Molecular markers for weevil (*Sitophilus zeamais*)

population genetics

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Summary

Sitophilus weevils are cosmopolitan pests of stored grain that harbor bacterial endosymbionts. Therefore an examination of weevil population genetics and phylogeny would provide insight into both pest management and symbiotic evolution. We tested molecular markers in mitochondrial DNA for their utility in such studies. Two overlapping gene fragments, corresponding to partial cytochrome subunit one (COI) gene and partial COI and leucine tRNA 2 genes (COI-TL2), were amplified by PCR using two degenerate primer sets from individual weevils (*S. zeamais*) collected in Indiana (IN), Kansas (KS), Nebraska (NE), and Pennsylvania (PA), and an *S. oryzae* individual. A 644-bp partial COI fragment and an 831-bp partial COI-TL2 were purified and sequenced. The two overlapping fragments were pieced into one long consecutive sequence. Populations from IN, KS, and PA showed sequence homology while NE population differed at a single base. Sequence differed substantially between *S. zeamais* and *S. oryzae*, with a 14% sequence divergence. Phylogenetic tree analysis reflected intra- and interspecific relationships. These markers would be useful in *S. zeamais* population genetics and interspecific phylogenetic studies.

Introduction

Weevil species of the genus of *Sitophilus* (Coleoptera: Curculionidae), are pests of stored cereals around the world (Rees 1996). Maize weevils (*S. zeamais*) and rice weevils (*S. oryzae*), are two of the three major pest species (Grenier et al. 1997). Although much effort has been made to control these pests (Guedes et al. 2006; Hansen and Steenberg 2007), biological control is not very successful; taxonomical and systematic studies on weevils are needed to promote biological control of these pests (Gillespie et al. 2006).

Sitophilus weevils are also hosts to many endosymbiotic bacteria (Wernegreen 2002). Endosymbiosis is a ubiquitous interspecific association and plays significant roles in many ecological and evolutionary processes (Moran 2006). Weevils provide symbionts with a stable environment and some metabolites, while the symbiont provides the weevil with nutrients

deficient in their diet. Compared with the extensive studies on their endosymbionts, our knowledge of the weevil is quite limited. Populations in different regions probably feed on different diets characteristic of their habitat environment. As a consequence, the endosymbionts residing within them may also differ in their ability to synthesize required nutrients. An endosymbiont undergoes substantial genomic change following host-restriction (Moran and Wernegreen 2000), and therefore the association could be distinct among isolated weevil populations. Some weevil species have shown genetic variations across large spatial scales (Laffin et al. 2004; Laffin et al. 2005a; Laffin et al. 2005b). Thus, a phylogenetic analysis of weevil populations would further our understanding of the weevil-bacterium endosymbiosis.

Several molecular markers have been developed for arthropod population genetic and phylogenetic studies. Mitochondrial genes have been the most widely employed molecular markers to study phylogeny within species (Simon et al. 1994). Mitochondrial genes exhibit relatively rapid rates of evolution, which would allow for detection of population-level genetic differences. The gene encoding cytochrome oxidase subunit one (COI) has been one of the most often used markers to study insect phylogeny (Simon et al. 2006). COI and COI-COII (sequences spanning partial COI and COII) have been used to study the origin of cabbage seedpod weevil *Ceutorhynchus obstrictus* (Laffin et al. 2005b), and population structure and phylogenetic relationships of *C. neglectus* (Laffin et al. 2005a). Molecular markers were also developed for internal transcribed spacer 2 gene of rice weevil *S. oryzae* (Jeong et al. 2006).

In this pilot study, we tested the COI and COI-TL2 (sequence spanning COI and leucine tRNA gene 2) regions for their utility in examining the population genetics of *S. zeamais*. A rice weevil (*S. oryzae*) was included in the study as an outgroup for the construction of a phylogenetic tree.

