

Stylet Penetration Behavior of Three Major Rice Planthoppers and Green Rice Leafhopper on Susceptible and Resistant Rice Varieties in Korea Using DC-EPG

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Three rice planthoppers, the brown planthopper (*Nilaparvata lugens*), the white back planthopper (*Sogatella furcifera*), and the small brown planthopper (*Laodelphax striatellus*) and the green rice leafhopper (*Nephotettix cincticeps*) are major homopteran sap-sucking rice pests in Korea. These insect pests commonly have highly modified mouthparts, the stylet bundle, for piercing and sucking. Two pairs of mandibular and maxillary stylets consist of the stylet bundle by interlocking each stylet, which forms the two canals, larger one for food canal and smaller one for salivary canal. Destructive damages result from direct feeding effects (hopperburing) with heavy infestation and/or transmitting virus diseases (Rice stripe virus and Rice black-streaked dwarf virus by *L. striatellus* and Rice dwarf virus by *N. cincticeps*). Damage level is closely related to the feeding behavior of sap-sucking insects, so generally honeydew excretion amount on the resistant rice variety is smaller than that on the susceptible. Therefore, the method to measure the honeydew excretion amount has been primarily used as an indirect way to compare the feeding amount between the susceptible and the resistant. On the other hand, the electrical penetration graph (EPG) technique was firstly developed by McLean and Kinsey (1964) to measure voltage changes during piercing and sucking of insect on the plant. Since specific voltage waveforms were identified and it was known that each waveform is commonly related to salivary and feeding behavior of insect stylets in the plant tissue, EPG technique has been used to real-timely and quantitatively measure feeding behavior of piercing and sucking insects on susceptible and resistant rice variety. However, identifying each different waveform distinctly and understanding biological function of each waveform are certainly necessary to analyze feeding behavior such as the feeding amount of phloem sap.

In this study, the stylet penetration behavior of *N. lugens*, *S. furcifera*, *L. striatellus*, and *N. cincticeps* on rice plants (*Oryza sativa*) was evaluated through the use of a direct current based electrical penetration graph (DC-EPG). To

accomplish this, we classified the EPG waveforms of planthopper group into seven different patterns, np, N1, N2, N3, N4-a, N4-b, and N5, according to their shapes, voltage amplitudes, voltage levels, and frequencies. The N4-b pattern was always preceded by N3 and N4-a, in that order. Continuous honeydew excretion only occurred during the N4-b period, and the honeydew deposited on a filter paper containing ninhydrin reagent during the N4-b period were stained violet. Based on the location of the stylets in the cross-section of rice tissue and honeydew excretion, the EPG waveforms for the stylet penetration behaviors of the three rice planthoppers were assigned to the following groups; np: non-penetration of stylets, N1: penetration initiation, N2: salivation and stylet movement, N3: an extracellular activity near the phloem region, N4-a: an intracellular activity in phloem region, N4-b: phloem sap ingestion, and N5: activity in the xylem region. Furthermore, we classified the EPG waveforms of the green rice leafhopper, *N. cincticeps* into seven different patterns, Nc1, Nc2, Nc3, Nc4, Nc5, Nc6, and Nc7 according to their shapes, voltage amplitudes, voltage levels, and frequencies. The Nc6 pattern was always preceded by Nc5 pattern. The Nc6 pattern of the leafhopper was carefully considered as a phloem sap feeding behavior based on regular honeydew excretion. On the other hand, the planthopper group and the leafhopper hardly showed the phloem sap feeding pattern on resistant rice varieties during an EPG-recording. In addition, the duration of the phloem sap feeding patterns was highly decreased on resistant rice varieties relative to susceptible ones. From these results, it is suggested that the phloem sap feeding related patterns are an important parameter to determine resistance of rice plant.

Key words: *Nilaparvata lugens*, *Sogatella furcifera*, *Laodelphax striatellus*, *Nephotettix cincticeps*, electrical penetration graph (EPG)

In vivo* function of acetylcholinesterase (AChE) in synaptic plasticity and pathogenesis of Alzheimer's disease in *Drosophila

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Dysfunctions of the cholinergic system caused by degeneration or loss of cholinergic neurons in the human brain have been observed in various progressive neurodegenerative disorders including Alzheimer's disease (AD). Acetylcholinesterase (AChE) inhibitors delay the progression of symptoms in mild and moderate AD patients. Recent studies have shown that AChE may form a complex with Presenilin-1 (Psn) and Amyloid precursor protein, genes linked with familial AD pathogenesis. Thus, understanding the role of AChE and Psn in synaptic plasticity is critical to improving our understanding of the onset and progression of AD.

