 2N_{AB}/(N_A+N_B)$, where N_A and N_B are the number of bands in the fingerprints of A and B and N_{AB} is the number of shared bands (Wetton *et al.* 1987). D is equivalent to Jeffreys' (1985a) *x* between unrelated individuals. All clear bands larger than 2 kilobase pairs (kbp) were scored. Poor resolution on some autoradiographs meant that a few individuals could only be scored for one probe ('no data' entries in Table 1). Comparisons between individuals on different gels were made by photocopying one autoradiograph to the exact size of the other. This was standardized by having control Blue Duck samples as well as a lambda-*Hind*III molecular weight standard on each gel. Individual fingerprints were cut out of the photocopy to allow side-by-side comparison. Genetic similarity among a group of birds was estimated by the mean of D values between each pair of individuals in the group.

Paternity and maternity were confirmed for each parent-offspring combination (Fig. 1) by checking that each band present in an offspring was also present in one or other putative parent and that values of genetic similarity were in the expected range for a parent-offspring (r=1/2) relationship. Offspring fingerprints were run adjacent to and between the putative parents to facilitate comparison of bands. In this way, band similarity could be compared even between some of the more poorly resolved individual fingerprints.

All statistics (mean ± standard deviation) were calculated independently for each of the two probes, 3'HVR and 33.15. The assumption of Mendelian inheritance of bands, was not examined in this study, but has been confirmed in similar studies (Burke & Bruford 1987, Jeffreys *et al.* 1987, Burke *et al.* 1989, Flint *et al.* 1989).

Results

A mean of 21.3 (± 3.2) bands greater than 2 kbp were scored per individual for the 3'HVR probe and 22.3 (± 4.3) bands for 33.15. A sample fingerprint is shown in Figure 2. The average genetic similarity (D; Table 1) between individuals not known to be related was 0.38 ± 0.11 (3'HVR, $n = 21$) and 0.46 ± 0.10 (33.15, $n = 42$). Therefore, the average expected D between individuals sharing half their genes (r = 1/2; e.g. parent-offspring, siblings) is approximately (0.38 + 0.62/2) = 0.69 (3'HVR) and (0.46 + 0.54/2) = 0.73 (33.15). Individuals related by r = 1/4 (e.g. grandparent *v* grandchild, aunt/uncle *v* niece/nephew) have an expected D of approximately (0.38 + 0.62/4) = 0.54 (3'HVR) and (0.46 + 0.54/4) = 0.60 (33.15).

Actual values of D (Table 2) between individuals thought to be related by r = 1/2 were D = 0.70 ± 0.04 (3'HVR, $n = 16$) and D = 0.73 ± 0.08 (33.15, $n = 42$). Between individuals related by r = 1/4, the values were D = 0.53 ± 0.06 (3'HVR, $n = 5$) and D = 0.59 ± 0.06 (33.15, $n = 8$). The average similarity between parent-offspring and siblings are consistent with these being the correct biological relationships. Parentage was confirmed by comparisons of the fingerprints of the four pairs of parents (ML1/2, ML9/10, ML11/12, ML13/20) and their 14 sampled offspring from a total of ten clutches (Fig. 1), as every band in each offspring was also found in either the mother or father. There was only one exception to this - one band in offspring ML21 was not shared by either parent (ML13, ML20). In this case all other bands matched and the D of 0.60, 0.72 (3'HVR) and 0.69, 0.76 (33.15) was consistent with a parent-offspring relationship, suggesting that the extra band was a new mutation. Mutations are relatively common in both mammalian and avian minisatellite DNA (Jeffreys *et al.* 1985a, Burke & Bruford 1987). In spite of the high degree of band sharing neighbouring adults could be excluded as parents on the basis of at least one or more non-shared bands.

In two other cases only one adult (ML3, ML17) was caught with the brood. In both cases the D of 0.70-0.73 (33.15) was consistent with

