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Mitochondrial DNA Sequence Variation of the Oriental Mole Cricket, *Gryllotalpa orientalis* (Orthoptera: Gryllotalpidae) in Korea

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The mole crickets, *Gryllotalpa*, are insect pest distributed in the world. In Korea, *G. orientalis* was reported to occur, but previous ecological studies suggested the presence of two ecological types. To test this hypothesis, we sequenced a portion of mitochondrial (mt) genome from 48 *G. orientali* individuals collected over five Korean localities: Busan, Suwon, Okchon, Wonju, and Gangneung. From the sequence analysis, only two haplotypes were obtained, but the sequence divergence between the two haplotypes was 11%, suggesting the presence of two distinct genetic groups in Korea. Although the population of Busan, Okchon, Wonju, and Gangneung was identified as a single haplotype, but that of Suwon was occupied by both hapotypes. Considering sequence divergence of other insect species occurring in Korea, the divergence estimate found between the two haplotypes seems to be too large to be considered as identical species. This result may suggest that the two differentiated haplotypes found in this study may reflect the previously reported two ecological types found in Suwon, Korea. To further understand the genetic divergence of the two phylogenetic groups, analysis of more variable regions of *G. orientalis* genome is required.

Key Words: Mitochondrial DNA, COI gene, *Gryllotalpa orientalis*, Oriental mole cricket, Genetic divergence

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Introduction

Mole crickets belong to a family of cricket, Gryllotalpidae, in an insect order Orthoptera. Approximately, 70 species, placed in 5 genera are distributed throughout temperate and tropical regions. Among them *G. orientalis* occurs in Korea, China, Japan, Taiwan, Australia, Netherlands, and even to the Hawaii (Frank *et al.*, 2006; http://molecrickets.ifas.ufl.edu/credits.htm). In Hawaii, the species arrived before 1896 from Asia, but mistakenly regarded as *G. africana*. In a similar fashion, mole crickets from a host of Asian countries, including Korea, have also been misidentified as *G. africana*, although they are actually *G. orientalis* (Frank *et al.*, 2006; http://molecrickets.ifas.ufl.edu/credits.htm). In Korea, *G. orientalis* is a monotypic species of a genus known to exert deleterious effects on ginseng crops (Jeong, 1994).

Although *G. orientalis* constitutes a serious pest in Korea, there have been relatively few studies regarding the field ecology of the species. Nevertheless, Kim (1995) made some pioneering study on the field ecology of the species and determined that the population of oriental mole crickets inhabiting Suwon, a mid-Korean locality, is composed of the *G. orientalis* species, confounded with two different life cycles: one that undergoes one generation annually and another that undergoes one generation biannually. However, this observation is somewhat difficult to reconcile with the fact that populations with obviously different life cycles may not be present without

reflection of underling genetic perspective, such as genetic isolation and divergence. On the other hand, another study reported that a population of *G. orientalis* originated from the same locality over-winters in the nymph phase and emerges as an adult in May, undergoing one generation per year (see Kim, 1995). Thus, the time of adult emergence and voltinism contradict to each other. Nevertheless, no study has yet definitively settled this discrepancy.

Therefore, we tested the hypothesis that the Korean populations of *G. orientalis* are composed of two ecological or possibly genetic groups, accompanying proper genetic background. Up to now, in fact, no population-level genetic studies of any mole cricket species are available, although some other species were subjected to this aspect. Thus, our study may be the first that aimed to determine genetic aspects of *Grylotalpa*. For the purpose of study, we sequenced a portion of mt COI gene from 48 individuals of *G. orientalis* collected over five localities in Korea.

Materials and Methods

Insects

Forty-eight individuals of *G. orientalis* were collected by digging in ridges of dry fields in five Korean localities, between December 2001 and October 2002 (Fig. 1). The