Materials and Methods

Weevil samples. Maize weevil *S. zeamais* (Coleoptera: Curculionidae) and rice weevil *S. oryzae* samples were obtained from Kevin Dougherty at Fordham University. *S. zeamais* was collected from four populations in different states of US: Indiana (IN), Kansas (KS), Nebraska (NE), and Pennsylvania (PA) (Fig. 1). Live weevils were frozen in liquid nitrogen, and stored at -80°C until DNA extraction.

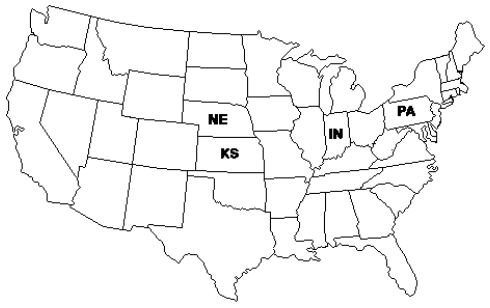

Figure 1. Locations of weevil (*S. zeamais*) populations used in this study.

DNA extraction. Individual weevils were ground in microcentrifuge tubes with a pestle; DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions for tissue samples.

Primers and PCR amplification. Two primer sets were used to amplify two overlapping regions of mitochondrial DNA. The first set, forward C1-J1709 (5'-AATTGGWGGWTTYGG AAAYTG-3') and reverse C1-N2353 (5'-GCTCGTGTATCAACGTCTATWCC-3'), was used to amplify COI. A second set of primers, forward C1-J2183 (5'-CAACATTTATTTTGATTTTTTG G-3') and reverse TL2-N3014 (5'-TCCAAT GCACTAATCTGCCATATTA-3'), was used to amplify partial COI-TL2. Touchdown PCR was performed as described in table 1. PCR products were purified using a Rapid PCR purification system (Marligen Biosciences, MD) and eluted with 30 μL hot deionized water.

Table 1 Touchdown PCR program

94 °C	5:00	
94 °C	0:30	13 cycles
58 °C	0:30	
	-1°C/cycle until 45°C	
72 °C	1:30	
94 °C	0:30	22 cycles
45 °C	0:30	
72 °C	1:30	
72 °C	7:00	
4 °C	HELD	

Sequence analysis. Purified PCR products were sequenced by Sanger dideoxy termination method, and sequences were analyzed using MacVector. The two sequences for COI and COI-TL2 was integrated into one long sequence. Sequence alignment and phylogenetic tree construction were performed using MEGA 3.1.

Results

Sequence analysis of mtDNA fragments from weevils. Two fragments were amplified from *S. zeamais* populations and *S. oryzae*, which were 644 bp and 831 bp in length (Fig. 2). The 644-bp fragment is a partial sequence of the COI gene, and the 831-bp fragment is a DNA fragment spanning partial COI and TL2.

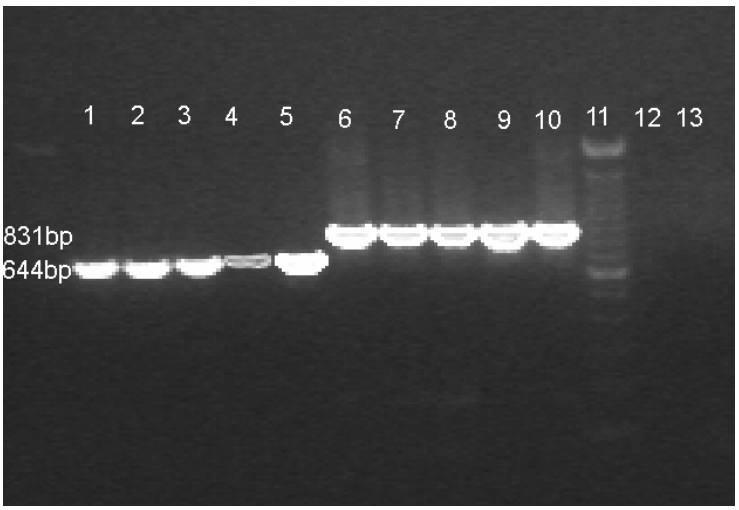

Figure 2. Partial COI and COI-CTL2 gene fragments amplified from four individual weevils (*S. zeamais*) from different states of the US and a *S. oryzae* individual. *S. zeamais* partial COI and COI-CTL2 segments from IN (lanes 1 and 6), KS (lanes 2 and 7), NE (lanes 3 and 8), and PA (lanes 5 and 10), and the *S. oryzae* partial COI and COI-CTL2 segments (lanes 4 and 9) were presented. Negative control: lanes 12 (COI) and 13 (COI-CTL2) were also included.

