

# 한국에 서식하는 아메리카동애등에 *Hermetia illucens* (Diptera: Stratiomyidae)의 개체군 구조<sup>1</sup>

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## Population Structure of the Exotic Black Soldier Fly, *Hermetia illucens* (Diptera: Stratiomyidae) in Korea<sup>1</sup>

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### 요약

외래생물인 아메리카동애등에는 뛰어난 유기물분해능으로 주목받아왔다. 이에 우리는 한국내 분포상과 유전적 다양성을 알아보기 위해 전국적으로 채집하여 미토콘드리아의 사이토크롬 C 산화효소 1 (*COI*) 유전자를 이용해 집단유전학분석을 실시하였다. 그 결과 이 곤충은 한국에 성공적으로 정착한 것으로 보이며 10개의 미토콘드리아 유전자형이 있었고 개체군 간 분화가 뚜렷했다. 이 결과는 국내정착한 아메리카동애등에가 특정 유전자형의 모계만이 도입된 후 지역 간 분산이 제한적임을 나타내는 것으로, 이는 이들의 단거리 비행 경향에 기인한 것으로 추정된다.

주요어: 아메리카동애등에, *COI* 유전자형, 유전적 구조, 외래종

### ABSTRACT

The exotic black soldier fly, *Hermetia illucens*, has been paid much attention as an excellent organic matter decomposer. We conducted the nationwide survey and the population genetic study using a mitochondrial *cytochrome C oxidase 1* gene to understand its genetic diversity and distribution pattern in Korea. The results show that it has successfully settled down in South Korea and there are only 10 haplotypes and the populations of the insect are highly differentiated. The results may indicate that their dispersal was restrained probably due to their short distance flying tendency since their introduction and settlement of only few maternal lineages with certain genotypes in Korea.

**KEY WORDS:** *Hermetia illucens*, *COI* HAPLOTYPE, GENETIC STRUCTURE, EXOTIC SPECIES

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## INTRODUCTION

The Food and Agriculture Organization of the United Nations reports that food resources for human consumption only are lost or wasted approximately one third of the total production equivalent to 1.3 billion ton/year globally (Gustavsson, 2011). Contrary to food loss mainly occurring in low-income countries during production and processing, in medium- and high-income countries, most of food waste is generated at the consumption stage largely due to human attitude (Gustavsson, 2011). For example, in the Netherlands, 9.5 million tons of food is wasted per year worth at 2.4-4.4 billion Euros equivalent to 3~5.6 billion dollars (Thonissen, 2010; Waarts *et al.*, 2011). In the USA, the estimates ascend steeply to 165.6 billion dollar loss per year (Buzby and Hyman, 2012). In Korea, 13,300 ton/day of food waste was generated and claimed about 15 billion dollars of the annual economic loss in 2006 (Lee *et al.*, 2009). Food wastes are largely treated by abandoning, landfilling and ocean disposal, which have caused severe environmental pollution (Heo *et al.*, 2011), even though most of household wastes are collected and recycled (Kim *et al.*, 2010).

One of the recycling methods of food wastes is composting (Adhikari, 2009; Kubota and Nakasaki, 1991; Yun *et al.*, 2000). The earthworm has been employed for processing organic compost (Elvira *et al.*, 1996). However, it is of particular difficulty to convert the wastes with high salt concentration to composts using earthworms, unless the additional desalination process is performed (Hernandez *et al.*, 2010). This applies to the Korean food wastes that usually contain high salts. The black soldier fly (*Hermetia illucens*; hereafter BSF) has been paid much attention, because of its ability to decomposing various biological matters such as manure, food wastes, and its forensic purpose (Kim *et al.*, 2008; Lord *et al.*, 1994; Sheppard *et al.*, 1994). The BSF originated from North America and is common in the southeastern USA (Newton *et al.*, 2005). The insect has spread to all over the world from tropical to temperate regions, as, most probably, intercontinental trade increased (McCallan, 1974; Sheppard *et al.*, 1994). In Japan, the species is regarded as one of the insects that have established after invasion (Mito and Uesugi, 2004). This may be invariably same in South Korea (hereafter Korea). In Korea, the fly was officially recorded in 1997 (Kim, 1997). The species is normally found in rural agricultural and dairy areas. Adults

are not attracted to human habitation or foods (Furman *et al.*, 1959). In addition, its competitiveness outcompetes that of house flies resulting in oviposition avoidance and house fly elimination (Newton *et al.*, 2005). The BSF has been strategically reared for decomposing food waste in Korea (Kim *et al.*, 2008). The rearing stock was established from a collection from one location i.e. Suwon in Table 1, which might cause severe inbreeding condition.

