Morphogenesis of the silkworm egg

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SUMMARY

Ovarian follicle development, which accompanies morphogenesis of the silkworm egg has three distinct phases: spheric, ellipsoidal and flattened-ellipsoid. Transitions between phases are rapid and form-stability (characterized by length/width ratio) is preserved from the beginning of the ellipsoidal phase. The geometric stability of the follicle-oocyte-ovariole system, the polarity of the egg and the determinism in form changes reveal strikingly coordinated spatial and temporal organization.

INTRODUCTION

Oogenesis is not only transmission of genetic information and energy stores, it is also a process of cellular morphogenesis (Waddington, 1967). The process is being elucidated by studies on the internal and external architecture of insect eggs (Anderson, 1972; Counce, 1973) and isolation of genetic factors for egg form in Drosophila (King, 1970) and Bombyx mori (Tazima, 1964).

The simply shaped silkworm egg develops entirely from pre-existing reserves during the nymphal stage. With polytrophic Bombyx mori, oocyte follicles constitute a relatively simple geometric family (Legay, 1974) whose development reveals two synchronous phenomena. The first is growth of remarkable magnitude (3000–100 000 times, depending on whether follicles at the top or bottom of ovarioles are measured). The other is progressive change of the egg shape, the subject of this paper. Past efforts in this field have been mainly qualitative. I try here to treat oogenesis as a quantitative process.

MATERIAL AND METHODS

In my experiments, chrysalids of Bombyx (univoltine European race) dated at nymphal moult were dissected. Oocytes or follicles were individually extracted upstream from the ovariole in physiological solution under a binocular microscope. Dimensions were taken at once with an ocular micrometer. The most critical measures concern the follicular space which is often confused at the end of oogenesis with the oocyte space. Of course, at the start of the oocyte cycle there are seven nurse cells as well as the oocyte in the follicle.

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RESULTS

Follicle-form development is in three phases. Spheric, of short duration, is characterized by a simple dimension, \( l \). Ellipsoidal begins at \( l = 0.06 \) mm (follicles in gonads of fifth stage larvae are already oval). During this phase the rapidly growing oocyte progressively fills the follicular space (at \( l = 0.33 \) mm it occupies 40\% of the space; at \( l = 0.5 \) mm, 50\%; this transition takes place in less than one day).

The mature oocyte \( l/w \) ratio of about 1.23 is reached rapidly and the flattene ellipsoid phase starts at \( w = 1 \) mm. The follicle then flattens and the oocyte space assumes three characteristic dimensions \( l, w \) and \( t \), which soon show \( l/t = 2.0 \) and \( w/t = 1.6 \) (with \( l/w \) unaffected). The overall egg form changes no further. We consider it an ellipsoid of elliptic cross section. This approximation facilitates surface area and volume estimates (Legay & Pernet, 1971); the latter correlate highly \((r = 0.91)\) with egg weight.

Flattening is in the plane of bilateral symmetry. In fact, the symmetry is not perfect. Thus the micropyle, which is in the plane of symmetry, falls short of

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Fig. 1. Geometric characteristics of the oocyte of *Bombyx mori*. (A) Geometry of the oocyte-follicle-ovariole system at start of ellipsoid phase; (B) oocyte, flattened ellipsoid phase, face view; (C) same oocyte, side view; (D) embryo position at beginning of development; (E) embryo at end of development. \( m \), Micropyle; \( nc \), nurse cells; \( fc \), follicle cells; \( tw \), wall of ovariole; \( o \), oocyte; \( os \), oocyte space; \( vs \), ventral side; \( ds \), dorsal side; \( e \), exit hole; \( u \) upstream; \( d \), downstream.
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corresponding to greatest egg length by 14% of the egg’s width. Also, in profile, the micropyle end of the egg is blunter and 13% thicker than the other end (see sketches 3 and 4 of Fig. 1).

