MITOCHONDRIAL DNA POLYMORPHISM IN INVASIVE AND NATIVE POPULATIONS OF *HARMONIA AXYRIDIS*

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ABSTRACT

The Asian ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), is one of the most invasive insects in the world. Originally introduced into the USA and Europe for the biological control of pest insects, it has recently gained the status of an invasive species. There is little data on the differences between invasive and non-invasive populations at the genetic level. In this research mtDNA sequences of the *COI* gene from specimens of native and non-native populations were compared. The results indicate that individuals from invasive populations are similar to those from Far Eastern native populations.

Keywords: *Harmonia axyridis*, Invasive species, *COI* gene

Introduction

Invasive species have recently become a global problem, attracting the attention of many researchers. Unfortunately, the reasons for these invasions in many cases are poorly understood. The process of invasion may be differentiated into three events: initial dispersal, establishment of self-sustaining populations and spread. Research should concentrate on the first stage, initial dispersal, because it is the stage in which effective management may be organized. In addition, initial dispersal is significant to community assembly and meta-community dynamics (Puth and Post 2005).

Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae) is believed to be native to China, Japan, Korea, Mongolia, and Russian Siberia (Dobzhansky 1933; Kuznetsov 1997), although its entire range is not fully recorded due to the lack of information from former republics of the Soviet Union.

At the beginning of the 20th century this species was introduced for the first time as a biological control agent against aphids and coccids in North America (Gordon 1985). Nevertheless, it did not become established in the USA until 1988 (Chapin and Brou 1991), despite many releases between 1964 and 1982 (Gordon 1985). As an alien invasive species, *H. axyridis* has recently been found in South America and two African countries – Egypt and South Africa (Brown et al. 2007).

Original use of *H. axyridis* for controlling pests in Europe began in 1968 in the eastern part, including Ukraine (Katsoyannos et al. 1997) and Belarus (Sidlyarevich and Voronin 1973). Then in 1982 it was introduced into Western Europe in France (Coutanceau 2006), again as a biological control agent. Commercially produced *H. axyridis* was released in many European countries, including Belgium, the Czech Republic, France, Germany, Greece, Italy, Netherlands, Portugal, Spain and Switzerland, as a control agent. Subsequently, these releases led to the beetle spreading very quickly all-around Europe. Its establishment in the wild was recorded in countries where *H. axyridis* was not introduced. There is strong evidence of *H. axyridis* in Austria, Denmark, Great Britain and the Channel Islands, Liechtenstein, Luxembourg, Norway and Sweden (Brown et al. 2007). Recently, *H. axyridis* was recorded in the city of Kaliningrad in the eastern part of Russia.

Analysis of mitochondrial (mt) DNA plays a crucial role in modern molecular phylogenetics and is an essential component of phylogeography. Due to its "phylogenetically favourable properties" (Avise 2000) of maternal transmission, general absence of intermolecular recombination and extensive variation within and among different species, mtDNA can be used to determine the relatedness of specimens from different populations (geographical locations) of one species and their genetic separation (Avise 2000). The cytochrome *c* oxidase gene (*COI*) exhibits a large range of phylogenetic signals (Hebert at al. 2003) among the various mtDNA and nuclear ribosomal (r) DNA molecular markers.

In order to understand the genetic differentiation of non-native and native populations of *H. axyridis*, populations from six geographical locations were surveyed: Italy, Germany, UK, USA, Japan and Russia. The *COI*-based haplotypes of the invasive (non-native) populations were compared with those of three native Russian and Japanese populations.

Material and methods

Insect sampling

Adult individuals and pupae of *H. axyridis* were collected in the wild from five locations: Denver (USA, 2004), Cambridge (Great Britain, 2009), Berlin (Germa-

Fig. 1 Map of collection sites (black circles).

ny, 2008), Turin (Italy, 2006), Gornoaltaysk, Vladivostok (Russia, 2008), and Kyoto (Japan, 2010) (Fig. 1).