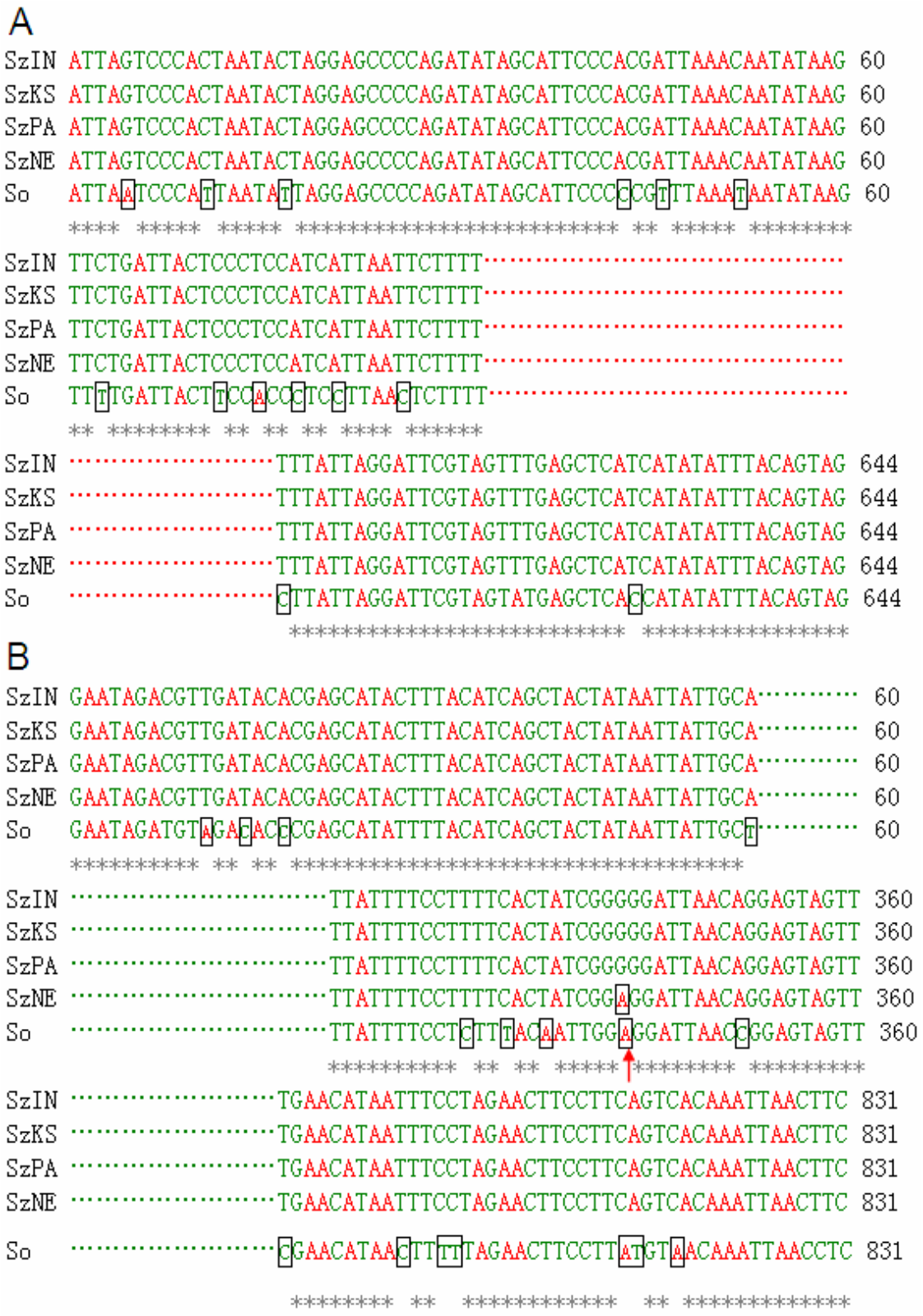

Figure 3. Alignment of partial COI (A) and COI-TL2 (B) sequences amplified from four individual weevils (*S. zeamais*) harvested in different states and a *S. oryzae* individual. * indicates identity among all five sequences. The base change in NE *S. Zeamais* sequence is pointed by arrow. Base changes in *S. oryzae* COI and COI-TL2 sequence are boxed.

Sequence analysis revealed that these four 644-bp sequences of COI were identical among the four *S. zeamais* populations (Fig. 3A), and there was only one base change in the 831-bp sequence of COI-TL2 in *S. zeamais* from NE, compared to the sequences from three other populations (Fig. 3B). As there is a 171-bp overlap between the COI and COI-TL2 segments, these two fragments were pieced together into a long consecutive sequence, 1304 bp in length. In contrast, the sequences between two weevil species were quite different (Fig. 3A and B) in both regions of amplified segments, showing a 14% sequence divergence.

Intra- and inter-specific phylogenetic relationships of weevils. Based on the integrated sequence from the two fragments, a minimum evolution phylogenetic tree was constructed to illustrate the relationship among the four *S. zeamais* populations and that between *S. zeamais* and *S. oryzae* (Fig. 4). *S. zeamais* populations from IN, KS, and PA showed the closest relationship, NE population was somewhat apart. Interspecific relationship between *S. zeamais* and *S. oryzae* was quite divergent as seen in Fig. 4.

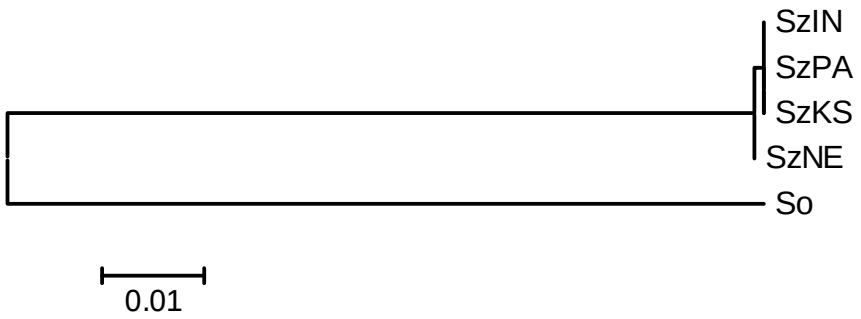

Figure 4. Phylogenetic relationship between *S. zeamais* populations and between *S. zeamaise* and *S. oryzae* based on the sequence of the integrated 1304-bp fragment.

Discussion

Two partial COI and COI-Tl2 segments were amplified in the study, which indicated their presence in the four *S. zeamais* populations from different states. As only one individual from each population was studied, it was not possible to calculate and compare allele frequencies for these genes among populations.

Low variability of the fragment COI through TL2 was both detected in our and other studies for populations at a small geographic scale (Laffin et al. 2004; Laffin et al. 2005a), and high variation was observed among populations from different continents (Laffin et al. 2005b). Generally, species with large-scale geographic distributions show considerable genetic variation (Avise 1994). However, low variability at the small geographic scale may be accounted for by their short residence time in those states, either as invading or introduced species, with not enough time for differences to accumulate (DeSalle et al. 1987). The four populations in our study may arise from a common ancestor.

In contrast, high variation between *Sitophilus* species was detected, using these mtDNA fragments, indicating they are good markers for interspecific phylogenetic studies. Significant difference in both COI and COI-TL2 regions were both observed. For intraspecific phylogeny, the tree reflected the sequence difference among different *S. zeamais* populations.

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