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# Preliminary analysis of the genetic structure in the fen raft spider *Dolomedes plantarius* (Araneae: Pisauridae)

# Предварительный анализ генетической структуры паука *Dolomedes plantarius* (Araneae: Pisauridae)

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ABSTRACT. The fen raft spider *Dolomedes plantarius* is one of the rarest, red-listed species in Europe. It faces the threat of extinction through the degradation of its wetland habitats. In the United Kingdom, there are only three known populations of *D. plantarius.* This research applies molecular genetic techniques to the study of population structure of *D. plantarius* within the United Kingdom. Our preliminary results of mitochondrial DNA analysis suggest a lower level of haplotype diversity in the endangered Redgrave and Lopham Fen populations than in the much larger population in the Pevensey Levels, West Sussex. The implications of these results are considered with respect to future conservation action and management decisions.

РЕЗЮМЕ. Паук *Dolomedes plantarius* относится к наиболее редким краснокнижным паукам Европы. Ему грозит вымирание из-за деградации заболоченных местообитаний. В Великобритании известно только три популяции *D. plantarius.* В данном исследовании молекулярно-генетические методы применены для изучения популяционной структуры *D. plantarius* в Великобритании. Наши предварительные результаты митоходриальной ДНК показывают более низкий уровень разнообразия гаплотипов в вымирающих популяциях локалитетов Redgrave и Lopham Fen чем в значительно более крупной популяции Pevensey Levels, West Sussex. Оценивается применимость этих данных с позиций будущих решений по охране и управлению популяций данного вида.

KEY WORDS: *Dolomedes plantarius*, genetics, bottleneck, mitochondrial DNA, conservation.
КЛЮЧЕВЫЕ СЛОВА: *Dolomedes plantarius*, генетика, ограничение популяции, митохондриальная ДНК, охрана.

## Introduction

The fen raft spider *Dolomedes plantarius* (Clerck, 1757) is an aquatic spider and one of two species of the genus *Dolomedes* (family Pisauridae) that live in Europe. It is a large species with adult females reaching body lengths of up to 23 mm and a leg span of 70 mm [H. Smith, unpubl. data]. The species inhabits lowland wetland and fen areas, and depends on a constant supply of freshwater throughout its life [Smith, 2000].

Dependence on aquatic habitats means the distribution of this species is naturally restricted and disjunct. In addition, despite its large size and distinctive features, the fen raft spider has been under-recorded due to the inaccessi-

bility of its habitat and because of taxonomic confusion with its congener *D. fimbriatus* [Duffey, 1995; Helsdingen, 1995]. Consequently, the exact geographical distribution of this species is not known. The latest estimates for the current distribution of *D. plantarius* were provided by Duffey [1995], Helsdingen [1995], and Gajdoš *et al.* [2000].

Despite such rough estimates of *D. plantarius* distribution, there is strong evidence that it has been declining throughout its range [Duffey, 1995; Helsdingen, 1995; Gajdoš *et al.*, 2000; Smith, 2000]. Dependence on lowland aquatic habitats, which themselves are susceptible to degradation by human activities, and low dispersal capabilities, make populations of this species vulnerable to extinction. Consequently, in many countries it has been Red Listed, and has either Vulnerable or Endangered status [The IUCN Red List of Threatened Species — http://www.redlist.org/].

In the United Kingdom there are three known populations of *D. plantarius* (Fig. 1). The spider was first recorded in 1956 by Duffey [1958] at Redgrave and Lopham Fen on the Norfolk/Suffolk border, subsequently on the Pevensey Levels in Sussex [Kirby, 1990] and more recently in 2003 from South Wales [H. Smith, unpubl. data]. The Redgrave and Lopham Fen population has since declined due to water abstraction and consequent habitat degradation, leaving population fragments occupying approximately 15% of their former range [Smith, 2000]. Consequently, the species has been classified as endangered, and is the subject of a Biodiversity Action Plan [UK Biodiversity Steering Group, 1999]. In 1991 English Nature initiated monitoring and management work for this population under the Species Recovery Programme [Smith, 2000].

