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# The Reproductive System of the Army-Ant Queen, *Eciton (Eciton)* Part 3. The Oöcyte Cycle[1]

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The present paper is the concluding part of a study of the reproductive system of *Eciton* queens and considers especially the activity of the individual ovariole during the period between ovulations. It is restricted to gross anatomical details, for other persons are conducting cytological investigations on the component cells present in the ovary, including the development of the oöcyte nucleus.

It had once been suspected that an examination of the ovariole and its contents might reveal abrupt and distinctive observable changes in relation to the reproductive cycle. If found, these stages could provide a calendar whereby minutely exact intervals in statary and nomadic phases of the colony could be determined by visible evidence in the queen alone. A most cursory inspection of the freshly prepared slides of the ovary

[1] For parts 1 and 2 of this series of papers, see Hagan (1954a, 1954b).

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The three studies of which this is the last have been carried out in collaboration with the Department of Animal Behavior of the American Museum of Natural History. The writer wishes to express his thanks to Dr. T. C. Schneirla, Curator in that department, for having made available a series of queens collected and preserved by him in connection with his field investigations on army-ant behavior and biology. Each queen referred to in this study will be identified by the annual series and symbol used for her colony in the original publication (Schneirla, 1947, 1949; Schneirla and Brown, 1950).

The writer also wishes to acknowledge the courtesy of Prof. Ephriam Hixon, Director of Resident Instruction, Prof. R. E. Hill, Head of the Department of Entomology, and Mr. L. W. Quate, Assistant Professor of Entomology, of the University of Nebraska School of Agriculture, for generously providing guest privileges of laboratory space, microscopes, and preservatives for the dissection of some of the specimens used in this study.

showed that no such simple and precise data would be available. As the pacemaker of the colony, the queen must influence colony behavior through far more subtle variations in the ovariole than can readily be detected except over considerable time intervals. Regulatory control of the colony by the queen must depend upon the initiation of physiological and hormonal conditions, the effects of which may be very slight but are cumulative in time. Visible physical changes in the ovariole and its contents are necessarily gradual and interdependent with physiological changes. The physiological activity of the reproductive system is unknown.

Fortunately, Schneirla's extensive observations and experimentation have provided a generous series of criteria by which colony behavior can be correlated with the reproductive cycle of the queen with considerable accuracy. An examination of the ovary, with which this paper is concerned, tends to verify his data in broader aspects. Some of his critical time tables in the nomadic and statary phases of colony activity and of the reproductive cycle of the queen are marked by:

Duration in days of the nomadic and statary phases (Schneirla, 1949; Schneirla and Brown, 1950)
Beginning and end of the nomadic and statary phases (Schneirla 1938, 1945, 1947, 1949; Schneirla and Brown, 1950)
Types and extent of raids (Schneirla, 1944, 1945)
Selection of bivouacs and their duration (Schneirla, 1933, 1938, 1949, 1952)
Contracted and physogastric conditions shown by the queen (Schneirla, 1938, 1944, 1947, 1949; Schneirla and Brown, 1950)
Presence or absence of eggs, larvae, pupae, and callows in the colony and the developmental age of such broods (Schneirla, 1933, 1938, 1944, 1945, 1947, 1949; Schneirla and Brown, 1950)

Even the series of events cited are not inflexible within the species, and environmental factors also cause some fluctuations in them. The schedules likewise are somewhat different in extent in the various species observed. They are perhaps not so uniformly constant as the changes within the ovariole. But at present such histological changes appear so gradually and are so smoothly integrated that minor distinctions are difficult to differentiate, as is shown below.

It would be desirable to study the oöcyte cycle in a single species, but none was collected at appropriate intervals throughout the cycle of colony behavior. *Eciton burchelli* collections most nearly fulfill the requirements both in number of queens and in respect to the time element. Queens of other species must fill in the gaps as required to complete the cycle. The entire colony behavior cycle varies slightly in different species, but this is felt to be of little significance in this study. For example, the *hamatum* colony is typically nomadic for 16 days and statary for 20 days. The *burchelli* colony averages 13 and 21 days for the same phases (Schneirla, 1949; Schneirla and Brown, 1950). *E. vagans* and *rogeri* phase durations seem to be unknown. However, a schedule of cyclic behavior in relation to the ovulation cycle, not the duration in days, is being studied. Thus it would be of little significance whether a specific point in any cycle is attained in a longer or briefer elapsed time in a contemporary species provided the sequence of events follows the same pattern. Colony activity phases may be correlated with the reproductive condition of each of the queens discussed in the following pages by referring to figure 20.

## *ECITON HAMATUM* ('45 G), A CONTRACTED QUEEN, STATARY PHASE

This queen, having deposited her eggs, is about five-eighths through the statary phase of colony function. In her former physogastric condi-

