Commun. Fac. Sci. Univ. Ank. Ser. C
V. 6, pp. 73–84 (1988)

STUDIES ON THE EXTERNAL MORPHOLOGY OF THE EGGS OF SOME *MELITAEA* SPECIES *(SATYRIDAE: LEPIDOPTERA)*

by ZEKİYE SULUDERE

Department of Biology-Faculty of Art and Sciences-University of Gazi, Ankara, Turkey

ABSTRACT

The eggs of four species of the genus *Melitaea* Fabricius were studied by scanning electron microscopy. To obtain the eggs from dried specimens, some methods were tested.

Key words: Lepidoptera, egg, morphology, SEM, technique.

INTRODUCTION

Studies carried out on the eggs of insects pointed out that they present morphological characteristics, which can be utilized in the taxonomic investigations. Especially their shape and chorionic structure vary greatly among the higher taxonomical groups. These features are less conspicuous among the closely related species but almost constant.

Observations by light microscopy on the morphology of the butterfly eggs were formerly carried out especially by Chapman (1896) and Clark (1900). Since only limited chorionic features are available by the light microscopy, this stage of many insect groups has been very little studied in the earlier period. On the other hand, the scanning electron microscopy (SEM) contributes to much better morphological definitions. It reveals detail of the chorion that is of considerable value in determining species, as demonstrated by a number of recent studies (Rowley and Peters, 1972; Salkeld, 1973; Ward and Ready, 1975; Suludere, 1977; Downey and Allyn, 1979, 1980; Chauvin and Chauvin, 1980; Regier et al., 1980; Arbogast and Byrd, 1981, 1982; Casperson et al., 1983; Viscuso and Longo, 1983; Wagener, 1983; Griffith and Lai-Fook, 1986).

In the literature, an extensive review was made by Hinton (1981) regarding the diversity in the sculpture and chemical composition of the chorion of insect eggs, including some Lepidoptera.

It should be noted that SEM micrographs of eggs have been frequently included in many works of systematics over last few years. Chorionic characters have been used successfully in determining species and by using these features, keys to some groups have been established (Salkeld, 1975, 1976; Arbogast et al., 1980; Davidova–Vilimova, 1987).

The aim of the author is to carry out SEM investigations on the little or unknown external morphology of the eggs of Lepidoptera, as its characteristics appear to be of high taxonomic value. Within the frame of this aim, the chorion morphology of some species of the genus *Melitaea* Fabricius have been investigated in this paper. Besides, new techniques for preparing the eggs have also been tested.

MATERIALS AND METHODS

In this paper, the eggs of *Melitaea didyma* and its closely related species *M. transcaucasica, M. persea,* and *M. fascelis* were examined by scanning electron microscopy.

The collecting data of each species are as follows:

Melitaea didyma ESPER

1 ♀ Turkey: Ankara, Elmadağ, 7.7.1971

1 ♀ Turkey: Ankara, Çiftlik, 29.5.1967

1 ♀ Turkey: Ankara, Çiftlik, 6.6.1967

1 ♀ Turkey: İstanbul, Pendik, 5.8.1968

1 ♀ Poland: Leg, E. Palik

Melitaea transcaucasica TURATI

1 ♀ Turkey: Trabzon, Zigana, 18.7.1973

1 ♀ Turkey: Artvin, Ardanuç, 2.8.1972

1 ♀ Turkey: Rize, Sivrikaya, 1.8.1972

Melitaea persea KOLLAR

1 ♀ Turkey: Diyarbakır, Çüngüş, 22.6.1972

Melitaea fascelis ESPER

1 ♀ Turkey: Ankara, Kepekli, 23.6.1970

The eggs were obtained from the dried specimens of the species mentioned above. The stages of the preparation are given below:

The abdomens were cut off and soaked in a very dilute solution of 0.2 % of tribasic sodium phosphate ($Na_3PO_412H_2O$) for about 6h prior to dissection (Salkeld, 1980). The abdomens were full of eggs which were glued to each other within the long axis of ovarioles. The eggs were separated from each other and from the ovariolar sheat and fat body etc., by using fine needles and tweezers. The cleaned eggs were prepared for SEM by using different methods. The best result was obtained from the emptied eggs; they may also be called as "egg ghost". They can be easily obtained by removing the egg content during the preparation. After dehydration in aseton, the empty eggs were dried in the air.

