Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Population genetic structure of the migratory rice leaf roller, *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae), inferred from the mitochondrial A+T-rich region and nuclear ITS2 sequences

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Genet. Mol. Res. 10 (1): 273-294 (2011)
Received September 9, 2010
Accepted November 10, 2010
Published February 22, 2011
DOI 10.4238/vol10-1gmr1005

ABSTRACT. The population genetics of the migratory rice leaf roller, *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae), was characterized using the maternally inherited mitochondrial A+T-rich region and bi-parentally inherited nuclear internal transcribed spacer 2 (ITS2). One hundred and eighty-seven specimens of the rice leaf roller collected from 13 Korean and Chinese localities revealed 94 A+T-rich region haplotypes, ranging in sequence length from 339 to 348 bp and 129 ITS2 sequence types, ranging from 444 to 450 bp, with maximum sequence divergences of 4.55 and 4.43%, respectively. The finding of almost no significant F_{ST}, even among Chinese and Korean localities, except for one

Chinese island population (ITS2 only), and the finding of genetic variance principally at the within-population level indicate the genetic structure characteristics of a migratory insect that is well connected among populations due to high gene flow. Detection of significant F_{ST} estimates of one offshore island population in China (Haikou) compared to most others only by ITS2 rather than by the mitochondrial A+T-rich region, as well as the somewhat higher degree of genetic differentiation seen on ITS2, suggest the importance of female dispersal. Structural analysis of the A+T-rich region revealed a poly-T stretch (10-16 bp), a microsatellite-like AT repeat (10-14 repeats), and a 5-bp long-motif "ATTTA". The typical 5-bp long conserved motif sequence (ATAGA) previously detected in other lepidopterans was found to be ATAG in the *C. medinalis* A+T-rich region.

Key words: *Cnaphalocrocis medinalis*; A+T-rich region; ITS2; Population genetics; Genetic diversity; Gene flow

INTRODUCTION

Genetic variability, the source of evolution upon which the maintenance and persistence of an organism depends, is affected by a variety of factors. These include mutation, population size, and gene flow (Amos and Harwood, 1998). Population size is of primary importance, as a reduction in population size may result in a loss of diversity due to genetic drift. This force, on the other hand, may be compensated by dispersal, via which individual populations can be interconnected to form integrated metapopulations (Hanski, 1996). On the other hand, the genetic distance among separated populations may be generated by differential selection forces specific to each population, habitat fragmentation, geographic isolation, limited gene flow, and so on (Nei and Feldman, 1972; Zhang et al., 2009). In particular, a species that occupies ephemeral habitats is likely to face extinction if it is incapable of dispersal, whereas dispersal is not expected to play as large a role in stable habitats (Denno et al., 1996).

The rice leaf roller, *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae), feeds on a broad range of graminaceous crops, but primarily on rice, and it is widely distributed throughout South and East Asia (Kisimoto, 1984). A substantial amount of research has been conducted on the dispersal of this species in Asia (Miyahara, 1981; Wada et al., 1988; Riley et al., 1995). Although an understanding of the genetic population structures of insect pests may provide us with important biological information, no such studies have yet been conducted on *C. medinalis*. Available examples include members of the same superfamily, Pyraloidea, including the European corn borer, *Ostrinia nubilalis* and the rice stem borer *Chilo suppressalis*.

It has been suggested that population genetics can be illustrated more accurately than was previously possible by analyzing both mitochondrial and nuclear markers (Ballard and Whitlock, 2004). This is because each molecule evidences its own evolutionary characteristics that allow for inferences of different aspects of population history. For example, mitochondrial DNA has an

effective population size one-quarter that of nuclear DNA, resulting in stronger effects of genetic drift, more rapid fixation, and stronger population subdivision (Birky Jr. et al., 1983). Further, inference of population structure on the basis of a single molecular marker could be either exaggerated or unrecognizable whenever dispersal is gender-biased (Birky Jr. et al., 1983).

We evaluated the population genetics of the migratory rice leaf roller, *C. medinalis*, for the first time, using the mitochondrial A+T-rich region and nuclear internal transcribed spacer 2 (ITS2). Each molecular sequence is widely regarded as a powerful tool for the study of population genetics and phylogenetic reconstruction of closely related taxa (Bravo et al., 2008; Depaquit et al., 2008). Considering that each molecule has its own evolutionary characteristics, using both molecules is expected to provide greater insight into more diverse aspects of population genetics than was previously possible (Ballard and Whitlock, 2004). In particular, by employing two molecular sequences we addressed the following questions: First, what is the magnitude of genetic diversity and gene flow among *C. medinalis* populations? Second, how is the gene flow reflected in these two molecules? Third, can any bias in dispersal be detected between the two genders in *C. medinalis*?

MATERIAL AND METHODS

Sampling

Larvae and adults of *C. medinalis* were collected from rice plants in 13 localities in Korea and China from 2008 to 2009 (Table 1; Figure 1). Each larva or adult specimen was collected at least one meter from the neighboring one, in order to cover a wide area of each collecting locality.

DNA extraction, primers, and amplification

Total DNA was extracted with a DNA purification kit (Promega, USA). In order to amplify the entirety of the mitochondrial A+T-rich region, a pair of primers was designed on the basis of the full-length mitogenome sequences of lepidopterans (Yukuhiro et al., 2002; Cameron and Whiting, 2008). The forward primer (ATF, 5'-AATAATAGGGTATCTAATCCTAG-3') and reverse primer (ATR, 5'-AATTTATCCTATCAGAATAATCC-3') are located between the 12S rRNA and $tRNA^{Ile}$ genes, respectively. The polymerase chain reactions (PCR) were conducted as follows: denaturation for 7 min at 94°C, followed by 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 55.6°C, and 1 min extension at 72°C, and a final extension for 7 min at 72°C. In order to amplify the ITS2, the primers NG02955, 5'-ATGAACATCGACATTTCGAACGCACA T-3' and AB052895, 5'-TTCTTTTCCTCCGCTTAGTAATATGCTTAA-3' (Ji et al., 2003) were used. After an initial 4-min denaturation step at 94°C, a 35-cycle amplification (95°C for 40 s, 64°C for 20 s, and 72°C for 40 s) was run. The final extension step was conducted for 2 min and 40 s at 72°C. PCR amplification was conducted using AccuPower® PCR PreMix (Bioneer, Korea) in a Biometra thermal cycler (model T-gradient Thermoblock, Germany).

The amplicons of the A+T-rich region and ITS2 were subsequently purified and cloned into pGEM-T Easy vector (Promega), and the plasmid DNA from one clone per individual was isolated with a Plasmid Miniprep Kit (Dyne Bio Inc., Korea). Both strands of the plasmid were sequenced with an ABI PRISM™ 3100 Genetic Analyzer (PE Applied Biosystems, USA).

Table 1. List of trapping localities, sample size, collecting date, animal number, sequence size, GenBank accession number, the mitochondrial A+T-rich region haplotypes and ITS2 sequence types of *Cnaphalocrocis medinalis*.