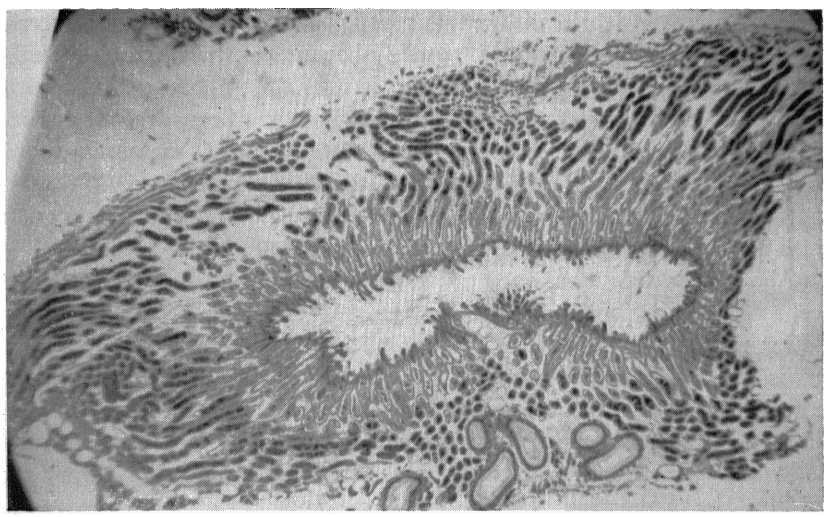


FIG. 1. Ovary ('45 B, *Eciton burchelli*) sectioned parallel to median flat surface and through calyx. Posterior surface of ovary to right, ventral below. Larger, black ovarioles are rarely continuations from pedicels in papillate calyx epithelium shown here but originate elsewhere. Gray debris is visible in bases of ovarioles and calyx; germaria, as thin gray extensions of ovarioles, especially in upper left. Terminal filaments numerous as fine lines and dots against top edge of ovary. Tracheae in section visible as thin-walled circles, particularly in lower left and in median indentation near center of calyx where paired oviduct enters on another section. Spermathecal duct with thick cuticular lining appears in five oblique sections and one cross section at lower center. Photograph by courtesy of Dr. Roman Vishniac.

tion, her ovary was very large and distended both in length and diameter by the multitude of contained oöcytes. This condition is no longer true, and the gaster and ovary have attained their minimum size. Within a few days the colony will begin its nomadic phase during which the contents of the ovary of the queen will be gradually altered. We therefore start the examination of the oöcyte cycle with this queen. Certain aspects of the changes within the ovarioles are followed through various specimens, until physogastry again is evident and ovulation is about to ensue.

The proximal end of an ovariole is empty. The pedicel is a round, straight tube. Distally the more flexible and unsupported ovariole is also devoid of contents for quite a distance, and its proximal portion tends to be thrown into slight folds as if it were partially telescoped. Farther up the ovariole the first debris from the previous ovulation is encountered (fig. 2). There is a considerable amount of this disintegrated, evenly granulated substance which fills the ovariole for a distance four or five times as long as the diameter of the organ at this point. Above this material is more debris with a different appearance. The amount seems to vary in individual ovarioles, possibly because of the degree of breakdown that has already occurred in it (fig. 3). This debris shows heterogeneity because the cells from which it has been derived are incompletely destroyed at this stage. Remnants of nuclei, membranes, and other organelles are visible but distorted in size and shape. The mass appears to be reticular, with clearer spaces containing faint granules. It also fills the ovariole for some distance.

Above the debris mentioned above lies a short plug of small interstitial cells remaining from those that formerly filled the vitellarium and the lower end of the germarium not occupied by other cells (fig. 4). This mass of cells will eventually lose its identity, as its constituent elements also disintegrate. The three stages in this disintegration of unused cells in the ovulation process will continue until all are dissolved and gradually absorbed by surrounding tissues as the oöcyte cycle unfolds. The oöcytes of the last generation, liberated a few days previously, apparently mechanically carried with them some of this debris, for after ovulation it may be seen in the calyx of the oviduct. The descent of mature oöcytes sweeps all traces of debris from the preceding ovulation into the calyx and leaves the lower end of the ovariole clean.

A long row of oöcytes is encountered immediately distal to the plug of interstitial cells. This series composes the present generation of oöcytes which will mature and be deposited in the fore part of the next statary phase. The oöcytes are estimated to be over 20 in number, possibly 25. Since the preceding oöcyte generation was laid by the queen, thus ending her physogastric condition, this is the only generation of oöcytes possessed by her and will be for a few more days. Before the present statary phase has ceased, however, another generation of oöcytes will appear higher up the ovariole. Thus the queen has two generations of oöcytes in the lower end of the germarium and in the vitellarium simultaneously throughout most of the cycles of colony behavior. Only the middle third and early portion of the last third of the statary phase have but one generation of oöcytes. Figure 4 shows three of the seven oldest oöcytes in this section lying just above the interstitial cell mass.

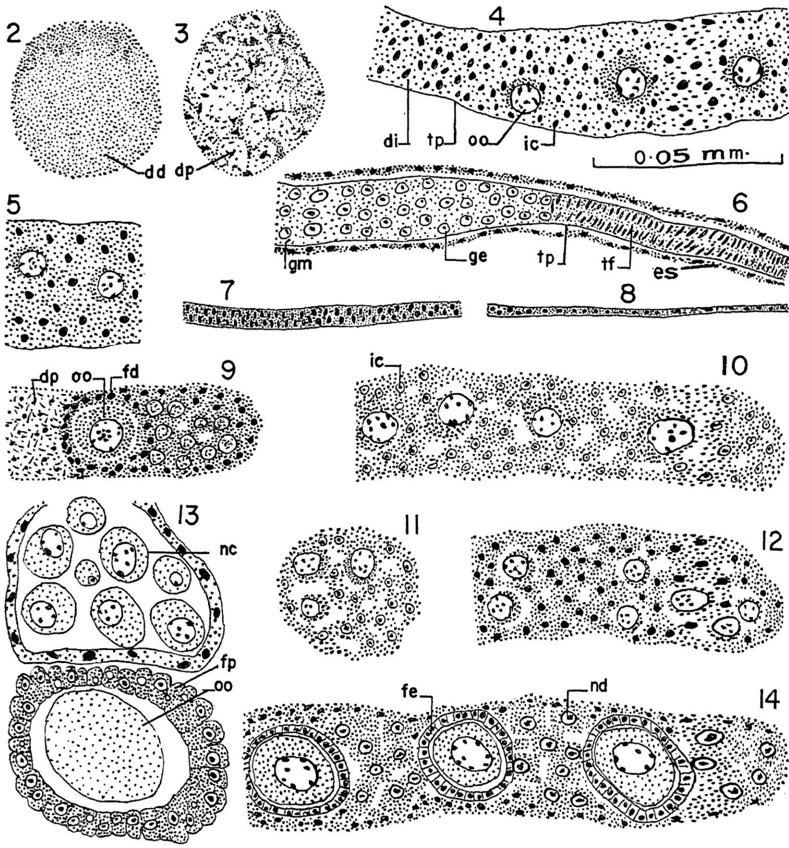
The oöcytes and their locations in relation to one another in the ovariole are discussed more in detail as the course of the oöcyte cycle unfolds in successive queens. Here it suffices to remark that the more distal oöcytes are seen to be younger as each is examined in the ascending series. A view of two oöcytes near the middle of the string illustrates this point (fig. 5). Since the oöcytes are often off center in the ovariole one can understand why the section may appear wider if a central rather than a peripheral section is used. Sections often are narrower as the ovariole bends out of the plane of the microtome knife. Many of the drawings also omit the wall of the ovariole but wherever a line covers the margin, this represents the delicate, and often invisible, tunica propria. The epithelial sheath is shown in one drawing of this specimen to provide a view of this structure. It often stands well away from the tunica and is generally found only around the terminal filament of the functional queen.