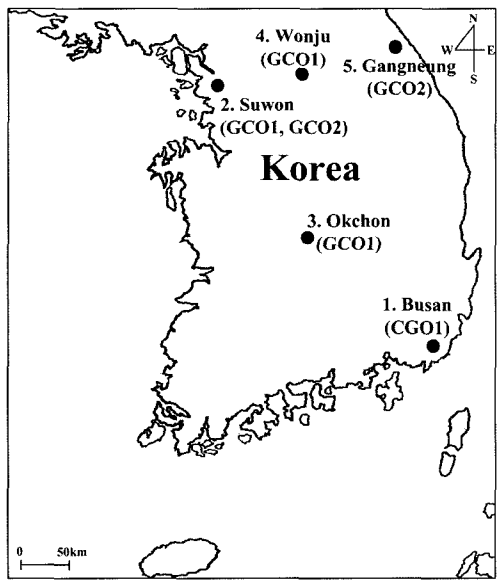

Fig. 1. A map of sampling locations of the oriental mole crickets in Korea and corresponding haplotypes found in each locality. General locality names are as follows: 1, Noksan, Busan; 2, Suwon, Kyunggi Province; 3, Okchon, Chungcheongbugdo Province; 4. Wonju, Gangwondo Province; and 5, Gangneung, Gangwondo Province.

total DNA was extracted using the Wizard Genomic DNA Purification Kit, in accordance with the manufacturer's instructions (Promega, USA).

Primer, PCR, and Sequencing

For the amplification of a portion of mt COI gene, a pair of primer was designed based on Simon *et al.* (1994): CI-J-1751, 5'-GGATCACCTGATATAGCATTCCC-3' and CI-N-2191, 5'-CCCGGTAAAATTAAAATATAAACTTC-3'. After an initial denaturation step at 94°C for 5 min, a 40-cycle amplification (94°C for 30 s, 50°C for 40 s, and 72°C for 45 s) was conducted. The final extension step was continued for 7 min and 45 s at 72°C. To confirm the successful DNA amplification, electrophoresis was carried out using 0.5× TAE buffer on 0.5% agarose gel. The PCR product was then purified using PCR purification Kit (QIAGEN, Germany). The COI gene fragments were directly sequenced from PCR products. DNA sequencing was performed using the ABI PRISM® BigDye® Terminator v1.1 Cycle Sequencing Kit under the ABI PRISM™ 310 Genetic Analyzer (PE Applied Biosystems, USA). All products were sequenced from both strands. Sequence alignment was performed using CLUSTAL X programs (ver. 1.8; Thompson *et al.*, 1997). When homologous sequences from two individuals differed by ≥ one nucleotide base, the sequences were considered as different haplotypes. Haplotype designations were applied to new sequences as they were discovered (*i.e.*, GCO1 and GCO2).

Phylogenetic analysis

Phylogenetic analysis was performed by maximum likelihood (ML) method (Felsenstein, 1981) using PAUP* (Phylogenetic Analysis Using Parsimony and Other Method*) ver. 4.0b10 (Swofford, 2002). Heuristic search was conducted using tree-bisection-reconnection (TBR) for branch-swapping algorithm, steepest descent option not in effect, stepwise addition option for starting tree, number of trees held at each step during stepwise addition for one, and initial "MaxTrees" setting for 100. Branches were collapsed if maximum branch length is zero. Negative branch lengths were allowed, but these were set to zero for tree-score calculation. Trees were evaluated using the bootstrap test (Felsenstein, 1985) by 100 iterations. To root the trees, the full-length mtDNA of *Locusta migratoria* (Flook *et al.*, 1995) was utilized for the corresponding region.

Results and Discussion

Sequence analysis of the mt COI gene from 48 individuals of *G. orientalis* collected over five Korean localities resulted in only two nucleotide-based sequence haplo-

Table 1. A list of trapping localities, animal numbers, and COI haplotypes of *G. orientalis*

Collecting locality	Collection date	Animal number	Haplotype
1. Noksan, Busan, Gyeongsangnamdo Province (4)	2001. 12. 19	GO-01	GCO1
	"	GO-02	"
	"	GO-03	"
	"	GO-04	"
2. Suwon, Kyunggido Province (23)	2001. 12. 19	GO-05	GCO2
	"	GO-06	"
	"	GO-07	"
	"	GO-08	"
	"	GO-09	GCO1
	"	GO-31	GCO2
	"	GO-32	"
	"	GO-33	"
	"	GO-34	"
	"	GO-35	"
	"	GO-36	"
	"	GO-37	GCO2
	"	GO-38	"
	"	GO-39	"
	"	GO-40	"
	"	GO-41	"
	2002. 08. 23	GO-42	"
	"	GO-43	"
	"	GO-44	"
	"	GO-45	"
	"	GO-46	"
	"	GO-47	"
	2002. 09. 02	GO-48	GCO2
3. Okchon, Chungcheongbugdo Province (9)	2002 .09. 02	GO-10	GCO1
	"	GO-11	"
	"	GO-12	"
	"	GO-13	"
	"	GO-14	"
	"	GO-15	"
	"	GO-16	"
	"	GO-17	"
	"	GO-18	"
4. Wonju, Gangwondo Province (9)	2002. 10. 01	GO-19	GCO1
	"	GO-20	"
	"	GO-21	"
	"	GO-22	"
	"	GO-23	"
	"	GO-24	"
	"	GO-25	"
	"	GO-26	"
	"	GO-27	"
5. Gangneung, Gangwondo Province (3)	2002. 10. 01	GO-28	GCO2
	"	GO-29	"
	"	GO-30	"

