Note

Mitochondrial DNA variation in four British populations of the white-clawed crayfish, *Austropotamobius pallipes*: implications for management

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Abstract

If recovery programmes are to be initiated in England and Wales, it is very important to obtain information on how genetic variation is partitioned between the remaining populations for the endangered species *Austropotamobius pallipes pallipes*. A survey of the mtDNA genome, using 12 restriction enzymes revealed 3 haplotypes in 4 British populations. The study reveals a low level of genetic variation amongst four geographically distant populations. Moreover, the most widespread haplotype found in the British populations is also similar to those found in French populations; one of the rare haplotypes found in one English population is also widespread in French populations. Management of crayfish stocks may be possible at a national level however, a more extended study of mtDNA variation is needed to prove this conclusively.

Keywords: Crayfish, *Austropotamobius pallipes*, genetic variation, mitochondrial DNA.

INTRODUCTION

Austropotamobius pallipes Lereboullet, the white-clawed crayfish, has a widespread distribution in Europe which stretches from the former Yugoslavia through Italy, France, Germany, Spain and into the British Isles where it reaches the limit of its northerly range (Albrecht, 1982; Laurent, 1988; Lowery and Holdich, 1988). Since the last century its distribution has been severely affected by habitat destruction, pollution, competition from foreign crayfish species and, most importantly, by the spread of crayfish plague, caused by the fungus, *Aphanomyces astaci* Schikora (Holdich and Reeve, 1991). This disease reached the British Isles in the early 1980s and has had a devastating impact on populations of *A. pallipes* (Alderman, 1993), the only native species of crayfish (Holdich and Reeve, 1991). Groombridge (1994) considers *A. pallipes* to be vulnerable and rare in Europe. However, many vigorous populations still exist, particularly in Britain and Ireland, but they are threatened and it is important that they are conserved.

The threat to the future survival of *A. pallipes* in England and Wales, both from crayfish plague and from the impact of foreign crayfish introduced for aquacultural purposes but which have invaded natural sites, has led the British Government to introduce additional legislation to that already in operation (Rogers and Holdich, 1995). This legislation includes the establishment of large-scale no-go areas where future crayfish farming developments will be banned as from May 29, 1996 (Holdich *et al.*, 1995; Rogers and Holdich, 1995). A number of action plans for

the conservation of *A. pallipes* in Britain have been published (Palmer, 1994; Rogers and Holdich, 1995; Wynne *et al.*, 1995). One of their recommendations is to initiate a programme of restocking waters from which *A. pallipes* has been eliminated. The first attempt made so far in Britain in this direction has prove, successful (Holdich *et al.*, 1995). The reintroduction involved stock from a nearby source (Frayling, M., pers. comm.) and it is likely that the genetic composition was very similar to that of the eliminated population.

Wayne *et al.* (1991) have highlighted the importance of obtaining information on how genetic variation is partitioned between remaining populations of an endangered species when recovery programmes are being designed. If a recovery programme is to be initiated in Britain then it is important to know how much genetic variation exists between populations.

Electrophoretic analyses of allozymes have not provided useful genetic markers for crayfish stock identification (Nemeth and Tracey, 1979; Brown, 1980; Albrecht and Von Hagen, 1981; Attard and Vianet, 1985; Busack, 1988, 1989; Agerberg, 1990; Fevolden and Hessen, 1989). Recently, a study of intraspecific mitochondrial DNA (mtDNA) polymorphism in crayfish has provided much useful information on population structure (Grandjean and Souty-Grosset, 1996). MtDNA analysis has been found to be one of the best approaches for studying evolutionary relationships among populations and (or) among closely related species and sub-species (Wilson *et al.*, 1985; Avise *et al.*, 1987; Moritz *et al.*, 1987; Ferris and Berg, 1987; Ashley *et al.*, 1990).

The objective of this study was to describe genetic variation in widely separated populations of *A. pallipes* in Britain by using mtDNA Restriction fragment length polymorphism (RFLP). To date, no genetic data have been reported for British populations. From a conservation point of view, the results of this study should allow managers to make restocking decisions.