The dried eggs were mounted on Jeol holders by means of double -sided tape, coated with gold and examined in an Jeol 100 CX II electron microscope at 20 KV.

Approximately 30 eggs of each species were examined. Height and width were measured from a sample of 10 eggs of each species on the display screen of the microscope at a magnification of x 100. The maximum dimension of micropylar pits on the different eggs was determined from screen or photographs at x 3000. Counts of primary cells and ridges were made on the examined eggs either from the screen or from the micrographs.

Results are presented in both descriptive and photographic form. It was not possible to show comparable SEM views of all egg samples examined. The photographs of the eggs and of the surface structures of their chorion are typical of the several eggs examined for each species.

The terminology used in describing the chorionic features of the *Melitaea* eggs follows that of Salkeld (1984).

OBSERVATIONS

1– *Melitaea didyma* ESPER

The eggs are spheroidal, more or less circular in outline, and somewhat flattened in the micropylar area (Fig. 1 a, b). They are 0.72 ∓ 0.01 mm in width and 0.74∓ 0.01 mm in height.

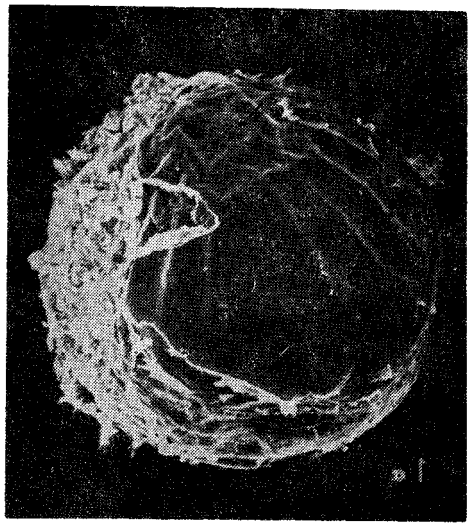

Fig. 1.a) The egg of *M. didyma*, arrow points to the micropylar area. L. Longitudinal ridge, Tr. Transverse wall. 100 X.

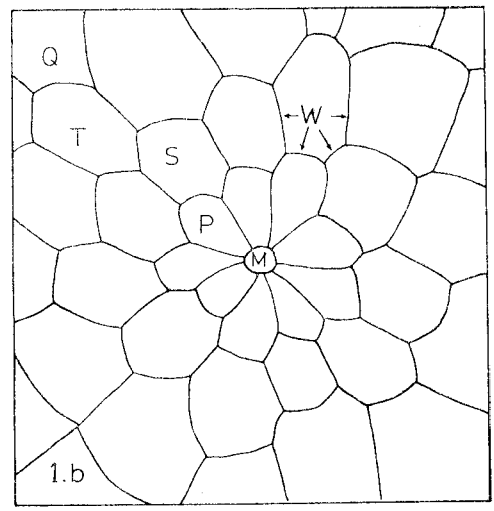

Fig. 1.b) A diagram of micropylar area. M. Micropylar pit, P. Primary cell, S. Secondary cell, T. Tertiary cell, Q. Quaternary cell, W. Cell walls.

From the micropylar area, 19–22 longitudinal ridges radiate and 1–2 ridges start from the equator, extend downward. All longitudinal ridges evanesce near the equatorial line. There are some transversal ribs, crossing the longitudinal ridges, which are often called as "transverse walls". In this species, these walls generally are much less distinct than the longitudinal ridges.

With an exception, the micropylar pit is surrounded by a rosette of 7–9 petal shaped primary cells which are more or less similar in size and in shape (Fig. 2). The micropylar pit is almost circular and about 6 μ in width and the micropylar openings are not discernable in it. The secondary cells that surround the rosette cells are clearly visible and easy to count. They are similar to each other being almost polygonal, and their length to width are nearly equal. The tertiary and qua-

ternary cells are also polygonal. Especially the primary and the secondary cells of micropylar area are arranged, and their walls are well marked.

Exceptionally the eggs obtained from one of the melanic female have 15–19 rosette cells contrary to 7–9 of the other *M. didyma* (Fig. 3). The central pit is about 11 μ in width and irregularly circular in outline of this sample. Besides, the primary and secondary cells are narrow and longer than those of the other *M. didyma*.

