THE SIGNIFICANCE OF GERMARIA IN DIFFERENTIATION OF OVARIOLES IN FEMALE APHIDS

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Introduction

Recognition of the fact that winged parthenogenetic female aphids produce both parthenogenetic and gamic female offspring differing in part in the structure of the ovarioles, invites an understanding of the mechanism that controls the development of the ovarioles. Such an undertaking may aid in determining how genes, presumably identical, can produce two types of individuals.

In studies on the development of aphids (Lawson, 1939), it was noted that the germaria are the first embryonic structures to mark a distinction between gamic and parthenogenetic females. Other differentiating characters do not appear until after birth. As determination of all differentiating characters occurs before birth (Shull, 1930*a*), it is possible that the germaria are instrumental in determining the adult nature of the individual at least in so far as the ovarioles are concerned.

In female aphids the essential reproductive organs consist of a pair of ovaries in which the eggs are developed, and an oviduct leading from each ovary to an external opening. Each ovary is made up of a number of loosely parallel ovarian tubes (ovarioles) which open into the oviduct. Three different regions are recognized in an ovariole (Fig. 1),—the terminal filament, the germarium, and the vitellarium. The terminal filament is a thread-like structure at the end of the ovariole farthest from the oviduct, which attaches the ovariole to the body wall. Next behind the terminal filament is the germarium which contains the germ cells from which the eggs develop, and nurse cells whose function is to furnish nutriment to the developing eggs. The vitellarium is a tubular structure which extends from the germarium to the oviduct and contains developing eggs in a gamic female and both eggs and embryos in a parthenogenetic female. A nutritive thread or yolk stream extends from the nurse cells to the youngest growing oöcyte in the vitellarium.

Differences between Gamic and Parthenogenetic Females

Gamic females are described by Shull (1930*b*) as follows: "Gamic females of this species of aphid have a wax yellow body color, dark

