

Genetic Structure of the Neglected Decomposer, *Ptecticus tenebrifer*, with an Introduced Decomposer, *Hermetia illucens*

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ABSTRACT

The era of mass production of agricultural and dairy systems inevitably causes a huge amount of biowastes during their processes. Modern consumption patterns of the general public also contribute to biowaste formation. Thus, processing biowastes has attracted much attention. The introduced black soldier fly (BSF) (*Hermetia illucens*) is considered as one of environmentally friendly management options for solving biowaste issues. However, an indigenous species, *Ptecticus tenebrifer*, is also a powerful decomposer that has been largely neglected. This species can be easily found on biowastes such as manure dump, agricultural wastes, and human food wastes. It can be also easily found in the field. It is even attracted to a food trap. To the best of our knowledge, this is the first ecological study on this species.

Keywords: Biowaste, Black soldier fly, Genetic structure, *Hermetia illucens*, *Ptecticus tenebrifer*

Introduction

In terms of nutrient cycling in the ecosystem, carbon fixation by photosynthesis has been paid much attention (Blodau, 2002; Nielsen *et al.*, 2011; Zhi *et al.*, 2015). However, when trophic level is considered, there are a lot more actors involved. For example, decomposers such as microbes have an essential role in the cycle (Moorhead *et al.*, 2014). In this regard, insects also play a pivotal role in degrading ingredients by simply consuming organic materials. In this way, organic materials are incorporated into nutrient cycling. Most notably, black soldier fly (BSF), *Hermetia illucens* (Diptera; Stratiomyidae), has been utilized for processing manures and other organic wastes such

as human food waste (Kim *et al.*, 2008; Lord *et al.*, 1994; Park *et al.*, 2017; Sheppard *et al.*, 1994). In Korea, this insect is in mass production since its accidental introduction in early 2000s (Kim *et al.*, 2008). This insect is also easily noticeable with its metallic black color. However, due to its sensitiveness to temperature and light availability, it is somewhat limited both in annual and diurnal activities.

Family Stratiomyidae is composed of at least 150 species worldwide (Rozkošný & Kovac, 2000). In Korea, 15 species belonging to this family have been identified (Paek *et al.*, 2010). Among them, *Ptecticus tenebrifer* is native to Korea. It seems to have remarkably similar ability to decompose organic materials as *H. illucens* does. Larvae of this insect consume wastes until they molt into pupae. Its adults usually hover over wastes. With this regard, we hypothesized potential competition between these two species in their adult stage to keep others from laying eggs in wastes. Thus, the objective of this study was to investigate their distribution and adult fighting to see their potential competition in the natural field.

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Materials and Methods

Locations of the insect

In 2019, we surveyed 22 sites based on administrative

district nationwide. Both adult insects were marked as presence/absence. Types of the wastes were also described (Table 1). These insects were also collected using insect nets for future genetic analyses.

Table 1. Observation sites and presence of *H. illucens*

Region	Location	Organic materials	Lat.	Long.	<i>H. illucens</i>
1	Anseong	AW	37.02	127.29	
	Seongnam	AW	37.38	127.13	
	Yangpyeong	AW	37.42	127.24	
	Gwangju	AW	37.42	127.24	
2	Seocheon	FW	36.03	126.73	
	Gunsan	AW	35.97	126.74	
	Cheongyang	M	36.45	126.70	Yes
3	Boeun	CC	36.59	127.71	
	Goesan	AW	36.66	127.74	
	Cheongwon	CC	36.58	127.51	
4	Hampyeong	AW	35.08	126.52	Yes
	Jangseong	FW	35.42	126.85	Yes
	Damyang	FW	35.31	126.92	
5	Haman	AW/FW	35.27	128.40	
	Goseong	FW	35.01	128.31	
	Changwon	FW	35.25	128.58	Yes
6	Bongwha	AW	35.27	128.40	Yes
	Youngju	AW	36.95	128.62	Yes
	Andong	FW	36.77	128.81	Yes
7	Inje	FW	38.03	128.47	
	Pyeongchang	AW	37.64	128.50	Yes
	Yangyang	FW	38.08	128.67	

Region 1: Gyeonggi, 2: Chungnam, 3: Chungbuk, 4: Jeonnam, 5: Gyeongnam, 6: Gyeongbuk, 7. Gangwon.

Mitochondrial (Mt) CO1 gene sequence and phylogenetic analysis of Stratiomyidae

Genomic DNA (gDNA) was extracted from the posterior end of each individual using a commercial kit (ExGen Tissue SV, Geneall, Korea, Cat No. 104-152) according to the manufacturer's instructions with slight modifications. Extracted gDNA was kept at -20°C until further analyses. PCR was performed on a thermocycler (Peqlab, Germany). LCO-1490 (GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO-2148 (TAA ACT TCA GGG TGA CCA AAA AAT CA) were used to obtain partial sequence of Mt-CO1 gene. PCR conditions were: pre-denaturing for 3 min at 94°C ; 35 cycles of 1 min at 94°C , 1 min at 55°C , and 1 min at 72°C ; and a final extension step for 4 min at 72°C . PCR reaction had a total volume of $20\ \mu\text{l}$, containing 0.5 mM of each primer and $1\ \mu\text{l}$ of gDNA in the premix (Intron, Korea).

A total of 196 individuals of *P. tenebrifer* were tested for this genetic study.