Several points merit emphasis:

(1) In every race the mature oocyte has a remarkably stable form (Legay & Tran-Bugnot, 1974). For the race studied here, coefficients of variation (standard deviation/mean) for \( l/w \) and \( l/t \) are weak (2-7% and 4-1%).

<table>
<thead>
<tr>
<th>Female number</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>21</th>
<th>42</th>
<th>44</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>( l/w )</td>
<td>1.29</td>
<td>1.22</td>
<td>1.25</td>
<td>1.22</td>
<td>1.24</td>
<td>1.24</td>
<td>1.22</td>
<td>1.24</td>
</tr>
<tr>
<td>c.v.</td>
<td>0.031</td>
<td>0.031</td>
<td>0.034</td>
<td>0.032</td>
<td>0.034</td>
<td>0.053</td>
<td>0.024</td>
<td>0.034</td>
</tr>
<tr>
<td>( r )</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>( l ) range</td>
<td>0.73</td>
<td>0.63</td>
<td>0.68</td>
<td>0.68</td>
<td>0.63</td>
<td>0.54</td>
<td>0.63</td>
<td>0.60</td>
</tr>
<tr>
<td>( w ) range</td>
<td>1.37</td>
<td>1.57</td>
<td>1.67</td>
<td>1.48</td>
<td>1.53</td>
<td>1.57</td>
<td>1.48</td>
<td>1.50</td>
</tr>
</tbody>
</table>

\( l/w \), Average length/width ratio among the follicles in an ovariole. c.v. Coefficient of variation (S/\( \bar{x} \)) for this ratio.

\( r \), Correlation coefficient between \( l \) and \( w \). Range of \( l \) and \( w \) is given for each ovariole. The range reflects the different stages of development of eggs in a female.

(2) The developing follicular space also has a very stable form (see Table 1). Thus, for the fast growing ellipsoid phase, the coefficient of variation for the \( l/w \) ratio (3.4%) is only slightly greater than that (2.7%) for mature oocytes. Furthermore, the coefficient of correlation for regression of \( l \) on \( w \) is very high (0.98–0.99) in the ovarian tubes (Fig. 2). This is an exceptional case of isometric growth. Follicle development is, therefore, strictly determined. We consider the constant \( l/w \) as a characteristic of the dual Waddington chreodes (Waddington, 1968), the ellipsoid of revolution and the flattened ellipsoid.

(3) The two form changes come abruptly and few (six or so, see Fig. 3) follicles are found in an intermediate state. These brief transitions between the stable phases can be considered elementary catastrophes in Thom’s sense (Thom, 1972). The second transition approximately coincides with the point of inflexion on the follicle growth curve (not shown, see Legay, 1974).

(4) Geometry of the oocyte–follicle–ovariole system is precise (Fig. 1). Major axes of the oocyte and the ovarian tube coincide. The micropyle position upstream on the oocyte and the above discussed asymmetry elements completely orient the egg well before the nuclear events of gametogenesis are finished. The embryo head always develops on the micropyle side. The embryo is at first (germinal-band stage) on the micropyle side but after turning over has its back against the opposite side. The exit hole for the newborn caterpillar is not terminal but subpolar, as is apparently the case for all univoltine races (Grandori, 1924).
Fig. 2. Follicle growth in an ovariole during nymphal stage. (A) 8 day; (B) 12 day; \( l \), length; \( w \), width. (A) and (B) show that succession of growing follicles follows their rank in the ovariole (see Fig. 3). Graph B shows accumulation of mature oocytes. (A) corresponds to ranks 1–40 and (B) to ranks 1–99. In each normal mother, egg age (rank) and dimensions are strongly linked (see Legay, 1974 and unpublished observations).