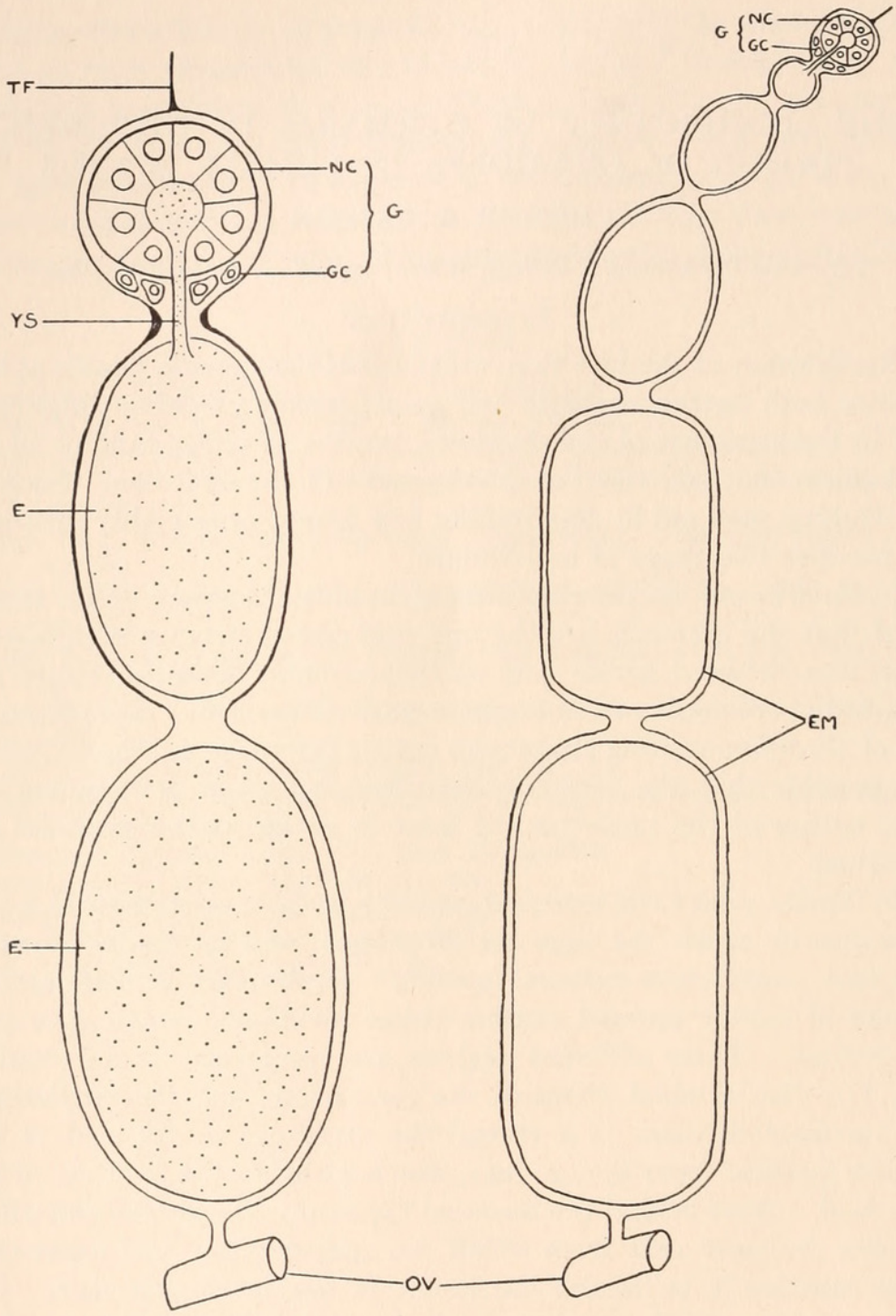

FIG. 1. Diagrams of aphid ovarioles, gamic at left, parthenogenetic at right. *E*, egg; *Em*, embryo; *G*, germarium; *GC*, germ cell; *NC*, nurse cell; *OV*, oviduct; *TF*, terminal filament; *YS*, yolk stream.

brown antennae, and greatly swollen hind tibiae of dark brown color, covered with hundreds of sensoria. Their reproductive systems consist of a vagina on which are borne a pair of colleterial glands and a seminal receptacle, a pair of short oviducts formed as branches of the vagina, and a variable number (usually ten) of ovarioles branching

from the oviducts. Each ovariole, in a mature female, contains usually one mature or nearly mature egg, distinctly opaque and of very regular ovoid form; beyond this often an oöcyte, in early growth stage, hence long and slender and not very opaque; and lastly a large, spherical germarium forming a conspicuous knob at the end of the ovariole. In old gamic females, especially those that have not been laying eggs, the second reproductive cell from the base of the ovariole may be large and opaque and regular in form, and is then presumably mature like the one posterior to it. Almost never, however, in typical gamic females (that is, those produced at low temperature by winged females whose other daughters are practically all gamic), are there more than two oöcytes in any stage in one ovariole. An ovariole of a gamic female may therefore be regarded as regularly consisting of a tube containing one or two eggs or oöcytes, and a large round germarium."

In stained sections of adult gamic females the ovarioles (Fig. 1) are prominent in the abdominal cavity. The germaria usually lie anterior to the large yolk-laden eggs. Each germarium is surrounded by the closed end of an ovariole tube composed of a single layer of thin, squamous epithelial cells. Posterior to the germarium the tube constricts, forming a short neck in which the lumen is quite narrow. The narrowing of the lumen is due in part to contraction of the tube, but also to an increase in the height of the cells which change from a squamous to a columnar type in the neck region. A constriction of the tube likewise occurs between the eggs contained in the vitellarium, but this is due to contraction only as there is no change in cell shape. Around the young growing oöcytes the ovariole wall is constructed of a single layer of cuboidal cells. In the posterior part of the ovariole the cells are rectangular with the greatest width parallel to the surface of the egg.

The germarium is composed of two types of cells, a round ball of large nurse cells and a small group of germ cells. Each nurse cell is roughly pyramidal in shape (triangular in section) with the base at the periphery of the germarium and the apex in the center. The nurse cells fit closely together and form a ball, in the center of which is a substance secreted by the nurse cells. This substance flows from the central area through the neck of the ovariole into the growing oöcyte within the vitellarium. The exact nature of this substance is unknown, however, as it flows directly into the growing oöcyte; it might be yolk. No better term is available, hence the term "yolk" is used to facilitate discussion, and the term "yolk stream" is used to indicate the distinct cord, or string of substance which passes from the germarium into the growing oöcyte.

Germ cells, smaller and fewer in number than the nurse cells, lie between the ball of nurse cells and the neck of the ovariole. Posterior to the neck the vitellarium contains ovoid oöcytes in various stages of growth. Because only young adults were used for this study, it is likely that no mature eggs were examined. Each oöcyte consists of a central mass of yolk surrounded by a thin peripheral layer of cytoplasm. Outside of this cytoplasm is a single egg membrane. In the smaller younger oöcytes the nucleus occupies the center of the cell, but in older ones it lies at the periphery halfway between the two ends of the egg. The nucleus is large and clear and contains but a few small bodies which stain heavily.