Collecting locality (sample size, collecting date)	Animal number	A+T-rich region					ITS2				
		Haplotype I*	AT content (%)	Sequence size (bp)	GenBank accession No.	Haplotype II**	Sequence type I*	GC content (%)	Sequence size (bp)	GenBank accession No.	Sequence type II**
1. Haikou, Hainan Island, China (15; 2009. 08. 15)	CM3241	CMAT01	96.0	344	HM755071	CMAT01	CMITS013	47.5	448	HM755258	CMITS009
	CM3242	CMAT06	95.9	342	HM755072	CMAT01	CMITS014	47.6	445	HM755259	CMITS010
	CM3243	CMAT11	95.9	345	HM755073	CMAT06	CMITS005	48.1	445	HM755260	CMITS004
	CM3244	CMAT01	96.0	344	HM755074	CMAT01	CMITS015	47.2	447	HM755261	CMITS011
	CM3245	CMAT01	96.0	344	HM755075	CMAT01	CMITS016	48.3	445	HM755262	CMITS012
	CM3246	CMAT01	96.0	344	HM755076	CMAT01	CMITS017	48.3	445	HM755263	CMITS013
	CM3247	CMAT12	95.6	343	HM755077	CMAT07	CMITS018	47.4	445	HM755264	CMITS014
	CM3248	CMAT13	95.6	345	HM755078	CMAT08	CMITS019	47.7	449	HM755265	CMITS015
	CM3249	CMAT05	95.9	345	HM755079	CMAT01	CMITS020	48.3	445	HM755266	CMITS016
	CM3250	CMAT14	95.6	339	HM755080	CMAT09	CMITS005	48.1	445	HM755267	CMITS004
	CM3251	CMAT01	96.0	344	HM755081	CMAT01	CMITS021	47.2	445	HM755268	CMITS017
	CM3252	CMAT04	95.9	345	HM755082	CMAT01	CMITS022	47.5	446	HM755269	CMITS018
	CM3253	CMAT01	96.0	344	HM755083	CMAT01	CMITS005	48.1	445	HM755270	CMITS004
	CM3254	CMAT15	95.9	344	HM755084	CMAT10	CMITS023	48.1	445	HM755271	CMITS019
	CM3255	CMAT01	96.0	344	HM755085	CMAT01	CMITS024	48.3	445	HM755272	CMITS020
2. Tonghai, Yunnan Province, China (15; 2009. 07. 16)	CM2903	CMAT04	95.9	345	HM755086	CMAT01	CMITS001	47.7	445	HM755273	CMITS001
	CM2904	CMAT16	95.6	345	HM755087	CMAT11	CMITS025	47.9	445	HM755274	CMITS021
	CM2905	CMAT02	95.9	343	HM755088	CMAT01	CMITS026	47.7	445	HM755275	CMITS022
	CM2906	CMAT02	95.9	343	HM755089	CMAT01	CMITS001	47.7	445	HM755276	CMITS001
	CM2907	CMAT01	96.0	344	HM755090	CMAT01	CMITS027	48.1	445	HM755277	CMITS023
	CM2908	CMAT17	95.7	344	HM755091	CMAT12	CMITS008	47.5	447	HM755278	CMITS001
	CM2909	CMAT03	95.9	342	HM755092	CMAT01	CMITS001	47.7	445	HM755279	CMITS001
	CM2910	CMAT02	95.9	343	HM755093	CMAT01	CMITS009	47.3	448	HM755280	CMITS001
	CM2911	CMAT05	95.9	345	HM755094	CMAT01	CMITS028	47.9	445	HM755281	CMITS024
	CM2912	CMAT18	95.6	342	HM755095	CMAT03	CMITS029	47.7	448	HM755282	CMITS025
	CM2913	CMAT03	95.9	342	HM755096	CMAT01	CMITS030	47.4	445	HM755283	CMITS026
	CM2914	CMAT19	95.7	344	HM755097	CMAT13	CMITS001	47.7	445	HM755284	CMITS001
	CM2915	CMAT03	95.9	342	HM755098	CMAT01	CMITS001	47.7	445	HM755285	CMITS001
	CM2916	CMAT01	96.0	344	HM755099	CMAT01	CMITS031	48.3	445	HM755286	CMITS027
	CM2917	CMAT06	95.9	342	HM755100	CMAT01	CMITS032	47.5	448	HM755287	CMITS028
3. Guangzhou, Guangdong Province, China (7; 2009. 07. 17)	CM2924	CMAT02	95.9	343	HM755101	CMAT01	CMITS002	47.9	445	HM755288	CMITS002
	CM2925	CMAT01	96.0	344	HM755102	CMAT01	CMITS033	47.4	445	HM755289	CMITS029
	CM2926	CMAT01	96.0	344	HM755103	CMAT01	CMITS002	47.9	445	HM755290	CMITS002
	CM2927	CMAT02	95.9	343	HM755104	CMAT01	CMITS001	47.7	445	HM755291	CMITS001
	CM2928	CMAT06	95.9	342	HM755105	CMAT01	CMITS001	47.7	445	HM755292	CMITS001
	CM2929	CMAT01	96.0	344	HM755106	CMAT01	CMITS006	47.8	445	HM755293	CMITS005
	CM2930	CMAT01	96.0	344	HM755107	CMAT01	CMITS006	47.8	445	HM755294	CMITS005
4. Changsha, Hunan Province, China (15; 2009. 07. 15)	CM2931	CMAT20	95.9	343	HM755108	CMAT14	CMITS034	47.6	448	HM755295	CMITS030
	CM2932	CMAT21	95.6	343	HM755109	CMAT15	CMITS001	47.7	445	HM755296	CMITS001
	CM2933	CMAT22	95.7	343	HM755110	CMAT03	CMITS035	47.8	445	HM755297	CMITS031
	CM2934	CMAT01	96.0	344	HM755111	CMAT01	CMITS036	47.9	445	HM755298	CMITS032
	CM2935	CMAT23	95.4	344	HM755112	CMAT16	CMITS037	47.9	445	HM755299	CMITS033
	CM2936	CMAT24	95.9	339	HM755113	CMAT01	CMITS001	47.7	445	HM755300	CMITS001
	CM2937	CMAT01	96.0	344	HM755114	CMAT01	CMITS038	47.1	448	HM755301	CMITS034
	CM2938	CMAT25	95.6	345	HM755115	CMAT03	CMITS039	48.0	445	HM755302	CMITS035
	CM2939	CMAT08	95.9	341	HM755116	CMAT01	CMITS001	47.7	445	HM755303	CMITS001
	CM2940	CMAT02	95.9	343	HM755117	CMAT01	CMITS040	47.4	445	HM755304	CMITS036
	CM2941	CMAT01	96.0	344	HM755118	CMAT01	CMITS041	47.7	445	HM755305	CMITS037
	CM2942	CMAT04	95.9	345	HM755119	CMAT01	CMITS042	47.4	445	HM755306	CMITS038
	CM2943	CMAT01	96.0	344	HM755120	CMAT01	CMITS043	47.9	445	HM755307	CMITS039
	CM2944	CMAT01	96.0	344	HM755121	CMAT01	CMITS044	48.1	445	HM755308	CMITS040
	CM2945	CMAT26	95.7	344	HM755122	CMAT17	CMITS045	47.7	445	HM755309	CMITS041
5. Xiaogan, Hubei Province, China (15; 2009. 07. 14)	CM2949	CMAT07	95.9	339	HM755123	CMAT01	CMITS002	47.9	445	HM755310	CMITS002
	CM2950	CMAT03	95.9	342	HM755124	CMAT01	CMITS046	47.4	447	HM755311	CMITS042
	CM2951	CMAT02	95.9	343	HM755125	CMAT01	CMITS047	47.7	445	HM755312	CMITS043
	CM2952	CMAT27	95.6	341	HM755126	CMAT18	CMITS001	47.7	445	HM755313	CMITS001
	CM2953	CMAT05	95.9	345	HM755127	CMAT01	CMITS048	47.7	445	HM755314	CMITS044
	CM2954	CMAT01	96.0	344	HM755128	CMAT01	CMITS001	47.7	445	HM755315	CMITS001
	CM2955	CMAT28	95.7	344	HM755129	CMAT04	CMITS002	47.9	445	HM755316	CMITS002

Continued on next page

Table 1. Continued.

Collecting locality (sample size, collecting date)	Animal number	A+T-rich region					ITS2				
		Haplotype I*	AT content (%)	Sequence size (bp)	GenBank accession No.	Haplotype II**	Sequence type I*	GC content (%)	Sequence size (bp)	GenBank accession No.	Sequence type II**
	CM2956	CMAT02	95.9	343	HM755130	CMAT01	CMITS001	47.7	445	HM755317	CMITS001
	CM2957	CMAT02	95.9	343	HM755131	CMAT01	CMITS049	47.6	445	HM755318	CMITS045
	CM2958	CMAT02	95.9	343	HM755132	CMAT01	CMITS050	48.3	445	HM755319	CMITS046
	CM2959	CMAT29	95.7	344	HM755133	CMAT05	CMITS051	48.1	445	HM755320	CMITS047
	CM2960	CMAT30	95.9	342	HM755134	CMAT19	CMITS052	47.7	447	HM755321	CMITS048
	CM2961	CMAT31	95.7	344	HM755135	CMAT20	CMITS053	47.7	447	HM755322	CMITS049
	CM2962	CMAT32	95.7	344	HM755136	CMAT21	CMITS001	47.7	445	HM755323	CMITS001
	CM2963	CMAT33	95.6	342	HM755137	CMAT04	CMITS054	48.1	445	HM755324	CMITS050
6. Hangzhou,	CM2980	CMAT34	95.9	341	HM755138	CMAT22	CMITS055	47.2	447	HM755325	CMITS051
Zhejiang Province,	CM2981	CMAT35	95.9	345	HM755139	CMAT01	CMITS056	47.6	445	HM755326	CMITS052
China	CM2982	CMAT36	95.6	345	HM755140	CMAT23	CMITS001	47.7	445	HM755327	CMITS001
(15; 2009. 07. 25)	CM2983	CMAT37	95.7	346	HM755141	CMAT24	CMITS057	47.2	447	HM755328	CMITS053
	CM2984	CMAT02	95.9	343	HM755142	CMAT01	CMITS004	47.7	447	HM755329	CMITS002
	CM2985	CMAT38	95.7	344	HM755143	CMAT25	CMITS001	47.7	445	HM755330	CMITS001
	CM2986	CMAT02	95.9	343	HM755144	CMAT01	CMITS058	47.9	445	HM755331	CMITS054
	CM2987	CMAT39	95.9	344	HM755145	CMAT26	CMITS059	48.1	445	HM755332	CMITS055
	CM2988	CMAT40	96.0	344	HM755146	CMAT27	CMITS060	47.4	445	HM755333	CMITS056
	CM2989	CMAT41	95.9	343	HM755147	CMAT01	CMITS061	47.4	445	HM755334	CMITS057
	CM2990	CMAT01	96.0	344	HM755148	CMAT01	CMITS001	47.7	445	HM755335	CMITS001
	CM2991	CMAT08	95.9	341	HM755149	CMAT01	CMITS062	47.7	445	HM755336	CMITS058
	CM2992	CMAT01	96.0	344	HM755150	CMAT01	CMITS063	47.9	447	HM755337	CMITS059
	CM2993	CMAT01	96.0	344	HM755151	CMAT01	CMITS064	47.5	448	HM755338	CMITS060
	CM2994	CMAT01	96.0	344	HM755152	CMAT01	CMITS001	47.7	445	HM755339	CMITS001
7. Naju,	CM3001	CMAT05	95.9	345	HM755153	CMAT01	CMITS009	47.3	448	HM755340	CMITS001
Chonnam	CM3002	CMAT03	95.9	342	HM755154	CMAT01	CMITS065	47.4	445	HM755341	CMITS061
Province, Korea	CM3003	CMAT03	95.9	342	HM755155	CMAT01	CMITS066	47.9	445	HM755342	CMITS062
(15, 2008. 09. 26)	CM3004	CMAT42	95.7	343	HM755156	CMAT28	CMITS010	47.9	445	HM755343	CMITS006
	CM3005	CMAT43	95.3	345	HM755157	CMAT02	CMITS010	47.9	445	HM755344	CMITS006
	CM3006	CMAT01	96.0	344	HM755158	CMAT01	CMITS003	47.7	445	HM755345	CMITS003
	CM3007	CMAT02	95.9	343	HM755159	CMAT01	CMITS067	47.4	445	HM755346	CMITS063
	CM3008	CMAT01	96.0	344	HM755160	CMAT01	CMITS068	47.9	445	HM755347	CMITS008
	CM3009	CMAT44	95.3	342	HM755161	CMAT29	CMITS069	47.7	448	HM755348	CMITS064
	CM3010	CMAT45	95.9	342	HM755162	CMAT01	CMITS070	47.6	445	HM755349	CMITS065
	CM3011	CMAT01	96.0	344	HM755163	CMAT01	CMITS001	47.7	445	HM755350	CMITS001
	CM3012	CMAT07	95.9	339	HM755164	CMAT01	CMITS001	47.7	445	HM755351	CMITS001
	CM3013	CMAT01	96.0	344	HM755165	CMAT01	CMITS003	47.7	445	HM755352	CMITS003
	CM3014	CMAT01	96.0	344	HM755166	CMAT01	CMITS002	47.9	445	HM755353	CMITS002
	CM3015	CMAT01	96.0	344	HM755167	CMAT01	CMITS011	47.5	447	HM755354	CMITS003
8. Chongju,	CM3020	CMAT04	95.9	345	HM755168	CMAT01	CMITS071	47.9	447	HM755355	CMITS066
Chongbuk	CM3021	CMAT01	96.0	344	HM755169	CMAT01	CMITS072	48.5	445	HM755356	CMITS067
Province, Korea	CM3022	CMAT01	96.0	344	HM755170	CMAT01	CMITS073	47.4	445	HM755357	CMITS068
(15; 2009. 08. 26)	CM3023	CMAT46	95.7	344	HM755171	CMAT30	CMITS074	47.9	447	HM755358	CMITS069
	CM3024	CMAT47	96.0	344	HM755172	CMAT31	CMITS075	47.5	446	HM755359	CMITS003
	CM3025	CMAT48	95.6	348	HM755173	CMAT01	CMITS007	47.3	448	HM755360	CMITS003
	CM3026	CMAT49	95.7	344	HM755174	CMAT32	CMITS004	47.7	447	HM755361	CMITS002
	CM3027	CMAT50	95.9	345	HM755175	CMAT33	CMITS076	47.2	447	HM755362	CMIT070
	CM3028	CMAT01	96.0	344	HM755176	CMAT01	CMITS077	47.3	448	HM755363	CMITS071
	CM3029	CMAT51	95.6	343	HM755177	CMAT04	CMITS006	47.8	445	HM755364	CMITS005
	CM3030	CMAT01	96.0	344	HM755178	CMAT01	CMITS078	47.7	447	HM755365	CMITS072
	CM3031	CMAT01	96.0	344	HM755179	CMAT01	CMITS079	47.4	445	HM755366	CMIT073
	CM3032	CMAT52	95.7	343	HM755180	CMAT34	CMITS003	47.7	445	HM755367	CMITS003
	CM3033	CMAT53	95.9	343	HM755181	CMAT35	CMITS080	47.9	447	HM755368	CMITS074
	CM3034	CMAT54	95.7	343	HM755182	CMAT36	CMITS081	47.9	444	HM755369	CMITS075
9. Andong,	CM3127	CMAT03	95.9	342	HM755183	CMAT01	CMITS082	47.9	445	HM755370	CMITS076
Kyongbuk	CM3128	CMAT01	96.0	344	HM755184	CMAT01	CMITS083	48.1	447	HM755371	CMITS077
Province, Korea	CM3129	CMAT55	95.9	342	HM755185	CMAT01	CMITS004	47.7	447	HM755372	CMITS002
(15; 2009. 09. 05)	CM3130	CMAT56	96.0	344	HM755186	CMAT37	CMITS084	47.9	445	HM755373	CMITS078
	CM3131	CMAT04	95.9	345	HM755187	CMAT01	CMITS012	47.9	445	HM755374	CMITS007
	CM3132	CMAT01	96.0	344	HM755188	CMAT01	CMITS085	48.5	445	HM755375	CMITS079
	CM3133	CMAT09	96.0	346	HM755189	CMAT01	CMITS086	48.1	445	HM755376	CMITS080
	CM3134	CMAT04	95.9	345	HM755190	CMAT01	CMITS087	47.8	445	HM755377	CMITS081
	CM3135	CMAT01	96.0	344	HM755191	CMAT01	CMITS001	47.7	445	HM755378	CMITS001
	CM3136	CMAT57	95.9	340	HM755192	CMAT01	CMITS001	47.7	445	HM755379	CMITS001
	CM3137	CMAT58	95.6	341	HM755193	CMAT38	CMITS088	47.4	447	HM755380	CMITS082
	CM3138	CMAT01	96.0	344	HM755194	CMAT01	CMITS001	47.7	445	HM755381	CMITS001
	CM3139	CMAT01	96.0	344	HM755195	CMAT01	CMITS089	47.5	447	HM755382	CMITS083

