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GENERAL GENETICS

Intraspecific and Interspecific Variation of the Mitochondrial Gene of Cytochrome Oxidase I in Ladybirds (Coleoptera: Coccinellidae)

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Abstract—Intergeneric, interspecific, and intraspecific genetic variation of the 310-bp 3'-end region of the mitochondrial gene of cytochrome oxidase I (COI) has been assessed in ladybirds (Coleoptera: Coccinnellidae). The phylogenetic distances between eight species of ladybirds have been determined. Mitochondrial DNA (mtDNA) nucleotide sequences have been compared in *Adalia bipunctata* L. differing in the elytron and pronotum colors that have been sampled from several geographically remote populations. The taxonomic statuses of two morphs from the genus *Adalia*, *A. bipunctata bipunctata* and *A. bipunctata fasciatopunctata*, have been identified.

INTRODUCTION

The interest in the origin, phylogenetic relationships, and species composition of ladybird (Coccinellidae) beetles is explained by their considerable phenotypic diversity and geographic variation [1, 2]. Many ladybird species are polymorphic with respect to the elytron and pronotum color and pattern; there are data on the dependence of the population structure of these species on ecological characteristics of their habitats. Systematics based on comparison of morphological traits is problematic for many ladybird species.

There is considerable controversy regarding the taxonomic status of species and geographic forms of the genus *Adalia* [3]. One of these forms is *Adalia bipunctata fasciatopunctata* Fald., which coinhabits with *A. bipunctata bipunctata* the western part of the Trans-Baikal region, Tyva, and Mongolia. The percentages of ladybirds with the *fasciatopunctata* phenotype in Tyva and Mongolia are 50–57 and as high as 75%, respectively. Some authors regard this form as a separate species [4–6]; others, as a subspecies or a geographic race (morph) [3, 7].

Comparison of nucleotide sequences is successfully used for estimating genetic distances between taxa and analyzing population structure [8]. The mitochondrial gene encoding cytochrome oxidase subunit I (COI) has been demonstrated to be suitable for determining phylogenetic relationships between closely related families, genera, and species of insects and for differentiating between subspecies [9–11]. The 3' end of the COI gene is its most variable region [9, 10]. Polymorphism of some mitochondrial DNA (mtDNA) genes, including the genes of COI, COII, and ND5, was found in a study on *A. bipunctata* populations [12]. For the COI gene, ten variants (haplotypes) of mtDNA were identified.

In this connection, we studied the intergeneric and interspecific variation of a region of the mtDNA COI gene in ladybirds. The data obtained were compared with the data on the variation of this mtDNA region in several geographically isolated populations of *A. bipunctata.* One of the purposes of this study was to determine the taxonomic statuses of and phylogenetic distances between *A. bipunctata fasciatopunctata* and *A. bipunctata bipunctata* (which has a characteristic pigmentation of the elytra and pronotum) with the use of molecular genetic methods.

MATERIALS AND METHODS

We studied the following species of ladybirds: *A. bipunctata* L., *A. decempunctata* L., *Coccinella distincta* Fald., *C. trifasciata* L., *C. transversoguttata* Fald., *C. septempunctata* L., *C. quinquepunctata* L., and *Harmonia axyridis* Pall. The population analysis of *A. bipunctata* included 9, 13, 1, and 1 beetles from Italy, Tyva, St. Petersburg (Russia), and Bulgaria, respectively. Total DNA was isolated as described earlier; the lysate was treated with proteinase K, and then DNA was extracted with a phenol–chloroform mixture [13]. Primers UEA9 and UEA10, which were specific to the 3' end of the COI gene [10], were used for amplification of and sequencing of its 310-bp fragment. The amplification consisted of 35 cycles performed in the following mode: 30 s at 94°C, 1 min at 50°C, and 1 min at 72°C.

The PCR products were purified using a Wizard PCR DNA Purification Systems kit (Promega, United States) and cloned in a pGEM-T Easy Vector (Promega,

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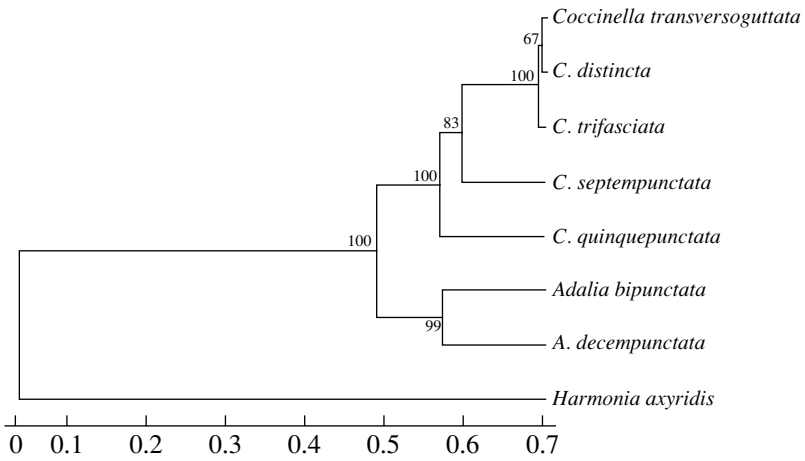