Despite the conservation efforts described, the isolated population units remained small and have experienced breeding failure in some years. Even after the restoration of Redgrave and Lopham fen's hydrology in 1999, the population failed to show any significant or sustained recovery [Smith, 2000, 2004].

The failure of this population to recover suggests that factors other than habitat size and quality might be important in influencing the current demographic trend. One possible explanation is that reduced genetic diversity within this population, which resulted from the recent bottleneck, has reduced fitness and decreased population viability. Bottlenecked populations that remain small are especially susceptible to the loss of genetic variability through random genetic drift and inbreeding [Lande, 1995; Crnokrak & Roff, 1999; Hedrick & Kalinowski, 2000].

The objective of this research is to complement ongoing conservation efforts by investigating the level of genetic variability and the distribution of genetic variants within and between *D. plantarius* populations. This will involve analysis of British and continental populations throughout their range. The issues that will be examined include:

(1) The amount of genetic variability within the focus populations in the UK.

(2) Levels of inbreeding in populations and potential effects on fitness components and thus on population viability.

(3) The level of genetic differentiation between United Kingdom and continental populations, and distribution of genetic variability across the species distribution range ('phylogeography').

The results of this research are expected to have important implications for future management decisions, through informing conservation strategies that involve population augmentation, translocations and reintroductions, all of which are being considered under the Species Action Plan [UK Biodiversity Steering Group, 1999], as well as predicting the future of the species in the face of changing environmental conditions.

This paper presents the first genetic data on *D. plantarius*, obtained in a preliminary study of mitochondrial sequences from two of the United Kingdom populations. These data provide some indication of a difference in the levels of genetic variability between those populations. We discuss the potential implications of these results and the future directions of this study.

## Material and methods

The issues of population genetics and intraspecific phylogeography addressed by this study require the analysis of appropriate molecular markers.


Fig. 1. Approximate locations of the three known *D. plantarius* populations in the United Kingdom. A — Redgrave and Lopham Fen, B — Pevensey Levels, C — south Wales.

Рис. 1. Примерное местонахождение трех известных популяций *D. plantarius* в Великобритании. A — Redgrave и Lopham Fen, B — Pevensey Levels, C — Южный Уэльс.

Many of the genes commonly used as markers in this kind of study are located in the mitochondrial DNA (mtDNA). This is a fast evolving, uniparentally (maternally) inherited DNA molecule, and its sequence variation has been shown to give a good measure of the genetic relationships within and between populations [Avise, 1987; Moritz *et al*.,1987; Hedin, 1997; Masta, 2000]. As such, it can provide data of practical significance that complement other demographic studies [Moritz, 1994]. Here we present the initial sequence data from the mitochondrial genes COI and Cyt b.

## Samples

This analysis involved 18 individuals from two United Kingdom populations. Eight were from Redgrave and Lopham Fen, and ten were from the Pevensey Levels (see Fig. 1). All were collected during 2002, and stored in absolute ethanol at 5°C.

## DNA Extraction

The tissue used for DNA extraction was taken from the leg segments of the specimens. The tissue was treated with Proteinase K and incubated overnight at a temperature of 55°C, prior to a column-based extraction method performed using the QIAGEN™ DNA Easy Tissue Kit according to the manufacturer's instructions. Extracted DNA was stored at –20°C.

## PCR

Fragments of mitochondrial DNA including 291 base pairs (bp) of cytochrome oxidase subunit 1 (COI) and 341bp of cytochrome b (Cyt b) were amplified by the polymerase chain reaction (PCR). Since no *D. plantarius* sequences previously existed, the primers for the PCR had to be specifically designed for this study. This was done by aligning the sequences of COI and Cyt b genes from different spider and other arthropod species, which were available at the GenBank [http://www.ncbi.nlm.nih.gov/]. Primer sequences were chosen from those alignments to match the short gene regions that were most conserved across all the aligned taxa and are given in the Table. The primers were synthesized by the commercial suppliers.