Above the vitellarium the germarium is an ill-defined separate portion of the ovariole. It is filled at the lower end with interstitial cells of the ovariole and may contain young oöcytes. Its upper end may at times reveal quite distinctly cell nuclei of intermediate size which are thought to compose the germinal epithelium. From this region, and tissue, the oöcytes are derived, as are possibly also the nurse cells (fig. 6).

Figures 6, 7, and 8 show the arrangement of the cells within the tunica propria of the terminal filament. In figure 6 the lower end of the terminal filament contains very flat cells with staggered, flat nuclei lying in two rows. At a higher elevation the tendency of the nuclei is to become more ovate as the cells elongate (fig. 7). Distally in the very long filament, a portion of which appears in figure 8, the cells are uniserial, much narrower but long, and the nuclei are round. The greater portion of the length of the filament is somewhat more slender than is shown.

## *ECITON BURCHELLI* ('44 B). A CONTRACTED QUEEN, STATARY PHASE

This queen is from a colony thought to be a little over three-fourths through the statary phase. Examination of the ovarioles in a series of sections show apparently 22 to 25 oöcyte nuclei to be visible in each. The ex-




FIGS. 2–14. Details of ovaries at different points in oöcyte cycle. In most o[f] these figures wall of ovariole is omitted and only contents shown. Dimension[s] same for all figures. Queen identifications (in parentheses) may be referred t[o] figure 20.

FIGS. 2–8. *Eciton hamatum* ('45 G). 2. Disintegrated debris in ovariol[e] near base; cross section. 3. Disintegrating interstitial cells and possibly nurs[e] cell remnants in ovariole distal to view in figure 2; dark objects are probably nuclear remains; cross section. 4. Three lowest and oldest oöcytes of seven visible in this section of ovariole; to left is debris consisting of interstitial cell[s] forming basal cell plug above debris shown in figures 2, 3; longitudinal section. 5. Two oöcytes situated at about center of series of oöcytes, longitudinal section. 6. Upper end of germarium and base of terminal filament; nuclei, showing no concentration of dense cytoplasm, probably represent germinal epithelium; longitudinal section. 7. Terminal filament some distance above view in figure 6; nuclei spaced farther apart and more ovate; longitudinal section. 8. More distal view of terminal filament; cell nuclei spherical, spaced farther apart, and uniserial; filament higher up even thinner.

FIGS. 9–13. *Eciton burchelli* (9–12, '45 B; 13, '45 D). 9. Lowest oöcyte in act number is difficult to ascertain, because the ovariole turns so frequently, often is in cross sectional view, and a confusingly large number of them are present. The lowest oöcyte possesses a definite layer of incompletely differentiated follicle tissue cells surrounding it (fig. 9). This layer may be one or two cells in thickness, and their long axes lie at right angles to the oöcyte. In addition, above the oöcyte but not distinctly separated from it is a cluster of cells of which the nuclei are slightly larger than adjacent cells. This is the potential nurse cell mass. They are still much smaller, however, than they will appear later in the oöcyte cycle. Below the oöcyte lies a mass of debris from the preceding ovulation resembling the debris described in the case of the preceding queen. The largest oöcyte nucleus found in the ovary is slightly larger than the one shown because the latter is drawn slightly off center. The oöcyte nucleus is almost always spherical.

Higher up the ovariole lie oöcytes that are successively younger and consequently possess smaller nuclei. Nearer the upper end of the vitellarium the oöcyte cytoplasm is much reduced in amount. Since these oöcytes are surrounded by interstitial cells, they are not usually discernible unless the ovariole is actually sectioned. The visible oöcytes appear to lie in a linear series (fig. 10). Five were present in the section from which this figure was drawn, but seven to nine are sometimes seen before the ovariole curves out of the section on the slide. That this impression of lineal assortment is incorrect at this stage of oöcyte development can be readily ascertained by studying cross sections of an ovariole. In such cases it is evident oöcytes are irregularly spaced and may occur side by side

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ovariole, showing definitive follicle and nurse cells; to left is debris in early stage of dissolution; longitudinal section. 10. Oöcytes apparently in linear arrangement; four of five visible in this section are shown, but 12 have been observed in younger oöcyte area; about midway in oöcyte series of this queen; follicle and nurse cells not distinguishable; longitudinal section. 11. Ovariole at virtually same elevation as in figure 10, to show that oöcytes are not strictly in linear series; cross section. 12. View near upper end of ovariole shown in figure 10; random assortment of oöcytes and their comparative size are evident; little cytoplasm present; slightly curved and oblique longitudinal section. 13. Oöcyte, primary follicile, and nurse cell body; oöcyte nucleus in other sections; composite, longitudinal section as nurse cells are from an adjacent ovariole with better view.

FIG. 14. *Eciton vagans* ('45 J). Young oöcytes in follicles near upper end of vitellarium, with definitive nurse cells enlarging; longitudinal section.