Despite of its ecological and economic importance, there was no nationwide survey on its distribution and genetic structure. Therefore, the goal of this study was 1) to investigate the distribution pattern and genetic diversity and 2) determine the population genetic structure of the Korean populations of BSF using the mitochondrial *cytochrome c oxidase subunit 1 (COI)*. The results could be a foundation of international cooperative work on the beneficial insect and on finding strains with more efficient decomposition ability.

## MATERIALS AND METHODS

### 1. The fly collection

Adult BSFs were easily distinguished from other fly species with its structural metal blue-colored compound eyes and club-shaped antennae. They were captured using an insect net near or on farms or organic waste stacks. The flies were collected in total 245 individuals from 30 localities nationwide during July to October in 2006, 2010 and 2011 (Table 1). Local populations were clustered into five regions based on the Korean vegetation map isothermal and isobaric lines (Yi, 2011) (Table 1). Once collected, they were freshly sacrificed in 100% EtOH and kept at around 4°C until they were brought into the laboratory. In the laboratory, the BSF specimens were kept at -20°C until genomic DNA extraction.

### 2. Genomic DNA extraction and PCR analysis

The genomic DNA was extracted from individuals using a commercial kit following the manufacturer's instructions (DNeasy Blood and Tissue Kit, Qiagen, Germany). The extracted genomic DNA was kept at -20°C until further analyses. The partial *COI* gene was obtained from each individual using the specific primer set developed for this study (forward: CAA CAA ATC ATA AAG ATA TTG

Table 1. Collection sites of BSF nationwide in Korea

Region*	Localities	Latitude	Longitude	Individuals collected
1	Suwon	37.15.08	126.57.34	12
	Yangyang	38.05.13	128.32.19	11
	Dangjin	36.49.29	126.47.19	5
	Icheon	37.10.58	127.28.35	8
	Koesan	36.40.22	127.48.13	15
	Seoul	37.37.06	127.04.27	3
	Sunghwan	36.55.30	127.07.50	3
	Yongin	37.13.21	127.05.37	13
	Eumsung	36.55.02	127.44.59	7
	Jincheon	36.52.11	127.27.58	10
	Pyongtaek	37.03.22	126.59.23	10
	Hwasung	37.13.03	126.48.46	5
	Kimpo	37.36.10	126.47.53	4
	Danyang	36.48.07	128.22.02	1
Hwayang	36.40.02	127.48.48	23	
Sum				130
2	Buyeo	36.18.06	126.48.05	12
	Jangsu	35.42.11	127.31.16	9
	Kunsan	35.57.26	126.42.49	7
	Boeun	36.31.59	127.49.31	2
Sum				30
3	Gurae	35.14.35	127.30.16	10
	Kwangyang	34.58.05	127.43.24	13
	Goksung	35.16.49	127.18.22	3
	Omoun	35.28.20	127.31.37	3
Sum				29
4	Youngcheon	35.58.10	128.44.40	8
	Bonghwa	36.54.38	128.45.19	11
	Jinju	35.12.25	128.06.49	10
	Munhyung	36.34.46	128.11.06	2
	Jinyoung	35.18.49	128.46.05	12
	Kimcheon	36.09.19	128.07.38	1
Sum				44
5**	Jeju	33.28.49	126.27.29	12
Sum				12
	Total			245

\*: The regions were defined by following Korean vegetation map isothermal and isobaric lines (Lee, 2011). Region 1: Deciduous broadleaved forest (Central temperate zone); Region 2: Deciduous broadleaved forest (Western South temperate zone); Region 3: Deciduous broadleaved forest (Eastern South temperate zone); Region 4: Evergreen broadleaved forest (Subtropical warm temperate zone); Region 5\*\*: Jeju island belongs to the evergreen broadleaved forest (Subtropical warm temperate zone), but the island is geographically isolated. Therefore, in the analysis, it was independently treated.