PCR reactions were performed in a MJ Thermocycler in a final volume of 25 µl, containing 0.625 units of Taq polymerase (ABGene™), 2.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 400 nM of each primer, in a buffer of 20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl (pH = 8.8 at 25°C). The DNA was initially denatured at 94°C for 45 s, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 45°C for 30 s, and extension at 72°C for 30 s, with the final extension at 72°C for 5 mins. Amplification products were visualized by electrophoresis on a 1.5% agarose gel.

## Sequencing

PCR products were cleaned with the QIAGEN™ PCR Purification Kit (columns) and eluted in 30 µl of 10 mM Tris-Cl buffer (pH = 8.5), before sequencing with the fluorescent BigDye™ terminator 3 sequencing kit (supplied by Applied Biosystems) and

Table.
The primers for amplifying the target genes of the *D. plantarius* mtDNA, with their respective sequences.
Таблица.
Праймеры для амплификации целевых генов в mtDNA *D. plantarius* и их соответствующие последовательности.

| mtDNA gene | Primer name | 5'–3' sequence |
|---|---|---|
| COI | Dp1I (forward) | CTATAGTAGAAATAGGAGTTGG |
| | Dp3I (reverse) | GTTGAAATAAAATAGGATCTCC |
| Cyt b | IX-5L-Cb (forward) | GACAAATATCATTTTGAGGAGC |
| | IX-6R-Cb (reverse) | AAGCAAATAAAAAATATCATTCTGG |

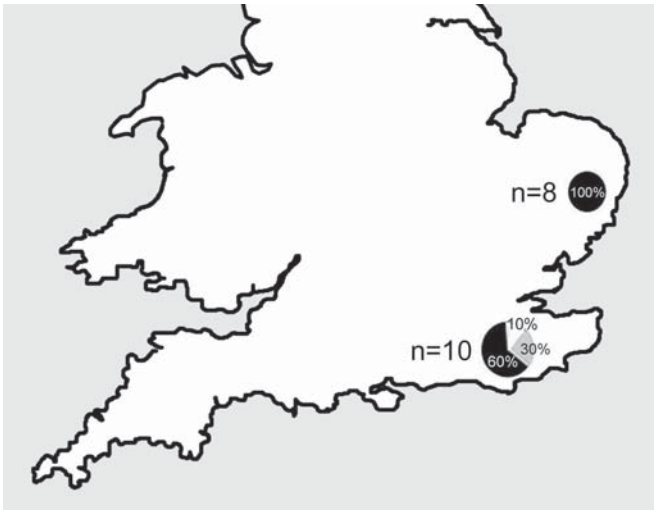


Fig. 2. The distribution of three haplotypes in two UK populations; n is the number of individuals analyzed in each population. The haplotypes are determined according to the 249bp alignment of the COI and Cyt b genes.

Рис. 2. Распространение трех гаплотипов в популяциях Великобритании; n означает число особей проанализированных в каждой популяции. Гаплотипы определялись в соответствии с последовательностью генов COI и Cyt b (249 оснований).

running on an ABI 3700 automated sequencer. The sequencing reactions were performed in a final volume of 10 µl, containing 3.5 µl of purified DNA, 0.5 µl of 10 µM primer and 2 µl BigDye™. The sequencing reaction protocol was as follows: 94°C for 1 min, followed by 24 cycles of 94°C for 10 s, 54°C for 5 s, and 68°C for 3 mins, with the final treatment at 20°C for 5 mins. The sequences obtained were aligned manually using the BioEdit software [Hall, 1999].

