Testing for evidence of philopatry: minisatellite DNA variation in Auckland Island teals

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Abstract

This study examined minisatellite DNA variation in three groups of Auckland Island teals from three locations (Boat Bay, Nellie Spit and East Coast). To determine whether teals showed high levels of natal philopatry, we were asked to test two hypotheses: 1) birds within each group are more closely related to each than to those in other groups; and 2) birds from nearby sites, Boat Bay and Nellie Spit, are more closely related to each other than to those from the more distant East Coast group. DNA was extracted from whole blood and the samples were digested with the restriction enzyme *Hae*III. The digested DNA was probed with three multilocus minisatellite DNA probes, namely 33.15, pV47-2 and 3'HVR. DNA profiles revealed high levels of bandsharing between individuals within each of the three groups of Auckland Island teal studied (range 0.645 - 0.765). Interestingly, bandsharing levels between individuals from different groups were very similar to those recorded within groups (range 0.633 - 0.739). Thus, there is no evidence to indicate that the Boat Bay and Nellie Spit samples are more closely related to each other than they are to birds from the East Coast group. One family of Auckland Island teals allowed bandsharing indices to be calculated for individuals of known relationships. The parent - offspring levels of bandsharing were generally higher than those obtained for within and between Ewing Island groups.The results presented here suggest that any philopatry in Auckland Island teals has not resulted in detectable genetic differences between groups, as revealed by minisatellite DNA markers.

Introduction

A subantarctic flightless teal *(Anas aucklandica aucklandica)* is restricted to the Auckland Islands where it now occurs on all substantial islands in the group, except main Auckland Island. It is one of four closely related teals of dark plumage in the Australasian region. Samples for this study were collected from three sites on Ewing Island: Nellie Spit, Boat Bay and East Coast (figure 1). Individuals are thought to exhibit a degree of philopatry and consequently it could be expected that such behaviour would result in a degree of genetic structuring of populations.

Minisatellite DNA variation was regarded as an appropriate tool to study such a problem, because it typically reveals very considerable amounts of genetic variation in birds, a group which is notoriously genetically invariant using conventional isozyme technology. "DNA fingerprinting" utilises probes specific to conserved regions within "minisatellite" DNA repeats. These regions are a category of variable number tandem repeats (VNTRs) and are highly repeated, comprising multiple copies of a short sequence of typically less than 65 base pairs. The hypervariability is the result of a high mutation rate for the loss or gain of repeat units. These regions, although being extremely variable in repeat copy number, have a highly conserved 'core' region. This is a sequence of the DNA to which the probe hybridises.

Individual minisatellite loci are considered 'hypervariable' and include the most polymorplfic sequences ever detected. For example, at the most variable locus yet discovered a sample of 79 humans was found to have at least 77 different alleles. Minisatellite DNA regions are however not exclusive to the human genome and have been identified in a wide variety of other mammals (Jeffreys and Morton 1987), birds (Burke and Bruford 1987), plants (Dallas 1988), fungi and protozoa. Furthermore, some of the human multilocus DNA fingerprinting probes have been shown to hybridise well to many of the different groups examined (ie they can be used as heterologous probes), in some cases detecting DNA fingerprints of similar complexity to those of humans. In particular, the original 33.15 probe isolated by Jeffreys et al. (1985) has been an extremely useful source of genetic markers in a diverse array of organisms. Many of the applications of DNA fingerprinting in humans have direct parallels in animal studies (see Lambert and Millar, 1994 for a review).

Minisatellite DNA techniques have been used to examine a broad array of problems in population biology. DNA fingerprinting, for example, was used by Kuhnlein et al. (1989) to calculate the genetic distances between five strains of chicken using DNA fingerprinting data and found that the genetic relationships determined from these data generally reflected the derivation of the strains. The two most genetically similar strains had been derived from a common stock, while strains of poultry derived from other stocks, and subject to different selective pressures, and different levels of inbreeding, showed greater genetic divergence. In a more extensive study Gilbert et al. (1990) examined six island populations of the California Channel Island fox *(Urocyon littoralis)* using multilocus DNA fingerprinting. These authors found relativity low levels of intra-island genetic variation, with one population monomorphic for all the restriction fragments detected. Furthermore, representatives of each population possessed a set of island-specific restriction fragments. Inter-population variation was, in contrast, high amongst the foxes examined. Both a phenetic and a phylogenetic analysis of the data grouped populations according to their likely historical patterns of dispersal and known vicariant events. Gilbert et al. (1990) therefore suggested that DNA fingerprinting could be used successfully for phylogenetic reconstruction in small recently isolated populations. In addition, DNA fingerprinting has been used to identify potential species, population and strain specific genetic markers in a number of organisms. For example, Meyer et al. (1991) were able to differentiate the strains of three species of filamentous fungi using DNA fingerprinting, while Nybom et al. (1989) could similarly distinguish cultivars of blackberries and raspberries.