DNA extraction and PCR

DNA was extracted from whole specimens using the DIA*tom*™ DNA *Prep* 100 kit and following the manufacturer's protocol (Isogene, Russia). Isolated DNA samples were stored at 20 °C until required. 710 base pairs, including the entire *COI* gene, were sequenced from the mitochondrial genome. PCR amplification was performed using forward LCO1490 (5′– GGTCAACAAATCATAAAGATATTGG – 3′) and reverse HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA–3′) primers (Folmer et al. 1994) under the following PCR conditions: hot start of 94 °C for 5 min; pre-PCR 5 cycles: denaturing at 94 °C for 1 min, annealing at 45 °C for 1 min 30 s, elongation at 72 °C for 1 min 30 s; 35 cycles: denaturing at 94 °C for 1 min, annealing at 50 °C for 1 min 30 s and elongation at 72 °C for 1 min. Amplification products were run on 1% TBE agarose gels (Sigma, USA). PCR products of 710 b.p. length were extracted from the agarose gels following the manufacturer's protocol of JETQUICK Gel Extraction Spin Kit 250 (Genomed, GmbH). The sequencing was done using sequenator ABI PRISM 310 and Applera kit (USA) reagents.

Sequence analysis

All 162 *COI* gene sequences of *H. axyridis* were initially assembled and analyzed using ChromasPro v. 13.3 and then consensus sequences were produced. Cluster analysis was performed using MEGA 4 (Tamura 2007) software with the ClustalW (Thompson et al. 1994) option. Haplotypes, based on *COI* sequence data, were determined using DnaSP v. 5 (Librado and Rozas 2009), the repeating haplotypes were removed from the data set. Sites with alignment gaps were not considered. Phylogeny was constructed on aligned haplotype sequences

Table 1 Polymorphic nucleotide positions defining the 13 mitochondrial COI haplotypes identified in *H. axyridis*.

			1	1	1	1	2	2	2	3	3	4	4	5
		2	4	5	5	8	0	0	5	6	8	8	8	6
		8	4	0	5	9	4	7	8	7	7	3	9	2
		T	A	A	T	T	C	A	C	G	T	A	T	T
H1		.	G	.	.	.	.	.	.	.	.	.	.	.
H2		.	.	G	.	.	.	.	.	.	.	.	.	.
H3		.	.	.	.	.	.	.	.	.	.	T	.	.
H4		.	.	.	.	.	.	.	.	.	.	.	.	.
H5		.	.	.	.	.	.	.	T	.	.	.	A	.
H6		.	.	.	.	.	T	.	T	.	.	.	A	.
H7		.	.	.	.	.	.	G	T	.	.	.	A	.
H8		.	.	.	G	.	.	.	.	.	.	.	.	.
H9		.	.	.	.	.	.	.	.	.	.	T	.	C
H10		.	.	.	.	.	.	.	T	C	.	.	A	.
H11		.	.	.	.	C	.	.	.	.	.	.	.	.
H12		C	.	.	.	.	.	.	.	.	.	.	.	.
H13		.	.	.	.	.	.	.	.	.	C	.	.	.

by the Unweighted Pair Group Method with Arithmetic Means (UPGMA) of Jukes-Cantor distances, based on 1000 bootstrap replications, using MEGA4.

Results

Ten variable sites were identified along the 567 bp of the *COI* sequence (Table 1). Thirteen distinct haplotypes of *H. axyridis* were identified (Table 2). The sequences were deposited in GenBank with accession numbers HM594302–HM594420 and HQ593064–HQ593106. Examination of the base composition revealed that this data set has the following empirical base frequencies: A = 0.309, C = 0.155, G = 0.140, T = 0.396. Of the 567 b.p. of the sequence, thirteen sites exhibited variation, four of which are parsimony-informative. Because of the low number of parsimony-informative sites, parsimony-based methods of phylogeny were considered to be inappropriate hence the UPGMA method was used to construct the haplotype phylogeny (Fig. 2). All *COI* haplotypes grouped into two main branches; the lower branch of the dendrogram included only individuals with haplotypes from the native population (H5–H7, H10). The upper cluster included the of halotypes from populations H1–H4, H8, H9, H11–H13, both native and invasive; no "invasive" halotypes were found in the "native" group (i.e. the lower branch of the tree).