Table 2. Frequency of haplotypes by localities and regions

Region	Localities	Hap 1	Hap 2	Hap 3	Hap 4	Hap 5	Hap 6	Hap 7	Hap 8	Hap 9	Hap 10	Sum
1	Suwon	3	2	1	5	1	-	-	-	-	-	12
	Yangyang	5	-	3	2	-	1	-	-	-	-	11
	Dangjin	2	-	3	-	-	-	-	-	-	-	5
	Icheon	3	-	2	3	-	-	-	-	-	-	8
	Koesan	2	-	10	2	-	-	1	-	-	-	15
	Seoul	2	-	-	1	-	-	-	-	-	-	3
	Sunghwan	-	-	2	1	-	-	-	-	-	-	3
	Yongin	5	-	7	1	-	-	-	-	-	-	13
	Eumsung	1	-	4	2	-	-	-	-	-	-	7
	Jincheon	-	-	3	7	-	-	-	-	-	-	10
	Pyeongtaek	3	-	5	2	-	-	-	-	-	-	10
	Hwasung	-	-	1	-	-	4	-	-	-	-	5
	Kimpo	1	-	-	-	-	3	-	-	-	-	4
	Danyang	-	-	-	1	-	-	-	-	-	-	1
	Hwayang	3	-	15	-	-	4	-	-	-	-	1
sum	30	2	56	27	1	12	1	-	-	-	1	130
2	Buyeo	8	-	-	3	-	1	-	-	-	-	12
	Jangsu	-	-	8	-	-	-	-	-	1	-	9
	Kunsan	-	-	5	2	-	-	-	-	-	-	7
	Boeun	-	-	2	-	-	-	-	-	-	-	2
	Goksung	-	-	3	-	-	-	-	-	-	-	3
	Gurae	-	5	5	-	-	-	-	-	-	-	10
	sum	8	5	21	5	-	1	-	-	-	1	43
3	Kwangyang	-	-	12	1	-	-	-	-	-	-	13
	Omoun	-	-	3	-	-	-	-	-	-	-	3
	Jinju	-	-	9	1	-	-	-	-	-	-	10
	Jinyoung	-	-	11	-	-	-	-	1	-	-	12
	sum	-	-	35	2	-	-	-	1	-	-	38
4	Youngcheon	1	5	2	-	-	-	-	-	-	-	8
	Bonghwa	2	-	9	-	-	-	-	-	-	-	11
	Munhyung	-	-	2	-	-	-	-	-	-	-	2
	Kimcheon	-	-	1	-	-	-	-	-	-	-	1
	sum	3	5	14	-	-	-	-	-	-	-	22
5	Jeju	6	-	3	3	-	-	-	-	-	-	12
	sum	6	-	3	3	-	-	-	-	-	-	12
Total	47	12	131	37	1	13	1	1	1	1	1	245

GAA C; reverse: GTC CGA AGA ATC AAA ATA AAT GYT G). The PCR condition was a pre-denaturing for 3 min at 94°C and 35 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C, and a final extension for 4 min at 72°C. In all PCRs, the total volume was 25  $\mu$ l with

1× PCR buffer, 0.5 mM of each primer, 0.1 mM of each dNTP, 0.2  $\mu$ l (1 unit) of Taq polymerase (EX Taq Polymerase, Takara, Japan) plus 2  $\mu$ l of genomic DNA. After the reaction, the *COI* amplicons were visualized under a UV transilluminator and purified using a

commercial kit (Qiaquick PCR Purification Kit, Qiagen, Germany). The purified PCR amplicons were sequenced. In all cases, the sequences were read in both directions for maximum clarity. Haplotypes with singletons were resampled and the sequences in both directions were verified.

### 3. Data analyses

The resultant *COI* sequences were aligned and analyzed using Clustal W embedded in MEGA (ver. 6) (Tamura *et al.*, 2013; Thompson *et al.*, 1994). The *COI* haplotypes were determined by DnaSP (ver. 5) (Librado and Rozas, 2009). Genetic distance and diversity among haplotypes were calculated after selecting the best fit substitution model for the haplotypes on MEGA. Parsimony split algorithm with 500 bootstrapping and median joining algorithm were used to infer phylogenetic relationships among the haplotypes using Splitstree (ver. 4.12.3) (Huson and Bryant, 2006) and Network (ver. 4.6.10) (Bandelt *et al.*, 1999) with fixed connection limit at 1000 steps between haplotypes. The haplotype networks created by the two different algorithms were manually consolidated and reconstructed with the regional distribution data on Adobe illustrator CS3 (Adobe Inc, USA).

Analysis of molecular variance (AMOVA) among regions including *Fst* statistics was performed for determining the population genetic structure with 1000 permutations. To test whether the sequence variation was congruent to the neutral evolution model, the neutrality tests were performed. Based on mismatch distribution, Harpending's raggedness was estimated with 100 bootstrapping to infer the population expansion in relation to the neutrality tests. Arlequin (ver. 3.5.1.2) was used for the three tests above (Excoffier and Lischer, 2010).

Due to no HKY (Hasegawa-Kishino-Yano) option on Arlequin, K80 (Kimura 80) with the same substitution rate was used for AMOVA and *Fst* statistics. Recently, it was suggested that *Fst* did not properly measure genetic differentiation among groups (Jost, 2008). Hence to estimate the degree of genetic differentiation between regions, the Jost's *D* (differentiation) value was calculated based on the haplotype diversity among regions (Crawford, 2010; Jost, 2008).