Fig. 3. Follicle dimensions as a function of rank in the ovariole during growth. \( l \), length; \( w \), width; \( t \), thickness. The oldest egg is rank no. 1. Transition from ellipsoid to flattened ellipsoid is seen (toward the left) from the 40th oocyte. Width and thickness cannot be distinguished in younger eggs. Flattening is accompanied by a sizeable increase in \( l \) and \( w \). At this age of morphogenesis (8–9 days), final egg dimensions have not yet been attained at the end of development (Legay, 1974), an overall shrinking is seen and \( t \) decreases with respect to \( l \) and \( w \).

**DISCUSSION**

The geometric stability of the above system reflects its organizational regularity which does not vary with normal physiological and genetic processes. The total geometric structure associated with an oocyte includes not only the follicular envelope, but also the ovarian tube itself. The tubes are wound and intermingled such that no privileged position in the abdomen of the female can be identified.
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The oocyte is orientated with respect to the ovariole and not to the mother’s body. 

Polarity of the system results from two energy flows toward the oocyte region. One, perpendicular to the follicle surface and roughly so to the ovarian tube, crosses the follicular cells, establishing a transverse gradient. The other, in the tube axis, sets up a longitudinal gradient parallel to follicle position (order) in the tube, to food cell position behind (nutritionally upstream from) the follicle, and to envelopment of the oocyte by follicular cells (which progresses upstream).

Form changes seem independent of the follicular space surface/volume ratio. In the transition from sphere to ellipsoid this ratio barely changes (from 77·4 to 77·7, assuming equal volumes). During the ellipsoid phase, oocyte growth lowers the ratio by a factor of 12, but then it remains stable at 6·25–6·52 during transition to the flattened ellipsoid phase. It is likely that mechanical pressure exerted on the follicle by the ovarian tube and peristalsis induce ovoid formation, analogous to processes described by d’Arcy Thomson for birds’ eggs (d’Arcy Thomson, 1942). But timing and characteristics of form change still need to be related precisely to functioning of the two above gradients. Passage from sphere to ellipsoid takes place during high food cell activity and under the influence of a dominating longitudinal gradient. Ellipsoid to flattened-ellipsoid transition occurs with disappearance of the food cells and, consequently, of most of the longitudinal gradient. Conversely, follicle cell activity continues and the transverse gradient persists and probably becomes dominant in the final phase.

Two kinds of verification for the above interpretation exist. The first is found in the characteristics of the end product of the system. The architecture of the chorion of the egg when examined under the electron microscope shows the regular pattern exhibited by the follicle cells and their regional differentiation (anterior and posterior poles, sides and periphery). Furthermore, laying down of the endochorion is accompanied by parabolic patterns always orientated toward the micropyle (Foussal & Legay, 1976). Such lamellate appearances have been found in arthropod integuments (Hackman, 1971), but not previously in insect eggshells.

The second confirmation comes from certain aspects of functioning of the system. High concentrations of ecdysone discovered in the oocytes, where this hormone cannot have been synthesized, and the demonstration of a gradient along the ovarian tube, established concurrently with the development of the oocyte (Legay, Calvez & De Reggi, 1976), also corroborate the above description of the organization of the ovariole–follicle–oocyte system. This last observation probably opens new possibilities for research on oocyte morphogenesis, especially since maximum ecdysone content coincides with the rapid form change to a flattened ellipsoid (Legay et al. 1976).

My results show that well before the oocyte attains full size the surrounding, partly empty, space has the morphological characteristics (for example, l/w) of the mature egg. This suggests that the follicle serves as matrix for the egg. Oocyte growth may then be considered an anti-, or complementary development. An
embryo grows from one cell and organizes its many cells into an organism. But ignoring possible interactions between the future gamete and the surrounding tissue, it is a complex population of cells that organizes a cell during development of the silkworm egg.

We cannot, however, eliminate the possibility that the follicle is pre-adapted to the egg, that the follicle assumes its shape because it is necessary that the egg have this shape. This determinism could come from material and geometrical constraints (Legay, in preparation).

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REFERENCES


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