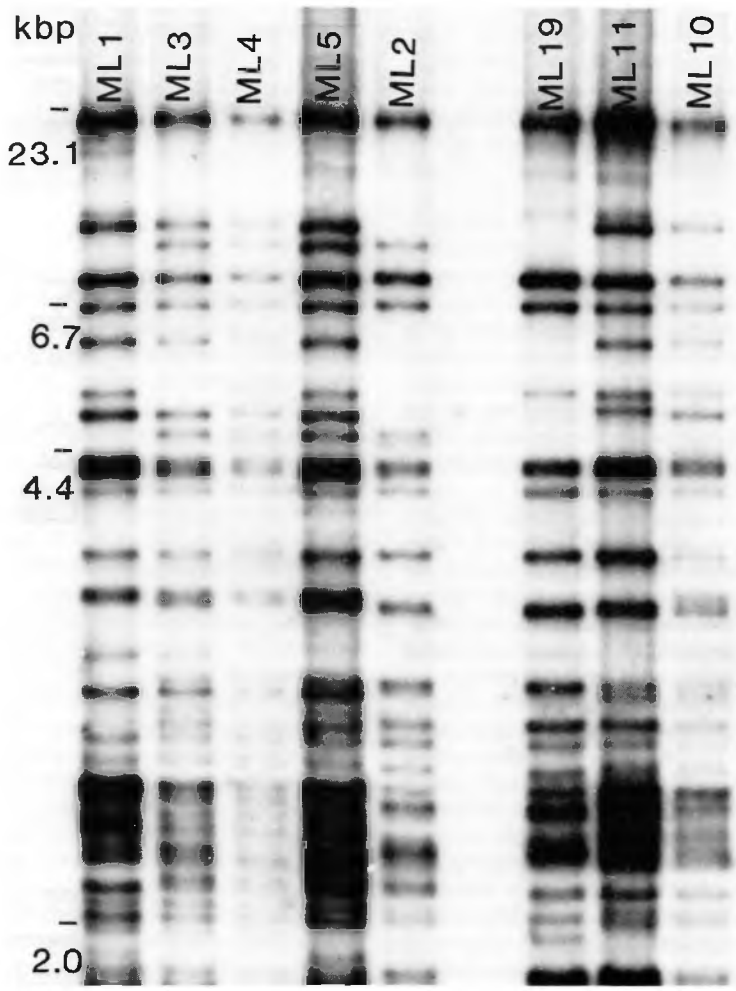

Figure 2. DNA fingerprints of Blue Ducks using probe 33.15. Individuals numbered as in Figure 1.

correct paternity. One offspring (ML8) is thought to have resulted from a pairing between brother (ML3) and sister (RM.RW) (Fig. 1). Although RM.RW was not caught, this relationship is consistent with the high D (0.66 ± 0.12; 33.15) between ML8 and his grandparents (ML1, ML2), aunts and uncles (ML4, ML5, ML6, ML7) compared to D = 0.70 (33.15) between ML8 and his father ML3.

A number of individuals not known to be related by observational studies also show a high degree of genetic similarity (Table 1). A single estimate yielding a high similarity can occur by chance, as the 95% confidence intervals around the means of 'unrelated' individuals are 0.16-0.62 (3'HVR) and 0.13-0.77 (33.15). However, these estimates are confounded by possible (but unknown) relationships between the birds in the study area. Comparisons between 42 Blue Ducks from three populations separated by several hundred kilometres (Triggs *et al.* in press) gave mean genetic similarities of only 0.23 (3'HVR) and 0.21 (33.15) with 95% confidence intervals of 0.05-0.41 and 0.07-0.35 respectively. These data suggest that values of D greater than 0.40 may indicate close relationship. All the individuals in Table 1 have some D values of 0.40 or more. In some comparisons genetic similarity is as high as that found between first or second degree relatives. True biological relationships, rather than chance similarity, can be confirmed when several known relatives all show a high degree of similarity to an individual of unknown origin. This is so in two cases (ML10

Table 2. Genetic similarity (D) among family groups of Blue Duck (3'HVR below and 33.15 above diagonal).

(a) ML1-ML2 family

	ML1	ML2	ML3	ML4	ML5	ML6	ML7	ML8
ML1	——	*	0.79	0.75	0.83	0.85	0.69	0.81
ML2	*	——	0.67	0.76	0.64	0.82	0.70	0.68
ML3	-	-	——	0.78	0.82	0.91	0.69	0.70
ML4	-	-	-	——	0.73	-	0.73	0.63
ML5	0.72	0.73	-	-	——	0.86	0.62	0.69
ML6	0.74	0.69	-	-	0.68	——	-	-
ML7	-	-	-	-	-	-	——	0.48
ML8	-	-	-	-	-	-	-	——

(b) ML17-GR

	ML18	ML19
ML17	0.73	0.70
ML18	——	-
ML19	-	——

(c) ML9-ML10 family

	ML9	ML10	ML11	ML12	MM1	ML13	ML14	ML15	ML16	ML20	ML21
ML9	——	*	0.74	*	0.69	-	-	-	-	*	-
ML10	*	——	0.83	*	0.81	0.72	0.54	0.63	0.64	*	-
ML11	-	0.72	——	*	0.67	0.67	0.65	0.72	0.71	*	-
ML12	*	*	*	——	*	*	0.77	0.84	0.76	*	-
MM1	-	-	-	-	——	0.62	0.56	0.50	0.62	*	0.68
ML13	-	0.70	0.68	*	-	——	0.55	-	-	*	0.76
ML14	-	0.55	0.70	0.75	-	-	——	-	-	*	-
ML15	-	-	0.72	0.64	-	0.50	-	——	-	*	-
ML16	-	-	0.67	0.70	-	0.50	-	-	——	*	-
ML20	*	*	*	*	*	*	*	*	*	——	0.69
ML21	-	0.62	0.48	*	-	0.72	-	-	-	0.60	——

- No Data.
* No known relationship.

with ML17 and ML2 with ML17) and is possible in a third case (ML9 with ML12).

