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Biological and genetic studies of Polish population of *Cinara tujafilina*

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Abstract

The bionomy and ecology of *Cinara tujafilina* (Del Guercio 1909) (Hemiptera Aphididae) on *Thuja orientalis* L. was studied under insectary conditions. The number of generations per year was determined, as well as the lifespan of a generation and fecundity of the females. DNA sequence data from the mitochondrial cytochome oxidase I (COI) gene was used to examine the level of genetic variability between specimens among natural populations of *C. tujafilina*.

Key words: Hemiptera, Aphididae, *Cinara* sp.

Introduction

The species of *Cinara* sp. are connected with conifers trees and shrubs, also ornamental shrubs in urban green areas. 27 species are known in Poland, but only 3 species are connected with *Thuja* sp.. *Cinara tujafilina* (Del Guercio 1909) (Hemiptera Aphididae) is a new aphid species in Poland. It was introduced with shrubs.

C. tujafilina is an anholocyclic and monoecious species of aphids feeding on *Thuja orientalis* L. (Blackman and Eastop, 1988). This species was considered rare in Poland and was only observed on few areas. In Poland it was observed on branches from the end of August until the beginning of February (Durak *et al*., 2006). Biology of this species is poorly known. *C. tujafilina* in Japan reproduces parthenogeneticly throughout the year, settling the branches also in winter (Furuta, 1988). In Italy and Poland it was observed on branches but in winter it migrated to shrub roots (Colombo and Parisini, 1984, Durak *et al*., 2006). The aim of this paper was to study the bionomy of *C. tujafilina* and determine the number of generations per season and aphid fecundity within particular generations. DNA sequence data from the mitochondrial cytochome oxidase I (COI) gene was used to examine the level of genetic variability between specimens among natural populations of *C. tujafilina*.

Materials and methods

The bionomy of the species was observed from the beginning of August until the end of January in 2005 and 2006. For this purpose aphids were bred on isolated *T. orientalis* twigs. One apterous virginoparae was placed under each insulator. The first larvae they gave birth to would mark the beginning of a new generation. They were then placed under another insulator where their fecundity and the length of particular developmental periods were observed and examined. In each generation the development of five females was observed and the observations were carried out five times a week.

DNA was extracted from whole aphids alcohol preserved using the DNAeasy Tissue Kit (Qiagen, www.qiagen.com) according to the manufacturer's instructions. Voucher genomic extracts are located in the Laboratory of Molecular Systematic in Madrid (Museo Nacional de Ciencias Naturales, MNCN), Spain. We generally did two PCR reactions to get 1450 bp of COI. The first half of COI is gotten with the primer pair LCO/HCO (Folmer *et al*., 1994), which gives about 650 bp, and the second half is PCRed with the primer pair Jerry/Pat (Simon *et al*., 1994) - gives about 800 bp. The temperature profile for the amplification of the COI gene fragment included an initial denaturation step of 95 °C for 5 min followed by 35 cycles of 95 °C for 30 sec, 47 °C for 30 sec, 72 °C for 1 min 30 sec and a final extension period of 72 °C for 10 min. Amplification products were resolved by electrophoresis in 2% agarose gels with TAE buffer (40 mM Tris-Acetate, 1 mM EDTA pH 8.0) containing ethidium bromide. A 100 bp ladder marker was used as a molecular size standard. Sequence gels were run on an ABI 377 DNA sequencer (Applied Biosystems). The sequences obtained were aligned and verified with the forward and reverse overlap sequences with the Sequencher program (Gene Code Corporation). The translation to proteins was also verified with this program and with MacClade (Maddison and Maddison, 1992), where we designated each position in the codons. Nucleotide saturation was analysed by plotting transition/transversion rate against uncorrected p divergence values.

Results and discussion

C. tujafilina feeding on *T. orientalis* has a brownish body with a slight waxy coating. They were observed in large colonies of up to 50 insects. This species is often visited by ants. *C. tujafilina* were nine generations in Poland (figure 1). First alate females were observed in September. The length of development of a generation of *C. tujafilina* aphids was determined. The average length of development of a generation was 27 days in variable

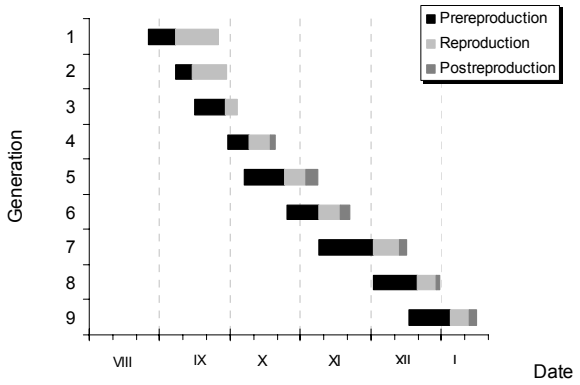

Figure 1. Average length of prereproduction, reproduction and postreproduction period.

temperatures. The longest prereproductive period occurred in the 7th generation (figure 1). For the virginoparae from summer generations the prereproductive period was slightly shorter and in the autumnal generations again prolonged. The reproductive period lasted the longest among first generation. This period was shortened in the subsequent generations. The postreproductive period usually lasted from 0 to 5 days. Females of the fifth generation were longest postreproduction period (figure 1).

The highest fecundity was found among the first generation (average of 51.4 larvae per female) (figure 2). The maximum fecundity per single aphid was 65 larvae. Fecundity decreased in subsequent generations. There was a raise in fecundity only in the 7th generation on November.

The number of generations observed on branches within a year amounted to nine, which confirms studies carried out other *Cinara sp*. i.e. *Cinara cupressi* (Buckton) (Mustafa, 1987; Kairo and Murphy, 1999; Durak *et al.*, 2007). The length of development of a single generation in Poland was 27 days. The prereproductive and reproductive periods were shorter in Polish populations then in Japan populations (Furuta, 1988). Both populations of *C. tujafilina* were similarity average fecundity per female.

The COI is considered one of the most conservative genes in the mitochondrial genome with respect to the amino acid substitutions (Black *et al*., 1997). DNA sequencing analysis was used to characterize the mitochondrial cytochrome oxidase I (approximately 1500 bp) from polish populations of the *C. tujafilina.* Till now the COI marker was used in the research of other species of the

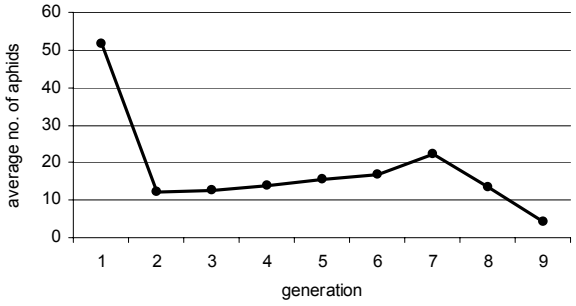

Figure 2. Average fecundity of wingless virginoparae from subsequent generations of *C. tujafilina.*

genus *Cinara* by Favret and Voegtlin (2004). These authors amplified the DNA fragment (approximately 700 bp) in the centre of this gene. We consider that our results represent a promising beginning for the use of DNA in the research of *Cinara* populations. The data generated via DNA sequence analysis, on the other hand, have applicability to future studies as the sequences can be directly compared and are readily accessible to other workers via GenBank.

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