MATERIAL AND METHODS

Specimens of *A. pallipes* were collected in September 1995 from four locations across the species' range in England and Wales; River Wye (R. Irfon, Powys) (NGR; SO 834556-SO 033512) (nine animals), River Avon (Broadmead Brook, Wiltshire) (NGR; ST 830774) (six animals), River Sprint (Cumbria) (National Grid Reference; SD 513959-NY 479074) (nine animals) and River Wharfe (Yorkshire) (NGR; SE 030 611) (seven animals). They were transported alive to the University of Poitiers for analysis.

Mitochondrial DNA was extracted from green glands, ovaries and heart according to the method developed by Grandjean and Souty-Grosset (1996).

Mitochondrial DNA samples were cleaved with 12 restriction endonucleases; six 6 base cutter (Bam HI, Bgl II, Eco RI, Hind III, Pst I Xho I); one 5 base cutter (Hinf I); and five 4 base cutters; Acc II, Hae III, Hpa II, Nde II and Taq I. Digestions were performed according to the manufacturer's instructions (BRL). The digested fragments were then separated electrophoretically in 1.2 % agarose gels at 30 V for 10 h. Gels were stained with SYBR™Green I (FMC Bioproducts) and examined with a UV light transilluminator.

The total proportion of shared fragments (S-value) between two individuals was calculated from the following equation (Nei and Li, 1979).

$$S_{ij} = \frac{2\,m_{ij}}{m_i + m_j}$$

where m_i and m_j are the numbers of restriction fragments in DNA sequences x and y, respectively, whereas m_{ij} is the number of fragments shared by the two sequences. The number of nucleotide substitutions per site d can be estimated by

$$\hat{d}_{ij} = \frac{-\ln S_{ij}}{r}$$

where r is the number of bases per restriction site (Nei and Li, 1979). When different kinds of enzymes with different r values are used, the mean number of nucleotide substitutions can be estimated by the formula given by Nei and Tajima (1981);

$$\hat{d}_{ij} = \frac{\sum_k m_k\, r_k\, d_{ij}(k)}{\sum_k m_k\, r_k}$$

where $m_k = \frac{m_{i(k)} + m_{j(k)}}{2}$ and k refers to the kth class of restriction enzymes.

The AMOVA (analyses of molecular variance) method and program developed by Excoffier *et al.* (1992) was used for analysing the distribution of genetic variation among populations. The AMOVA program was also used for calculation of Φ-statistics; Φst, the correlation of random haplotypes within population, relative to that of random haplotypes drawn from the whole of population.

RESULTS

The size of *A. pallipes* mtDNA was estimated from patterns showing a low number of bands and was estimated at approximately 17750±580 base pairs (pb).

Among the 12 restriction enzymes used, only Bam HI lacked restriction sites on the mitochondrial genome. The endonucleases Hind III, Hpa II, Hae III, Nde II, Hinf I, Taq I produced a large number of bands, many of small size (Table 1, Fig. 1). The sum

Table 1. – Estimated sizes (in base pairs) of mtDNA fragments resulting from digestion with restriction endonucleases in *A. pallipes*. For each enzyme, letters refer to the different revealed patterns.

	Hind III	Hind III	Hpa II	Hpa II	Hae III	Acc II	Nde II	Hinf I	Taq I	Bgl II	Pst I	Xho I	Eco RI
	A	B	A	B	A	A	A	A	A	A	A	A	A
	5 520	5 520	4 330	4 330	2 730	1 0475	1 770	1 930	2 340	4 710	8 235	9 200 × 2	1 3545
	2 710	1 590	1 730	1 730	1 380	2 160	1 450	1 600	2 070	3 070	7 460		1 865
	1 425	1 425	1 360	1 460	1 310	1 395	1 250	1 170	1 120	2 520	935 × 2		935 × 2
	1 050	1 130	1 250	1 160 × 2	1 230	1 330	950	1 100	950				
	935	1 050	1 160 × 2	980	770	870	790	980	860				
	845	935	885	885	710		740	790	770				
	615	845	630	630	680		690	700	660				
	530	615	560	560	610		630	650	590				
		530			530		530	570	570				
								500	490				
								460	460				
Total	13 630	13 630	13 165	12 895	9 930	16 230	8 800	10 450	10 880	10 300	17 565	18 400	17 280

