Metamorphosis of the Micropylar Chorion of the Honey Bee (Hymenoptera: Apidae) Egg[1]

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Ann. Entomol. Soc. Am. 79: 971–974 (1986)

ABSTRACT The chorion of the micropylar area of eggs dissected from oviducts of mated queens of *Apis mellifera* L. had a prominent network of ca. 116 canals of varied shape and size, whereas most eggs laid by virgin or mated queens had indistinct, shallow depressions. The micropylar pattern of a few eggs treated with fixative immediately after being laid by mated queens was intermediate between that of oviducal eggs and older eggs laid by queens. The micropylar chorion of eggs laid by laying worker bees had an intermediate pattern similar to that of some newly laid queens's eggs. The change in chorionic morphology was fertilization-independent, for it was observed on all fertilized eggs and unfertilized eggs laid by mated queens.

KEY WORDS *Apis mellifera*, egg, micropylar chorion

EGGS PRODUCED by honey bee, *Apis mellifera* L., queens have a thin transparent chorion (<0.1 μm) (Du Praw 1967). Except for small areas at the poles, the chorion is covered with a network of hexagonal ridges composed of single rows of granules. The micropyle, at the anterior or free end, is a region of shallow depressions or pits (width 1 μm) interspersed with small openings (diam 0.2 μm) (Dietz et al. 1978). Bronskill & Salkeld (1978) stated that this portion of the honey bee egg is a "more or less circular patch of chorion . . . imprinted with a shallowly ridged circular pattern" and lacks "pores or other structures that might be considered micropyles."

Except for the behavioral mechanism controlling sperm release (Koeniger 1970), the coordination of oviposition and fertilization in the queen honey bee is unknown, including timing of egg movement through the genital tract, egg location, and positioning in relation to sperm contact and egg/sperm interaction (Camargo & Mello 1970). Consequently, additional morphological information on the micropylar area, the site at which sperm enter eggs, should enhance our knowledge of the honey bee reproductive process. This report presents the results of further studies of the micropylar chorion of honey bee eggs.

Materials and Methods

Slow dehydration of honey bee eggs in ethanol without prior fixation usually produced satisfactory results. Most eggs were transferred one at a time from brood combs directly into a modified Beem capsule (Postek et al. 1974) standing in 40% ethanol. Capsules containing eggs then were moved to clean 40% EtOH and held ca. 20 min. Next, eggs were passed through a graded series of ethanol (40–90%), in 10% increments (30 min each), to 95% ethanol (20 min), two changes of absolute ethanol (10 min each), and then 100% acetone (10 min). Finally, eggs were critical-point dried with liquid CO_2 (chamber pressurization and exhaustion times increased to 30–40 min each). Freshly laid eggs were obtained by removing from colonies a brood comb with an actively laying queen on it, holding the comb in subdued light at ca. 30°C, and then adding fixative (10–15 drops of 2.5% glutaraldehyde in 40% ethanol) to a cell as soon as the queen withdrew her abdomen after depositing an egg. Additional preparation of newly laid eggs was as above.

Critical-point dried eggs were mounted on aluminum stubs with double-stick tape and coated with gold-palladium in a sputter coater (Denton Vacuum Desk-1). Coated eggs were examined and photographed with scanning electron microscopes (SEM) (Hitachi S-500 and International Scientific Instruments Super III-A).

Observations included eggs deposited in worker and drone cells by three unrelated laying virgin queens and 11 unrelated naturally mated queens, eggs deposited in combs by laying workers of three unrelated queenless colonies, and eggs dissected from the oviducts of nine unrelated mated queens. In addition to chemically fixed, newly laid worker eggs, SEM examinations of mated queens's worker eggs included timed eggs (15–90 min old) and eggs of random age (<3 days old). More than 100 of each of these seven types of eggs and 23 eggs treat-