At first, we investigated the consequence of altered AChE activity in the nervous system by utilizing *ace* hypomorphic mutant animals. We found that the amount of *ace* mRNA and the activity of AChE both *in vivo* and *in vitro* were significantly reduced compared with those of controls in *ace* hypomorphic mutant fly larvae. Reduced *ace* in *Drosophila* larvae resulted in significant down-regulation of branch length and the number of boutons in Type 1 glutamatergic neuromuscular junctions (NMJs). In pharmacological inhibition experiments of AChE activity, we found that controls exposed to a sublethal dose of DDVP phenocopied the synaptic structural defects of the *ace* hypomorphic mutant.

In addition, we also found that AChE activity in a functionally null *psn* mutant (*psn*^{B3}) was significantly reduced and that *psn*^{B3} mutant NMJs had smaller synaptic boutons and altered localization of Discs large (DLG), a synaptic scaffolding protein at the synaptic terminals compared to wild-type controls.

These results indicate that down-regulation of AChE activity results in altered synaptic architecture and that Psn is important for regulation AChE activity, the size of synaptic boutons, and the localization of DLG at synaptic terminals. Our study suggests that these changes may underlie or contribute to the onset and progression of AD.

Key words: Acetylcholinesterase, Alzheimer's disease, Synapse development

감귤원 네눈썹가지나방(*Ascotis selenaria*) 발생 및 개체군 모형

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감귤원에서 7종의 자나방이 조사되었고, 이 중 네눈썹가지나방(*Ascotis selenaria*)의 발생량이 많았다. 감귤 과실 피해는 주로 네눈썹가지나방 유충에 의해 발생하는데, 노지 온주밀감은 1~3령의 저령기 유충이 주로 가해하여 무정형의 피해흔을 남기는 반면, 하우스 부지화는 6령 유충에 의해 크게 피해를 입는다. 이러한 피해증상의 차이는 감귤의 부지화(*Shiranuhi*: [*C. unshiu* × *C. sinensis*] × *C. reticulata*)와 온주밀감(*Citrus unshiu*)의 착과 특성에 따른 차이로 보인다. 감귤원에서 자나방류 감귤 과실 피해율은 2008년부터 2010년까지 평균 4.5%였다. 감귤원에서 네눈썹가지나방 유충의 공간분포는 무작위분포에 가까운 수치의 군집지수를 보였다.

온주밀감의 신초와 과실의 피해양상을 보면, 5월 중순에 신초피해가 처음 발생하였고, 6월 상중순에 최성기를 보였다. 이후 봄순이 경화되는 시기인 6월 하순부터 급격히 감소하여 여름순(7~8월)과 가을순(9~10월)에는 피해가 적었다. 과실은 봄순이 경화되는 시점(6월 하순 또는 7월 초)부터 증가하여 7월 말경 급증하였다. 실제 유충 발생시기는 잎과 과실 피해와 비슷하게 발생하는데, 유충은 5월 중순에 처음 발생하여 6월 중순에 발생최성기를 보였다. 이후 불규칙한 양상을 보이는데 7월 하순과 9월 상중순에 약한 발생피크를 보였다. 성충 발생은 5월 중순에 발생최성기를 보였고, 7~9월은년도에 따라 복잡한 발생양상을 보이거나, 대개 7월 상순부터 하순, 8월 하순~9월 상순에 발생 피크를 보였다.

효과적인 네눈썹가지나방의 방제시기를 예측하기 위하여 실내에서 온도별 네눈썹가지나방의 발육기간과 산란을 조사하여 네눈썹가지나방의 개체군 모형을 구축하였다. 네눈썹가지나방의 발육영점온도와 유효적산온도는 각각 알이 10.98°C와 83.7 DD, 유충이 9.68°C와 340.7 DD, 용이 9.07°C와 200.7 DD였다. 비선형 모형으로 알, 유충, 용의 발육속도(1/median day)에 Hilbert와 Logan 모형을, 생리적 연령에 따른 발육완료 분포 값에 Weibull 함수를 적용하여 매개변수를 추정하였다. 산란모형은 총산란수 모형, 생리적 연령에 따른 누적산란율 모형, 생리적 연령

에 따른 생존율 모형으로 구성하였다. 네눈썹가지나방의 개체군 모형은 알, 유충, 용, 산란 모형을 구성 요소로 하여 각 단계가 완료 될 때마다 전이 되도록 구성하였다. 월동용을 2 : 3 : 3 : 2 비율로 나눈 그룹을 만들고, 각각의 그룹에 생리적 연령을 -0.3, 0, 0.3, 0.6을 할당하였다. 이 시나리오 조건으로 모의 실행하였을 때 실제 포장에서 1령 유충과 성충의 발생양상과 발생 최성기가 유사하게 모의되었다.