The rest of the chorion has no remarkable surface pattern.

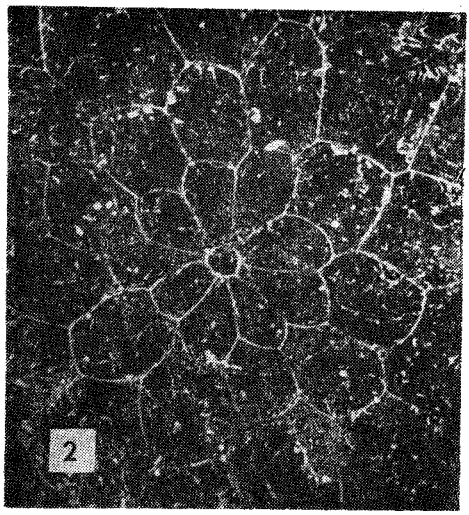

Fig. 2. The micropylar area of normal *M. didyma* with a rosette of 9 petal shaped primary cells. 1 000 X.

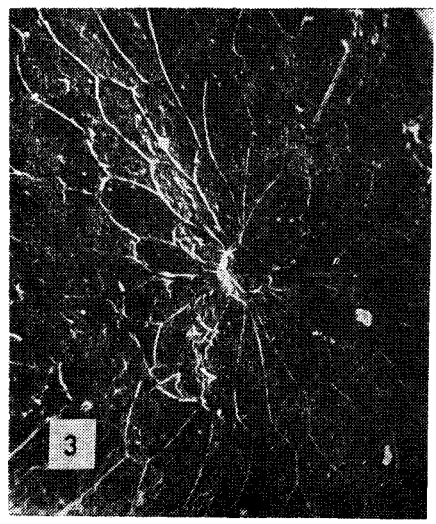

Fig. 3. The micropylar area of one of the melanic *M. didyma* with a rosette of 16 petal shaped primary cells. 1000 X.

2– *Melitaea transcaucasica* TURATI

The eggs are spheroidal, more or less circular in outline, somewhat flattened in the micropylar area (Fig. 4). The eggs have an average width of 0.72 ∓ 0.01 mm and height of 0.72 ∓ 0.01 mm.

18–19 longitudinal ridges radiate from the micropylar area and between some of them 2–3 more ridges originate from above the equator. All of them are faint and gradually disappear below the equator. The transverse walls are much less distinct than the longitudinal ridges.

The micropylar pit is surrounded by a rosette of 10–12 petal shaped primary cells (Fig. 5). It is irregular in shape without a wall. It is about 11 μ in width and has some openings of the micropylar canals which are difficult to count. The primary cells are surrounded by the secondary cells which are broader and longer than the primary ones. The primary cells and the other series of cells are distinct but they are delinated by extremely fine walls. The tertiary and quaternary cells are polygonal in various sizes and arranged irregularly.

The rest of the chorion is almost unmarked.

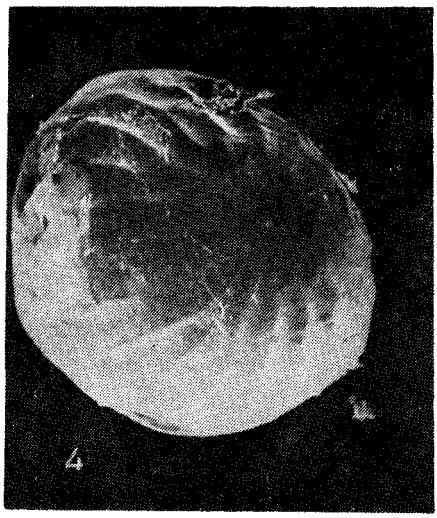

Fig. 4. The egg of *M. transcaucasica*. 100 X.