## RESULTS

The BSF was found in almost all localities we surveyed as shown in Table 1. Therefore we may conclude that it is distributed nationwide in Korea.

### 1. Haplotype diversity by localities

From the 245 BSF specimens, a total of 657bp were analyzed. The sequences were deposited on GenBank (GenBank accession number: FJ794325~FJ794425, HQ541184~HQ541321, and KF500241~KF500278). There were 41 variable sites, and among them 35 nucleotide sites were parsimony informative. There was a total of 10 distinct haplotypes of which the amino acid sequence was identical among them caused by synonymous mutation (data not shown). Among them, the haplotype 3 consists of 53.5% (131 individuals) and is followed by the haplotypes 1 (19.2%) and 4 (15.1%) (47 and 37 individuals respectively). On the other hand, the haplotypes 5, 7, 8, 9 and 10 appeared locally (Table 2). Additionally, the BSF (KC192965) collected in the USA (Wiegmann *et al.*, 2011) belongs to the haplotype 4.

### 2. Population genetic structure

The 8 haplotypes except Hap 5 and 9 can be grouped into 3 major clusters. Within each cluster, mean genetic distances are extremely low at 0.002, 0.002 and 0.003 respectively. Genetic distances between clusters are 0.022 (Cluster1 to Cluster 2), 0.042 (Cluster1 to Cluster 3), and 0.04 (Cluster2 to Cluster3) (Fig. 1).

The AMOVA test indicates that populations are genetically highly isolated ( $F_{st}=0.32$ ,  $P<.05$ ), even though the degree of genetic isolation is diminished at the regional level ( $F_{ct}=0.15$ ,  $P<.05$ ) (Table 3). The result of Jost's *D* value shows low differentiation among regions (Table 4), similar to that of the AMOVA test (Table 3).

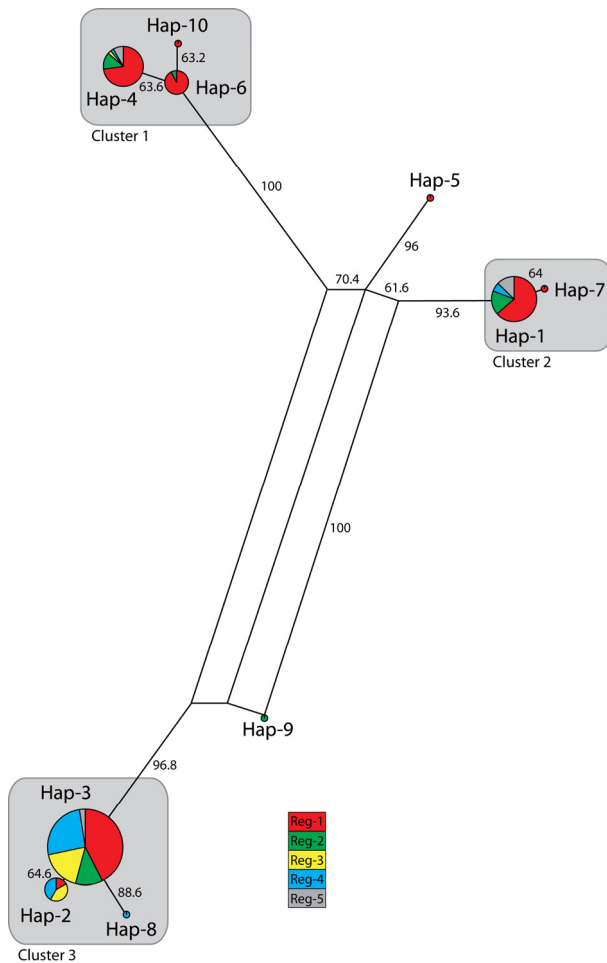


Fig 1. Haplotype network and the regional distribution of the 10 haplotypes with bootstrap values, bootstrap branch support values on each branch.

### 3. Neutrality and population expansion

Under a neutral model of constant population size, the expected value of  $D$  is close to zero (Tajima, 1989). A positive  $D$  value results, when more than the expected number of polymorphic sites has low frequencies in the sample, a pattern that can be explained by either a recent population size expansion or recent selection. Our results show positive values ( $D=0.7070$ ) for the total samples and no statistical significance ( $P>0.1$ ) (Table 5). Therefore the haplotype diversity is not the result of selection. The raggedness index (0.2708) and SSD (sum of squared deviations) (0.1163) are not statistically significant ( $P>0.1$  for both). This means that the null hypothesis of static population status is rejected. The ratio of  $\theta$  ( $\theta = 1/\theta_0 = 27992.1$ ) indicates that population expansion model may be accepted (Table 6). Therefore the its population has grown under the genetic circumstance.