*Abbreviations*: dd, disintegrated debris; di, debris, interstitial cell plug; dp, debris, partially disintegrated; es, epithelial sheath; fd, follicle, definitive; fe, follicular epithelium; fp, follicle, primary; ge, germinal epithelium; gm, germarium; ic, interstitial cell; nc, nurse cell; nd, nurse cell, definitive; oo, oöcyte; tf, terminal filament; tp, tunica propria.

across the width of the ovariole (fig. 11). In the figure provided, two oöcytes are seemingly together while a third is only slightly above them. The nucleoplasm is clear except for a few coarse chromatin granules eccentrically placed.

The cytoplasm of the oöcyte is very finely and densely granular and stains deeply. A cellular membrane is not visible but must be present, because the cell outline is definitely pronounced and regular, especially where slight shrinkage has occurred through the technique employed. The cytoplasm of the interstitial and definitive nurse cells in the ovariole stains very faintly, or not at all. The nuclei of the interstitial cells are small, mostly spherical or ovate, stain darkly but reveal a central, larger clump of chromatic material, either a nucleolus or a chromatin mass.

The oöcytes are more closely aggregated near the distal end of the vitellarium than are the older oöcytes lower down, but there is no regularity in the spacing shown. The youngest show only a scanty cytoplasm to be present. A slightly curved section of the contents of an ovariole shows the conditions at this point (fig. 12). The ovariole appears wider than normal owing to its curvature and to the fact that it is sectioned through the longitudinal midline while those in other drawings are not.

Below the oldest and proximal oöcyte (fig. 9) the ovariole is pleated in about seven folds before it joins the pedicel and calyx. Between this region and the oldest oöcyte is a long portion, perhaps a third or a half of the length of the ovariole that contains no oöcytes. This lower portion of the ovariole is not empty but partly filled with detritus from the previous ovulation. This material is dense, and its breakdown is still in an early stage, for in the upper portion nuclei still preserve their identity although cellular differentiation is completely lost.

The calyx of the oviduct, which receives the pedicels of the ovarioles, possesses exceptionally tall epithelial papillae projecting into its lumen. This is thought to be caused by collapse and slow recovery of its expanded walls during and following the previous ovulation.

## *ECITON BURCHELLI* ('45 E), A CONTRACTED QUEEN, NOMADIC PHASE

This queen was collected at a time representing the elapse of a little over one-fourth of the nomadic time phase. Few characteristics of the ovary readily distinguish her from the queen previously discussed but these are here mentioned. The oöcytes possess a little more cytoplasm, and in actual measurement the oldest oöcyte nucleus is slightly larger.

Their linear distribution is more pronounced in that the lower oöcytes lie in a single row. Their longitudinal spacing in the ovariole, nevertheless, is still irregular, and intervening cells lying between them vary greatly in number. This is apparently a random assortment, for two oöcytes separated by a few cells may be spaced from others on either side by relatively many more cells and at considerably greater intervals. At the distal end of the vitellarium, the youngest of the current oöcytes are less regularly and linearly spaced, recalling their position in the preceding queen.

Several of the oldest oöcytes in each ovariole show definitive follicles and clusters of attendant nurse cell masses similar to those in figure 9. Exactly how many are in this condition could not be determined, but the number very definitely exceeds those previously mentioned. However, they are no farther advanced in specialization. Occasional groups of interstitial cells, typical of the vitellarium, just above these differentiating nurse cells and oöcytes showed mitotic figures. Their destiny was undetermined. Those seen were in groups of three to five cells.

The entire string of oöcytes, in the spacing procedure, has moved down the ovariole so that the oldest oöcytes have approached but not reached its pleated part. The pleated portion of the ovariole is still recognizable, but the folds are not so deep as formerly and the abrupt edges of folds appear more smoothed out into gentler curves. The debris has preceded the oöcytes and further disintegration has taken place, but some remnants of nuclei persist. Its anterior end has reached the pedicel. The calyx wall is still thrown into long papillae of epithelium, as mentioned above.

In the preceding queen occasional clear spaces existed in the germarium. This fact was not previously mentioned because the sections revealed nothing to describe. In this queen these areas now show very definitely several, often three to eight, relatively large, very pale, unstained cells with larger nuclei than the great mass of cells in this region. These are thought to be the early appearance of some of the oöcytes of the next generation. They seem too young to belong to the present series of oöcytes. This point is clarified below.

## *ECITON BURCHELLI* ('45 I). A CONTRACTED QUEEN, NOMADIC PHASE

This queen was collected when the colony had just passed one-third of the nomadic phase. A photograph of a section of the ovary of this queen is reproduced in figure 1 for several reasons. It gives some idea of the complexity involved in tracing ovarioles through a series of sections strewn with them. This view shows more than 1600 parts of ovarioles yet was selected because this number is fewer than usually appears in a section. From another aspect the papillate condition and the size of the calyx can be seen. Also, several structures and details that have previously been mentioned are shown here quite clearly.

When compared with the preceding queen, very little difference appears in the oöcyte cycle. The older oöcytes show nuclei of the same size, and their cytoplasm, too, remains about the same in bulk. A few more possess definitive follicular cells but neither these nor potential nurse cells seem to have developed much further. Intervals between oöcytes are still variable, and the intervening nurse cells show no sign of becoming approximately equal in number. The debris in the ovariole is dense as before and extends from the lowest oöcyte to the pedicel, but the latter is empty.