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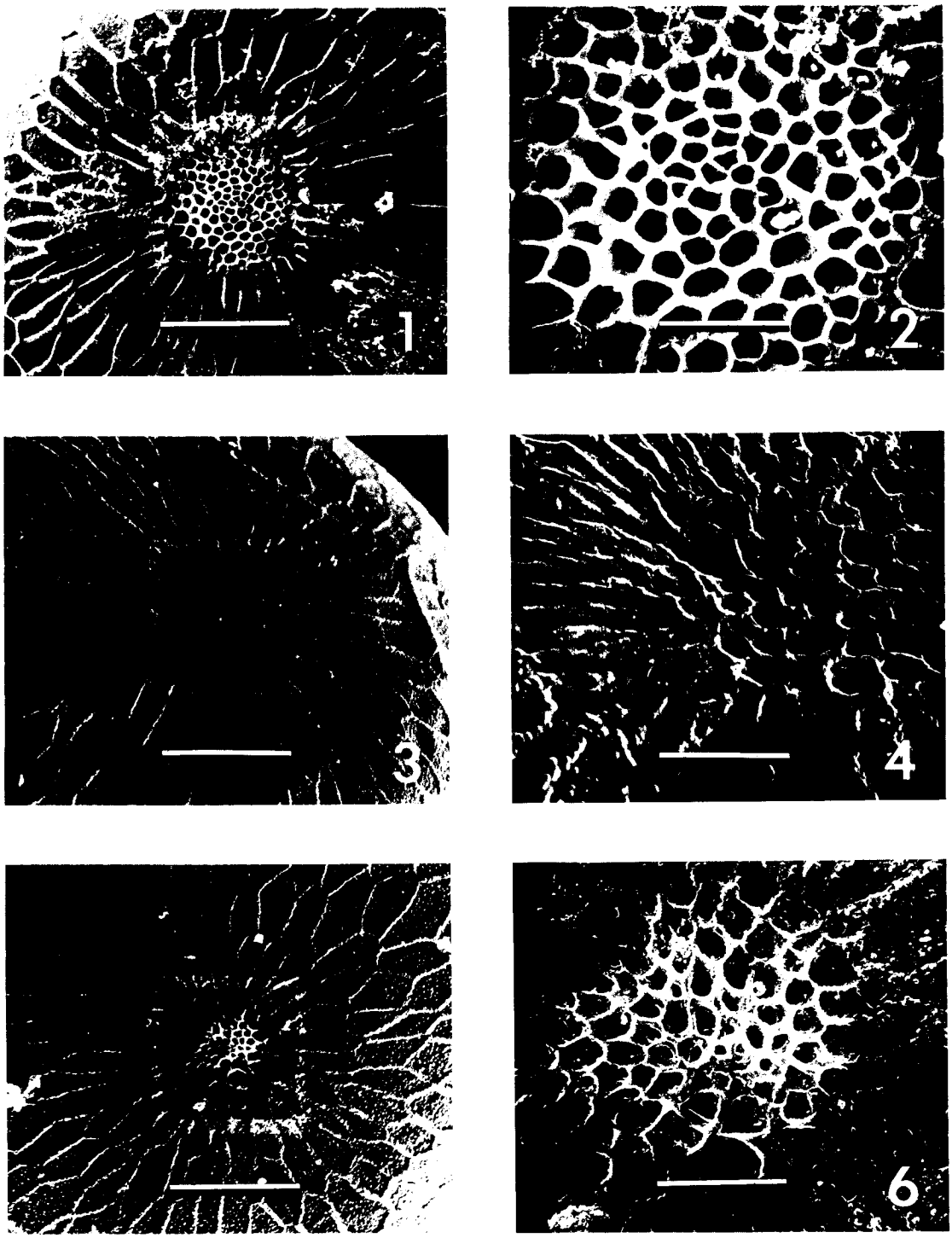
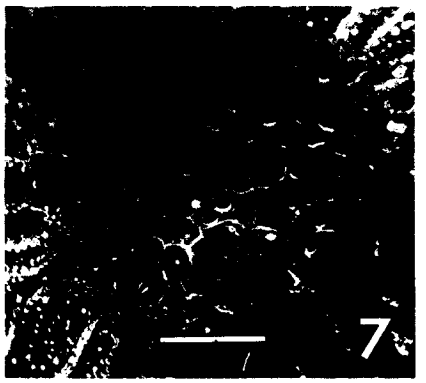


Fig. 1–6. SEM photographs of micropylar area on eggs of *A. mellifera*. (1 and 2), oviducal egg dissected from mated queen; (3 and 4) fertilized egg oviposited by queen, <1.5 h old; (5 and 6) egg oviposited by laying worker, <1.5 h old. Reference bars: (1, 3, and 5) 30 μm; (2, 4, and 6) 100 μm.