Within parenthesis indicates total number of individual collected from each locality.

```
                                          30                                  60
GC01(Go01)      CGAATAAATA ATATAAGCTT CTGGCTCCTC CCCCCTTCAT TAACTCTTCT TCTTGCATCA
GC02(GC05)      .......... .......... T..A..T..T ..T......C .......... C.........
L. migratoria   .......... .C.....A.. T..AT.AT.A ..A..A...T ....A..C.. A..AATG..T
                                          90                                 120
GC01(Go01)      AGTATAGTTG ACGTCGGAGC AGGAACTGGT TGAACTGTAT ATCCCCCTTT ATCCTCTAAT
GC02(GC05)      ........G. .TA....... ...G.....C .......... .......C.. ..........
L. migratoria   TC.G....A. .TAAT..... T..T..A..A .....A.... ....T..AC. .G..AGAGTC
                                         150                                 180
GC01(Go01)      ATTGCCCATG CAGGATCCTC TGTAGACCTA ACTATTTTTT CTTTACATTT AGCAGGAGTA
GC02(GC05)      ..C..T.... .T.....T.. .........G .......... .A.....C.. .........T
L. migratoria   ..T..T...A GA...G.T.. ......TT.A G.A....... .A.....TC. ......T..T
                                         210                                 240
GC01(Go01)      TCTTCTATTC TAGGGGCAGT TAACTTTATT ACTACAATAA TTAATATACG TTCCCCAGGA
GC02(GC05)      ........C. ....A..... ...T...... .......... .......... C.....T...
L. migratoria   ..C..A..TT ....A..TA. ...T..C... ..A...GC.. .C........ A..AAATAAT
                                         270                                 300
GC01(Go01)      ATATCTCTAG ATCAAACCCC TTTATTTGTT TGAGCTGTAG GTATCACTGC ACTTCTACTT
GC02(GC05)      ......T... .......... .........C ..G.....T. .......... ......C...
L. migratoria   ...A.CC.T. .......A.. A........T ..AT.A..A. CA..T..A.. CT.AT.A...
                                         330                                 360
GC01(Go01)      TTATTATCTT TACCAGTTTT AGCAGGGGCT ATTACTATAC TTCTTACTGA TCGTAATCTA
GC02(GC05)      ........C. .......C.. ......A... ..C....... .C..C..... .......T..
L. migratoria   ........A. .......A.. ...T..A..A ..T......T .AT.A..... ...A..CC.T
                                         390              405
GC01(Go01)      AATACATCCT TTTTTGATCC TGCTGGAGGA GGTGATCCCA TCCTA
GC02(GC05)      ........T. .C........ ...C.....T .....C..T. .T...
L. migratoria   .....G..A. .C..C..C.. G..A.....A .....C..A. .TT..
```

Fig. 2. Sequence alignment of three mt COI haplotypes (designated as GCO1 and GCO2) obtained from 405-bp COI gene sequences and one homologous sequence of the *Locusta migratoria* (Flook *et al.*, 1995) utilized as an outgroup for phylogenetic analysis.
Only nucleotide positions that differ from haplotype GCO1 are indicated.