## DISCUSSION

There are two major international ports, one in region 1 and the other in region 4. It could be thought that either of them would be the starting point of its spread in Korea. Gradual decrease of the haplotype diversity is correlated with the distance from the origin. However, taken together with the population genetic structure data, haplotype distribution does not support the scenario. One potential explanation for this is immediate spread after introduction.

Table 3. Molecular variance (AMOVA) of the five regions based on the mt-*COI* sequences of BSF (significance level: .05)

Source of variation	<i>d.f.</i>	Variance components	% variation	fixation indices
Among regions	4	1.0815	14.95	F <sub>ct</sub> =0.14948
Among localities within regions	24	1.22715	16.96	F <sub>sc</sub> =0.19942
Within localities	205	4.92642	68.09	F <sub>st</sub> =0.31909

Table 4. Estimation of genetic differentiation among regions (significance level: .05)

Diversity Parameter	Bootstrapped Est.	Variance±S.E	95 % CI Min/Max
Hs <sub>est</sub>	0.532	0.0005±0.001	0.492/0.566
Ht <sub>est</sub>	0.552	0.0003±0.001	0.521/0.582
G <sub>est</sub>	0.036	0.0002±0.001	0.015/0.066
G <sub>Hedrick</sub>	0.086	0.0009±0.001	0.038/0.146
D <sub>est</sub>	0.052	0.0003±0.001	0.023/0.086

Table 5. Tests of the model of neutrality of regions

Region	1	2	3	4	5	Mean±s.d.
No. Sample	130	30	29	44	12	49±46.6798
Tajima's <i>D</i>	3.31156	2.84278	-2.51047	-1.51943	1.41069	0.7070±2.6053
<i>p</i> -value	>0.1	>0.1	<0.01	<0.05	>0.1	
Fu's <i>FS</i>	26.01726	15.43143	4.05856	6.16156	11.49735	12.6332±8.7129
<i>p</i> -value	>0.1	>0.1	>0.1	>0.1	>0.1	

Table 6. Demographic and spatial history of BSF

		mean±s.d.
Demographic	$\tau$	17.28672±13.04231
	$\theta_0$	0.00105±0.00157
	$\theta_1$	31.26066±30.73311
	SSD	0.11627±0.09617
	Model (SSD) <i>p</i> -value	>0.1
	Raggedness index	0.2708±0.1068
	Raggedness <i>p</i> -value	>0.1
Spatial	$\tau$	20.78798±11.4052
	$\theta$	0.18955±0.2548
	M	20001.17±44720.14889
	SSD	0.0613±0.05514
	Model (SSD) <i>p</i> -value	>0.1
	Raggedness index	0.2708±0.1068
	Raggedness <i>p</i> -value	>0.1

raggedness index: mismatch distribution index between hypothetical population and experimental population

This might be achieved by rapid logistics of imported goods from USA with hitchhiking BSF, as pointed out by Hulme (2009). The AMOVA test result of high genetic differentiation among populations reflects the limited dispersal ability of the insect after regional settlement. In fact, in the field, the insect's activity may largely be affected by the daily weather condition (unpublished). Paradoxically, this limited dispersal may help the BSF establish locally and a genotype with higher efficiency can be confined with less effort.

The demographic expansion may have taken place in BSF probably due to the absence of its natural enemies in the new habitat. However, this analysis may not be appropriate for exotic species, especially to a species that is recently introduced. The reason is that such analysis is solely dependent on the genetic data that might be distorted during the invasion process mainly by a genetic

bottleneck (Muirhead *et al.*, 2008). Alternative potential explanation may be that the expansion already took place in its origin before its worldwide spread and such prior expansion was embedded as a genetic signature in the locally settled Korean population.

In general, an exotic species has a negative impact on native species (for review, See Sakai *et al.*, 2001). A community diversity alteration or native species extinction is attributed to an exotic species (Gurevitch and Padilla, 2005). The closely related native *Ptecticus tenebrierfer* fly species also decomposes organic matters at the larval stage. There may be a mutual competitive exclusion of BSF and *P. tenebrierfer*. Future efforts should investigate the potential adverse effect of the BSF on the native species.

In conclusion, this study shows that the insect has spread nationwide and there are only 10 haplotypes of the insect in Korea that is rather typical to exotic species. Due

to its potentially limited dispersal ability, the populations are genetically highly isolated. The population genetic study can contribute the current effort on finding the insect lineages with improved decomposition capability.

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