Fig. 1. The phylogenetic tree of representatives of seven species from tribe *Coccinellini* constructed by the neighbor-joining method with the use of Kimura's algorithm, taking into account nucleotide substitutions at all of the three positions. Bootstrap analysis was performed in 500 duplicates.

United States). Sequencing was performed in a cyclic mode using an ABI PRISM 3100-Avant. The nucleotide sequences were compared using the CLUSTAL-W software [14]. Phylogenetic trees were constructed by the neighbor-joining (NJ) method using Kimura's algorithm, taking into account substitutions at all of the three positions [15]. Bootstrapping [16] was performed in 500 replicates. The degree of the COI gene variation was estimated using the Mega software [16].

RESULTS AND DISCUSSION

We determined the primary structure of the 3'-end region of the COI gene in five species of the genus *Coccinella* and two species of the genus *Adalia*. When comparing nucleotide sequences, the number of variable sites was 27% of the total number of nucleotides. The Mega software was used to calculate the average nucleotide composition. The percentages of A, T, G, and C were 38.9, 11.1, 15.6, and 34.4%, respectively. We did not find differences between species with respect to the A + T proportion. The transition-to-transversion ratio was 0.974. The maximum divergence both within the genus Coccinella and within the genus *Adalia* was 10%.

Figure 1 shows the phylogenetic tree of the tribe *Coccinellini* constructed by the NJ method. The values of the bootstrap coefficients for the main clusters demonstrate significant resolution of phylogenetic relationships at the interspecific level. Thus, analysis of the nucleotide sequences of the COI gene may be used to demonstrated distinct interspecific differences between the species studied and to determine interspecific genetic distances within clusters. The representatives of the genus *Coccinella* that are widely spread in northeastern Asia (*C. distincta* Fald., *C. trifasciata* L., and *C. transversoguttata* Fald.) are grouped together in the cladogram, which indicates close phylogenetic relations between them. *C. quinquepunctata* L. is the most distant genetically.

One of the goals of this study was to assess genetic divergence within the genus *Adalia*, in particular, to the degree of genetic differentiation within *A. bipunctata*. In our study, the genus *Adalia* was represented by two polymorphic species, *A. bipunctata* L. and *A. decempunctata* L. Taking into account that the mtDNA evolution rate is 1.5–2% of nucleotide substitutions per 10^6 years [9], the divergence between the two species corresponds to 5 Myr.

The taxonomic status of Central Asian populations of *A. bipunctata* had until recently remained a controversial point. These populations have a special type of polymorphism (f. *fasciatopunctata*) including characteristic color and pattern of the pronotum and elytra. This polymorphism serves to distinguish the "Asian" *A. bipunctata* from other forms of this species. We performed molecular genetic analysis of two populations that were the most remote geographically, the Italian and Tyvan ones. The sample from the Tyvan population comprised ladybirds of the forms f. *bipunctata* and f. *fasciatopunctata*. In addition, we used the nucleotide sequences of samples from Bulgaria and St. Petersburg (Russia) and one sample from Moscow (Russia) [12] (the latter was obtained from the GenBank database). Figure 2 shows the comparison of the nucleotide sequences of the COI gene fragment studied.

Our data indicate that one variant of mtDNA, which we called typical, is prevalent in *A. bipunctata* from various populations. It was found in 16 out of 27 beetles from Italy, Moscow, St. Petersburg, and Tyva. In total, we detected 11 mtDNA variants (mt haplotypes), most of them found in one beetle each. Nine of these variants differed from the typical variant in one or two nucleotide substitutions. Two haplotypes (haplotypes 25 and 26 found in Tyva and Bulgaria, respectively) were most different from each other. They differed from the typi-