types (GCOI and GCO2; Fig. 2). Sequence alignment revealed 48 variable nucleotides: 32 were T⇔C transitions, seven G⇔A transitions, two C⇔A transversions, one T⇔G transversion, and six A⇔T transversions (Fig. 2). No length variation among haplotypes was detected (Fig. 2). Uncorrected pairwise distance between the two haplotypes was 11% (Table 2). This estimate is very high considering the estimates obtained from other insects occurring in Korea and even including neighboring countries, wherein the homologous portion of mt COI gene sequence was employed. For example, the maximum sequence divergence was 0.2% for the domestic silkworm (Kim *et al.*, 2000), 0.2% and 1.2% for two species of mushroom fly (Bae *et al.*, 2001), 0.9% in the diamondback moth *Plutella xylostella* (Li *et al.*, 2006), 4.0% for the firefly, *Luciola lateralis* (Kim *et al.*, 2001), and 5.5% for the Korean firefly *Pyrocoeria rufa* (Lee *et al.*, 2003). Considering the divergence estimates of the two firefly species, which may have stemmed from genetic subdivision and speciation the estimate obtained from *G. orientalis* is substantially high to consider the two haplotypes as identical species. Nevertheless, the difference of amino acid sequence between the two haplotypes was only one (Valine → Isoleucine) (Fig. 3). Thus, the remaining 47 nucleotides substitution were synonymous position. Phylogenetic analysis of the *G. orientalis* mt COI haplotypes also provided no informative clustering between the two *G. orientalis* haplotypes, including the outgroup *L. migratoria* (Fig. 4). Maybe this analysis has some limitation

Table 2. Uncorrected pairwise distance of three *G. orientalis* COI haplotypes

	1	2	3
1. GCO1 (Go01)	-	0.11	0.28
2. GCO2 (Go05)	46	-	0.27
3. *L. migratoria*	112	111	-

The *Locusta migratoria* COI sequence (Flook *et al.*, 1995), which was utilized as an outgroup for phylogenetic analysis was also included.
Percent sequence divergence is presented above diagonals and their numbers of nucleotide differences are given below diagonals.

```
                                                30                                  60
GC01(Go01)      RMNNMSFWLL  PPSLTLLLAS SMVDVGAGTG WTVYPPLSSN IAHAGSSVDL TIFSLHLAGV
GC02(Go05)      ..........  .......... ....I..... .......... .......... ..........
L. migratoria   ..........  ........M. .V..N..... .......A.V ...S.A.... A.........

GC01(Go01)      SSILGAVNFI  TTMINMRSPG MSLDQTPLFV WAVGITALLL LLSLPVLAGA ITMLLTDRNL
GC02(Go05)      ..........  .......... .......... .......... .......... ..........
L. migratoria   ......I...  ..A.....NN .T........ .S.A...... .......... ..........

GC01(Go01)      NTSFFDPAGG  GDPIL
GC02(Go05)      ..........  .....
L. migratoria   ..........  .....
```

Fig. 3. Amino acid sequence alignment of the two mt COI haplotypes.

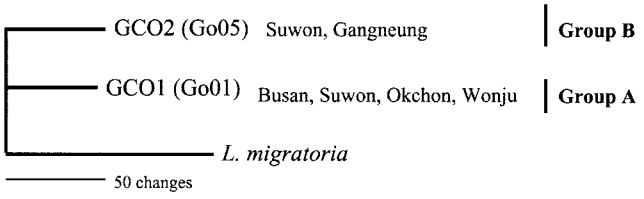

Fig. 4. Phylogenetic analysis of mt COI gene haplotypes of *G. orientalis*. The tree was obtained by ML method incorporated in PAUP* (Phylogenetic Analysis Using Parsimony and Other Method*) ver 4.0b10 (Swofford, 2002). The migratory locust, *L. migratoria* (Flook *et al.*, 1995), belonging to the same Orthoptera was incorporated in the analysis to root tree.

because the outgroup, which is the only available orthopteran species in the GenBank, is too distant to clearly distinguish the two haplotypes from outgroup. Nevertheless, lack of clustering of the two haplotypes obtained from supposedly identical species from another orthopteran suborder Caelifera may suggest genetic divergence of the two haplotypes obtained from *G. orientalis*.

