SHORT COMMUNICATIONS

Fingerprinting Birds' DNA with a Synthetic Polynucleotide Probe $(TG)_n$

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Unambiguous determination of genetic relationships is important to understand mating patterns and population structures. For example, thorough social studies of lekking or cooperatively breeding species require that parentage and other close genetic relationships be determined. Similarly, although extrapair copulations (EPC) have been documented in several bird species (e.g. Birkhead 1987, Westneat et al. 1990), some details about the behavior cannot be evaluated unless the result of illegitimate matings can be quantified reliably. Until recently these have been serious obstacles, and although some attempts to use protein variants as genetic markers to determine genetic relationships have been successful, many have been inadequate. Basically this is a consequence of the low degree of polymorphism in protein systems (Mumme et al. 1985, Romagnano et al. 1989). Highly polymorphic markers are required for effective studies of genetic relationships.

The discovery of families of hypervariable DNA regions in the human genome led to the development of DNA fingerprinting. The technique screens several hypervariable loci simultaneously to produce individual-specific fingerprints of the DNA (Jeffreys et al. 1985a, b). The technique is also applicable to birds (Burke and Bruford 1987, Wetton et al. 1987), plants (e.g. Dallas 1988), fungi (Braithwaite and Manners 1989), and nematodes (Uitterlinden et al. 1989). In recent avian studies, DNA fingerprinting has provided a powerful method for analyzing parentage (Burke et al. 1989, Gelter 1989, Birkhead et al. 1990, Gibbs et al. 1990, Gyllensten et al. 1990, Rabenold et al. 1990, Westneat 1990; see also Quinn et al. 1987).

DNA fingerprinting has traditionally been performed by using genetically cloned human minisatellite probes (e.g. Jeffreys' clones 33.6 and 33.15) or by a tandemly repeated DNA segment present in the wild-type genome of the bacteriophage M13 (see Vassart et al. 1987). Minisatellites derived from other species, including the Willow Warbler (*Phylloscopus trochilus*), have been used (e.g. Gyllensten et al. 1989). However, synthetic oligonucleotides (15–25 base pairs) with simple repeat motifs (e.g. di- or trinucleotide repeats) also produce individual-specific fingerprints in a variety of species (Epplen 1988, Schäfer et al. 1988, Kashi et al. 1990). Simple repeat motifs are probably present in all eukaryotic genomes and their polymorphism is due to a varying number of repeated units (Tautz 1989).

The synthetic polynucleotide probe $(TG)_n$ detects highly specific DNA fingerprints in horses (Ellegren et al. 1991). This polynucleotide type of probe offers several advantages compared with oligonucleotides and minisatellites (see below). I demonstrated that the $(TG)_n$ probe also detects individual-specific hybridization patterns in birds.

Five unrelated individuals of the Barn Swallow (*Hirundo rustica*) were analyzed. DNA was prepared from blood as described by Gelter and Tegelström (1990). Ten micrograms of DNA from each individual was digested with 50 U of restriction endonuclease *Hin*fI (Promega) according to the manufacturer's instruction. DNA was precipitated with ethanol, dissolved in 1 × TE (10 mM Tris; 1 mM EDTA, pH 7.0) and separated in 20 cm 0.7% agarose gels at 40 V for 24 h. After electrophoresis, DNA was transferred to nylon filters (Biodyne B, Pall) under alkaline conditions. The filters were prehybridized in 7% SDS; 0.263 M Na_2HPO_4; 1 mM EDTA pH 8.0; 1% BSA for 2 h at +65°C. The probe was a synthetic, double-stranded polynucleotide with the simple repeat dT-dG × dA-dC, "$(TG)_n$," (Pharmacia LKB Biotechnology). Agarose electrophoresis indicated an average probe length of 750 base pairs, but whether this was the actual length of the individual polymer or the length of multimer annealed products was unknown. DNA (100–200 ng) was radioactively labeled by standard nick translation. Hybridization was performed in the prehybridization solution overnight at +65°C. The filters were washed twice in 2×SSC (0.3 M NaCl; 0.03 M sodium citrate); 0.1% SDS at +65°C and exposed to Kodak X-AR film for 1–3 days at −70°C with intensifying screens.

The $(TG)_n$ probe revealed highly polymorphic hybridization patterns on Barn Swallow DNA digested with *Hin*fI (Fig. 1). Based on the limited sample of 5 unrelated birds, the average probability that 2 random individuals would display identical fingerprints was estimated to be 8.1×10^{-12} (calculated according to Jeffreys 1985b). This estimate is derived from a mean band frequency of 0.36 and an average of 24.8 scoreable bands (larger than a fragment size of 2 kilo base pairs) per individual. In total, I scored 66 resolvable bands among the 5 individuals. Distinct and variable hybridization patterns were also obtained for Canada Geese (*Branta canadensis*) and chickens (*Gallus gallus*) (data not shown). The probability that two Barn Swallows would have identical DNA fingerprints with the $(TG)_n$ probe (8×10^{-12}) is somewhat higher than that described for other avian species and fingerprinting probes (e.g. Burke and Bruford 1987, Gyllensten et al. 1990, Meng et al. 1990). Because of the small

sample size (i.e. band frequencies could not be less than 0.2), however, the probability estimate is likely to be an underestimate.