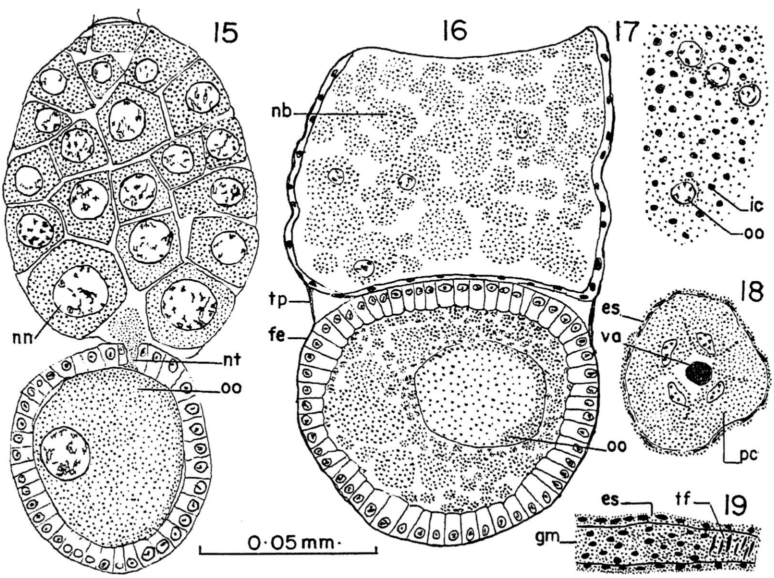
The sections were very favorable from one aspect. In the germaria, of which several were fortunately sectioned longitudinally for some distance before they turned out of view, the pale, large nuclei mentioned for the preceding queen are here quite visible. In the above description these were mentioned as probably being the second-generation oöcytes, destined to follow the present generation. This fact can here be established. In several germaria from five to 14 were readily counted. In one instance, where an attempt was made to follow a germarium through two adjacent serial sections, it seemed that 22 very young oöcytes of the second generation had already made their appearance before the current generation had matured. They possess nuclei much larger than the majority of the cells in the germarium, are very pale, and the cytoplasm is scanty and unstained by eosin.

## *ECITON BURCHELLI* ('45 D), A CONTRACTED QUEEN, NOMADIC PHASE

The consideration of this queen concerns one in the last few days of the nomadic phase of colony behavior. The ovariole shows no new structures within it, but those already described have altered in several respects. The second-generation oöcytes in the germarium possess a thin layer of cytoplasm around each nucleus that readily takes a stain. It is finely granular, and the nucleus is centrally situated.

The oöcytes in the vitellarium are all much larger because of an increase in the cytoplasm each possesses. They still exhibit a graded series in size, the smallest being the most distal. The oldest oöcyte or oöcytes (for several appear almost alike in size) seem to have accumulated the full quota of cytoplasm. They therefore are complete but lack the bulk of maturity, for they have not yet acquired their necessary yolky reserves. Some show a vacuolated margin. Whether this is due to initial yolk storage or to partial disintegration of the cytoplasm through imperfect fixation it is impossible to say. Certainly fixation in this specimen was faulty.

The follicular cells of the higher oöcytes are still in the definitive stage but are more regular and have their long axes extending parallel to the oöcyte surface. Near the lower end of the ovariole the follicular cells have changed their orientation so that their long axes lie in radial arrangement




FIGS. 15–19. Details of ovaries at different points in oöcyte cycle. In most of these figures wall of ovariole is omitted and only contents shown. Dimensions same for all figures. Queen identifications (in parentheses) may be referred to figure 20.

FIGS. 15–16. *Eciton vagans* ('45 J). 15. Oöcyte in follicle ready to receive nutriment from mature nurse cell body; longitudinal section. 16. Nurse cells breaking down and liberating nutriment to oöcyte; only a few nurse cell nuclei persist at this stage; longitudinal median section of nurse cell body, parasagittal view of oöcyte.

FIGS. 17–19. *Eciton hamatum* (17, '45 N; 18, 19, '48 H). 17. Germarium with some of next-generation primary oöcytes following present oviposition stage in physogastric queen; longitudinal section. 18. Vitellarium of ovariole in virgin queen; cross section. 19. Junction of germarium and terminal filament, virgin queen; longitudinal section.

*Abbreviations*: es, epithelial sheath; fe, follicular epithelium; gm, germarium; ic, interstitial cell; nb, nurse cell degenerating; nn, nurse cell nucleus; nt, nutrient transfer; oo, oöcyte; pc, preovulation cell; tf, terminal filament; tp, tunica propria; va, vacuole.

around the oöcyte (fig. 13). Intercellular membranes are not clearly defined but are indicated by fairly wide separation lines of denser cytoplasm. Cellular polar membranes are sharply defined. Their nuclei are large, not yet entirely regularly situated basally in the cells, and within them appears a single, dark mass of chromatin or a nucleolus.

Oöcytes are spaced farther apart in the ovariole, because nurse cell masses and their component cells are enlarging (fig. 13). This applies particularly to the older oöcyte locations, for the youngest are less affected by much nurse cell differentiation at present. Many of the nurse cells have almost reached their maximum size; when they do they will completely fill their allotted space. In the figure only those in one elevation are shown. They are still widely spaced. The nurse cell mass is typically more elongate and somewhat narrower in the ovariole than the oöcyte it accompanies. These proportions will alter quickly, early in the statary phase.

The lower oöcytes have progressed down the ovariole as they space themselves farther apart. Some debris is present below them, however, so they do not reach the pedicel. This debris is far less in quantity than formerly and has undergone further disintegration. Most of it has been absorbed but by which tissues is unknown. The accordion-like pleats at the lower end of the ovariole are inconspicuous although some waviness is observable.

## *ECITON VAGANS* ('45 J), A CONTRACTED QUEEN, STATARY PHASE

This queen is in the early statary phase and still a few days before the attainment of complete physogastry and oviposition. Internal conditions relating to the ovary are almost the same as described for the preceding queen, but they are in more advanced stages towards the fulfilment of the reproductive function.

All oöcytes are now provided with a follicle and a nurse cell body in some degree of development. At the distal end of the vitellarium the oöcyte is small and consists entirely of cytoplasm without yolk (fig. 14). The nurse cell body is not fully formed, but nurse cells are clearly distinguishable above each oöcyte. The interstitial cells of the vitellarium are still distributed at random all along this portion of the ovariole.

Approximately the middle third of the vitellarium shows completed nurse cell bodies and fully enlarged oöcytes composed only of cytoplasm (fig. 15). It would appear that the nurse cell body varies in the number of nurse cells it may contain. Most of them seem to possess about 20 cells, but the figure referred to above shows more. All cells in a single focal plane are drawn to reveal their close proximity, but only a few are sectioned through their centers. It was early noticed that spaces between oöcytes differed in most of the ovarioles studied and in whole mounts of single ovarioles. Wider spacing and a large nurse cell body may be correlated.