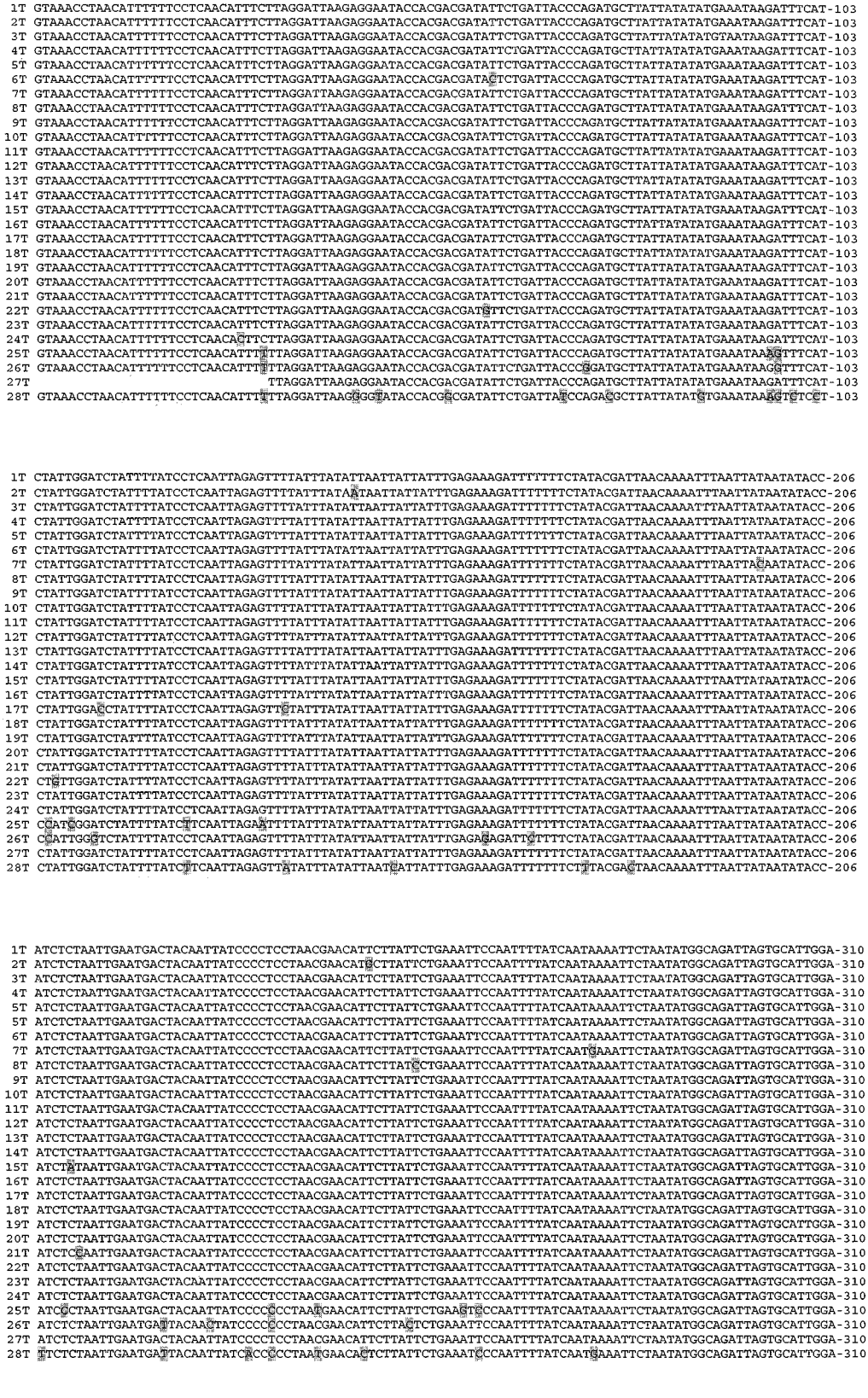

Fig. 2. Comparison of the nucleotide sequences of the 310-bp 3'-end fragment of the COI genes of *Adalia bipunctata* from different populations (1T–9T, Italy; 10T, St. Petersburg; 11T–25T, Tyva; 26T, Bulgaria; and 27T, Moscow) and *Adalia decempunctata* (28T).

cal variant in 12 and 10 nucleotides, respectively; however, this was considerably less than the difference between *A. bipunctata* and *A. decempunctata* (24 nucleotides). Thus, notwithstanding drastic morphological differences between beetles from European and Central Asian populations of *A. bipunctata*, analysis of mtDNA variation did not show differences between these populations (i.e., genetic characters with respect to which the populations studied could be differentiated into clusters). The data obtained are additional evidence for the conclusion made by Lusis [3] that there are no grounds for regarding the form *fasciatopunctata* as a separate species. To determine whether different populations of *A. bipunctata* differ from one another in haplotype frequencies, it is necessary to analyze more material than we used in this study. We may conclude that analysis of the variation of the COI gene makes it possible to reveal important evolutionary mechanisms of species formation in ladybirds. This analysis permits, first, determining phylogenetic distances between species and genera of the family studied and, second, studying microevolutionary processes, i.e., relatively recent events of interpopulation divergence.

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