I believe that the polynucleotide probe $(TG)_n$ would be suitable for parentage testing in birds. But for a correct assessment of the amount of information that can be received by this fingerprinting probe, family studies must demonstrate to which degree the restriction fragments are independently inherited. However, little or no allelism or linkage has been found in the species/fingerprinting probe combinations so far tested (see Burke 1989). A rough estimate of the usefulness of the $(TG)_n$ probe for paternity testing in Barn Swallows can be obtained as follows. The probability that an autosomal band from an offspring's fingerprint is present in the mother equals

$$(1 + q - q^2)/(2 - q),$$

where q is the mean allelic frequency derived from

$$q = 1 - (1 - x)^{0.5},$$

and x is the mean band frequency (Georges et al. 1988). From this probability the proportion of an offspring's fragments expected to be present in the mother can be determined. The remaining fragments in the offspring must be of a paternal origin. For the Barn Swallow, $q = 0.20$, and the probability to make an incorrect paternity determination can thus be approximately estimated to 1.1×10^{-4} ($0.36^{8.8}$; 8.8 is the average number of paternal bands). It must be emphasized, however, that the number of paternal bands is likely to vary and the estimate is just for an average offspring. For offspring carrying fewer paternal bands, the confidence in excluding and assigning parentage will be lower.

Synthetic polynucleotides are easy to obtain and are convenient as DNA probes because they are in a ready-to-use form for hybridization. Minisatellite probes are usually cloned into plasmid vectors and must regularly be prepared on a large scale. This is not difficult, but it is time-consuming and requires basic biochemical equipment, often not easily available. Another attractive feature of polynucleotides with simple repeat motifs is that they, in contrast to some common minisatellite probes, are not protected by patents. Moreover, in comparison with oligonucleotides, polynucleotide probes offer several technical advantages during the labeling and hybridization procedures. They can be labeled by standard nick translation, unincorporated labeled nucleotides can easily be separated, and hybridization and washing conditions are similar to that used for genomic clones.

The synthetic polynucleotide $(TG)_n$ detected individual-specific fingerprints in Barn Swallow DNA, and is likely to be suitable for parenthood testing in other birds. This type of probe offers several advantages compared with cloned minisatellites and oligonucleotides, and would thus facilitate the use of DNA fingerprinting in birds.

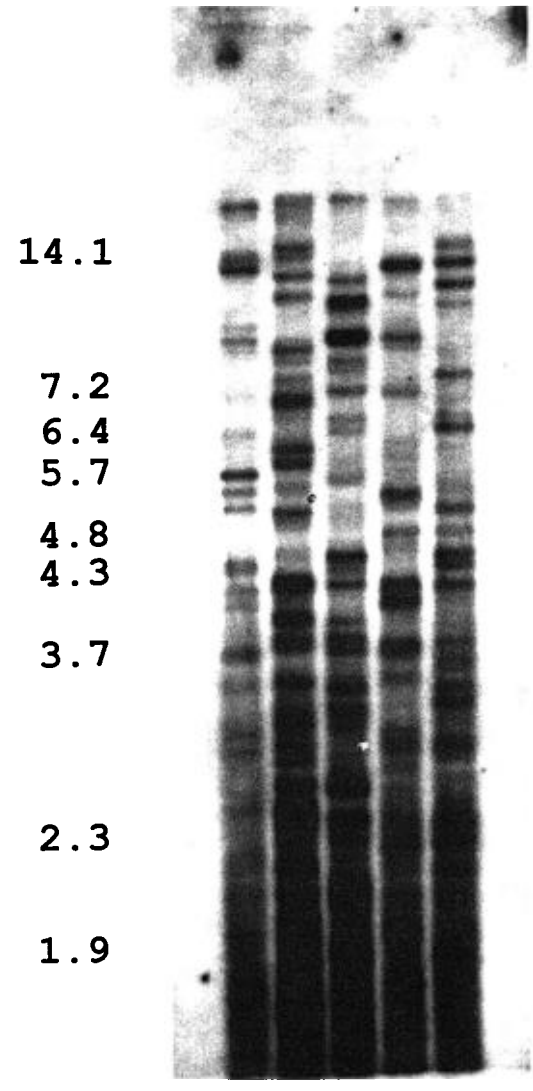

Fig. 1. DNA fingerprints of five unrelated individuals of the Barn Swallow (*Hirundo rustica*). The probe was the synthetic polyunucleotide $(TG)_n$. Fragment sizes (in kilo basepairs) are indicated to the left.

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