Zeh et al. (1992) found that, despite high levels of intra-population variation in the minisatellite regions, they could clearly differentiate two populations of the pseudoscorpion *Cordylocheres scorpioides*. As a result of differences found, individuals could be correctly assigned to either population. On the basis of these results and other behavioural and morphological data, Zeh et al. (1992) have suggested that *C. scorpioides* is likely to represent a complex of cryptic species.

This report details an investigation into the genetic relationships between groups of Auckland Island teals using three minisatellite DNA probes known to reveal typical multilocus profiles under appropriate hybridisation and wash

conditions. Both within and between similarity analyses were conducted using minisatellite DNA fingerprinting data from individuals belonging to three groups of contiguous territorial birds on Ewing Island. The specific hypotheses we were asked to test were:

- that birds within each sample grouping will be more closely related to each other than to birds in the other groupings.
- as a grouping, birds in Boat Bay and Nellie Spit will be more closely related to each other than to birds in the East Coast grouping.

Methods

SAMPLES

Blood samples from Auckland Island teals from three sites on Ewing Island in the Auckland Island group were available. For the within and between bandsharing analysis, five individuals from Boat Bay, six from East Coast and five from Nellie Spit were examined. A further four blood samples, two juvenile individuals and their presumptive parents were provided. Details of the samples available for this study are presented in appendix 1.

DNA EXTRACTION AND DIGESTION

High molecular weight DNA was extracted by either of two techniques. In some cases 50gL of blood was resuspended in up to 600µL of SET buffer (0.1M Tris HCI pH 8.0, 0.01 M NaCl 1.0 mM EDTA) to which SDS was added to a final concentration of 0.5% and incubated with 5 units of proteinase K at 65 °C overnight. DNA was extracted twice with phenol and twice with phenol/chloroform. Alternatively, approximately 60gL of blood cells were lysed by being resuspended in 1mL of lysing buffer (144mM NH_4Cl; 10mM NH_4HCO_3). This was centrifuged at 16,000 for approximately 10 mins and the supernatant discarded. The pellet was then resuspended in 1mL of lysing buffer and the centrifugation repeated.The pellet was resuspended in 400µL of SET buffer, 5 units of proteinase K and 0.5% SDS and incubated overnight at 65 °C. The remaining procedure was as above, except that only one phenol extraction was used. Precipitation was performed according to Sambrook et al. (1989). All samples were subsequently digested with the restriction enzyme *Hae* III initially overnight with 20 units of enzyme in the presence of 4mM spermadine trichloride.

ELECTROPHORESIS AND SOUTHERN BLOTTING

Digested DNA was separated on a 0.8% agarose gel in TBE running buffer (2mM Tris, 1 mM acetate, 10mM EDTA pH 8.3) for approximately 48hrs at 2V/cm.All gels were run with molecular weight markers which allowed each gel to be standardised with respect to the distance run. The DNA was trans-

ferred by Southern blotting to a nylon membrane (Hybond-N, Amersham) by successively soaking in 0.25M HCl for 15 mins, 0.5M NaOH, 1.5M NaCl for 45 mins and 1.5M NaCl, 0.5M Tris pH 7.2 and 1mM EDTA twice for 15mins.The gels were then capillary blotted overnight in 6 x SSC.

DNA HYBRIDISATIONS AND AUTORADIOGRAPHY

After blotting the membranes were washed briefly in 3 x SSC before fixing the transferred DNA by baking at 80°C for 2 hours. Probes pV47-2 (Longmire, 1990), 33.15 and 33.6 (Jeffreys et al., 1985), and 3'HVR (Fowler et al., 1988; Goodbourne et al., 1983) were radioactively labelled by random priming with $^{32}P\alpha CTP$, according to suppliers instructions (Amersham). Filters were prehybridized at either 55 °C or 61 °C in 0.5M Na phosphate, 1M EDTA, 7% SDS pH 7.2, then hybridized in the same solution at either 55 °C or 61 °C in a hybrization chamber. Hybridized filters were washed twice for 30 mins at either 55°C or 61°C in 5 x SSC, 0.1 %SDS or 3 x SSC, 0.1 %SDS and exposed with X-ray film at -80°C for 1-10 days in a cassettes with an intensifying screen.

