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## A MICROSCOPIC STUDY ON THE BURSA COPULATRIX OF KOREAN PIERIDAE (LEPIDOPTERA)\*

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### Abstract

The innersurfaces of corpus bursae of Korean Pieridae were observed. On the innerwall of bursa copulatrix, many fine processes were partly distributed and the shapes were varied to the species. The conditions and the shapes were almost similar to the genus. The foldings and processes seems to be related to the efficiency of holding the spermatophore. The developmental trend of processes was opposite to that of Signum, and the strong processes in some species were considered as the substitution of signum. The phenograms for female Pieridae were obtained. Through the four phenograms, each genus was closely combined. From the results, it was clear that the characters from the female genitalia were very significant to the classification of Pieridae.

### Introduction

The study on the bursa copulatrix of Korean Pieridae has been carried out by Cheong, Park and Lee (1988), on the histology of the reproductive system of female moth was by Outram (1971), and a cluster analysis on the Korean Pieridae was by Cheong, Park and Lee (1988).

Authors found that many processes except for the signum were distributed on the innersurface of bursa copulatrix on the way of stereoscopic observation, and compared fine structures among species.

In this study, the bursa copulatrix of Korean Pieridae were observed with scanning electron microscope, and the cluster analyses on the basis of female genitalia were carried out. Thus, the phenomenal classification system using the characters selected from female genitalia were compared with reference systematic relationship.

Moreover, the role of the processes in the reproduction, the correlation between the processes and the Signum, and the evolutionary trends of them were inferred.

### Materials and Methods

Specimens of twelve Pierid species were collected from various parts of Korea. The dry

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specimens of females were used for this study and the abdomens were dissected after boiled in 10% KOH. The bursa copulatrix were turned inside out in saline for the observation of innersurfaces. They were undergone alcohol series for the dehydration. After coated with 200 Å gold, observed with ISI-SS40 SEM (Dawes, 1984).

For the cluster analyses of female, forty-one morphological characters were selected from bursa copulatrix. The character states used for the analyses were both qualitative and quantitative (Table 2). The clustering methods employed in the study were single linkage, complete linkage, between group method and ward method (Sokal & Sneath, 1963 ; Tadauchi, 1977).

Table 1. The conditions and the shapes of processes on the bursa copulatrix.


species	average length of the processes unit : $10^{-5}$ cm	average distance among processes unit : $10^{-4}$ cm	shape of process
<i>P. napi</i>	6.6	1.4	
<i>P. melete</i>	6.5	1.7	
<i>P. rapae</i>	5.2	1.5	
<i>P. canidia</i>	4.8	1.5	
<i>G. rhamni</i>	8.3	1.4	
<i>G. aspasia</i>	7.6	1.1	
<i>E. hecabe</i>	4.1	0.8	
<i>E. laeta</i>	2.3	1.2	
<i>C. erate</i>	5.2	1.2	
<i>P. dapludice</i>	5.5	2.1	
<i>L. amurensis</i>	22.3	2.1	
<i>A. scolymus</i>	6.9	1.7	

Table 2. The characters from female genitalia of Korean Pieridae.

1. existence of <b>signum</b>	22. apex of innersurface process round
2. direction of <b>signum</b> horizontal	23. apex of innersurface process sharp
3. direction of <b>signum</b> vertical	24. processes with basal ridges
4. median scierite of <b>signum</b>	25. process shaped like a spine
5. condition of <b>signum</b>	26. process shaped like a papilla
6. <b>signum</b> attached on the dorsal	27. process shaped like a spindle
7. <b>signum</b> attached on the entrance	28. process shaped like a horn
8. <b>signum</b> shaped like a heart	29. innersurface without foldings
9. <b>signum</b> elongated	30. ratio length to width 1.0-1.2
10. <b>signum</b> shaped like a ribbon	31. ratio length to width 1.3-1.9
11. <b>signum</b> shaped like a belt	32. ratio length to width above 2.0
12. <b>signum</b> with horns on both poles	33. distance among processes shorter than $0.8 \times 10^{-4}$ cm
13. <b>signum</b> with free leaves in bursa	34. distance among processes 0.9-1.9
14. appendix bursa > corpus bursa	35. distance longer than 2.0
15. appendix bursa < corpus bursa	36. ratio length to width of <b>signum</b>
16. appendix bursa $\approx$ corpus bursa	37. length of corpus b/appendix b.
17. median duct long (c. b. & a. b.)	38. length of process/width of process
18. corpus bursa swelled dorsally	39. average distance among processes
19. corpus bursa shaped spherical	40. length of <b>signum</b> 's spine/length of <b>signum</b>
20. corpus bursa shaped like a stomach	41. length of attached site/length of <b>signum</b>
21. corpus bursa with deep foldings	

### Results

The innersurfaces of bursa copulatrix had one **signum** and many processes. The processes on the corpus bursa were observed in this study, but they were also distributed on the ductus bursa and appendix bursa. The processes on the latter two parts had almost same shapes with that of the former part. Processes were partly distributed on the bursa, and were not distributed around the **signum**.