검색어: 감귤, 네눈썹가지나방, *Ascotis selenaria*, 개체군 모형, 발생 생태

Comparative genomic analysis of the UDP-glycosyltransferase multigene family in insects

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UDP-glycosyltransferases (UGT) catalyze the conjugation of a range of diverse small lipophilic compounds with sugars to produce glycosides, playing an important role in the detoxification of xenobiotics and in the regulation of endobiotics in insects. Recent progress in genome sequencing has enabled an assessment of the extent of the UGT multigene family in insects. Here we report over 310 putative UGT genes identified from genomic databases of eight different insect species together with a transcript database from the lepidopteran *Helicoverpa armigera*. Phylogenetic analysis of the insect UGTs showed Order-specific gene diversification and inter-species conservation of this multigene family. Only one family (UGT50) is found in all insect species surveyed (except the pea aphid) and may be homologous to mammalian UGT8. Three families (UGT31, UGT32, and UGT305) related to Lepidopteran UGTs are unique to baculoviruses. A lepidopteran sub-tree constructed with 40 *H. armigera* UGTs and 44 *Bombyx mori* UGTs revealed that lineage-specific expansions of some families in both species appear to be driven by diversification in the N-terminal substrate binding domain, increasing the range of compounds that could be detoxified or regulated by glycosylation. By comparison of the deduced protein sequences, several important domains were predicted, including the N-terminal signal peptide, UGT signature motif, and C-terminal transmembrane domain. Furthermore, several conserved residues putatively involved in sugar donor binding and catalytic mechanism were also identified by comparison with human UGTs. Many UGTs were expressed in fat body, midgut, and Malpighian tubules, consistent with functions in detoxification, and some were expressed in antennae, suggesting a role in pheromone deactivation. Transcript variants derived from alternative splicing, exon skipping, or intron retention produced additional UGT diversity. These findings from this comparative study of two lepidopteran UGTs as well as other insects reveal a diversity comparable to this gene family in vertebrates, plants and fungi and show the magnitude of the task ahead, to determine biochemical function and physiological relevance of each UGT enzyme.

Key words: UDP-glycosyltransferase, *Helicoverpa armigera*, *Bombyx mori*, multigene family, phylogenetic analysis, detoxification

Population Genetics and Disease Ecology of Mosquito Species in Galapagos

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The introduction of new pathogens and disease vectors has been recognized as a major threat to Galapagos Island biodiversity. Here I focus on mosquito species of the Galapagos archipelago, using population genetic and phylogenetic data to understand their historical and current population dynamics. I show that two mosquito species found in the archipelago have very different historical and contemporary evolutionary histories: one species, *Aedes taeniorhynchus*, naturally colonized the archipelago 200,000yrs ago and is now found widely in the islands, having adapted and spread to a range of different habitats. It has also changed its feeding-behaviour and now frequently feeds on reptiles in addition to mammals, unlike the continental progenitor populations. These properties potentially make *Aedes taeniorhynchus* a key bridge-vector in the archipelago for any new invading mosquito borne diseases. In contrast, I show that *Culex quinquefasciatus*, a major vector of diseases such as West Nile virus and avian malaria, has been introduced on multiple occasions since 1985 via human transportation networks and that its distribution and movement in the archipelago depend greatly on human activities. These two species might play an important role in the introduction and spread of new diseases in the Galapagos archipelago.

Evolution of the Aphidini aphids (Hemiptera: Aphididae): integrating phylogenetic and population genetic approaches

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Aphids (Hemiptera: Aphididae) are well known as micro-insect pests, which are very specific to their host plants, sucking phloem for acquiring nutrients, and most of them have successfully maintained parthenogenetic generations cyclically or permanently. In the world, the approximately 5,000 described aphid species belong to the family Aphididae, which has taxonomically been subdivided into 27 subfamilies in current. The diversification of host plants, especially angiosperms, has played an important role in their evolution. Major questions about aphid evolution include origins of host alternation as well as age and patterns of diversification in relation to host plants. To address these, I did both macroscale (phylogenetics) and microscale (population genetics) researches on aphids.