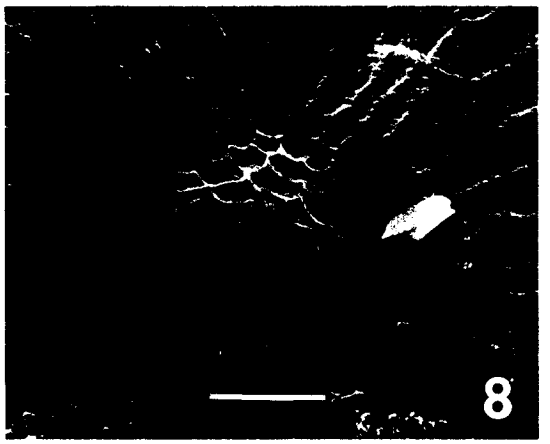


Fig. 7 and 8. SEM photographs of micropylar area on eggs of *A. mellifera*. Eggs fixed (2.5% glutaraldehyde) at moment queen withdrew from cell. Reference bars: (7 and 8) 30 μm.

ed with fixative immediately after being laid were examined by SEM.

Results and Discussion

SEM examination of oviducal eggs, newly oviposited eggs, and older oviposited eggs revealed that the morphology of the micropylar chorion varied according to physiological age of the egg and whether the egg source was a honey bee queen or laying worker. The micropylar area chorion on mated queens's eggs comprised three patterns (reticulate, transitional, and rudimentary), but only one pattern each was observed on eggs from laying virgins (rudimentary) and workers (transitional).

Mated Queens's Eggs

Reticulate Pattern. The micropylar area of all oviducal eggs consisted of a dense network of canals ($\bar{x} \pm$ SEM = 116 ± 11.2; $n = 12$) of varied shape and size (Fig. 1 and 2), as pictured by Erickson et al. (1986). For a short distance from the egg surface, the central canals are at a 90° angle to the margin of the micropylar area, whereas the outer canals generally slant toward the center. These canals appeared to decrease in size distally, but the termini were not visible, even at high magnification (5,000×). The number of eggs found in the lateral oviducts ranged from 0 to 15 and occasionally an egg also was present in the median oviduct.

Transitional Pattern. A few newly laid eggs (3 of 23 chemically fixed immediately after oviposition) exhibited a micropylar pattern intermediate between that of oviducal eggs and all older oviposited eggs (Fig. 7 and 8), but most newly laid eggs had the rudimentary pattern seen on all older eggs.

Rudimentary Pattern. Eggs taken from worker and drone cells 15 min or more postoviposition had a rudimentary pattern of 20–30 poorly defined depressions or cells on 30–70% of the micropylar area. The remainder of the micropylar area was covered by low ridges radiating toward the center of the area (Fig. 3 and 4), although a few eggs had only the shallow depressions shown by Dietz et al. (1978), and Bronskill & Salkeld (1978). The micropylar areas of fertilized and unfertilized eggs (i.e., those laid by mated queens in worker- and drone-sized cells, respectively) were indistinguishable.

Virgin Queens's Eggs

Eggs laid by virgin queens all had the same rudimentary micropylar pattern as mated queens's eggs processed ≥15 min postoviposition. Although the oviducts of mated queens usually contained eggs, those of virgin queens contained none because egg production was reduced.

Laying Workers's Eggs

All eggs laid by laying workers had a characteristic latticework pattern (ca. 60–80 shallow cells of varied depths and with rounded walls) (Fig. 5 and 6) similar to that of some eggs newly laid by mated queens (Fig. 7). Examination of bees from queenless colonies revealed no eggs in the oviducts of 31 workers that displayed queenlike behavior (presumed laying workers).

The micropylar chorion of all mated queens's eggs, both fertilized and unfertilized, had the reticulate pattern on preovipositional eggs and the rudimentary pattern on older postovipositional eggs; thus, metamorphosis of the micropylar chorion was fertilization-independent.

The micropylar area of eggs laid by a bumble bee species likewise is circular and inconspicuous (Salkeld 1978), bearing an outer border of shallow arches and low ridges radiating toward a flattened center. Neither the honey bee nor bumble bee egg has conspicuous material added to the micropylar area, as in the case of the cap substance of house fly eggs, that could function as a sealant (Leopold et al. 1978).