*Pieris napi* : Foldings of inner-wall distinct. Ridges on the basis of process developed. Apex sharp (Fig. 1).

*P. melete* : Foldings not distinct. Process shaped like a weak spine, the basis of process appear thin and easily flexible (Fig. 2).

*P. rapae* : Foldings distinct. Apex sharper than *P. napi*, but weak. Ridges developed (Fig. 3).

*P. canidia* : Folding not distinct. Apex sharp, but weak and shaped like a scale. Ridges not developed (Fig. 4).

*Gonepteryx rhamni* : Foldings very distinct. Process shaped like a tongue. Apex rounded. Ridges not developed (Fig. 5).

*G. aspasia* : Foldings distinct. Process shaped like a spindle. Ridges weakly developed (Fig. 6).

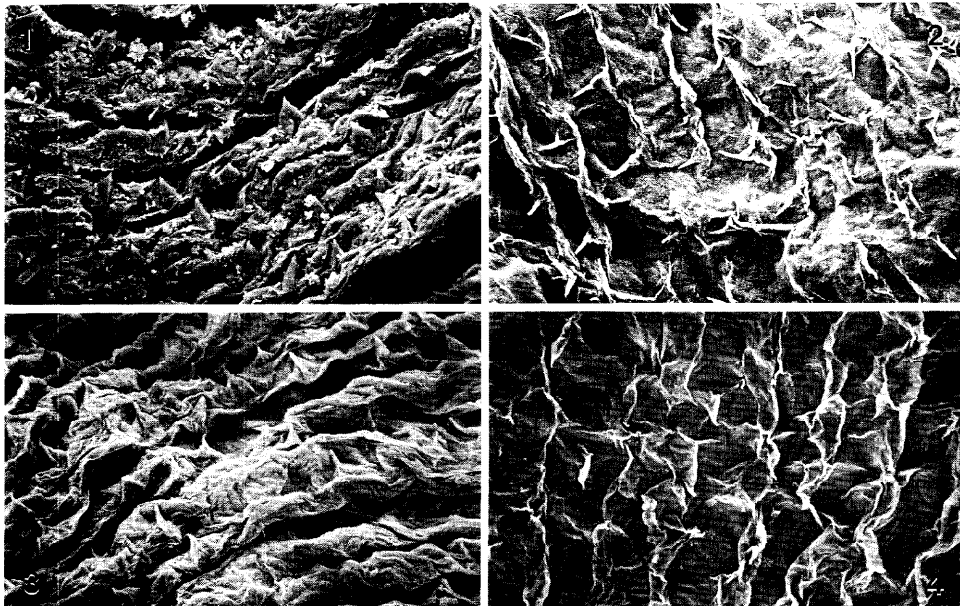
*Eurema hecabe* : Foldings distinct. Process shaped like a papilla. Ridges not developed (Fig. 7).

*E. laeta* : Foldings distinct. Tip of process flat. Ridges not developed (Fig. 8).