Data analyses

Resultant CO1 sequences were aligned and analyzed initially on MEGA (Tamura *et al.*, 2013; Thompson *et al.*, 1994). Haplotype frequency was determined using DnaSP version 5 (Librado & Rozas, 2009). Genetic distance and diversity among haplotypes were calculated after selecting the best fit model for haplotypes on MEGA. Maximum likelihood was adopted to infer phylogenetic relationship of insects within the family using 1000 bootstrapping on MEGA (Tamura *et al.*, 2013). Analysis of molecular variance was conducted to determine the population genetic structure with 1000 permutations among regions including *Fst* statistics on Arlequin (ver. 3.5.1.2) (Excoffier &

Lischer, 2010). Additionally, neutrality tests were performed to determine whether the gene was subjected to a neutral evolution model Arlequin (ver. 3.5.1.2) (Excoffier & Lischer, 2010; Tajima, 1989). All other sequences were retrieved from GenBank.

Competition over resources

To test whether there was a potential conflict between the two insects, we observed contact interactions between adults of both insects. In this study, we were unable to examine larval stage competition.

Results and Discussion

Distribution

Results showed that these insects were distributed nationwide. Both insects were located on or near food wastes, organic wastes from various farm lands, and manures from cattle sheds (Fig. 1). Contrary to *P. tenebrifer*, BSF was found in eight localities (Table 1). This might mean that the introduced BSF is still in its expansion process. We were unable to estimate local population sizes of the two in this study.



Fig. 1. Typical types of habitats of *P. tenebrifer*
A: Food waste trap (Seochon), B: Agro waste (Seongnam), C: Chicken coop (Cheongju), D: Manure (Cheongyang).

Genetic analysis of partial CO1 gene

Results showed that there was no genetic isolation among seven regions (F_{st} : 0.01935, $p > 0.05$). The genetic diversity of the insect can be explained by the diversity within populations (Table 2). In fact, the insect has very robust wings and flies actively. Therefore, it may fly or spread long distance and exchange genes in a frequent manner. Two neutrality tests showed negative values with

opposite statistical values (Table 3). It is too early to conclude that the population has expanded recently. Further study is needed to reach conclusion on its population size change. Compared to *P. tenebrifer*, BSF showed a remarkably lower genetic diversity (Table 4). This indicates that the insect might have experienced a genetic bottleneck in the introduction period, although there is no conclusive evidence (Park *et al.*, 2017).

Table 2. Genetic differences among regions (Fst)

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among 7 groups	6	5.721	0.00071Va	0.09
Among populations within groups	15	13.94	0.01502Vb	1.85
Within populations	174	138.681	0.79702Vc	98.07
Total	195	158.342	0.81274	

Table 3. Neutrality test for population expansion

Neutrality		Mean ± s.d.
Tajima's D	No. samples	8.9±1.6
	S	5.59±2.19
	Pi	1.59±0.51
	Tajima's D	-0.91147±0.60203
	Tajima's D p-value	>0.1
Fu's Fs	No. alleles	5.5±1.3
	Theta pi	1.59308±0.50839
	Exp. No. of alleles	3.40424±0.57807
	FS	-2.2768±1.21631
	FS p-value	<0.05

Table 4. Comparison between *P. tenebrifer* and BSF

	<i>P. tenebrifer</i>	<i>H. illucens</i>
No. tested	245	196
No. haplotypes	10	46
Genetic diversity	low	high

Phylogenetic status of *P. tenebrifer*

Results of phylogenetic analysis showed that *P. tenebrifer* was grouped with *P. trivittatus*, a Stratiomyidae sp. and *H. illucens* (Fig. 2). This was unexpected since *H. illucens* was placed as an outgroup. This result may reflect that partial gene sequence is not appropriate to resolve the phylogenetic status of this taxa. More sequence data are needed to further understand their evolutionary relationship.

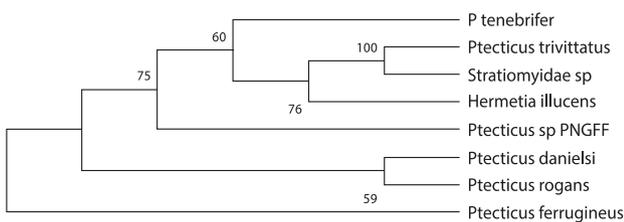


Fig. 2. Phylogenetic relationships of *P. tenebrifer* with other Stratiomyid insects

Competition over the resources

We initially hypothesized that the exotic BSF might encounter severe competition with the endemic *P. tenebrifer* over resources (Park *et al.*, 2017). Although both insects frequently flied over organic matters, there was no detectable competition between the two. Instead, intra-specific interaction, flight interruption, was routinely observed, especially for *P. tenebrifer* adults. This indicates that the introduced BSF might not have experienced any barrier in its settling period. Whether BSF competitively exclude the endemic *P. tenebrifer* in the future remains unknown.

In the field, *P. tenebrifer* should play a pivotal role in decomposing organic wastes. In the future, its ability to decomposition should be assessed. Recently, larvae of the insect have been tested as a food source for pigs (Ao & Kim, 2019). Therefore, this insect should be taken care of for future insect industry. This also will help protect invaluable genetic resources. In conclusion, this study shows that *P. tenebrifer* inhabits nationwide and that its genetic diversity is higher than that of BSF. There may be no interruption between these two insects. However, this needs to be elaborately examined in the future.

Conflict of Interest

The authors declare that they have no competing interests.

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