In terms of geographic distribution, Busan, and Okchon, and Wonju were occupied by haplotype GCO1, but Gangneuong was occupied by GCO2. On the other hand, Suwon was occupied by both haplotypes GCO1 and GCO2. Considering the genetic divergence of the two haplotype (11%) it was not expected to find such different haplotypes in a locality. Considering the *G. orientalis* individuals from Suwon were collected at the same place by digging in ridges of dry fields, it was very unusual to find such divergent ones within one habitat. The previous ecological study indicated that the *G. orientalis* population of Suwon is composed of species with two different life cycles (Kim *et al.*, 1995). Thus, it seems that the haplotypes found in this study reflect the two ecological types found previously in Suwon (Kim *et al.*, 1995). Currently, we do not know whether the *G. orientalis* with different life cycles are the two haplotypes found in this study or not. Nevertheless, it seems that the two previous ecological types are reflected in our genetic result considering the fact that populations with obviously different life cycles may not be present without reflection of underling genetic perspective, such as genetic isolation and divergence. In the future, the indoor rearing of *G. orientalis* individuals from the two halotypes to determine species status, coupled with field observation, is expected to solve this problem. Meanwhile, we can tentatively conclude that the *G. orientalis* species inhabiting Korea may be composed of two monophyletic groups with substantial sequence divergence between them, although the species status of these remains unclear. Because we were able to obtain only two haplotypes, the power of phylogenetic analysis was limited. Therefore, analysis of more variable portion of *G. orientalis* genome is required.

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References

Bae, J. S., I. Kim, S. R. Kim, B. R. Jin and H. D. Sohn (2001) Mitochondrial DNA sequence variation of the mushroom pest flies, *Lycoriella mali* (Diptera: Sciaridae) and *Coboldia fuscipes* (Diptera: Scatopsidae), in Korea. *Appl. Entomol. Zool.* **36**, 451-457.

Felsenstein, J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17**, 368-376.

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**, 783-791.

Flook, P. K., C. H. Rowell and G. Gellissen (1995) The sequence, organization, and evolution of the *Locusta migratoria* mitochondrial genome. *J. Mol. Evol.* **41**, 928-941.

Frank, J. F., Thomas, R. F., Short. D. E., and Weed, A. S. The MCricket Knowledgebase (http://molecrickets.ifas.ufl.edu/credits.htm).

Jeong, H. K. (1994) Check list of insects from Korea, Kon-Kuk Univ. Press, Seoul.

Kim, I., J. S. Bae, H. D. Sohn, P. D. Kang, K. S. Ryu, B. H. Sohn, W. B. Jeong and B. R. Jin (2000) Genetic homogeneity in the domestic silkworm, *Bombyx mori*, and phylogenetic relationship between *B. mori* and wild silkworm, *B. mandarina*, using mitochondrial COI gene sequences", *Int. J. Indust. Entomol.* **1**, 9-17.

Kim, J. G., I. Kim, J. S. Bae, B. R. Jin, K. Y. Kim, S. E. Kim, Y. C. Choi, J. Y. Choi, K. Y. Lee, H. D. Sohn and S. K. Noh (2001) Genetic subdivision of the firefly, *Luciola lateralis* (Coleoptera: Lampyridae), in Korea detected by mitochondrial COI gene sequences. *Korean J. Genetics* **23**, 203-219.

Kim, K. W. (1995) Seasonal changes in age structure and fecundity of the African mole cricket (*Gryllotalpa africana*) populations in Suwon, Korea. *Korean J. Appl. Entomol.* **34**, 70-74.

Lee, S. C., J. S. Bae, I. Kim, W. J. Yang, J. K. Kim, K. Y. Kim, H. D. Sohn and B. R. Jin (2003) Mitochondrial DNA sequence-based population genetic structure of the firefly, *Pyrocoelia rufa* (Coleoptera: Lampyridae). *Biochem. Genetics* **41**, 427-452.

Li, J., F. Zhao, Y. S. Choi, I. Kim, H. D. Sohn and B. R. Jin (2006) Genetic variation in the diamondback moth, *Plutella zylostella* (Lepidoptera: Yponomeutidae) in China inferred from mitochondrial COI gene sequence. *Eur. J. Entomol.* **103**, 605-611.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu and P. Flook (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a composition of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* **87**, 651-701.

Swofford, D. L. (2002) PAUP*. Phylogenetic analysis using parsimony (*and other methods). ver. 4.10., Sinauer Associates, Sunderland, M. A.

Thompson, J. D., T. J.Gibson, F. Plewniak, F. Jeanmougin and D. G. Higgins (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Res.* **24**, 173-216.