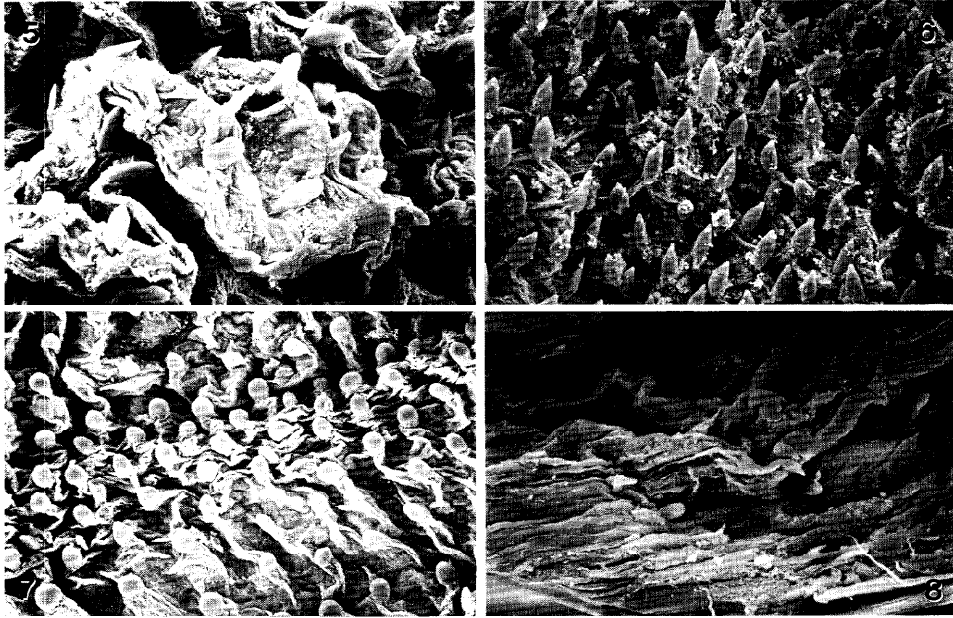
*Colias erate* : Similar to *G. aspasia*. Foldings not distinct. Ridges not developed (Fig. 9).

*Pontia daplidice* : Foldings not exist. Process shaped like a triangle scale. Apex not sharp. Ridges not developed (Fig. 10).

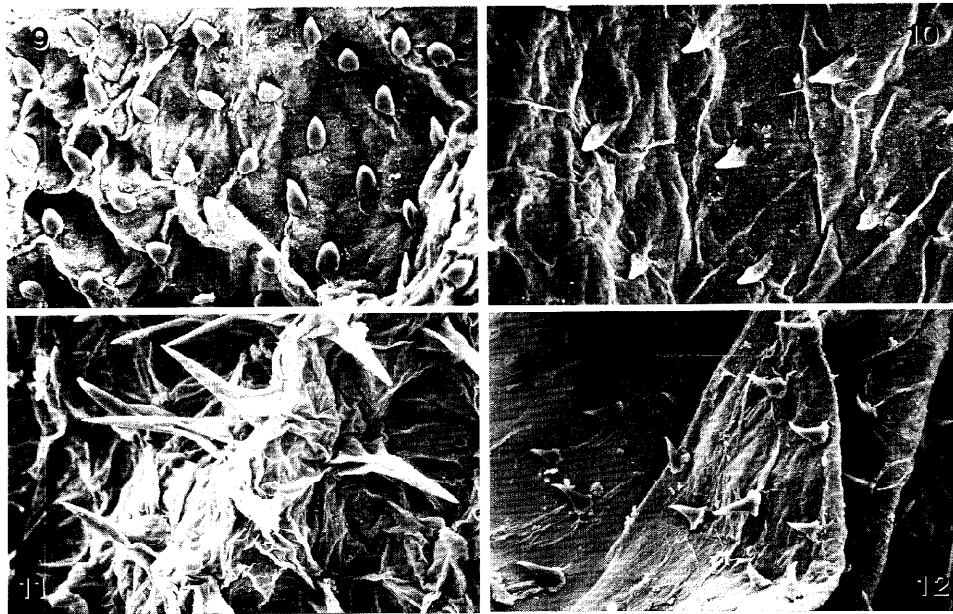
*Anthocharis scolymus* : Foldings not exist. Process shaped like a rose's thorn. Ridges not developed.



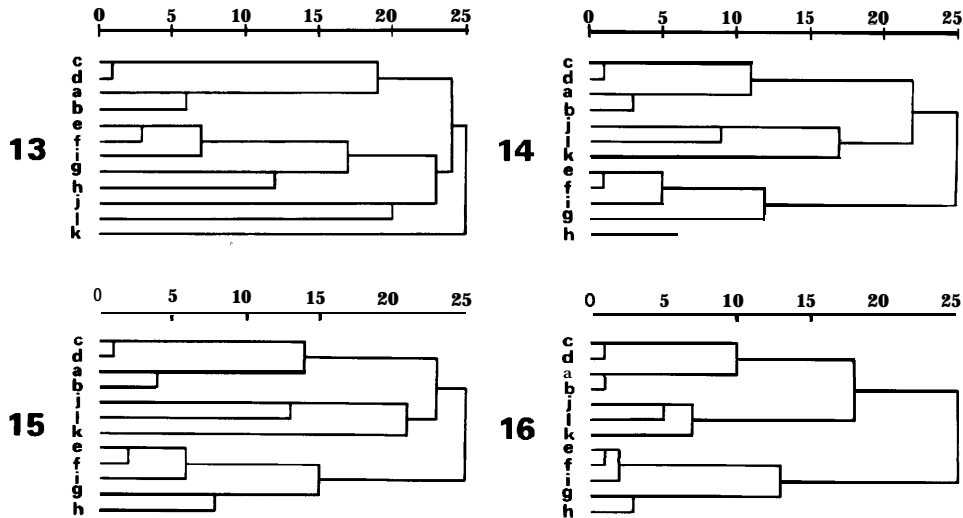
**Figs. 1-4.** The innersurfaces of the bursa copulatrix of Pieridae. 1 : *P. napi*, 2 : *P. melete*, 3 : *P. rapae*, 4 : *P. canidia*.