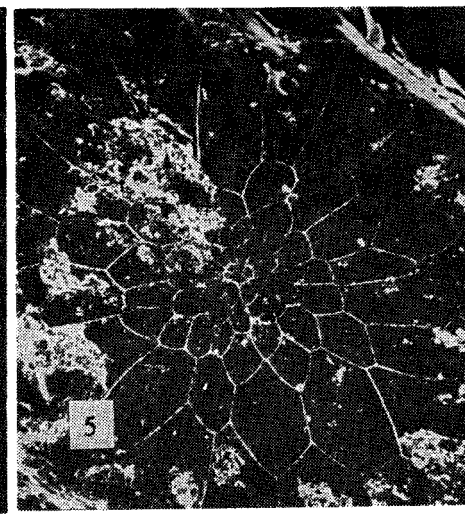

Fig. 5. The micropylar area of *M. transcaucasica*. 500 X.

3– *Melitaea persea* KOLLAR

The eggs are spheroidal with somewhat flattened micropylar area and are more or less circular in outline (Fig. 6). They are 0.69 ∓ 0.01 mm in width and 0.71 ∓ 0.01 mm in height.

22–23 of the 24–26 longitudinal ridges radiate from the micropylar area. The other ridges originate from above the equator. All longitudinal ridges are slightly elevated and gradually disappear below the equator. The transverse walls, which are less distinct than the longitudinal ridges, are slightly raised causing especially upper part of the eggs to appear pitted.

The cells of the micropylar area are delinated by extremely fine walls (Fig. 7). The 8–11 petal-shaped primary cells or rosette cells surround the central micropylar pit which varies in shape. The central pit is about 10μ in width at its broadest point and the openings of micropylar canals are not discernable in it. The rosette cells are always surrounded on their outer edges by another series of secondary cells. The secondary cells are variable in shape, being usually roughly quadrate, elongate and longer and broader than the primary cells. The tertiary and quaternary cells are poorly defined that their shape and size are difficult to determine.

The rest of the chorion is almost smooth, without any remarkable feature.

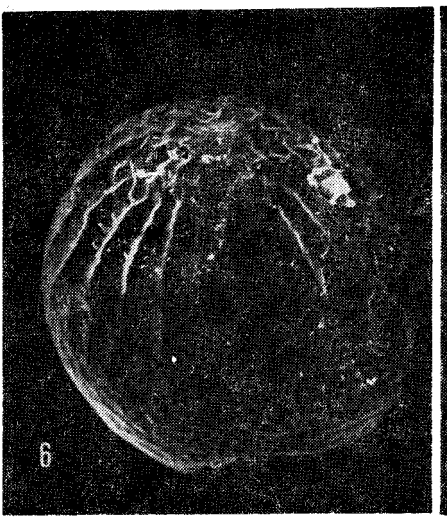

Fig. 6. The egg of *M. persea*. 100 X.

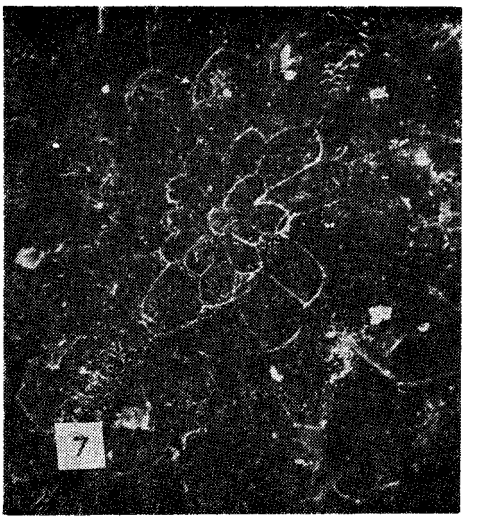

Fig. 7. The micropylar area of *M. persea*. 500 X.

4– *Melitaea fascelis* ESPER

The eggs are spheroidal, more or less circular in outline, and somewhat flattened in the micropylar area (Fig. 8). They are 0.62 ∓ 0.01 mm in width and 0.67 ∓ 0.01 mm in height.

21–23 longitudinal ridges originate from the micropylar area. All of them are slightly elevated and evanesce below the equator of the egg. The transverse walls are much less distinct than the ridges.

The micropylar rosette is composed of 8–10 petal shaped primary cells of the examined eggs (Fig. 9). The central micropylar pit which has a circular wall is about 6μ in width and the openings of micropylar canals are not discernable in it. All cells of the micropylar area are distinct with fine walls and each series is different from the others in shape and size. The secondary cells are broader and longer than the primary cells. The tertiary and quaternary cells are also broader and longer than the primary and secondary cells.