First I reconstructed the phylogeny of the three major aphid groups, Aphidini, Macrosiphini, and Pterocommatinae, which are the most diverse in the world and constitute more than 60% of the total species. These major lineages demonstrate the evolutionary history of aphids interacting with their host plants. I also used molecular dating method to calculate reasonable divergence time on each clade. Based on phylogenetic and dating analyses, most generic divergences in Aphidinae occurred in the Middle Tertiary when primary hosts, mainly Rosaceae, were diverging, whereas species-level divergences were related with diversification of secondary hosts such as Poaceae in the Middle to Late Tertiary. Most generic divergences in Aphidini occurred in the Middle Tertiary, and species-level divergences occurred between the Middle and Late Tertiary. The divergence times of aphid lineages at the generic or subgeneric levels are close to those of their primary hosts.

Second I performed population genetics of the polyphagous cotton-melon aphid, *Aphis gossypii* Glover. I analyzed population genetic structure between 570 aphids collected from 41 plant species of primary and secondary, mostly wild, hosts using 9 microsatellite loci. As results, population structure of *A. gossypii* revealed that several genetic affinities in common use of some secondary and primary hosts are detected. Host preference in secondary host is higher than that in primary host, and

woody plants share same genetic structure. This species might speciated by the related mechanisms such as host alternation and loss of primary host.

I will propose macro- and micro-evolutionary patterns of the Aphidini aphids based on integrating phylogenetic and population genetic approaches.

Key words: aphid, Aphididae, Aphidini, *Aphis*, phylogeny, population genetics

Entomopathogenic Fungi: Biology and Applications

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Entomopathogenic fungi have high potential in controlling harmful pests in agriculture and forest, but their slow progress in insect killing and low thermotolerance are major impediments to successful industrialization. Two possible efforts were done to overcome these problems. First the use of supernatant of a *Beauveria bassiana* isolate reduced the population of cotton aphid with a dosage-dependant manner, which allowed a quality control factor to be determined for the evaluation of the supernatant as the first step of a development. Chitinase was one of the major pathogenesis-related enzymes in the supernatant. A mineral material-mediated chitinase precipitation method was established to enhance the thermotolerance of chitinase. The use of supernatant can be a quicker way to control aphids. Secondly, to increase thermotolerance the effects of nutritional manipulation of culture media and oil-coating of conidia of *B. bassiana* and *Metarhizium anisopliae* isolates were investigated, followed by pairing of similar isolates. Recently, thermo-susceptible *B. bassiana* mutants were generated by a fungal transformation platform to discover thermotolerance-related genes, which are now underdetermination. Genetic information on fungal thermotolerance is going to be clear in near the future.

Key words: entomopathogenic fungi, chitinase, thermotolerance, *Beauveria bassiana*, *Metarhizium anisopliae*

Suppression of Pheromone Biosynthesis via RNA interference

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Sex pheromone production is regulated by pheromone biosynthesis-activating neuropeptide (PBAN) in many lepidopteran species. A cDNA isolated from female adult heads of *Plutella xylostella* encodes 193 amino acids including PBAN, designated as Plx-PBAN. When female adults were injected with synthetic Plx-PBAN, pheromone production showed a maximal increase 1h post-injection. RT-PCR screening revealed that Plx-PBAN cDNA was expressed in all examined body parts, with the highest expression level in the head of female adults. The PBAN receptor (Plx-PBANr) gene was also cloned from the female pheromone gland and has conserved structural motifs implicating in promoting G protein coupling and tyrosine-based sorting signaling along with seven transmembrane domains. The expression of Plx-PBANr was found only in the pheromone gland of female adults among examined tissues and developmental stages. Heterologous expression in human uterus cervical cancer cells revealed that Plx-PBANr induced significant calcium elevation when challenged with Plx-PBAN. Female *P. xylostella* injected with double-stranded RNA specific to Plx-PBANr showed suppression of the receptor gene expression and exhibited significant reduction in pheromone biosynthesis, which resulted in loss of male attractiveness. In addition, to assess molecular events occurring downstream of PBAN signaling, partial sequences of $\Delta 9$ and $\Delta 11$ fatty acid desaturases of *P. xylostella* were cloned. Phylogenetic analysis indicated that these two desaturase genes were highly clustered with other desaturases associated with sex pheromone biosynthesis in other insects. RT-PCR analysis showed that $\Delta 9$ desaturase was dominantly expressed in adult females, whereas $\Delta 11$ desaturase was expressed in all developmental stages. When PBANr expression was suppressed by PBANr-RNAi, the treated females also showed significant suppression of expression of both desaturases. These results suggest that expressions of the two desaturases are controlled by PBAN and that the two desaturases may be involved as downstream components in sex pheromone biosynthesis of *P. xylostella*.

Key words: PBAN, PBAN receptor, RNA interference, desaturase, pheromone, *Plutella xylostella*