**Figs. 5-8.** The inner surfaces of the bursa copulatrix of Pieridae. 5 : *G. rhamni*, 6 : *G. aspasia*, 7 : *E. hecabe*, 8 : *E. laeta*.



**Figs. 9-12.** The inner surfaces of the bursa copulatrix of Pieridae. 9 : *C. erate*, 10 : *P. daplidice*, 11 : *L. amurensis*, 12 : *A. scolymus*.



**Figs. 13-16.** Phenograms obtained from female genitalia of Korean Pieridae. 13 : Single linkage, 14 : Complete linkage, 15 : Between-group, 16 : Ward. a : *P. napi*, b : *P. melete*, c : *P. rapae*, d : *P. canidia*, e : *G. rhamni*, f : *G. aspasia*, g : *E. hecabe*, h : *E. laeta*, i : *C. erate*, j : *P. daplidice*, k : *L. amurensis*, l : *A. scolymus*. Scales : Rescaled Distance Coefficient

The detail conditions and shapes unnoted above is described in Table 1. The longest processes were those of *L. amurensis*, and the most densely distributed were those of *E. hecabe*.

From the analyses using four clustering methods, the phenograms of female Pieridae were obtained (Figs. 13-16). In the phenogram from single linkage, genus *Pieris* and subfamily Coliadinae were closely combined respectively, and *L. amurensis*, subfamily Dismorphinae was well separated. But from the other methods, genus *Pieris*, subfamily Coliadinae and three species, *P. daplidice*, *L. amurensis* and *A. scolymus* were closely combined respectively. In all methods, of course, the species of each genus were very closely combined. *C. erate* was combined with genus *Gonepteryx* in all methods, and the three species, *P. daplidice*, *L. amurensis* and *A. scolymus* were together combined with genus *Pieris* in three methods except for single linkage.

### Discussions

The roles of bursa copulatrix in Lepidoptera are holding of the spermatophore and scrapping out of it, thus helping the release of sperm. Of course the *signum* carry out the main function in tearing out the spermatophore (Chapman, 1982). But the processes observed in this study seemed to holding in order to the *signum* tear out the spermatophore by contraction of the muscles.

The processes on the innerwall is varied to the species, and it seems to related to the existence of *signum*. In the species having not *signum*, very strong horn-like processes were replaced with *Signum*. For example, *L. amurensis* and *A. scolymus* have big, sharp and rigid processes instead of *signum*. In these cases, the processes were seemed to carry out scrapping as well as holding.

The *signum* is considered a special chitinous organ that is more sclerotized and specialized in a group of processes. Therefore, the conditions that the *signum* is not *differenci*ated and having strong

processes were considered as the plesiomorphic state. In the case of the ridges developed on the basis of process, they were raised, but in the weak or having not the ridges, the processes laid down. And in the strong processes it could supported by itself without the help of ridges. In conclusion, as the bursa copulatrix was originated from the epidermis, and have not any gland by Outram, the processes seems mainly carry out physical role.

The trend of the development of processes is considered opposite to that of signum and the ridges are considered as having the supporting role of it.

As the solidity of each spermatophore may be different to the species, it is desirable, if we can, that all the conditions of process, signum and spermatophore to be considered.

From the phenograms of female Pieridae, it is enough to reflects the phylogeny. Though it is a result of phenomenal classification, is considerably agreed to that of phylogenetic analysis. *P. rapae* and *P. canidia* were most closely combined in Pieridae, and genus *Pieris* formed most stable cluster. In cluster formation, the unstabilities of *P. daplidice*, *L. amurensis* and *A. scolymus* may be caused by using only one species to each genus.

In this study, it is clear that the characters from female genitalia greatly contribute to classification. Consequently, as the shapes of male genitalia are very important in classifying most Lepidoptera, the structures of female genitalia are also important and diagnostic in classification of Pieridae.

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