In figure 15 one may find nutriment just beginning to pass from the nurse cell body to the follicle. In fact, a slightly denser marginal cytoplasm, over about half of the oöcyte, may indicate the latter has already absorbed some of it. It apears that the nurse cells have reached their fullest growth. From this point they will rapidly diminish in size as they discharge their contents, disintegrate, and cease contributing to the yolk of the oöcyte. In a very short time only remnants of the nurse cells will remain as debris in a very limited space. The adjacent oöcyte, enlarging by virtue of the accumulation of the nurse cell body contribution to yolky substances, will eventually take over most of the space formerly occupied by both.

In general, the early complete nurse cell body is elongate but no wider than its adjacent follicle. As it reaches maturity, however, it tends to become more truncate and wider than the follicle. But as observed before regarding the number of nurse cells and spacing between oöcytes, the shape varies, too, so this observation only applies to most of these structures. Of course, with the acquirement of yolk from nurse cell body activity, and possibly from the haemolymph via the follicular epithelium, the oöcyte is finally so distended as eventually to exceed in bulk the spaces formerly occupied by both of them.

The lowest third of the vitellarium encloses the oldest oöcytes which, at this stage of growth, are actively engaged in the absorption of nutriment. To this effort the nurse cell body is the important and obvious contributor (fig. 16). It will be seen that the nurse cells are breaking down and their contents have already almost enclosed the oöcyte by occupying all the available space within the follicle. It is arriving faster than the oöcyte can absorb it. The figure given shows only parts of the few nurse cell nuclei remaining, their disintegrating cytoplasm, and an almost polar section of the oöcyte. Naturally, the lower, or proximal, end of the ovariole will possess fewer oöcytes, for their bulk is relatively greater. Their spacing is closer as one observes them in ascending order. About four to six show this phase of enlargement in all the ovarioles of this queen.

It may likewise be noted that the follicle is rapidly growing in size. At present the follicular cells are columnar, regular in size, with basally situated nuclei. As oöcyte enlargement continues, these cells will be greatly stretched in order to encompass the future oöcyte. They will then revert more or less to the squamous condition and cease whatever metabolic activity they may have at present. Before their shape is too greatly modified they will secrete the delicate chorion, typical of ant eggs, over the oöcyte.

The lowest and oldest oöcyte now is situated at the upper end of the pleated portion of the ovariole. The pleats have almost entirely disappeared. The debris which formerly filled so much of this end of the ovariole has been almost absorbed so that only mesh-like traces of it can be found.

In field observations of this colony, Schneirla (1947, p. 5) could not determine with certainty, in the time available, whether this queen had entered the egg-laying stage or was just leaving it. Physogastry was not evident, but eggs were being deposited in the bivouac. With the foregoing data on hand regarding the appearance of the oöcytes in the ovary during the oöcyte cycle, it can now be concluded that physogastry is still to come. Here is the evidence. Old debris has not yet been entirely swept from the lower end of the ovariole; it is estimated visually that too many thousands of oöcytes approaching maturity are still unlaid; only the oldest nurse cell masses have begun to break down and contribute their products to the oöcytes; nurse cell masses in the upper parts of the ovarioles show successively younger degrees of development; no oöcytes were found in the calyx; the second, or succeeding, generation of oöcytes is well developed in the upper ends of the vitellaria and lower parts of the germaria. All of these conditions would be different or absent after ovulation.

## *ECITON HAMATUM* ('45 N), A PHYSOGASTRIC QUEEN, STATARY PHASE

For the physogastric individual a *hamatum* queen will suffice. She is not quite fully physogastric, for not all of the oöcytes are completely supplied with yolk. She will shortly reach maximum distention when the fat body reserves are largely converted into yolk and stored in the oöcytes. When this is achieved, the oöcytes will be largely mature in the ovarioles and the calyx and oviducts will be greatly inflated by those that have already descended and been stored within these organs.

At present one oöcyte, or possibly two oöcytes, have reached the calyx from some of the ovarioles. There are not enough of them there to assert that each ovariole has discharged even one. The calyx is not dilated nor is its cavity filled; none of the oöcytes appear in the paired oviducts.

The oöcytes in the calyx measure approximately 0.21 by 0.40 mm. in over-all breadth and length and are of course supplied with a chorion. One factor is somewhat against the unqualified acceptance of these dimensions and should be mentioned. The oöcytes, being surrounded by the chorion, had plasmolyzed by routine histological reagents and methods that are quite satisfactory for the maternal tissues and achorionic oöcytes. Several oöcytes in both cross and longitudinal sections were measured, however, and their choria only were considered. Absolute dimensions would be more pertinent if freshly deposited eggs were used, or before any appreciable embryonic development had occurred in them. Embryos of ants tend to lengthen the eggs which contain them as development proceeds. To check these figures, a few oöcytes from a physogastric queen, also *hamatum* ('38 H), which had squirted oöcytes from the oviducts when opened for dissection as described in part 1 (Hagan, 1954a), were measured. The largest oöcytes found averaged 0.24 by 0.57 mm. These figures fit into the range cited by Schneirla (1944).

The mature oöcyte exhibits a reticular cytoplasm with vast reserves of nutriment for the future embryo within its meshes. This is also true for the first few still confined to the ovariole where they are closely spaced. The first four or five abut end to end. Above these very little residual nutriment remains between the oöcytes to be absorbed. In spite of this remnant, some 10 or 12 seem to be almost full-sized oöcytes. This remnant increases slightly in quantity as the ovariole is ascended until more or less of the original nurse cell body is encountered. Near the upper end of the vitellarium the oöcytes are still smaller than the nurse cell body.

