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Multilocus DNA Profiling in the Red Grouse (*Lagopus lagopus scoticus*)

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ABSTRACT. *Multilocus DNA fingerprints were obtained from individual samples of red grouse (British game bird) genomic DNA that had been digested with a range of different restriction enzymes and probed with various ^{32}P-labelled oligonucleotides. The best combinations of enzyme and probe were found to be BamHI/$(GTG)_5$, BamHI/$(GT)_8$ and HinfI/$(GTG)_5$. This fingerprinting strategy was then used to analyze the relatedness of male red grouse on an individual moorland and to discover the relatedness of birds with adjacent territories.*

INTRODUCTION

Multilocus DNA fingerprinting (Jeffreys *et. al.*, 1985a; Jeffreys *et. al.*, 1985b) is a technique by which individual-specific variation in hypervariable, tandem-repetitive minisatellite DNA sequences can be represented as a series of bands. These sequences are inherited in a Mendelian fashion (Jeffreys *et. al.*, 1985a), and hence, each offspring has a characteristic subset of the polymorphisms of its parents. Moreover, amongst organisms where relationships are not known, by comparing the fingerprints of different genomes and by seeing how many of the bands are shared by each fingerprint, it is possible to detect levels of relatedness. This heritability of minisatellites has proved a useful tool in tackling a number of questions in population biology. In birds, it has been used to detect the monogamy or polygamy of different species and the success of various reproductive

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strategies (Bruford *et. al.*, 1992; Gelter and Tegelström, 1992; Graves *et. al.*, 1991; Kempenaers *et. al.*, 1992; Lifjeld *et. al.*, 1991; Rabenold *et. al.*, 1991).

The numbers of birds in red grouse populations cycle periodically on individual moors, such that in some years there are many birds while in others there are few (Middleton, 1934; Potts *et. al.*, 1984). One theory as to how this may occur suggests that the aggression of each bird, and therefore the size of each territory, is dependent upon whether adjacent birds are kin or non-kin (Watson *et. al.*, 1994). Hanotte *et. al.*, (1992) published fingerprints of red grouse using the Jeffreys minisatellite probes, but many of the bands were found not to segregate independently or to be present in both parents. While this would be adequate for parentage analysis, it makes other relationships, in which one or both of the parents cannot be detected, less easy to interpret. The aim of this project was to develop a method by which different probes would give informative fingerprints for male red grouse with adjacent territories on a moor.

MATERIALS AND METHODS

Between 80 and 100 μl of blood were extracted from the wing (brachial) vein of birds and stored in sterile isotonic buffer at -70°C (Bruford *et. al.*, 1992). The extraction was carried out as described by Bruford *et. al.*, (1992), except that 2/3 volume of 5 M NaCl was added instead of 1/3 to 1/2 volume of 6 M NaCl, and following salt precipitation of the digested protein, extra phenol/chloroform extraction steps were carried out. For each bird, 50 μg of DNA were digested with the chosen restriction enzyme. After stopping the reaction with a phenol/chloroform extraction step, the DNA was salt-ethanol precipitated at -20°C for at least sixteen hours. The DNA was then recovered and redissolved in Tris-EDTA buffer (Sambrook *et. al.*, 1989).

Between 4 and 10 μg of digested DNA from each bird was loaded into separate wells of a 20x25 cm 0.8% agarose gel containing Tris-Borate-EDTA buffer (Sambrook *et. al.*, 1989). The gel was run for 36-42 h at 35 volts, such that the DNA fragments remaining in the gel were at least 2 kilobases in length (compare with Buitkamp *et. al.*, 1991). The gel was prepared, and pre-hybridized for a minimum of half an hour, after which the probe and the hybridization solution were added (Buitkamp *et al.*, 1991). Probes were labelled as described by Schäfer *et al.* (1988). In addition to

the short repetitive sequences under study ($(GT)_8$, $(GTG)_5$, $(GGAT)_4$ and $(TTC)_5$), the core sequences of the 33.6 and 33.15 Jeffreys probes were also tested.