ML10 was caught on the territory of ML17 as a newly independent juvenile, so it is possible that ML10 is the son of ML17 and half-brother of ML18 and ML19. The genetic data support a close (r = 1/2) relationship between ML10 and ML17 (D = 0.69, 3'HVR; 0.89, 33.15), but also suggest a closer relationship between ML10 and ML 18, ML19 than half siblings (D = 0.68, 0.86; 33.15), implying that the two female parents (GG and GR) were also related. The relationship between ML10 and ML17 is also supported by the similarity between ML17 and ML10's offspring, ML11, ML13, and MM1 (D = 0.44, 3'HVR; 0.57, 33.15; r = 1/4).

ML2 is of unknown origin, but the genetic data suggest that ML2 and ML17 could be brothers, as D = 0.67 (33.15) between ML2 and ML17. This is supported by a suggested relationship of r = 1/4 between ML2 and ML17's offspring (ML10, ML18, ML19) (D = 0.50, 3'HVR; 0.57, 33.15) and between ML17 and ML2's offspring (ML3-8) (D = 0.50, 33.15) .

ML9 and ML12 may also be related (D = 0.63, 33.15; r = 1/4?). This is supported by the similarity between ML12 and ML9's offspring, ML11, ML13, MN1 (D = 0.44, 33.15; r = 1/8?), although the resolution of the technique is not adequate to discriminate positively between r = 1/8 and r = 0. If this relationship is correct then the paired birds ML11 and ML12 are approximately third degree relatives and their offspring ML14-ML16 are inbred.

An additional two territory holders are of unknown origin, ML1 and ML20. ML1 appears to share a general similarity to other ducks in the population, particularly ML17 (D = 0.59, 33.15), but a close relationship is not supported by similarity between ML1 and ML17's offspring (D = 0.30, 3'HVR; 0.35, 33.15). ML20 has a low average similarity to other ducks in the population (D = 0.31, 3'HVR; 0.32, 33.15).

Discussion

The use of DNA fingerprinting has allowed confirmation and extension of observational data on genetic relationships collected during a long-term ecological and behavioural study of Blue Ducks. The two probes used showed similar levels of variability and gave similar results, thereby reinforcing the findings. No evidence of multiple paternity or maternity was found in a total of ten clutches (14 offspring), thereby supporting the prediction of mate fidelity in this territorial, riverine species.

The limited dispersal of Blue Ducks has, as predicted, resulted in a highly interrelated local

population. The birds sampled in this study came from seven contiguous territories along the river. Some territory holders were known to be descendants of others and our genetic data indicate that the three major family groups are interrelated through the males ML2, ML10, and ML17. This accounts for the high average genetic similarity amongst individuals not previously known to be related. An additional two territory holders have unknown backgrounds. One, ML20, was an original territory holder when the observational study began in 1981 (Williams 1991) and appears to be an outsider, while the other, ML1, arrived as a new partner for an existing bird and may be distantly related to birds in the study area.

A much lower genetic similarity is found between Blue Ducks from populations separated by several hundred kilometres (D = 0.17-0.24; Triggs *et al.* in press) than within the Manganuiateao population. Genetic similarity values between isolated Blue Ducks are comparable to those found within populations of four avian species probed with 33.15 (D = 0.17-0.28; Burke & Bruford 1987), House Sparrows probed with 33.6 (D = 0.14; Wetton *et al.* 1987), and humans (D = 0.21, 33.15; Jeffreys *et al.* 1985b).

The genetic structure of the Manganuiateao population will inevitably lead to a relatively high level of inbreeding as most individuals within a local population are interrelated. Two examples of inbreeding documented by observational and genetic studies are presented in this paper. In Blue Duck, inbreeding appears to be a natural part of the social system, although the Manganuiateao population has a higher degree of genetic similarity than larger populations in a less modified habitat (Triggs *et al.* in press). Although inbreeding is usually deleterious in outbred populations (Ralls *et al.* 1986), it is unlikely to affect a species in which a degree of inbreeding is part of the natural social structure, as deleterious recessive genes are rare (Templeton & Read 1983).

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