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Table 1. Continued.

Collecting locality (sample size, collecting date)	Animal number	A+T-rich region					ITS2				
		Haplotype I*	AT content (%)	Sequence size (bp)	GenBank accession No.	Haplotype II**	Sequence type I*	GC content (%)	Sequence size (bp)	GenBank accession No.	Sequence type II**
	CM3140	CMAT10	94.0	341	HM755196	CMAT01	CMITS090	47.5	447	HM755383	CMITS084
	CM3141	CMAT59	96.0	344	HM755197	CMAT39	CMITS001	47.7	445	HM755384	CMITS001
	CM3090	CMAT04	95.9	345	HM755198	CMAT01	CMITS091	47.2	447	HM755385	CMITS085
10. Yesan,	CM3091	CMAT06	95.9	342	HM755199	CMAT01	CMITS001	47.7	445	HM755386	CMITS001
Chungnam	CM3092	CMAT60	95.7	344	HM755200	CMAT02	CMITS092	48.1	445	HM755387	CMITS086
Province, Korea	CM3093	CMAT61	95.7	344	HM755201	CMAT40	CMITS093	48.3	445	HM755388	CMITS087
(15; 2009. 08. 29)	CM3094	CMAT01	96.0	344	HM755202	CMAT01	CMITS094	47.9	444	HM755389	CMITS008
	CM3095	CMAT03	95.9	342	HM755203	CMAT01	CMITS001	47.7	445	HM755390	CMITS001
	CM3096	CMAT01	96.0	344	HM755204	CMAT01	CMITS095	48.3	445	HM755391	CMITS088
	CM3097	CMAT62	95.6	341	HM755205	CMAT02	CMITS003	47.7	445	HM755392	CMITS003
	CM3098	CMAT01	96.0	344	HM755206	CMAT01	CMITS007	47.3	448	HM755393	CMITS003
	CM3099	CMAT63	95.7	344	HM755207	CMAT41	CMITS001	47.7	445	HM755394	CMITS001
	CM3100	CMAT64	95.9	343	HM755208	CMAT01	CMITS096	48.1	445	HM755395	CMITS089
	CM3101	CMAT65	95.9	345	HM755209	CMAT01	CMITS097	47.8	445	HM755396	CMITS090
	CM3102	CMAT66	95.9	345	HM755210	CMAT42	CMITS098	48.1	445	HM755397	CMITS091
	CM3103	CMAT01	96.0	344	HM755211	CMAT01	CMITS099	47.7	447	HM755398	CMITS092
	CM3104	CMAT01	96.0	344	HM755212	CMAT01	CMITS008	47.5	447	HM755399	CMITS001
11. Geosan,	CM3042	CMAT67	95.3	340	HM755213	CMAT43	CMITS100	47.9	445	HM755400	CMITS093
Chungbuk	CM3043	CMAT06	95.9	342	HM755214	CMAT01	CMITS101	48.1	445	HM755401	CMITS094
Province, Korea	CM3044	CMAT68	95.3	341	HM755215	CMAT03	CMITS001	47.7	445	HM755402	CMITS001
(15; 2009. 08. 27)	CM3045	CMAT01	96.0	344	HM755216	CMAT01	CMITS003	47.7	445	HM755403	CMITS003
	CM3046	CMAT01	96.0	344	HM755217	CMAT01	CMITS102	47.3	444	HM755404	CMITS095
	CM3047	CMAT09	96.0	346	HM755218	CMAT01	CMITS001	47.7	445	HM755405	CMITS001
	CM3048	CMAT69	95.6	343	HM755219	CMAT44	CMITS001	47.7	445	HM755406	CMITS001
	CM3049	CMAT10	95.9	341	HM755220	CMAT01	CMITS103	47.7	445	HM755407	CMITS096
	CM3050	CMAT70	95.6	343	HM755221	CMAT45	CMITS104	47.4	445	HM755408	CMITS097
	CM3051	CMAT71	95.9	345	HM755222	CMAT46	CMITS001	47.7	445	HM755409	CMITS001
	CM3052	CMAT72	95.9	342	HM755223	CMAT47	CMITS105	47.9	445	HM755410	CMITS098
	CM3053	CMAT06	95.9	342	HM755224	CMAT01	CMITS106	47.9	447	HM755411	CMITS002
	CM3054	CMAT73	95.1	343	HM755225	CMAT48	CMITS005	48.1	445	HM755412	CMITS005
	CM3055	CMAT74	95.6	342	HM755226	CMAT02	CMITS107	48.0	448	HM755413	CMITS099
	CM3056	CMAT75	95.6	343	HM755227	CMAT02	CMITS108	47.4	445	HM755414	CMITS100
12. Suwon,	CM3069	CMAT76	95.9	340	HM755228	CMAT01	CMITS001	47.7	445	HM755415	CMITS001
Kyonggi	CM3070	CMAT09	96.0	346	HM755229	CMAT01	CMITS109	47.7	448	HM755416	CMITS101
Province, Korea	CM3071	CMAT01	96.0	344	HM755230	CMAT01	CMITS110	47.7	444	HM755417	CMITS102
(15; 2009. 08. 28)	CM3072	CMAT77	95.7	347	HM755231	CMAT05	CMITS111	47.6	445	HM755418	CMITS103
	CM3073	CMAT09	96.0	346	HM755232	CMAT01	CMITS112	47.4	445	HM755419	CMITS104
	CM3074	CMAT06	95.9	342	HM755233	CMAT01	CMITS113	47.7	445	HM755420	CMITS105
	CM3075	CMAT02	95.9	343	HM755234	CMAT01	CMITS114	47.9	445	HM755421	CMITS106
	CM3076	CMAT02	95.9	343	HM755235	CMAT01	CMITS115	47.6	445	HM755422	CMITS107
	CM3077	CMAT78	95.7	344	HM755236	CMAT49	CMITS001	47.7	445	HM755423	CMITS001
	CM3078	CMAT04	95.9	345	HM755237	CMAT01	CMITS001	47.7	445	HM755424	CMITS001
	CM3079	CMAT09	96.0	346	HM755238	CMAT01	CMITS116	47.9	445	HM755425	CMITS108
	CM3080	CMAT04	95.9	345	HM755239	CMAT01	CMITS004	47.7	447	HM755426	CMITS002
	CM3081	CMAT79	95.9	344	HM755240	CMAT50	CMITS117	47.5	448	HM755427	CMITS003
	CM3082	CMAT80	95.9	341	HM755241	CMAT01	CMITS118	47.7	445	HM755428	CMITS109
	CM3083	CMAT81	95.9	341	HM755242	CMAT01	CMITS119	47.9	445	HM755429	CMITS110
13. Kangreung,	CM3150	CMAT01	96.0	344	HM755243	CMAT01	CMITS002	47.9	445	HM755430	CMITS002
Kangwon	CM3151	CMAT82	95.7	344	HM755244	CMAT51	CMITS007	47.3	448	HM755431	CMITS003
Province, Korea	CM3152	CMAT83	95.7	344	HM755245	CMAT52	CMITS120	47.7	447	HM755432	CMITS111
(15; 2009. 09. 05)	CM3153	CMAT01	96.0	344	HM755246	CMAT01	CMITS121	47.5	447	HM755433	CMITS003
	CM3154	CMAT84	95.7	344	HM755247	CMAT53	CMITS122	47.1	448	HM755434	CMITS112
	CM3155	CMAT85	95.6	345	HM755248	CMAT54	CMITS123	47.9	445	HM755435	CMITS113
	CM3156	CMAT86	95.7	343	HM755249	CMAT55	CMITS124	47.9	445	HM755436	CMITS114
	CM3157	CMAT87	95.6	342	HM755250	CMAT56	CMITS012	47.9	445	HM755437	CMITS007
	CM3158	CMAT88	95.3	340	HM755251	CMAT57	CMITS011	47.5	447	HM755438	CMITS003
	CM3159	CMAT89	95.9	343	HM755252	CMAT58	CMITS125	47.5	448	HM755439	CMITS115
	CM3160	CMAT90	95.9	343	HM755253	CMAT01	CMITS001	47.7	445	HM755440	CMITS001
	CM3161	CMAT91	95.7	344	HM755254	CMAT59	CMITS126	47.7	445	HM755441	CMITS116
	CM3162	CMAT92	95.6	344	HM755255	CMAT60	CMITS127	47.4	445	HM755442	CMITS117
	CM3163	CMAT93	95.7	343	HM755256	CMAT61	CMITS128	47.2	447	HM755443	CMITS118
	CM3164	CMAT94	95.7	344	HM755257	CMAT62	CMITS129	47.1	445	HM755444	CMITS119