The rest of the chorion shows no remarkable structure.

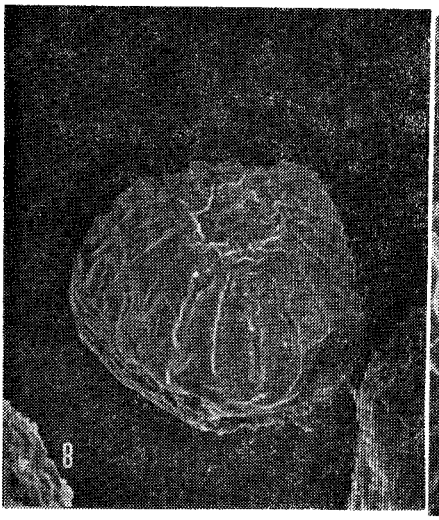

Fig. 8. The egg of *M. fascelis*. 100 X.

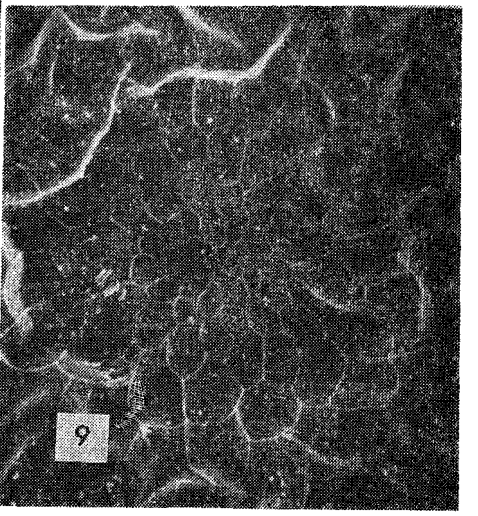

Fig. 9. The micropylar area of *M. fascelis*. 500 X.

RESULTS AND DISCUSSION

Generally, the authors obtained the eggs either from the reared females in the laboratory or from the fresh specimens catched in the field and made the eggs laid on a piece of paper or on the fine sands in the cages (Salkeld, 1973, 1976, 1984; Suludere, 1977; Edlich et al., 1981; Arbogast and Byrd, 1982; Arbogast et al., 1983, 1984; Lambdin and Lu, 1984). The eggs obtained in this way always require cleaning in order to remove the adherent sand particles, scales and hairs etc.. To avoid this inconvenience, some eggs are obtained from the gravid

females by dissection. According to Salkeld (1975), Downey and Allyn (1981), chorionic sculpturing was the same on both laid and dissected eggs, provided that the dissected eggs were obtained from the fully developed oviduct.

The eggs used in this study were extracted from the females in the collection. But a different method was followed for our preparations. Tribasic sodium phosphate, which was a chemicals used by Salkeld (1980) to obtain the eggs of some Diptera, also was used in this study, but other steps of preparation were not satisfactory for the eggs of *Melitaea*. As most of the eggs were collapsed and shrivelled, the content of the eggs were drawn out with fine tweezers by opening a small hole. The egg ghost obtained by this way returns to the normal shape even if it collapses during the preparation. But the rate of surface pollution of the eggs increases in this method. This pollution are removed by agitation in the solution reducing the surface tension, before washing in water and dehydration in aceton.

As to the observations, all the eggs studied are spheroidal with somewhat flattened micropylar area and are more or less circular in outline.

Egg size varies with the species from the smallest *(M. fascelis)* with an average width of 0.62 mm and height of 0.67 mm to the largest *(M. didyma)* with an average width of 0.72 mm and height of 0.74 mm. Salkeld (1975), Downey and Allyn (1981) suggest that the egg size is useful for separating only those species whose eggs are either very small or very large. Size may prove to be a useful character since *M. didyma* and *M. transcaucasica* have larger eggs than the other two species.

All eggs studied are not boldly marked. The basic pattern of sculpturing consists of slightly elevated longitudinal ridges joined by the transverse walls. This pattern sometimes are poorly developed such as in *M. transcaucasica* and transverse walls are almost imperceptible. The lower part of the chorion is usually slightly convex and unpatterned. The longitudinal ridges radiate from micropylar area and a few ridges originate from above the equator, except in *M. fascelis*. All of them disappear at the equatorial region. The number of longitudinal ridges was found to be of little value in separating the species examined here because of the variability of the number within a species and their similarity among the species.