Table 2 Thirteen (H1–H13) haplotypes detected among populations of *H. axyridis*.

Location	Status	No. tested	Haplotypes												
			H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13
USA (Denver)	invasive	17	15		1	1									
UK (Cambridge)	invasive	34	33	1											
Germany (Berlin)	invasive	22	21			1									
Italy (Turin)	invasive	20	15			2				2	1				
Russia (Gorno-Altaysk)	native	26	9				14	1	1			1			
Russia (Vladivostok)	native	24	21			2									1
Japan (Kyoto)	native	19	16										1	2	
Total		**162**	**130**	**1**	**1**	**6**	**14**	**1**	**1**	**2**	**1**	**1**	**1**	**2**	**1**

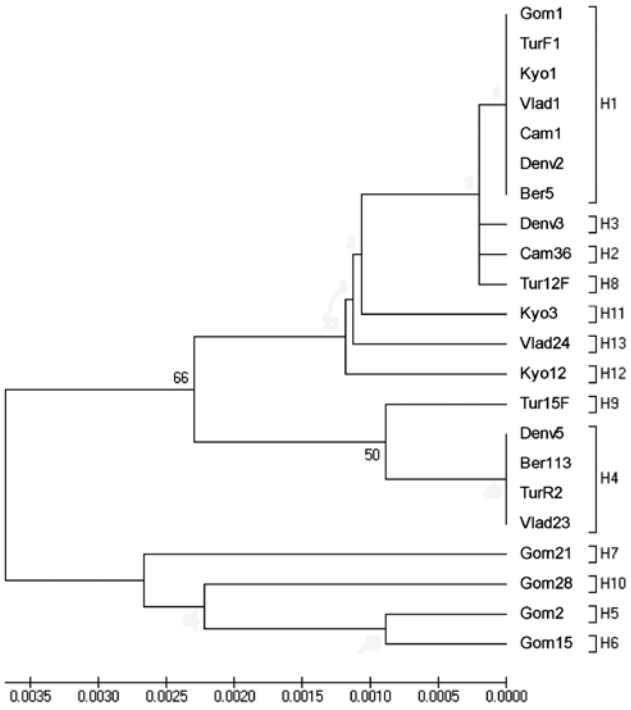

Fig. 2 UPGMA tree of the relationships among the COI haplotypes. The percentage of replicate trees in which the associated haplotypes clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Bootstrap scores less than 50 are not shown.

Discussion

Some researchers suggest that successful invasions emerge from a particular invasive population. In a recent article on *H. axyridis* this phenomenon is called the "invasive bridgehead effect", although the model needs to be improved (Lombert et al. 2010). In this work an attempt was made to identify the genetic variation among five invasive populations and three non-invasive populations. Some individuals from the native range are closely related to those from the non-native range, suggesting that the former could potentially form invasive populations. In addition, there is genetic differentiation within invasive populations that is not correlated with geographical distribution. For example, the H1 haplotype includes specimen from all of the populations studied. It is believed that North America was invaded by specimen originating from the Eastern Asia (Krafsur et al. 1997; Lombert et al. 2010). In addition, the *H. axyridis* commercially used for biological control in Europe also came from the Asian (Chinese) populations. Unfortunately, we got no specimen from that region, so the specimen from non-invasive populations in Russia and Japan were used instead. The comparison of the "invasive" haplotypes indicates that their gene pools are similar, with the H1

haplotype being prevalent. Thus, it appears that invasive populations in the Old and New Worlds may have a common origin.

Western Siberia populations, including those in Altai, consist almost entirely of the *axyridis* form, which rarely occurs in populations in the Far East (Dobzhansky 1933; Blekhman et al. 2010). There are differences among these groups of populations in mtDNA sequences. The H5 in particular, but also the H6, H7 and H10 haplotypes were frequent in the Altai population, but did not occur in the rest of the populations studied.

Author Contributions

Collections: AS – Cambridge, IAZ – all other locations. AS and IG did the experiments, AS analyzed the data and IAZ and AS wrote the paper.

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