of these lengths gave a comparatively low number; it is likely that the smallest fragments were undetected. Two of the eleven restriction enzymes used to screen for variation among the four *A. pallipes* populations revealed polymorphisms in the mtDNA (Table 1). Two patterns were observed for each of two restriction enzymes Hind III and Hpa I (Figs. 1, 2). For the four populations, only three haplotypes composed of eleven letters were observed (each letter corresponding to a given pattern of the enzyme profile). Of the 31 crayfish examined with 12 restriction enzymes, 29 shared a common mtDNA haplotype type (haplotype 1; AAAAAAAAAAA). Two crayfish had a unique haplotype type (haplotype 2; BAAAAAAAAAA and haplotype 3; ABAAAAAAAAA) with one additional restriction site for each of the enzymes Hind III and Hpa II. The rare haplotype (3) was found exclusively in the R. Wye population and the other rare haplotype (2) has only been detected in the R. Avon population.

Mitochondrial DNA nucleotide diversity values within species ranged from 0.28 to 0.69 %. Estimate of the Φst for mtDNA of *A. pallipes* was 0.023 (not significant).

DISCUSSION

In general, few population genetic data are available for crayfish species (Agerberg, 1990). In *A. pallipes*, the few reported data concerning genetic studies are based on the study of electrophoretic analyses and have revealed a relatively low level of genetic variation within and between natural populations (Albrecht and Von Hagen, 1981; Attard and Vianet (1985). The primary causes of reduced variation in small populations include founder effect (Waller *et al.*, 1987), genetic drift (Lacy, 1987) and inbreeding (Packer *et al.*, 1991).

No information on genetic data for English and Welsh *A. pallipes* populations has been published

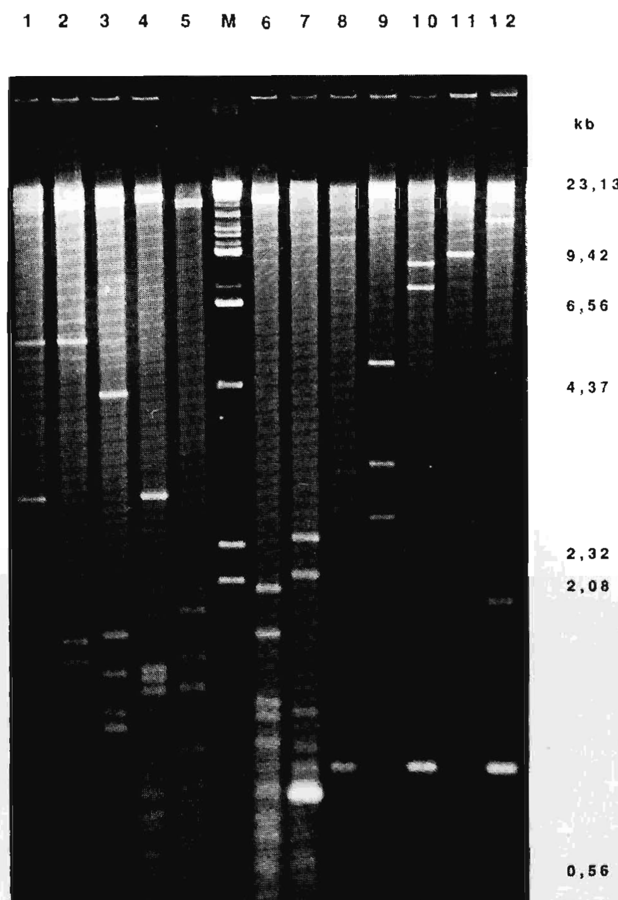

Figure 1. – Example of SYBR™Green I stained agarose gel of mtDNA digestion patterns of *A. pallipes* from R.Wye population. On the right side is given (Lambda-phage DNA digested by Hind III) fragment size markers in base pairs (bp). Lanes; 1-2 = Hind III (pattern A and B, respectively), 3 = Hpa II, 4 = Hae III, 5 = Nde II, M = standard size, 6 = Hinf I, 7 = Taq I, 8 = Acc II, 9 = Bgl II, 10 = Pst I, 11 = Xho I, 12 = Eco RI.