*Haplotype or sequence type I: sequences obtained before the Gblocks analysis (Castresana, 2000). **Haplotype or sequence type II: sequences obtained after the Gblocks analysis (Castresana, 2000), which removed non-conserved blocks in the alignment.

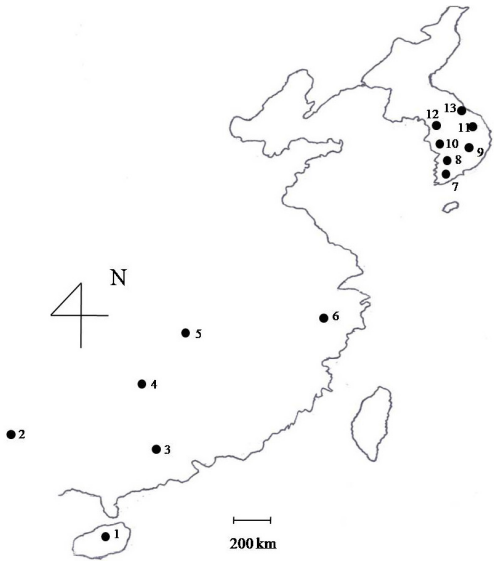

Figure 1. Sampling locations of *Cnaphalocrocis medinalis* in Korea and China. General locality names are as follows: 1. Haikou, Hainan Province, China; 2. Tonghai, Yunnan Province, China; 3. Guangzhou, Guangdong Province, China; 4. Changsha, Hunan Province, China; 5. Xiaogan, Hubei Province, China; 6. Hangzhou, Zhejiang Province, China; 7. Naju, Chonnam Province, Korea; 8. Chongju, Chongbuk Province, Korea; 9. Andong, Kyongbuk Province, Korea; 10. Yesan, Chungnam Province, Korea; 11. Geosan, Chungbuk Province, Korea; 12. Suwon, Kyonggi Province, Korea; 13. Kangreung, Kangwon Province, Korea.

Sequence analysis

The entire A+T-rich region was delimited by aligning the sequences with the full-length mitogenome sequences of lepidopterans (Yukuhiro et al., 2002; Cameron and Whiting, 2008), whereas the entire ITS2 was delimited via HMM-based ITS2 annotation (Keller et al., 2009). All A+T-rich region and ITS2 sequence alignments were conducted using MAFFT ver. 6 (Katoh et al., 2002), with the gap opening penalty set to 1.53 and the offset value (≈ gap extension penalty) set to 0.5. When the homologous sequences from two individuals differed by ≥1 nucleotide base or one insertion/deletion (indel) position, the sequences were regarded as different haplotypes (the A+T-rich region) or sequence types (ITS2). Haplotype or sequence type designations were applied to new sequences as they were discovered (i.e., CMAT01, CMAT02, and so on for the A+T-rich region; CMITS001, CMITS002, and so on for ITS2). Finally, 94 haplotypes from the A+T-rich region and 129 sequence types from the ITS2 region were acquired and deposited in the GenBank database under the accession Nos. HM755071-HM755257 for the A+T-rich region and HM755258-HM755444 for ITS2, respectively (Table 1).

In order to more accurately align the A+T-rich and ITS2 region sequences for the subsequent phylogenetic and population level analyses, the Gblocks (ver. 0.91b) software (Castresana, 2000) was employed for the selection of conserved regions. The alignment option, "allow the smaller final blocks", was used for both the A+T-rich region and ITS2; as a result, the following parameters were used: minimum number of sequences for a conserved position = 45 (A+T-rich region) and 65 (ITS2), minimum number of sequences for a flanking position = 74 (A+T-rich

region) and 109 (ITS2), maximum number of contiguous nonconserved positions = 8, minimum length of a block = 5, and gap positions = zero. After the Gblocks analysis, all indels were eventually removed. Finally, 62 new A+T-rich region haplotypes with 312 bp from 94 haplotypes and 119 new ITS2 sequence types with 439 bp from 129 ITS2 sequences were obtained.

Phylogenetic analysis

To determine the relationships among the 62 A+T-rich region haplotypes and 119 ITS2 sequence types acquired from the 187 individuals of *C. medinalis* and to detect any groups, phylogenetic analysis was conducted using the PAUP ver. 4.0b10 software (Swofford, 2002) via the maximum-parsimony method (Fitch, 1971), using a heuristic search strategy starting with stepwise addition trees replicated 100 times, and a random input order of sequences to generate the initial tree for each replicate. The robustness of maximum-parsimony topologies was assessed via bootstrapping with 1000 replicates (tree-bisection-reconnection heuristic search) of 100 random stepwise addition replicates each, for all analyses. The A+T-rich region and ITS2 of the within-family species *Locastra muscosalis* and *Teliphasa elegans* were utilized as outgroups to root the trees.

Estimates of population characteristics

Haplotype diversity and nucleotide diversity, both of which are reflective of genetic diversity within each locality, were determined using the Arlequin ver. 3.0 software (Excoffier et al., 2005). Maximum sequence divergence within each locality was estimated via extraction of the within-locality estimates of unrooted pairwise distances in PAUP ver. 4.0b (Swofford, 2002).

The genetic distance was estimated using the Arlequin ver. 3.0 software (Excoffier et al., 2005). Population pairwise genetic distance (F_{ST}) and a permutation test of the significant differentiation of the pairs of localities (1000 bootstraps) were evaluated in accordance with the approach described by Excoffier et al. (1992), wherein the distance between DNA sequences was calculated via the Tamura method (Tamura, 1992), which is an extension of the Kimura 2-parameters method (Kimura, 1980), allowing for unequal nucleotide frequencies. This option computes transition-transversion ratios, as well as the overall nucleotide frequencies from the original data. Pairwise N_m (the product of the effective population size, N_e, and migration rate, m) values were employed to estimate the pairwise F_{ST} based on the equilibrium relationship: $F_{ST} = 1 / (2N_m + 1)$ (1) for the A+T-rich region and $F_{ST} = 1 / (4N_m + 1)$ (2) for ITS2.

In order to determine whether any isolation-by-distance (IBD) effect has occurred in *C. medinalis* populations, matrices of genetic distance data [$F_{ST} / (1 - F_{ST})$] and the logarithms of geographical distance data (ln km) among the Chinese population and Korean population sampling sites were respectively constructed. These matrices were then analyzed to determine their degree of correlation via a Mantel test, with significance tests conducted over 10,000 randomizations (Mantel, 1967). The analysis was conducted using the isolation-by-distance software package, with the negative genetic distance set to zero (Bohonak, 2002).

Hierarchical analysis

In order to determine the degree of hierarchical subdivision between specified sets of lo-

calities (i.e., Chinese localities and Korean localities), analysis of molecular variance (AMOVA) was also performed using Arlequin ver. 3.0 (Excoffier et al., 2005). A hierarchical analysis of variance was conducted to partition total variance into variance components attributable to inter-population differences. Variance components were then employed to compute the fixation indices, and their significance was assessed by 1000 permutations, as described by Excoffier et al. (1992).

RESULTS

Sequence analysis

Ninety-four mitochondrial A+T-rich region haplotypes (CMAT01-CMAT94), ranging in size from 339 to 348 bp, were obtained from 187 *C. medinalis* specimens. The average A+T content of the 94 haplotypes was 95.6%, with a range of 95.1-96.0% (Table 1), suggesting very strong A+T bias; this is consistent with what has been found for other lepidopterans (Cameron and Whiting, 2008). The sequence alignment of 94 haplotypes revealed 112 variable sites, accounting for 31.82% of the A+T-rich region sequences; this total includes 33 sites of mono- or di-nucleotide insertions/deletions (indels) and 79 nucleotide substitution sites. Uncorrected pairwise distance among 94 haplotypes evidenced a maximum sequence divergence of 4.55%, with 16 variable positions. After the Gblocks analysis, which only selects conserved blocks, 62 A+T-rich region haplotypes were newly obtained, and the length was truncated to 312 bp (Table 1). The sequence alignment of these newly generated haplotypes evidenced 79 nucleotide substitution sites, with no indels. Uncorrected pairwise distance among the new 62 haplotypes evidenced a maximum sequence divergence of 1.60%, with five variable positions. We conducted a preliminary sequencing of the 658 bp of the mitochondrial COI genes corresponding to the "DNA Barcode" region, using the primers LepF (5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') (Hajibabaei et al., 2006) from 20 samples collected from each Korean (Naju, locality 7) and Chinese population (Xiaogan, locality 5). However, all sequence analyses of the samples revealed identical sequences. Thus, the A+T-rich region of *C. medinalis* exhibited substantially higher sequence variation than the COI gene.