The stringency of washing was dependent on the base composition of each probe. An initial wash with 6xSSC at 18°C was performed. This was followed by two more washes, each of the duration of 2 minutes, using 6xSSC and at the same temperature as the first wash. If the radioactivity count on the gel was still high, a further 6xSSC wash was performed at 45°C. If there was still a radioactive count of above 50 counts/s on the gel after this wash, a further wash was done at 45°C using 2xSSC, 0.1% SDS. This procedure usually washed off enough of the radioactive background on a gel for the DNA profiles to be clear. The gel was then exposed to photographic film for a day, or until such a period as gave clearly discernible banding patterns.

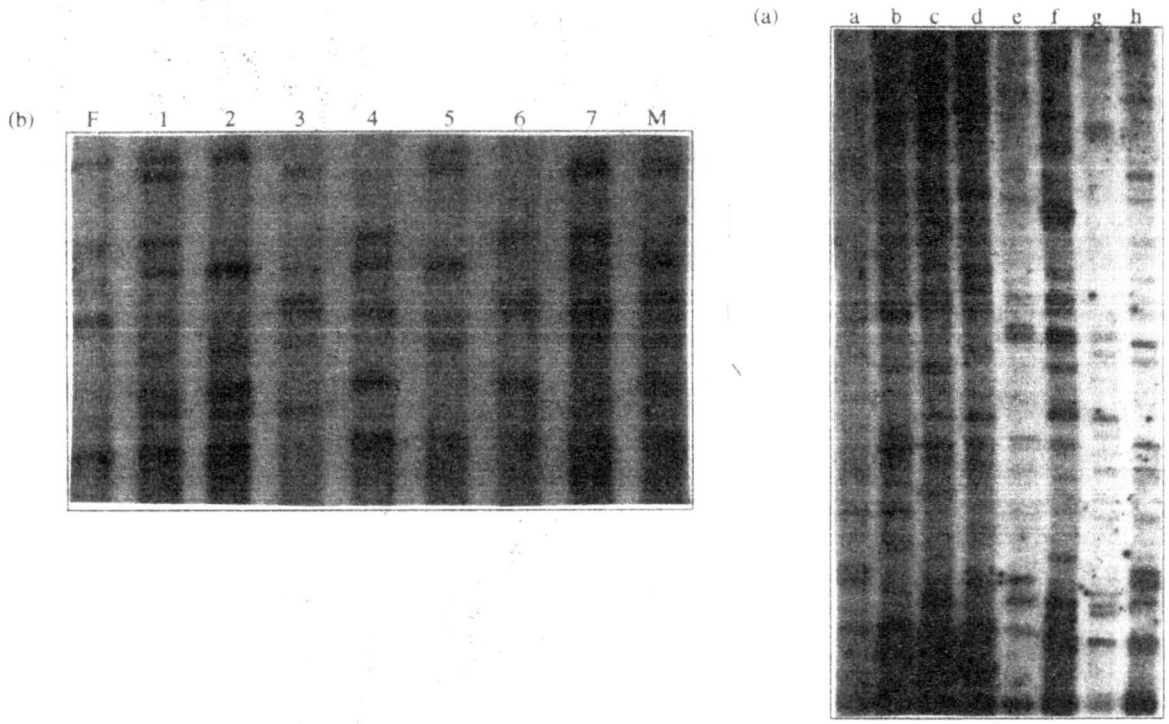
RESULTS AND DISCUSSION

The development of the method

Six probes with up to 13 restriction enzymes were tested in an attempt to determine the most informative combinations. Some combinations gave rise to fingerprints with too few bands, some resulted in profiles with too many bands to be readable, and some gave an adequate number of bands; but the hybridization of the probes was so weak, that they could not be routinely scored (data not shown). Of the various combinations tested, $(GTG)_5$/*Bam*HI, $(GTG)_5$/*Hin*fI and $(GT)_8$/*Bam*HI gave the most useful results. Analysis of these three combinations showed that they gave rise to only a very small number of allelic, linked and parentally-homozygous bands, which meant that the possibility of non-mendelian bands giving misleading indices of similarity could be discounted.