The timing and mechanism of micropylar-area chorion metamorphosis of honey bee eggs were not determined. Source queens often spent 1–2 min to lay an egg or ceased laying altogether when removed from the hive, whereas naturally mated queens conditioned to observation hives averaged ca. 12.6 s in the egg-laying process (Dietz 1969). Therefore, delayed egg laying by semi-isolated queens may have altered the timing of the micropylar chorion change; perhaps normally it is not completed until after the egg is laid. In any event, micropylar chorion alteration probably is initiated by chemical or physical means soon after an egg passes from the queens's median oviduct into the genital chamber (vagina). Sperm entry apparently is completed before eggs are laid because no traces of sperm were observed on any of the fertilized eggs examined.

Potential mechanisms for altering the soft micropylar chorion of preovipositional honey bee eggs are physical pressure from the genital chamber, especially the valve fold, or chemicals that might occlude or lyse the micropylar canal area. Preliminary attempts to fertilize honey bee eggs instrumentally indicate that reduction of the micropylar chorion probably is not a physical process. Sometimes micropylar canals (up to ⅛) were flattened and overlapped laterally, following addition of spermathecal fluid (containing sperm) from actively laying queens, but no reduction of the canals was observed (unpublished data). In the house fly, both female accessory gland secretion and micropyle cap substance are necessary to elicit a spermatozoan acrosome response (Degrugillier 1985). Thus, perhaps, chemical substances, singly or in combination, in the spermathecal fluid, the oviducts, genital chamber, or honey bee eggs themselves also reduce the micropylar area canals.

Precise methods for timing ovipositional events and collecting eggs immediately before they are laid need to be developed to elucidate the process of fertilization in honey bees. Nevertheless, rapid alteration of the micropylar chorion at about the time of oviposition suggests that spermatozoa penetrate the egg membranes before the micropylar area is altered. If so, it may be possible to fertilize honey bee eggs in vitro by instrumental application of spermathecal spermatozoa to the micropylar area of oviducal eggs.

Acknowledgment

The assistance provided by J. B. Graves and A. M. Hammond (Louisiana State Univ., Baton Rouge) and W. A. Hagstad and M. T. Postek (formerly of Louisiana State Univ., Baton Rouge) are appreciated. The use of facilities at Louisiana State Univ. is gratefully acknowledged. This article is published in cooperation with the La. Agric. Exp. Stn.

References Cited

Bronskill, J. F. & E. H. Salkeld. 1978. The micropylar area of some hymenopterous eggs. Can. Entomol. 110: 663–665.

Camargo, J. M. F. & M. L. S. Mello. 1970. Anatomy and histology of the genital tract, spermatheca, spermathecal duct and glands of *Apis mellifera* queens (Hymenoptera: Apidae). Apidologie 1: 351–373.

Degrugillier, M. E. 1985. *In vitro* release of house fly (*Musca domestica:* Diptera: Muscidae) acrosomal material after treatments with secretion of female accessory glands and micropyle cap substance. Int. J. Insect Morphol. Embryol. 14: 381–391.

Dietz, A. 1969. Performance of laboratory-reared and artificially inseminated honey bee queens. J. Econ. Entomol. 62: 251–253.

Dietz, A., W. J. Humphreys & J. J. Waldon. 1978. Polystyrene replicas of honey bee eggs for use in studying the fine structure of the shell surface. J. Ga. Entomol. Soc. 13: 187–192.

Du Praw, E. J. 1967. The honeybee embryo, pp. 183–217. *In* F. H. Wild & N. K. Wessells [eds.], Methods in developmental biology. Crowell, New York.

Erickson, E. H., Jr., S. D. Carlson & M. B. Garment. 1986. A scanning electron microscope atlas of the honey bee. Iowa State Univ., Ames.

Koeniger, N. 1970. Factors determining the laying of drone and worker eggs by the queen honeybee. Bee World 51: 166–169.

Leopold, R. A., S. Meola & M. Degrugillier. 1978. The egg fertilization site within the house fly *Musca domestica* (L.) (Diptera: Muscidae). Int. J. Insect Morphol. Embryol. 7: 111–120.

Postek, M. T., W. L. Kirk & E. R. Cox. 1974. A container for processing delicate organisms for scanning or transmission electron microscopy. Trans. Am. Microsc. Soc. 93: 265–267.

Salkeld, E. H. 1978. The chorionic structure of the eggs of some species of bumblebees (Hymenoptera: Apidae: Bombinae), and its use in taxonomy. Can. Entomol. 110: 71–83.

Received for publication 12 May 1986; accepted 24 July 1986.