In the case of ITS2, 129 sequence types were obtained via the sequencing of ITS2 rDNA from the 187 *C. medinalis* specimens (Table 1). The 129 ITS2 sequences of *C. medinalis* ranged in size from 444 to 450 bp, and they harbored an average G+C content of 47.8%, with a range from 47.1 to 48.5%. Sequence alignment revealed 165 variable sites, consisting of 10 sites of mono- or dinucleotide indels and 155 sites of nucleotide substitutions. Uncorrected pairwise distance among 129 sequence types (CMITS001-CMITS129) evidenced a maximum divergence of 4.43%, with 20 variable sites. Following the Gblocks analysis, the sequence length and the number of sequence types were reduced to 439 bp and 119, respectively, with a maximum sequence divergence of 3.19%, containing 14 variable positions.

Analysis of A+T-rich region structure

Substantial efforts have been made to understand the role of the A+T-rich region, but only limited success has been achieved thus far. Some of the progress made to date has involved the A+T-rich region for the replication origin for both mtDNA strands in *Drosophila* species (Fauron and Wolstenholme, 1980; Saito et al., 2005) and for minor-strand mtDNA

only in a few insect species, including the lepidopteran *Bombyx mori* (Saito et al., 2005). Other sources of information were consulted to look for conserved sequences associated with the regulatory role of the A+T-rich region; such signals are known to be engraved on the nucleotide sequences of the region, in the form of structural elements. These have included descriptions of the sequence region from the orthopterans *Schistocerca gregaria* and *Chorthippus parallelus* (Zhang et al., 1995), and partially from the lepidopteran *Parnassius bremeri* (Kim et al., 2009). Therefore, we searched such conserved sequences using a representative of the *C. medinalis* haplotype (CMAT01) that was detected in all populations, along with two additional haplotypes (Figure 2). The very obvious structural element detected was the poly-T stretch, which was expected to be present at the 5'-end (upstream of srRNA) of the A+T-rich region (Saito et al., 2005). This poly-T stretch has been suggested to be composed of a minimum of 10 thymidine nucleotides, to function as a possible recognition site for the initiation of replication of the major-strand mtDNA (Saito et al., 2005). In the haplotype CMAT01, one poly-T stretch of 13 bp was detected in the beginning region, starting at nucleotide position 28, as 5'-TTTTTTTTTTTTT-3' (Figure 2). In other haplotypes, the lengths of the poly-T stretches range from 10-16 bp. Besides the poly-T stretch, a 4-bp long conserved motif sequence, 5'-ATAG-3', was detected at nucleotide position 24 (Figure 2). This sequence has been detected in the A+T-rich region of all sequenced lepidopterans, but in most cases, the 5-bp long motif sequence 5'-ATAGA-3' was identified. This motif has also been suggested to perform some regulatory role together with the poly-T stretch (Kim et al., 2009).

```
                                    Motif 1  Poly-T stretch
CMAT01  ATTTATATATAGTAATTTTTAATATAGTTTTTTTTTTTTTTATATATTAAAATATTTATTA  60
CMAT02  ATTTATATATAGTAATTTTTAATATAGTTTTTTTTTTTTT-ATATATTAAAATATTTATTA  59
CMAT03  ATTTATATATAGTAATTTTTAATATAGTTTTTTTTTTT--ATATATTAAAATATTTATTA  58
        ************************************** ********************

CMAT01  TAAATTATTAAATTTTAAATATTTTCTTTTTCTTTCTTATTTATAATATTAATTTTAAAA  120
CMAT02  TAAATTATTAAATTTTAAATATTTTCTTTTTCTTTCTTATTTATAATATTAATTTTAAAA  119
CMAT03  TAAATTATTAAATTTTAAATATTTTCTTTTTCTTTCTTATTTATAATATTAATTTTAAAA  118
        ************************************************************

CMAT01  ATTAATATAAATTATAAATCAATTTATAATCATTGAAATATTTAATTAATTATAAAAATT  180
CMAT02  ATTAATATAAATTATAAATCAATTTATAATCATTGAAATATTTAATTAATTATAAAAATT  179
CMAT03  ATTAATATAAATTATAAATCAATTTATAATCATTGAAATATTTAATTAATTATAAAAATT  178
        ************************************************************

CMAT01  ATTAATTATTTTAATACCTATTTTTTAATATATTTTTTATTTTAATTTAATAATAAATTT  240
CMAT02  ATTAATTATTTTAATACCTATTTTTTAATATATTTTTTATTTTAATTTAATAATAAATTT  239
CMAT03  ATTAATTATTTTAATACCTATTTTTTAATATATTTTTTATTTTAATTTAATAATAAATTT  238
        ************************************************************
                                                Motif 2  Microsatellite-like A/T repeats
CMAT01  TTATAATATTATTTAATTTATATAAATTAAATATTTAATATATATATATATATATATAT  301
CMAT02  TTATAATATTATTTAATTTATATAAATTAAATATTTAATATATATATATATATATATAT  300
CMAT03  TTATAATATTATTTAATTTATATAAATTAAATATTTAATATATATATATATATATATAT  299
        ************************************************************

CMAT01  AACCATTCTTAATTTTTTTTCATTAAATAATATAAAATAATAA  344
CMAT02  AACCATTCTTAATTTTTTTTCATTAAATAATATAAAATAATAA  343
CMAT03  AACCATTCTTAATTTTTTTTCATTAAATAATATAAAATAATAA  342
        *********************************************
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Figure 2. Sequence alignment of three haplotypes of the *Cnaphalocrocis medinalis* A+T-rich region. Asterisks indicate identical nucleotides among the three haplotypes. One stretch of the poly-T, microsatellite-like AT repeats, and two conserved motif sequences are indicated by boxes in the sequence. The sequences are listed in the 5' to 3' direction.

Another element, referred to as the microsatellite-like AT repeat stretch, which was supposed to be preceded by the ATTTA motif detected in most other sequenced lepidopteran A+T-rich regions (Cameron and Whiting, 2008), was apparent in the *C. medinalis* A+T-rich region. One region harbored such stretches, which differed in length and core repeats among haplotypes, but was composed principally of the $(AT)_n$ sequence (Figure 2). Such tandem repetition in the A+T-rich region has been suggested to be the relevant site of the origin of DNA replication and the formation of the stem-and-loop structure, which is important in the initiation of replication (Zhang et al., 1995).

Phylogenetic analysis

In order to evaluate the relevant phylogenetic relationships and to detect any discernable groups in connection with geographic distribution, phylogenetic analysis was conducted via the maximum-parsimony method. The analysis based on the 62 A+T-rich region haplotypes revealed a major body of unresolved haplotypes, but only a few haplotypes were clustered in the four phylogenetic groups that were weakly supported by bootstrap analysis (<65%, Figure 2). Each of these groups was composed of a maximum of two haplotypes originating from different localities, respectively. For example, one group that was composed of haplotypes CMAT11 and CMAT47 was corroborated by a bootstrap support of 65%, and these haplotypes originated from localities 2 and 11, respectively (Figure 3), which were separated by a linear stretch of at least 2800 km (Figure 1). A similar clustering pattern can also be detected in other groups. Thus, the overall results of phylogenetic analysis indicate a random association of haplotypes, independent of geographic origin, possibly implying substantial gene flow in this species.

According to the phylogenetic results obtained from the ITS2 sequence types, only one discernable group (61%) was detected among the 119 analyzed sequence types (Figure 4). This group was composed of two sequence types obtained from localities 2 and 9, which are also quite distant from one another (Figure 4). Overall, the phylogenetic results obtained from the ITS2 sequence types were quite similar to those obtained from the A+T-rich region.

Genetic diversity, genetic distance, and gene flow