The micropylar pits are surrounded by a rosette of petal like primary cells, each of which is outlined by fine walls. According to Downey and Allyn (1981), the micropylar region of the chorion often reflect more of the electronic charge back through the micropylar openings. For that reason, the micropylar openings of *Melitaea* species are not discernable in it. The micropylar pit is about 6μ in width and circular in *M. facelis* and *M. didyma,* is about 10–11μ and irregular in shape in the others. In *Lycaenidae,* the rosette of primary cells seems to show a great deals of interspecific variability which may believe its taxonomic usefullness (Downey and Allyn, 1981). The design of this rosette is a useful diagnostic character, even though there is often considerable intraspecific variation in the shape and the number of primary cells (Arbogast et al., 1980). In *Melitaea* species, the number of primary cells varies and overlaps both intra–and interspecifically; none has less than 7 or more than 12. In the eggs obtained from the only one melanic female of *M. didyma,* the number of primary cells is higher (Fig. 3) than those of the other *M. didyma,* including melanic and normal individuals, which were collected from the same or different localities. This result leads us to conclude that the variability of primary cells in number is individual than geographical. *M. didyma* differs from the other species of *Melitaea* in having almost equal primary cells and in having regularly arranged and easily countable secondary cells. In *M. persea,* the secondary, tertiary, and quaternary cells are less distinct than those of the other species.

In conclusion, the results reveals that the chorionic structures of eggs of the closely related species in the genus *Melitaea,* appear to be significant. Further researches among the other species of *Melitaea,* and also other related genera of the family, will contribute certainly not only to the morphology of eggs, but also to the relationships of the taxa used in the classification.

ACKNOWLEDGMENTS

I wish to express my gratitude to Doç. Dr. A.Ö. Koçak for allowing me to examine and dissect the identified specimens of his collection. I am greatly indebted to Prof. Dr. S. Karol for her criticism and SEM facilities at EM Laboratory, Faculty of Sciences, Ankara University. I would like to thank Miss N. Gül and R. Verimli for their kind cooperation in the Laboratory.

REFERENCES

ARBOGAST, R.T. and BYRD, R.V., 1981. External morphology of the eggs of the meal moth, *Pyralis farinalis* (L.), and the murky meal moth, *Aglossa caprealis* (Hübner) (Lepidoptera: Pyralidae). Int. J. Insect Morphol. Embryol., 10: 419–423.

————, 1982. The eggs of the cadelle, *Tenebroides mauritanicus* (L.) (Coleoptera: Trogositidae): Fine structure of the chorion. Ent. News, 93 (3): 61–66.

ARBOGAST, R.T., BYRD, R.V., CHAUVIN, G., and STRONG, R.G., 1984. The eggs of *Hofmannophila pseudospretella* (Oecophoridae): Fine structure of the chorion. Journal of the Lepidopterists' Society, 38 (3): 202–208.

ARBOGAST, R.T., CHAUVIN, G., STRONG, R.G., and BYRD, R.V., 1983. The eggs of *Endrosis sarcitrella* (Lepidoptera: Oecophoridae): Fine structure of the chorion. J. Stored Prod. Res., 19 (2): 63–68.

ARBOGAST, R.T., LeCATO, G.L., and BYRD, R.V., 1980. External morphology of some eggs of stored-product moths (Lepidoptera: Pyralidae, Gelechiidae, Tineidae). Int. J. Insect Morphol. Embryol., 9: 165–177.

CASPERSON, G., OTTO, D., EDLICH, C.D., NITSCHMANN, J., and SCHMIDT, H.B., 1983. Rasterelektronenmikroskopische Untersuchungen am Ei der Wintersaateule *(Agrotis segetum* Schiff.) (Lep., Noctuidae). Entomolgische Nachrichten und Berichte, 27: 173–174.

CHAPMAN, T.A., 1896. The characters of the eggs of Lepidoptera, as affording a basis for classification. The Entomologist's Record, 8 (12): 287–289.