The mature oöcyte is ovate, with bluntly rounded ends and quite typical of ant oöcytes in shape. The convex anterior end is parabolic rather

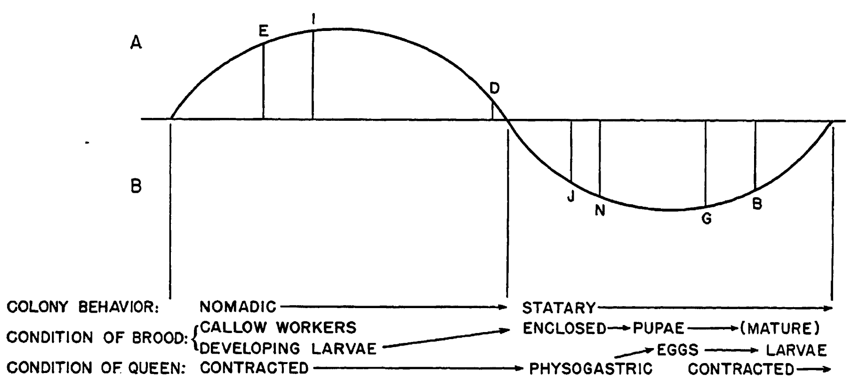



FIG. 20. Schematic diagram of reproductive condition of queens described in this paper, as estimated on the basis of functional condition of their respective colonies at the time of field study. A, nomadic phase; B, statary phase. The placement of letters on the curve represents condition of the respective queens: E, '45 E, *Eciton burchelli*; I, '45 I, *E. burchelli*; D, '45 D, *E. burchelli*; J, '45 J, *E. vagans*; N, '45 N, *E. hamatum*; G, '45 G, *E. hamatum*; B, '45 B, *E. burchelli*. (Modified from Schneirla, 1947, fig. 2.)

than symmetrically rounded. The chorion is very delicate, and the oöcyte is almost white in color. The nucleus is small and inconspicuous. Because of its conformity to general expectations, it has not been figured.

Before the physogastric queen is dispensed with, one further note should be made regarding the oöcytes of the next generation (fig. 17). Examination of the germarium shows large, typical oöcyte nuclei to be present there in large numbers in the physogastric queen. They have also been recognized and mentioned in some of the other queens described herein. Some of them seem to have a very scanty cytoplasm around the nucleus although most visible on one side so even at this stage of growth the nucleus is eccentrically placed. Apparently they will move into the vitellarium in the wake of the oöcytes of the present generation shortly to be deposited. With oviposition, a considerable number of interstitial cells, debris, nurse cell remnants, etc., are left behind in the ovariole. It might at once occur to the physiologist to inquire if this material could have any value as nutriment to the young oöcytes as they descend, or if it is all reabsorbed by maternal tissues.

## *ECITON HAMATUM* ('48 H-12), A VIRGIN QUEEN

While not strictly a part of this study, the virgin queen is undoubtedly of great interest to those desiring all the available data on the reproductive system of the *Eciton* queen. One is fortunate indeed to be able to make some comparisons with the same system in the functional individual.

The virgin queen need not be placed in the brood cycle or the nomadic and statary phases, for she has not as yet assumed her future role as the reproductive individual of the colony. She always appears as part of a small sexual brood during the dry season (Schneirla and Brown, 1950). A virgin queen in many respects is intermediate in ovarian development, although she more nearly approaches the pupal stage in appearance than she does that of the functional queen.

In the second part of the study (Hagan, 1954b) on the histology of the reproductive system, a distinction was made between the condition of the spermathecae of virginal and of functional queens. Other differences also are observable. The epithelial sheath is relatively thicker and more uniformly present over the tunica propria. Its nuclei are rounded and widely spaced over the terminal filament but are more ovate, numerous, and prominent over the vitellarium (fig. 19). The germarium contains the typical small cell in both virgin and functional queens but there are fewer in the former. The vitellarium as a whole is very much thicker than it is in the functional queen and, as is usual for insects, is far shorter.

Follicle cells, nurse cells, and oöcytes are not present in the vitellarium of the virgin queen. Instead, the lumen is filled with comparatively few huge cells with large nuclei which seem to be undergoing dissolution. In some cases the nuclei still show chromatin masses, but the nuclear shape is irregular. In most instances they appear to be pycnotic (fig. 18). This appearance may have been due entirely to technique, for fixation was incomplete.

The cytoplasm of these cells generally shows almost no sign of rupture in the cellular membrane. However, no organelles, except nuclei, are visible within them. They possess a very homogeneous, stainable, uniform content. In a few instances cytoplasmic breakdown is indicated by an irregular or broken membrane near the center of the ovariole.

The cells line the wall of the vitellarium as a single layer and stain so much like the tunica propria that the latter is usually impossible to distinguish from them. Their external border, next to the tunica, shows no indentations marking cellular boundaries. In the median portion of the vitellarium, these cells reveal a distinct border and often show cell membranes where the cells arch towards one another. Since the inner, free ends of the cells fail to touch, this leaves a central free space in the vitellarium in which, at intervals, bluish, translucent spheres of some unknown substance may be seen. These vacuoles vary in size and are startlingly distinct, because a reddish brown haematoxylin and eosin were employed as stains.

Neither the origin nor the fate of these cells is known. It is reasonable to assume, however, that they cannot become any of the three types that are due to appear later on, hence they probably break down and their remains pass into the calyx. In view of the fact that they seem to be present only once in the life of the queen and that they disappear upon the descent of the first oöcytes, they have been identified by the term "preovulation cells" in this paper.

## DISCUSSION

Biotic Potential of the Queen: The actual count of the ovarioles in the *burchelli* queen as reported in part 1 (Hagan, 1954a) of this series of papers came to 1149 in number at the minimum. The accidental loss of ovarioles in this manual dissection and count under the binocular microscope was estimated to be between 10 per cent and 15 per cent owing to manipulation and possible injury to some of them in the attempt to separate the two ovaries. This would place the total number in a single ovary of this species somewhere between 1264 and 1322 ovarioles. For the two ovaries of the queen it would mean a minimum of 2528 and a possible maximum of 2644 ovarioles.