Within-locality diversity was estimated in terms of haplotype or sequence type diversity (H), maximum sequence divergence, mean number of pairwise differences, and nucleotide diversity (π) (Table 2). With regard to the H values, the highest estimate in the A+T-rich region was observed in Kangreung (locality 13) at 0.9714, whereas in ITS2, the H values in Haikou (locality 1), Changsha (locality 4), Chongju (locality 8), Suwon (locality 12), and Kangreung (locality 13) were the same as detected in the A+T-rich regions. The lowest H in the A+T-rich region was detected in the Guangzhou (locality 3) population as 0.0000, whereas that of ITS2 was detected in the Tonghai population (locality 2) as 0.8000. In terms of π, the lowest and highest estimates in the A+T-rich region were detected in Guangzhou (locality 3) and Kangreung (locality 13) as 0.0000 and 0.0073, respectively. In ITS2, however, they were found in Hangzhou (locality 6) and Xiaogan (locality 5), at 0.0054 and 0.0097, respectively. These results demonstrate that the populations with the extreme estimates frequently evidence substantial differences between the two molecules. This may reflect population dynamics related to the organism's behavior, population history, or different evolutionary modes of each molecule.

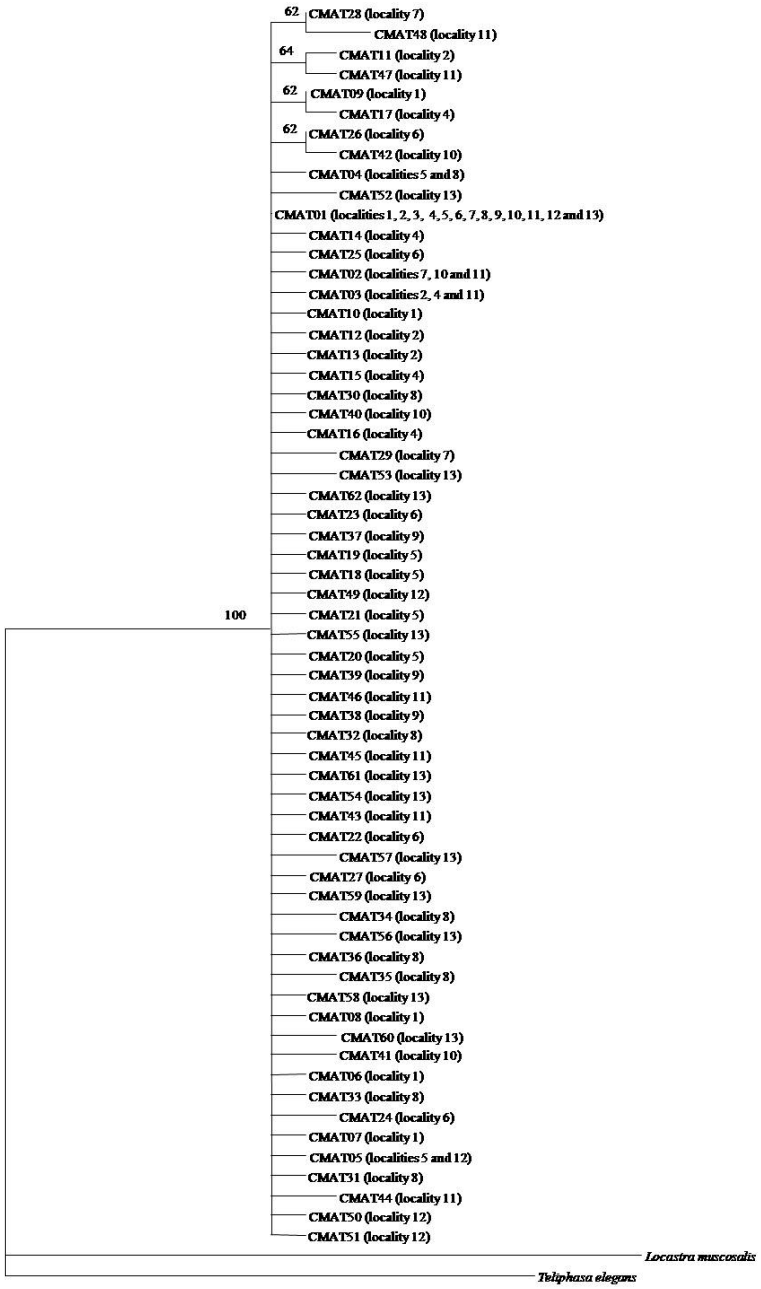

Figure 3. Phylogenetic analysis of 62 haplotypes of the *Cnaphalocrocis medinalis* A+T-rich region. The tree was acquired via the MP method incorporated in the PAUP ver. 4.0b10 software (Swofford, 2002). The tree length is 178 steps, the consistency index is 0.949, and retention index is 0.839. *Locastra muscosalis* and *Teliphasa elegans* belonging to the same family were incorporated in the analysis in order to root the tree. The numbers on the branches represent bootstrap values of 1000 replications.

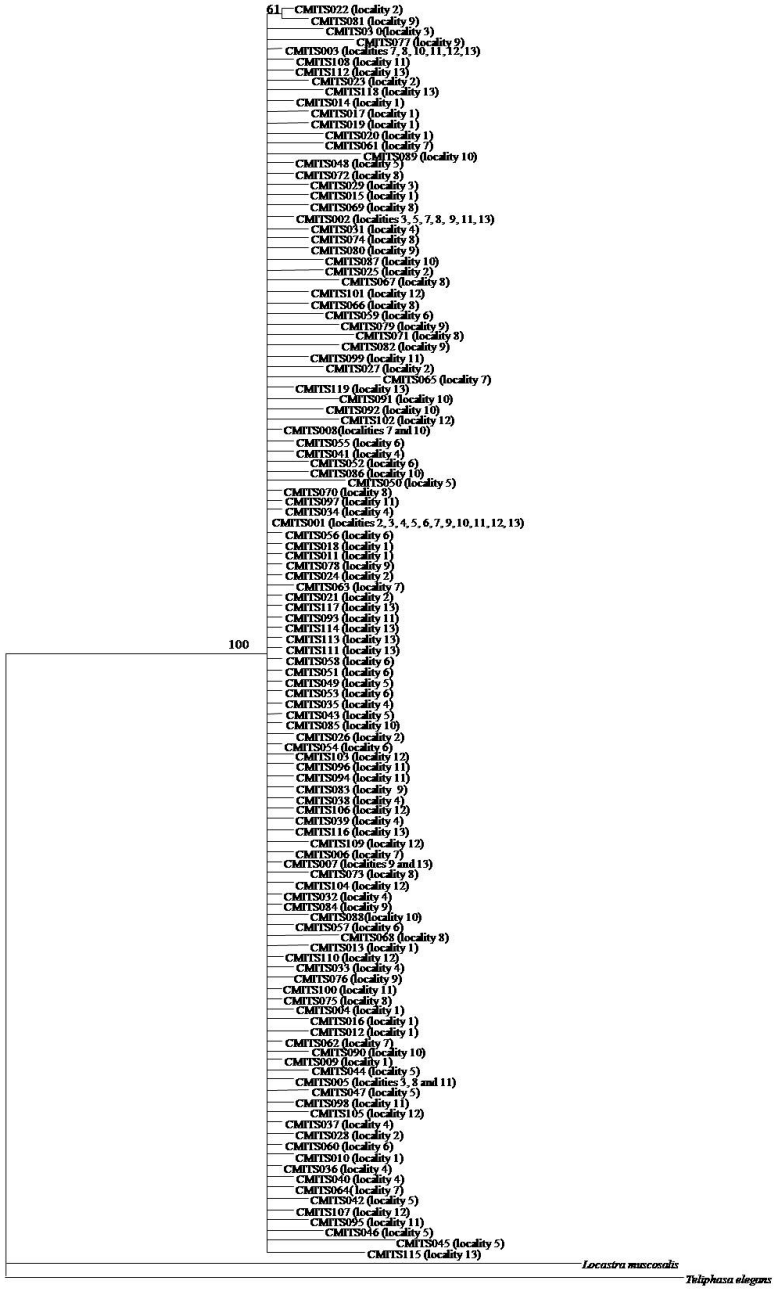

Figure 4. Phylogenetic analysis of 119 sequence types of *Cnaphalocrocis medinalis* ITS2. The tree was acquired via the MP method incorporated in the PAUP ver. 4.0b10 software (Swofford, 2002). The tree length is 533 steps, the consistency index is 0.689, and the retention index is 0.311. *Locastra muscosalis* and *Teliphasa elegans* belonging to the same family were incorporated in the analysis in order to root the tree. The numbers on the branches represent bootstrap values of 1000 replications.

Table 2. Within-locality diversity estimates of *Cnaphalocrocis medinalis* A+T-rich region and ITS2.

	SS	NH		H		NP		MSD (%)		MPD		π	
		A+T	ITS2	A+T	ITS2	A+T	ITS2	A+T	ITS2	A+T	ITS2	A+T	ITS2
1. Haikou	15	6	13	0.5714	0.9714	5	19	1.4097	2.6246	0.6667	4.0000	0.0021	0.0091
2. Tonghai	15	5	9	0.4762	0.8000	5	15	1.3237	2.4327	0.6667	2.4000	0.0021	0.0055
3. Guangzhou	7	1	4	0.0000	0.8571	0	5	0.0000	1.9909	0.0000	2.4762	0.0000	0.0056
4. Changsha	15	6	13	0.6476	0.9714	6	18	1.7237	2.4327	0.9143	2.8381	0.0029	0.0065
5. Xiaogan	15	7	11	0.7238	0.9333	6	27	1.4097	3.6815	0.9143	4.2667	0.0029	0.0097
6. Hangzhou	15	7	12	0.6571	0.9429	7	14	1.7237	2.2233	0.9333	2.3905	0.0030	0.0054
7. Naju	15	4	10	0.3714	0.9333	4	17	1.7237	3.1261	0.5333	3.1619	0.0017	0.0072
8. Chongju	15	9	13	0.8000	0.9714	10	24	1.9871	3.1261	1.3333	3.9238	0.0043	0.0089
9. Andong	15	5	12	0.5619	0.9429	6	25	1.9871	3.1261	0.9143	3.9619	0.0029	0.0090
10. Yesan	15	4	11	0.3714	0.9333	3	21	1.4097	2.8015	0.4000	3.6381	0.0013	0.0083
11. Geosan	15	9	12	0.8476	0.9429	12	16	2.2181	2.2233	1.7143	2.5333	0.0055	0.0058
12. Suwon	15	4	13	0.3714	0.9714	3	19	1.4097	2.8025	0.4000	3.4286	0.0013	0.0078
13. Kangreung	15	13	13	0.9714	0.9714	17	17	1.9871	2.9691	2.2667	3.0952	0.0073	0.0071

SS = sample size; NH = number of haplotypes; H = haplotype/sequence type diversity; NP = number of polymorphic sites; MSD = maximum sequence divergence; MPD = mean number of pairwise differences; π = nucleotide diversity.

The genetic distances (F_{ST}) between pairs of populations in the A+T-rich region and ITS2 are provided in Tables 3 and 4, respectively. For the A+T-rich region, F_{ST} ranged from -0.061 (comparison between localities 2 and 3), which is effectively zero (due to negative value), to 0.020 (comparisons between localities 4 and 5, between 4 and 10, and between 5 and 10), with a range of migration rate (N_m) from 24.00 to infinite. No significant genetic differentiation whatsoever was detected ($P > 0.05$) in 78 comparisons, suggesting a high interrelationship of the *C. medinalis* populations in terms of the maternally inherited mitochondrial genome (Table 3). In the case of ITS2, the F_{ST} value ranged from -0.026 (comparison between localities 5 and 12) to 0.082 (comparison between localities 1 and 8), with a range of N_m from 5.601 to infinite. Unlike the F_{ST} estimates obtained from the A+T-rich region, 13 population pairs evidenced significant differentiation ($P < 0.05$) among 78 comparisons. Among them, the remote island of Haikou (locality 1), was significantly different from all other populations, except for Guangzhou (locality 3) (Table 4). The two additional population pairs are localities 4 and 8, and localities 6 and 8 (Table 4). These results indicate genetic subdivision in a few *C. medinalis* populations, including Haikou (locality 1) in terms of the bi-parentally inherited nuclear genome. Nevertheless, those population pairs that evidenced significant F_{ST} values still had somewhat high estimates of N_m, ranging from 5.601 to 11.40, meaning an exchange of approximately 5 to 11 individual migrants of *C. medinalis*. Overall, the genetic distance and gene flow data indicate that *C. medinalis* populations are connected to one another quite well, but the nuclear ITS2 sequence reveals a somewhat higher degree of genetic differentiation relative to the A+T-rich region sequence.

Isolation by distance

The results of the Mantel test (10,000 randomizations) for the detection of IBD provided an r (correlation coefficient) value of 0.135 ($P = 0.88$) and 0.209 ($P = 0.86$) in the A+T-rich region among Chinese populations and among Korean populations, respectively. On the other hand, the r value based on ITS2 was 0.001 ($P = 0.49$) and 0.192 ($P = 0.86$) among the Chinese populations and Korean populations, respectively. These results indicate no IBD effect among the Chinese and Korean populations (Figure 5).

Table 3. Fixation indices (F_{ST}) and migration rate (N_m) between pairs of populations of *Cnaphalocrocis medinalis* based on the A+T-rich region.

	2. Tonghai	3. Guangzhou	4. Changsha	5. Xiaogan	6. Hangzhou	7. Naju	8. Chongju	9. Andong	10. Yesan	11. Geosan	12. Suwon	13. Kangreung
1. Haikou	F_{ST} = 0.000 N_m = inf	F_{ST} = -0.061 N_m = inf	F_{ST} = 0.001 N_m = 622.5	F_{ST} = 0.012 N_m = 41.500	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.006 N_m = 77.81	F_{ST} = 0.008 N_m = 62.50	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf
2. Tonghai		F_{ST} = -0.061 N_m = inf	F_{ST} = -0.011 N_m = inf	F_{ST} = 0.012 N_m = 41.50	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.012 N_m = 41.50	F_{ST} = -0.007 N_m = inf	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf
3. Guangzhou			F_{ST} = -0.044 N_m = inf	F_{ST} = -0.044 N_m = inf	F_{ST} = -0.061 N_m = inf	F_{ST} = -0.061 N_m = inf	F_{ST} = -0.061 N_m = inf	F_{ST} = -0.061 N_m = inf	F_{ST} = -0.044 N_m = inf	F_{ST} = -0.052 N_m = inf	F_{ST} = -0.061 N_m = inf	F_{ST} = -0.061 N_m = inf
4. Changsha				F_{ST} = 0.020 N_m = 24.00	F_{ST} = 0.010 N_m = 48.50	F_{ST} = 0.013 N_m = 38.00	F_{ST} = 0.008 N_m = 59.00	F_{ST} = 0.014 N_m = 34.50	F_{ST} = 0.020 N_m = 24.00	F_{ST} = 0.001 N_m = 517.5	F_{ST} = 0.014 N_m = 34.50	F_{ST} = 0.006 N_m = 83.50
5. Xiaogan					F_{ST} = 0.010 N_m = 48.50	F_{ST} = 0.013 N_m = 38.00	F_{ST} = -0.007 N_m = inf	F_{ST} = 0.008 N_m = 64.69	F_{ST} = 0.020 N_m = 24.00	F_{ST} = 0.014 N_m = 34.50	F_{ST} = 0.001 N_m = 517.5	F_{ST} = 0.000 N_m = inf
6. Hangzhou						F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.001 N_m = 727.5	F_{ST} = 0.007 N_m = 69.50	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf
7. Naju							F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf	F_{ST} = -0.012 N_m = inf	F_{ST} = -0.015 N_m = inf	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf
8. Chongju								F_{ST} = 0.000 N_m = inf	F_{ST} = 0.008 N_m = 59.00	F_{ST} = 0.006 N_m = 80.00	F_{ST} = 0.000 N_m = inf	F_{ST} = -0.003 N_m = inf
9. Andong									F_{ST} = 0.014 N_m = 34.50	F_{ST} = 0.009 N_m = 55.50	F_{ST} = 0.000 N_m = inf	F_{ST} = 0.000 N_m = inf
10. Yesan										F_{ST} = -0.013 N_m = inf	F_{ST} = 0.014 N_m = 34.50	F_{ST} = 0.006 N_m = 83.50
11. Geosan											F_{ST} = 0.009 N_m = 55.50	F_{ST} = 0.005 N_m = 104.5
12. Suwon												F_{ST} = 0.000 N_m = inf

inf = infinite.

Table 4. Fixation indices (F_{ST}) and migration rate (N_m) between pairs of populations of *Cnaphalocrocis medinalis* based on the ITS2 region.

	2. Tonghai	3. Guangzhou	4. Changsha	5. Xiaogan	6. Hangzhou	7. Naju	8. Chongju	9. Andong	10. Yesan	11. Geosan	12. Suwon	13. Kangreung
1. Haikou	F_{ST} = 0.055*	F_{ST} = 0.064	F_{ST} = 0.061*	F_{ST} = 0.043*	F_{ST} = 0.071*	F_{ST} = 0.050*	F_{ST} = 0.082*	F_{ST} = 0.042*	F_{ST} = 0.048*	F_{ST} = 0.054*	F_{ST} = 0.050*	F_{ST} = 0.058*
	N_m = 8.571	N_m = 7.265	N_m = 7.737	N_m = 11.07	N_m = 6.527	N_m = 9.527	N_m = 5.601	N_m = 11.40	N_m = 9.828	N_m = 8.750	N_m = 9.435	N_m = 8.181
2. Tonghai		F_{ST} = 0.039	F_{ST} = -0.004	F_{ST} = -0.011	F_{ST} = -0.007	F_{ST} = -0.006	F_{ST} = 0.050	F_{ST} = -0.015	F_{ST} = -0.002	F_{ST} = -0.022	F_{ST} = 0.002	F_{ST} = 0.006
		N_m = 12.33	N_m = inf	N_m = inf	N_m = inf	N_m = inf	N_m = 9.468	N_m = inf	N_m = inf	N_m = inf	N_m = 255.0	N_m = 81.65
3. Guangzhou			F_{ST} = 0.048	F_{ST} = -0.028	F_{ST} = 0.055	F_{ST} = 0.010	F_{ST} = -0.011	F_{ST} = -0.003	F_{ST} = 0.003	F_{ST} = 0.003	F_{ST} = 0.021	F_{ST} = 0.020
			N_m = 9.896	N_m = inf	N_m = 8.536	N_m = 49.74	N_m = inf	N_m = inf	N_m = 173.8	N_m = 173.8	N_m = 23.57	N_m = 25.89
4. Changsha				F_{ST} = 0.010	F_{ST} = -0.009	F_{ST} = 0.003	F_{ST} = 0.075*	F_{ST} = -0.005	F_{ST} = 0.014	F_{ST} = -0.005	F_{ST} = -0.011	F_{ST} = 0.028
				N_m = 51.81	N_m = inf	N_m = 168.8	N_m = 6.206	N_m = inf	N_m = 34.930	N_m = inf	N_m = inf	N_m = 17.12
5. Xiaogan					F_{ST} = 0.007	F_{ST} = -0.018	F_{ST} = 0.002	F_{ST} = -0.021	F_{ST} = -0.020	F_{ST} = -0.019	F_{ST} = -0.026	F_{ST} = -0.006
					N_m = 73.84	N_m = inf	N_m = 293.2	N_m = inf	N_m = 8.205	N_m = inf	N_m = inf	N_m = inf
6. Hangzhou						F_{ST} = 0.015	F_{ST} = 0.070*	F_{ST} = -0.002	F_{ST} = 0.010	F_{ST} = -0.002	F_{ST} = 0.004	F_{ST} = 0.040
						N_m = 33.38	N_m = 6.621	N_m = inf	N_m = 49.97	N_m = inf	N_m = 138.9	N_m = 11.93
7. Naju							F_{ST} = 0.007	F_{ST} = -0.017	F_{ST} = -0.020	F_{ST} = -0.003	F_{ST} = -0.016	F_{ST} = -0.008
							N_m = 68.05	N_m = inf	N_m = inf	N_m = inf	N_m = inf	N_m = inf
8. Chongju								F_{ST} = 0.016	F_{ST} = 0.005	F_{ST} = 0.047	F_{ST} = 0.038	F_{ST} = 0.013
								N_m = 29.86	N_m = 99.25	N_m = 10.217	N_m = 12.59	N_m = 38.12
9. Andong									F_{ST} = -0.015	F_{ST} = -0.012	F_{ST} = -0.010	F_{ST} = -0.004
									N_m = inf	N_m = inf	N_m = inf	N_m = inf
10. Yesan										F_{ST} = -0.002	F_{ST} = -0.005	F_{ST} = 0.003
										N_m = inf	N_m = inf	N_m = 151.5
11. Geosan											F_{ST} = -0.001	F_{ST} = 0.006
											N_m = inf	N_m = 83.63
12. Suwon												F_{ST} = 0.008
												N_m = 60.44

*P < 0.05; inf = infinite.

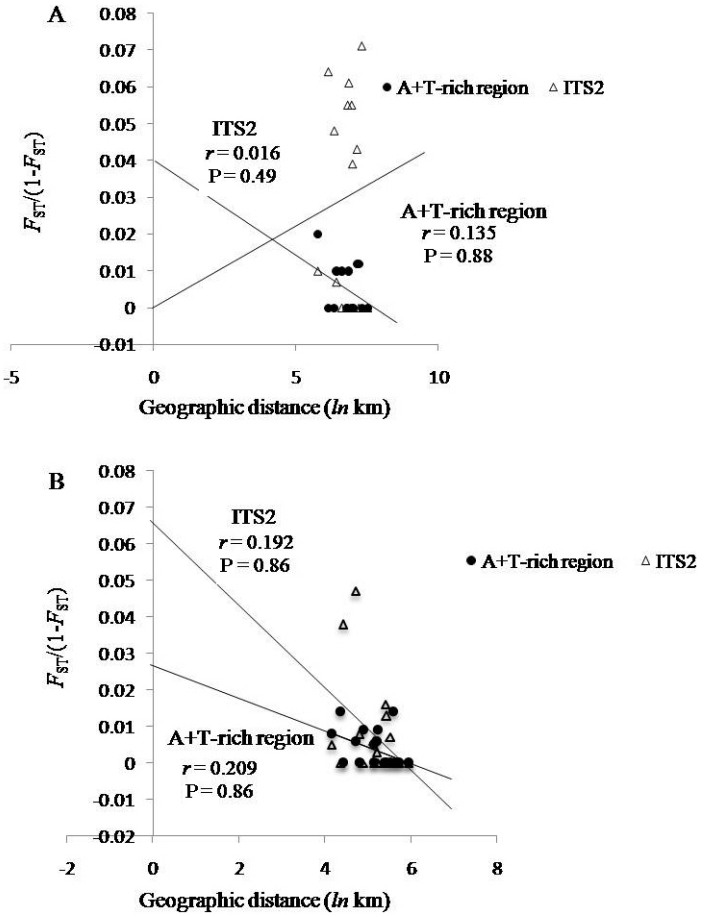

Figure 5. Scatter plots of genetic distance *vs* geographical distance (ln) for pairwise Korean and Chinese *Cnaphalocrocis medinalis* population comparisons based on two molecular markers. **A.** Isolation by distance among Chinese populations (the A+T-rich region: r = 0.135, P = 0.88; ITS2: r = 0.001, P = 0.49). **B.** Isolation by distance among Korean populations (the A+T-rich region: r = 0.209, P = 0.86; ITS2: r = 0.192, P = 0.86).

Population genetic structure

The hierarchical partition of genetic variance and fixation index of each hierarchical level by AMOVA is shown in Table 5. The analyses based on both the A+T-rich region and ITS2 showed that the majority of variance was present in the within-populations (99.93 and 98.48%, respectively), rather than the among-population within-regions or the among-regions. This indicates that each population of *C. medinalis* is composed of heterogeneous individuals. In ITS2, although it is not particularly high, a significant amount of the among-population variance component (1.20%) was detected in the among-population within-regions, and the estimate was significant ($P < 0.005$) (Table 5). This probably is reflective of a degree of divergence of certain *C. medinalis* from others, in that some population pairs, including the Haikou

(locality 1) and Chongju (locality 8) pairs, showed significant F_{ST} estimates in ITS2 (Table 4; Figure 1). On the other hand, the A+T-rich region evidenced no significant variance component of the among-population within-regions (Table 5). This result is consistent with the F_{ST} estimates obtained from the A+T-rich region (Table 3). Finally, the variance components of the among-regions based on both the A+T-rich region and ITS2 were only -0.03 and 0.32%, respectively, with no significance (Table 5), thus suggesting absence of a populational genetic structure between China and Korea.

Table 5. Hierarchical analysis based on the A+T-rich region and ITS2 in *Cnaphalocrocis medinalis*.

Sequence	Source of variation[a]	d.f.	Sum of squares	%	Φ	P
A+T-rich region	Among-regions	1	0.508	0.09	0.00094	NS
	Among-population within-regions	11	5.138	-0.03	-0.00028	NS
	Within-populations	174	81.600	99.93	0.00067	NS
ITS2	Among-regions	1	2.413	0.32	0.00316	NS
	Among-population within-regions	11	21.152	1.20	0.01202	*
	Within-populations	174	284.895	98.48	0.01515	*

[a]The scheme for among-region was established by subdividing six Chinese localities (localities 1, 2, 3, 4, 5, and 6) as one group and seven Korean localities (localities 7, 8, 9, 10, 11, 12, and 13) as another group; d.f. = degrees of freedom; % = percentage of variation; Φ = fixation indices; P = significance of percentage of variation and fixation indices estimated from permutation tests (1000 permutations) (Excoffier et al., 1992). NS = not significant; *P < 0.05.

DISCUSSION

Genetic diversity

For the rice leaf roller, *C. medinalis*, the leaf part of rice can be considered a major resource for both egg-laying and larval feeding. During their generations they run rampant, damaging rice leaves; the reduction of output has been reported in a range between 20-60% in China (Riley et al., 1995). Their dispersal capacity is also quite well known; insect monitoring radar has shown that *C. medinalis* can migrate in large numbers (Riley et al., 1995). Thus, the relatively high dispersal capacity, coupled with the abundance of rice fields in China and Korea, allow us to expect a high genetic diversity in *C. medinalis*. In fact, the pairwise comparisons between pairs of populations obtained both from the A+T-rich region and ITS2 sequences evidenced an overall relatively high estimate of N_m, primarily without any statistically significant F_{ST}, although one Chinese population, the Haikou population (locality 1), evidences a significantly high F_{ST} compared to most other populations, according to the ITS2 data only (Tables 3 and 4). Furthermore, the results obtained in the AMOVA test indicate that the majority of genetic variance was within-populations, rather than at any other hierarchical level (99.93% in the A+T-rich region and 98.48% in the ITS2 sequence, respectively; Table 5), showing that each population of *C. medinalis* is composed of genetically heterogeneous individuals, as a consequence of high dispersal and the characteristics of large populations.