## Preliminary results

MtDNA fragments of COI and Cyt b genes were obtained by the PCR. Sequences for 18 individuals were obtained from the fragments of COI and Cyt b genes. Of this sequence 165bp were from the Cyt b and 84bp were from the COI gene. The combined alignment of 249bp reveals two variable positions, one in each of the sequenced genes. Both changes are silent transitions at third codon positions. There are a total of three different haplotypes. The most common haplotype is shared by all eight individuals from the Lopham and Redgrave Fen population and six individuals from the Pevensey Levels population. The two other haplotypes are only found in the Pevensey Levels population (Fig. 2).

Longer sequences (153bp of COI and 324bp of Cyt b) were obtained from ten out of the 18 individuals; eight from the Pevensey Levels and two from the Fen population. This 477bp alignment contained one further variable site at the third codon position in the COI gene, indicating the presence of a fourth haplotype within one individual from the Pevensey Levels population.

## Discussion

The data reported in this paper identify four different genetic haplotypes in the two populations studied. Three of the haplotypes are seemingly confined to the Pevensey Levels, and one is shared between both populations. All individuals from the Redgrave and Lopham Fen population shared the same haplotype. These data are consistent with the expectation of a greater genetic diversity within the Pevensey Levels population because, unlike the Fen population, this population has not experienced a recent bottleneck. Population bottlenecks are known to reduce genetic diversity through the loss of alleles [Frankham *et al*., 2002]. MtDNA is particularly sensitive to bottlenecks and subsequent genetic drift because it has one-fourth

the effective population size of biparentally inherited nuclear DNA, as a consequence of its haploidy and uniparental inheritance [Birky *et al*., 1989]. The finding of only a single mitochondrial haplotype in the Fen population could reflect the loss of genetic diversity in that population following the bottleneck. However, the apparent difference in haplotype composition between the two populations must be interpreted cautiously because sample sizes were small.

A further cautionary note on the interpretation of those results is required because the analyzed sequence was short (249bp). Overall genetic diversity within the studied region was low (three variable nucleotide positions), which at this level of analysis is not unexpected [Moritz *et al*., 1987; Simon *et al*., 1994].

Based on only these results, it is therefore not possible to determine whether this low level of diversity is a feature of the species' mitochondrial DNA in general, or whether it reflects a loss of genetic diversity resulting from recent population history. Furthermore, the data presented in this study are from a single, uniparentally inherited genetic marker and there is no information about variation in biparentally inherited genes, which may be less sensitive to the effects of population bottlenecking.

Further studies on *D. plantarius* population structure and history will include more individuals from a wider range of populations and will use longer mitochondrial gene sequences. Additional genetic markers, such as nuclear genes and microsatellites, will also be used. Nuclear genes with sensitivity comparable to that of the mtDNA (such as internally transcribed spacers of ribosomal genes) provide information about biparentally inherited genetic variation and are analyzed in the same manner as mtDNA. Their analysis can be informative about relationships among populations within the species as a whole.

Microsatellites are biparentally inherited hypervariable regions of nuclear DNA with high mutation rates and, as a consequence, high levels of polymorphism [Tautz, 1989; Hancock, 1999]. This makes them highly suitable for detailed investigations of gene flow among populations, levels of inbreeding and paternity analysis. A suite of microsatellite markers is currently being developed by collaborators from the Chinese Academy of Sciences (Prof. Zhang's research group).

The immediate focus of this study is to obtain longer sequences of the target mtDNA genes and to include more individuals from other *D. plantarius* populations in the analysis. Specimens from several continental populations have been obtained and are currently being studied. The analysis of the nuclear DNA sequences and microsatellites is expected to give insights into the population dynamics and natural history of the United Kingdom populations, and their relationship to those on the continent.

Furthering the analysis of the genetic composition of *D. plantarius* in such a way is expected to provide important guidance for translocation and reintroduction schemes aimed at preserving the genetic diversity of the species. This should help conserve the United Kingdom, and possibly also continental populations of *D. plantarius*.

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