CHAUVIN, J.T. and CHAUVIN, G., 1980. Formation des reliefs externes de l'oeuf de *Micropteryx calthella* L. (Lepidoptera: Micropterigidae). Can. J. Zool., 58: 761–766.

CLARK, F.N., 1900. Photographing the eggs of Lepidoptera. The Entomologist's Record, 12 (11): 281–282.

DAVIDOVA-VILIMOVA, J., 1987. The eggs of two *Captosoma* species, with a review of the eggs of the Plataspidae (Heteroptera). Acta Entomol. Bohemoslov., 84: 254–260.

DOWNEY, J.C. and ALLYN, A.C., 1979. Morphology and biology of the immature stages of *Leptotes cassius theonus* (Lucas) (Lepid.: Lycaenidae). Bull. Allyn Mus., 55: 1–27.

————, 1980. Eggs of Riodinidae. Journal of the Lepidopterists' Society, 34 (2): 133–145.

————, 1981. Chorionic sculpturing in eggs of Lycaenidae. Part 1. Bull. Allyn Mus., 61: 1-29.

————, 1984. Chorionic sculpturing in eggs of Lycaenidae. Part 2. Bull. Allyn Mus., 84: 1-44.

EDLICH, C.D., CASPERSON, G., NITSCHMANN, J., and SCHMIDT, H. B., 1981. Die Eischalenoberflache von *Agrotis segetum* Schiff. (Lepidoptera). Biol. Rdsch. 19: 346–351.

GRIFFITH, C.M. and Lai-FOOK, J., 1986. Structure and formation of the chorion in the butterfly, *Calpodes*. Tissue and Cell, 18 (4): 589–601.

HINTON, H.E., 1981. Biology of Insect Eggs. 3 Vols., 1125 p., Pergamon Press, Oxford.

LAMBDIN, P.L. and LU, G.Q., 1984. External morphology of eggs of the spined soldier bug, *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae). Proc. Entomol. Soc. Wash., 86 (2): 374–377.

REGIER, J.C., MAZUR, G.D., and KAFATOS, F.C., 1980. The silkmoth chorion: Morphological and biochemical characterization of four surface regions. Developmental Biology, 76: 286–304.

ROWLEY, W.A. and PETERS, D.C., 1972. Scanning electron microscopy of the eggshell of four species of *Diabrotica* (Coleoptera: Chrysomelidae). Ann. Ent. Soc. Ame., 65: 1188–1191.

SALKELD, E.H., 1973. The chorionic architecture and shell structure of *Amathes c-nigrum* (Lepidoptera: Noctuidae). Can. Ent., 105: 1–10.

————, 1975. Biosystematics of the genus *Euxoa* (Lepidoptera: Noctuidae). IV. Eggs of the subgenus *Euxoa* Hbn. Can. Ent., 107: 1137–1152.

————, 1976. Biosystematics of the genus *Euxoa* (Lepidoptera: Noctuidae). VII. Eggs of the subgenera *Chorizagrotis*, *Crassivesica*, *Longivesica*, *Orosagrotis*, and *Pleonectopoda*. Can. Ent., 108: 1371–1385.

————, 1980. Microtype eggs of some Tachniidae (Diptera). Can. Ent., 112: 51–83.

————, 1984. A catalogue of the eggs of some Canadian Noctuidae (Lepidoptera). Memoirs of the Entomological Society of Canada, 127: 1–167.

SULUDERE, Z., 1977. *Locusta migratoria migratorioides* R.F. (Orthoptera: Acrididae)'nin genç ve yaşlı yumurta örtülerinin ince yapısının incelenmesi. Unpublished Master Thesis, University of Ankara.

VISCUSO, R. and LONGO, G., 1983. The egg of Leaf insect, *Phyllium pulchrifolium* Serv. (Phasmatodea: Phyllidae): Morphology and ultrastructure. Arch. Biol. (Bruxelles), 94: 441–457.

WAGENER, S., 1983. Struktur und skulptur der eihüllen einiger *Melanargia*-Arten (Lepidoptera, Satyridae). Andrias, 3: 73–96.

WARD, R.D. and READY, P.A., 1975. Chorionic sculpturing in some sandfly eggs (Diptera, Psychodidae). J. Entomol., 50 (2): 127–134.