Genetic similarity among distant populations

Our F_{ST} data from both the A+T-rich region and the ITS2 sequences showed almost no significant genetic distance between pairs of populations, even the Chinese and Korean localities (Tables 3 and 4). The results demonstrate substantially high gene flow among the

C. medinalis populations; this was confirmed by the results of phylogenetic analysis, which showed a random association of haplotypes, regardless of geographic origin (Figures 3 and 4) and IBD, which was not statistically supported (Figure 5).

These results were consistent with field observations. *Cnaphalocrocis medinalis* can breed all year round in the tropical rice-growing areas, corresponding to locations south of approximately 22°N latitude (Zhang et al., 1981). These source populations migrate to southwestern China, and subsequently expand into other parts of China in a northeastern direction, and subsequently into the southern part of the Korean peninsula (Zhang et al., 1981). In fact, several lines of evidence obtained from direct observation support the abundance of moths migrating long distances. For example, Zhang et al. (1981) determined, via a mark-and-recapture method, that *C. medinalis* migrates for over 800-1100 km in a northeastward direction in China. Additionally, it has also been determined that *C. medinalis* migrates from China to Japan every year during June-July, with assistance from winds (Miyahara et al., 1981). Similar observations have also been reported regarding migration from southwest China to the southwestern part of the Korean peninsula (Kim and Choi, 1984). Once arriving in Korea during June-July, *C. medinalis* spends two or three generations inhabiting sites in Korea (Kim and Choi, 1984). Considering that the cold temperatures of Korean winter do not allow for *C. medinalis* to overwinter (Kim and Choi, 1984), populations newly migrated from China are considered to be the source population. Thus, our data in collaboration with the information collected in the field support the supposition of this study, namely that *C. medinalis* possesses sufficient flight capacity for dispersal, resulting in a substantial amount of gene flow among populations and an absence of a populational genetic structure, even between China and Korea.

Genetic differentiation of the Haikou population only by ITS2

Our F_{ST} data generated both from the A+T-rich region and the ITS2 sequences showed almost no significant genetic distance between pairs of populations. Exceptions to this observation were seen only with the sequence analysis of ITS2 (Table 4), wherein comparisons of the Haikou population (locality 1) to all other populations, except for Guangzhou (locality 3), were made (Figure 1). This may imply that the *C. medinalis* individuals collected from Haikou (locality 1) were divergent from other populations; this finding requires some explanation. From a geographical perspective, Haikou is located on an offshore island, Hainan Island, which is located ~29.5 km south of the southern coast of mainland China (Figure 1). It has been determined that the majority of *C. medinalis* occurring in southwestern China including Hainan Island, where Haikou (locality 1) is located, is composed of emigrants dispersed from the source of the tropical rice-growing areas (Zhang et al., 1981). These southwestern Chinese populations are then expanded into China in a northeastern direction and subsequently into the southern part of the Korean peninsula (Zhang et al., 1981). In the case of Haikou (locality 1), where breeding is possible year-round (Chen and Guo, 1996), the pre-existing local subpopulations mingle with the newly dispersed immigrants from June to July for the first time and from August to September for the second time (Chen and Guo, 1996). Because our sample collection in Haikou (locality 1) was conducted on August 15, 2009, which is within the infestation period for both pre-existing populations that have bred independently and newly arrived populations that originated from the tropical rice-growing areas, the population genetic characteristics of the Hainan Island including Haikou population (locality 1) would represent

a mixture of the populations. Thus, the genetic differentiation of the Haikou population (locality 1) from most other Chinese populations, obtained via ITS2 sequence analysis (Table 4), appears to be the consequence of such a phenomenon.

However, the detection of the genetic differences between the Haikou population (locality 1) and most other populations only by ITS2 sequence analysis may require further explanation. Theoretically, the maternally inherited mtDNA would have an effective population size approximately one-quarter the size of the effective population size based on nuclear makers that are inherited bi-parentally, if the sex ratio is equal (Birky et al., 1983). This has led some to observe that the effects of genetic drift could be substantially stronger in the mtDNA, resulting in more rapid fixation and more profound population subdivision (Birky et al., 1983). However, dispersal functions as a counterforce against genetic drift (Hanski, 1996). In situations in which dispersal is biased to a single gender, the typical expectation of population structure would be falsified (Birky et al., 1983).

The observation of a somewhat higher degree of genetic differentiation in the ITS2 (Table 4) and the detection of significant genetic differentiation of the Haikou population (locality 1) only by bi-parentally inherited ITS2, rather than mtDNA sequence analysis, appears to indicate that female dispersal is more important than male dispersal. No previous studies have found evidence for gender-biased dispersal of *C. medinalis*, with the exception of some studies on the dispersal capacity of each gender within-superfamilial species. For example, Shirai (1998) has demonstrated a longer and continuous flight of the female of the European corn borer, *Ostrinia nubilalis* (family Crambidae), during the pre-ovipositional period, which lasts three to four days after emergence. Although their analysis did not involve within-familial species, Tu et al. (2010) also demonstrated that the total flight duration and distance of 1-day-old female *Spodoptera litura* moths during a 72-h period were substantially lengthened, by 24 h and 105.4 km; this suggests more effective dispersal of females than males. Although it may not be direct evidence, this circumstantial information still implies the importance of female-biased dispersal, considering that insect migration is closely associated with flight ability (Drake and Gatehouse, 1995).

Nevertheless, lack of support for significant F_{ST} estimates in most population pairs in ITS2 at the same time may also indicate that female-biased dispersal would be relevant only under temporary conditions such as the beginning of infestation, during which it is important to find further abundant host plants for egg-laying and feeding. Instead, overall high within-population genetic diversity, very low variance components of the among-population within-regions and the among-regions, lack of statistical support for isolation by distance, and no significant female-biased dispersal in other population pairs in both mitochondrial and nuclear DNA collectively indicate that migratory *C. medinalis* populations are well connected to one another by high gene flow, but do not necessarily reveal gender-biased dispersal.

ACKNOWLEDGMENTS

Research supported by a grant from the Agenda Program (#200901FHT020609413) from the Rural Development Administration, Republic of Korea.

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