The present study of the oöcyte cycle seems to place the number of oöcytes delivered from an ovariole at a single oviposition somewhere between 22 and 25 in number. There is no assurance that the number in an ovariole is invariably fixed, and some of the observations seem to show that a slight variation does exist. Thus, the *burchelli* queen appears to be

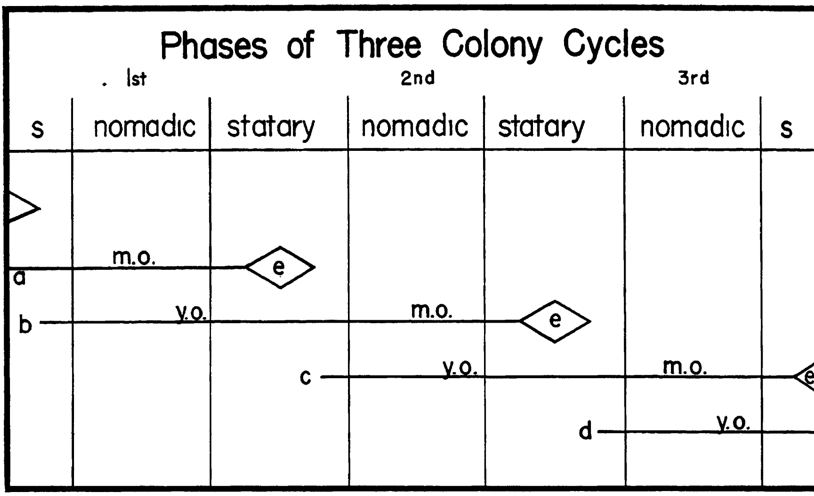



FIG. 21. Complete oöcyte cycles, with parts of two more, all in relation to three colony cycles. Colony cycles beginning and ending in middle of colony statary phases arbitrarily selected. Oöcyte cycle culminates in egg stage, represented by polygon, the widest part of which indicates most prolific deposition of ova. Diagrammatic, with approximate time intervals.

*Abbreviations*: a, d, parts of oöcyte cycles; b, c, complete oöcyte cycles; e, egg stage; m. o., maturing oöcytes; y. o., young oöcytes.

able to deposit about 58,168 oöcytes. Of these, of course, some will not become ova or develop embryos later. It has been observed, in the examination of eggs of a physogastric queen, that some were defective in yolk content. Some will fail to receive sperm, and in others embryos will not survive. It has already been noted (Hagan, 1954a) that non-functional ovarioles may exist, at least for the current ovulation cycle. Nevertheless, the biotic potential of approximately 58,000 eggs is far more than sufficient to account for the brood developed by the queen of the colony. It may also be construed as supporting evidence that but one functional queen is present and essential in the colony. Schneirla's observations show that the worker brood consists of 20,000 to 35,000 individuals produced at any one time.

COLONY AND OÖCYTE CYCLES: The cycle of colony behavior consists of a statary and a nomadic phase with a total duration of about 35 days. As previously mentioned, the statary phase lasts 20 or 21 days, while the nomadic phase extends over 13 to 17 days for the *burchelli* and *hamatum* colonies, respectively. In other words, *burchelli* requires approximately 34 days and *hamatum* takes 37 days to complete a functional cycle.

The oöcyte cycle has a different rhythm of greater duration, although it harmonizes perfectly with colony behavior. However, an oöcyte cycle requires almost all of the time consumed by two colony cycles. To trace the oöcyte cycle briefly one should begin with the nomadic phase of the colony behavior cycle in order to start with a queen in the contracted condition and at the appropriate time interval (fig. 21).

At a time corresponding to the lapse of about one-fourth of the nomadic phase, examination of the germarium of the queen reveals the presence of several young oöcyte nuclei, probably four to eight, which are clearly differentiated from smaller, adjacent nuclei. This is the beginning of a new oöcyte generation that probably started a few days earlier. When the queen and the colony are through one-third of the nomadic phase, 20 or more oöcyte nuclei may be seen in each germarium. In the early statary phase before physogastry has advanced to a very noticeable degree, all the oöcytes are formed and traces of stainable cytoplasm encompass each of the lower ones. While most of them lie in the germarium, some seem to have migrated into the upper end of the vitellarium. Their nuclei are also somewhat larger than before. They do not seem to migrate lower in the physogastric queen, but the nuclei enlarge and in the lower oöcytes take eccentric positions within their slightly increased amount of cytoplasm.

After the queen has oviposited and returned to the contracted condition, these oöcytes descend farther into the upper portion of the vitellarium, preceded by the debris derived from the late oviposition. One complete colony cycle will end at the close of the statary phase. With the beginning of the next nomadic phase, the oöcytes are largely, or entirely, in the vitellarium. They now become the oöcytes of the current generation, and a few days later a new, or second, generation of young oöcyte nuclei begin to appear in the germarium above them. The oöcytes of the present, or current, generation slowly mature, acquire a definitive follicle and a definitive nurse cell mass. Their development and maturity seem to be hastened at the close of this nomadic cycle. The nurse cell masses become nurse cell bodies, and the follicles are fully developed as the lowest oöcytes mature. When several of the oöcytes absorb their nutriment from the nurse cells, the beginning stage of physogastry is quite evident. As this activity extends upward to the other oöcytes of this generation and the oldest oöcytes pass into the oviducts, physogastry is completely achieved. With the deposition of the current generation of oöcytes and the return of the queen to the contracted condition, the second generation oöcytes, lying principally in the germarium, will descend to replace them. Upon the approach, or beginning, of the nomadic phase again, a third generation of oöcyte nuclei will appear in the germarium. Thus, for the short period between ovulation and nomadism, there will be but one generation of oöcytes high up in the vitellarium and lower end of the germarium. At all other times two will be present.

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