Analysis of captively-bred birds

Fingerprints of known unrelated captive birds showed that very few bands shared the same profiles (Figure 1a). It can be seen that the number of bands for each individual varies from 6 to 19 with a mean number of 13. This clearly represents a high level of polymorphism of potential use for population analysis.



(a) DNA was digested for over 16 h with *Hin*fI, and the gel was probed with $(GTG)_5$ at 45°C for over 2 h. The gel was exposed to the film for five days. It can be seen that there is a wide amount of variation between individuals.

(b) DNA was digested with *Bam*HI, and the gel was probed with $(GTG)_5$ at 45°C for over 2 h. It can be seen that every band present in the offspring (1-7) is also present in one of the parents (m=male, f=female).

Figure 1. DNA fingerprints of eight unrelated captive birds (a) and of a captively bred family of grouse (b).

In contrast, analysis of parents and offspring in a captively-bred family demonstrates that: bands represent DNA sequences inherited in a Mendelian fashion, all the bands in the offspring can be traced to one or other of the parents and that these bands are segregating independently (Figure 1b). In this case, the male and female parents are known to have 6 and 5 scorable bands respectively. All of their bands are represented in at least one of the progeny, and no bands are observed in the progeny that are not present in one of the parents. The number of bands in the individual offspring ranges from 4 to 7 with a mean of 6.

A comparison of any of the individual offspring with either parent reveals that on average, they have around half their bands in common. This can be represented mathematically by calculating a band-sharing coefficient of a parent and one of its offspring, namely, the Index of Similarity (s). This has been defined as,