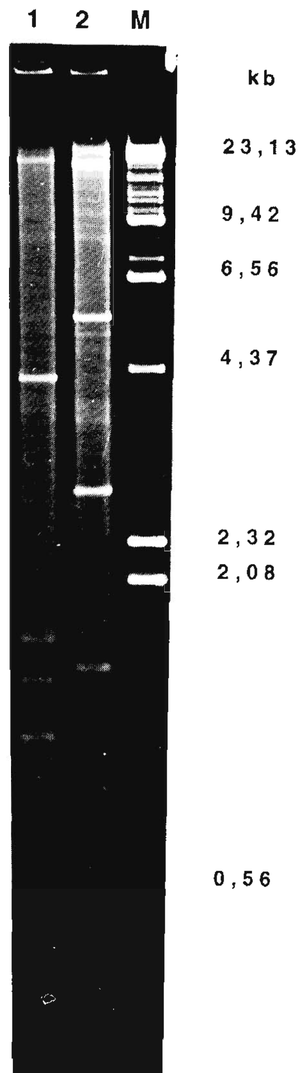

Figure 2. – Restriction pattern (B) produced by the endonuclease Hpa II of rare haplotype 3 (lane 1). Restriction pattern (A) produced by the endonuclease Hind III (lane 2). On the right side are given the fragment size markers in base pairs (Bb) of Lambda-phage DNA digested by Hind III.

previously. Generally, genetic variation among populations is often wider for mtDNA than for nuclear DNA or nuclear gene products, such as isozymes (Zwanenburg *et al.*, 1992).

Our study has revealed a low level of genetic variation among four geographically distant *A. pallipes* populations in England and Wales. Our results are in accordance with the general pattern of genetic diversity which often shows a predominance of one mtDNA composite phenotype within a population sample in combination with rarer mtDNA haplotypes (Avise *et al.*, 1983; Ashley and Will, 1987). However, the rare haplotypes come from a single location where only very few specimens have been analyzed (six animals for Avon and nine animals for Wye). Future work will be needed to confirm this suggestion. The Phist value (0.023) has could let suppose a high level of genetic homogeneity amongst English and Welsh stocks. According to Albrecht (1982), *A. pallipes* could have reached England either by naturally throught post-glacial stream connection with France or by an introduction by human. Insufficient time for the accumulation of mutations due to their recent establishment may explain the low genetic variability found in *A. pallipes* in Britain. However, this low level of genetic diversity within and between populations could be also explained by the small sample sizes; hence, the probability of finding a new mtDNA type within a given population is reduced (Avise *et al.*, 1979*a,b*; Saunders *et al.*, 1986).

In recent study, Grandjean and Souty-Grosset (1996) found three haplotypes based only on six endonucleases (Eco RI, Xho I, Bam HI, Pst I, Hind III and Hpa II) from three French populations of *A. pallipes*. In our study, each haplotype was characterized by 12 enzymes because we used a more sensitive staining method. If we compare the results obtained only from the six endonucleases used in the two studies (Eco RI, Xho I, Bam HI, Pst I, Hind III and Hpa II) we observe that the most widespread haplotype found in the English populations was similar to those revealed in French populations. Moreover, one of two rare haplotypes found in one English population was also widespread in French populations. These results could confirm the French origin of British stock.

The application of genetics to conservation issues is a practical endeavour and should yield concrete recommendations for management strategies. In addition to monitoring and protecting extant populations, a primary component of any recovery plan for *A. pallipes* would be an extensive reintroduction programme. Our results provide evidence for a very close genetic relationship among English and Welsh populations. Based on mtDNA analysis, each population could not be designated as a separate conservation unit. Therefore management at the national level should be suitable.

However, the mtDNA data presented here is preliminary. Confirmation of the genetic stock uniformity among English, Welsh and French populations will depend upon a more extensive mtDNA analysis, which should include a greater number of populations and individuals. In this case, the use of PCR techniques from nuclear or/and mitochondrial genomes could allow characterization of genetic variation without having to sacrifice the animals. Nuclear DNA diversity may exist in populations in the absence of mtDNA diversity, although the reverse is improbable (Franzin and Clayton, 1977; Ferris and Berg, 1987; Kristofferson and Clayton, 1990).

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