DATA ANALYSIS

Restriction fragments larger than 6kb were scored for probes pV47-2 and 33.15, while fragments in the 2 to 5kb size range were detailed in the case of membranes hybridised with the 3'HVR probes. For the resulting multilocus profiles the presence or absence of bands on autoradiographs was coded using a 1.5mm bin. These data were then recorded, in tabulated form and transferred to a spreadsheet format. An Excel macro was then used to determine the mean number of bands per individual, band sharing between individuals and the number of unattributable bands in offspring and their parents.

Results and Discussion

High resolution minisatellite DNA profiles were obtained for all the blood samples of Auckland Island teals (figures 2 and 3). Appendix 1 provides details of the samples examined in this study. Over the size ranges scored (see materials and methods), the mean number of restriction fragments recorded in Auckland Island teals was: 11.44 ±2.13 (SD) (probe 33.15), 7.44 ± 1.09 (SD) (3'HVR) and 15.13 ± 1.20 (SD) (pV47-2). The levels of bandsharing within and between the three breeding sites on Ewing Island are presented in table 1. These generally high levels are exceeded only by the values recorded for the Chatham Island black robin (0.79-0.87). This is perhaps not surprising in that both the Auckland Island teals and the black robins are confined to isolated offshore islands and a degree of enforced inbreeding is inevitable.

Table 2 compares the number of bands scored and the levels of bandsharing recorded in this study of Auckland Island teals, with other New Zealand avian

species. Care must be taken in interpreting these results since the number of fragments is affected by a broad range of variables eg the restriction enzyme used (note that all the studies used in table 1 employed *Hae* III restriction enzyme),the size range over which the autoradiographs were scored, the stringency at which the membrane was washed after hybridization and finally the general clarity of the DNA profiles obtained. Generally however it is clear that the minisatellite DNA profiles of Auckland Island teals will reveal significant data for use in a study of the relationships among individuals and of parentage in the case of the family material. This will then provide some data on the levels of bandsharing that could be expected from close order relatives. Limited information is available from this study in that a single family comprising the two adults and two offspring were available for genetic analysis. Figure 2 illustrates the multilocus DNA profiles for these individuals. All bands in the offspring were attributable to one or other of the adults. Levels of bandsharing between juveniles and parents were as follows: 0.82 ± .024 (SD) for probe 33.15; 0.819 ± .043 (SD) for probe pV47-7 and 0.55 ± .058 (SD) for probe 3'HVR.These are generally high in relation to both within and between bandsharing levels recorded in this study.The exception, probe 3'HVR, is low simply because of a paucity of bands in the size range scored.

There is clearly a very high level of bandsharing both within and among the groups examined (Table 1). The bandsharing indices both within and between groups are represented for each of the minisatellite DNA probes 33.15, 3'HVR and pV47-2 in figures 4, 5 and 6 respectively. There is no clear difference between the bandsharing levels both within and between the three groups examined. Within group bandsharing levels fall in the range 0.645 - 0.765, while between groups the values range from 0.633 to 0.759. The three minisatellite probes show very similar within and between group patterns, despite having exhibited quite different mean number of fragments per individual.

Conclusion

We were asked to test the following hypotheses.

1 That birds within each group are more closely related to each than to those in other groups.

2 Birds from nearby sites, Boat Bay and Nellie Spit, are more closely related to each other than to those from the more distant East Coast group.

The genetic data presented here show that the differences between individuals of the same group, are similar to those between individuals belonging to different groups. Moreover, there is no evidence to indicate that the Boat Bay and Nellie Spit samples are more closely related to each other than they are to birds from the East Coast group.

The results presented here suggest that any philopatry in Auckland Island teals has not resulted in detectable genetic differences between groups, as revealed by minisatellite DNA markers. It should be noted however, that since there is a high level of bandsharing within and between the groups studied, any effects of philopatry would be masked.

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