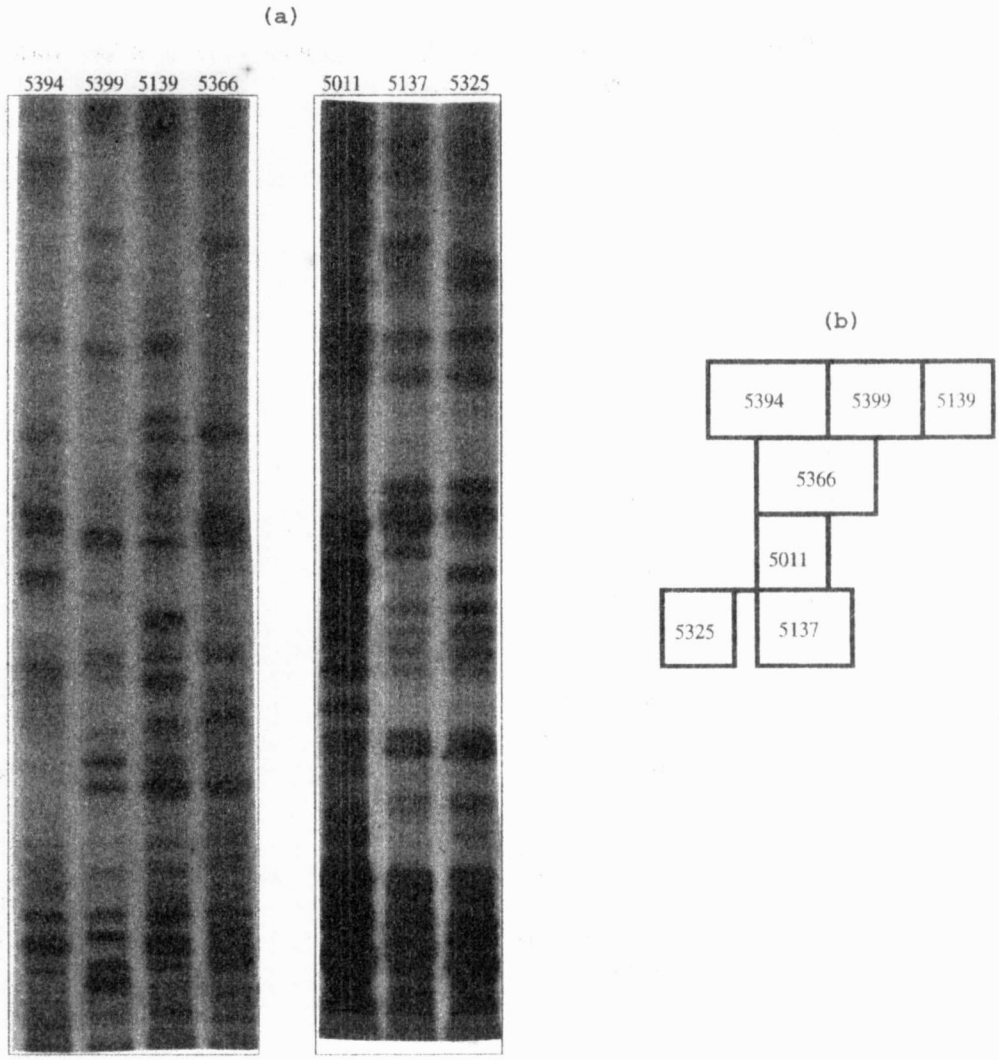
$$s = 2N_{ab} / (N_a + N_b)$$

where, N_{ab} = number of bands of similar intensity and electrophoretic mobility in individuals a and b; N_a = total number of bands in a that could be scored, if present, in b; and N_b = total number of bands in b that could be scored, if present, in a (see Bruford *et. al.*, 1992). The average value of s for any parent and offspring comparison is, thus, about 0.5. Similar calculations for band-sharing between offspring gives a mean of 0.47, but range from 0 to 0.67.

Analysis of a natural grouse population

We have initiated a study of a grouse population on Glass Choilie Moor. Figure 2 (a) shows the DNA profiles of these male grouse. Figure 2 (b) shows one small part of the moor, indicating the males holding territories of various sizes. The hypothesis under test is that related male grouse tend to occupy adjacent territories. The indices of similarity calculated from the fingerprints in Figure 2 are shown in Table 1. These data suggest that 5139, 5366, 5399 and 5394 are related to each other, and that 5137, 5325 and 5011 are also related to each other.

It is not possible to accurately determine the exact nature of their relationships. Where indices are around 0.5, the most likely relationships are father-son or brother-brother. Where indices are significantly lower than this, father-son relationships can be excluded, but grandfather-grandson,



(a) DNA was digested with *Hin*fI and the gel was probed with $(GTG)_5$ at 45°C for over two hours. Indices of similarity were calculated from these fingerprints, and are shown in Table 1.

(b) A map of part of the moor showing the seven territories of the birds analyzed in (a). The approximate sizes of the territories are indicated.

Figure 2. DNA fingerprints of wild birds (a), sampled from a part of the Glass Choilie moor (b).

Table 1. Indices of similarity calculated from DNA profiles shown in Figure 2(a).

		Bird Number				
		5399	5139	5366	5137	5325
Bird Number	5394	0.25	0.23	0.24		
	5399		0.35	0.40		
	5139			0.49		
	5011				0.27	0.26
	5137					0.77

The table only includes pair wise combinations from the data in Figure 2(a). The 5011/5366 index of similarity has yet to be determined, because pair wise comparisons can only be made where the DNA of both birds has been run on the same agarose gel.

uncle-nephew and brother-brother are all possible. With very low indices of similarity, it is difficult to distinguish chance band-sharing in unrelated birds from distant members of a family, *e.g.* cousins, whose estimated coefficient of similarity is 0.125.

These preliminary data correlate with field observations (Watson *et. al.*, 1994) that genetically related birds often do occupy adjacent territories, and, show that the enzyme/probe combinations used in this study are appropriate for a complete analysis of this population.

CONCLUSIONS

We have demonstrated multilocus DNA profiling in the red grouse using oligonucleotide DNA probes in conjunction with certain combinations of restriction-digested genomic DNA. This technique can distinguish related birds from unrelated birds. In a preliminary analysis, two groups of related male birds from adjacent territories were found on a moor.

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