Parthenogenetic females are described by Shull (1930*b*) as follows: "The parthenogenetic females have bright green body color, antennae quite pale except in the distal segment, and very slender, pale hind tibiae bearing no sensoria. The reproductive system consists of a vagina, without collegerial glands or seminal receptacle, two short oviducts branching from the vagina, and a variable number (apparently up to ten) of ovarioles branching from the oviducts. Each ovariole is a very delicate tube containing, in healthy individuals, usually six to nine embryos or eggs or oöcytes, and bearing at the end a very small germarium which is usually not much larger, and is often smaller, than the oöcyte or egg next behind it. All of these reproductive elements except one (the one next to the germarium) are as a rule embryos in some stage of development. They are all translucent or transparent, unless dead, and if dead they have a clouded appearance not at all like the opaque gamic eggs. Only the smaller embryos are ellipsoidal; the medium and larger ones always possess angles which correspond to the form of the young aphids. The six to nine embryos or eggs in one ovariole, in a typical healthy female, are of regularly decreasing size from oviduct to germarium, so that they resemble a tapering string of beads."

In stained sections of parthenogenetic female aphids the entire abdominal cavity is crowded with embryos of varying sizes and stages of development. In general, the larger more developed embryos lie in the posterior region while the smaller less developed embryos lie anterior to them. The germaria (Fig. 1), small and difficult to locate, usually lie in the anterior abdominal region squeezed among young embryos or between the embryos and the lateral body wall. The wall of the ovariole tube around the germarium is a single squamous epithelium which constricts, forming a neck just posterior to the germarium. The cells become somewhat cuboidal in this region. Extending posteriad from the neck the ovariole wall encloses embryos,

and is composed of a thin single layer of squamous cells. The tube is always constricted between the embryos within any one ovariole. The parthenogenetic germarium consists of two types of cells, a round ball of nurse cells and a small group of germ cells. Each nurse cell is roughly pyramidal in shape with the base at the periphery of the germarium and the apex in the center. The nurse cells fit closely together and form a ball. This ball of nurse cells contains yolk, and a yolk stream extends from the nurse cells through the ovariole neck into the youngest growing oöcyte. The germ cells lie in the germarium between the ball of nurse cells and the neck of the ovariole. Young oöcytes are found immediately posterior to the germarium. Progressing caudad, the next in line is usually an egg in cleavage followed in turn by a young developing embryo. Thereafter each succeeding embryo is larger and more fully developed.

Major Differences between Adult Gamic and Parthenogenetic Ovarioles

The primary difference between gamic female and parthenogenetic female ovarioles is in the development of the germ cells. In gamic female ovarioles development of germ cells consists of growth through accumulation of yolk and possibly meiosis, though no divisions have been observed in this species of aphid. In the parthenogenetic female ovarioles the germ cells are stimulated to develop parthenogenetically.

The size difference in the germaria presumably is secondary to and correlated with the germ cell difference. Gamic female ovarioles contain eggs that undergo embryonic development outside of the mother's body, are dependent on a large yolk supply and consequently accumulate this yolk supply during growth in the ovariole. The large size of the gamic female germarium (about three times larger than a parthenogenetic female germarium in fixed material) is evidently correlated with the necessity of producing much yolk. Eggs produced by parthenogenetic females develop within the body of the mother, and it is probable that nourishment for this growth and development is supplied directly by the mother by body fluid. Thus a large quantity of yolk is unnecessary for parthenogenetic eggs, and the small size of parthenogenetic female germaria may be correlated with this decreased secretory activity.

Ovarioles of Gamic Female and Parthenogenetic Female Embryos

The ovarioles of gamic female and parthenogenetic female embryos were studied and compared in those embryos of both types which

showed the greatest degree of development. These embryos were well developed, occupied the posterior abdominal region of the mother and, presumably, would have been born very shortly had the mother been allowed to live.

Each ovariole in gamic embryos of this late stage of development consists of a terminal filament, a germarium and a vitellarium (Fig. 2). Each germarium contains nurse cells and germ cells. The nurse cells are roughly pyramidal in shape and form a ball at the end of the

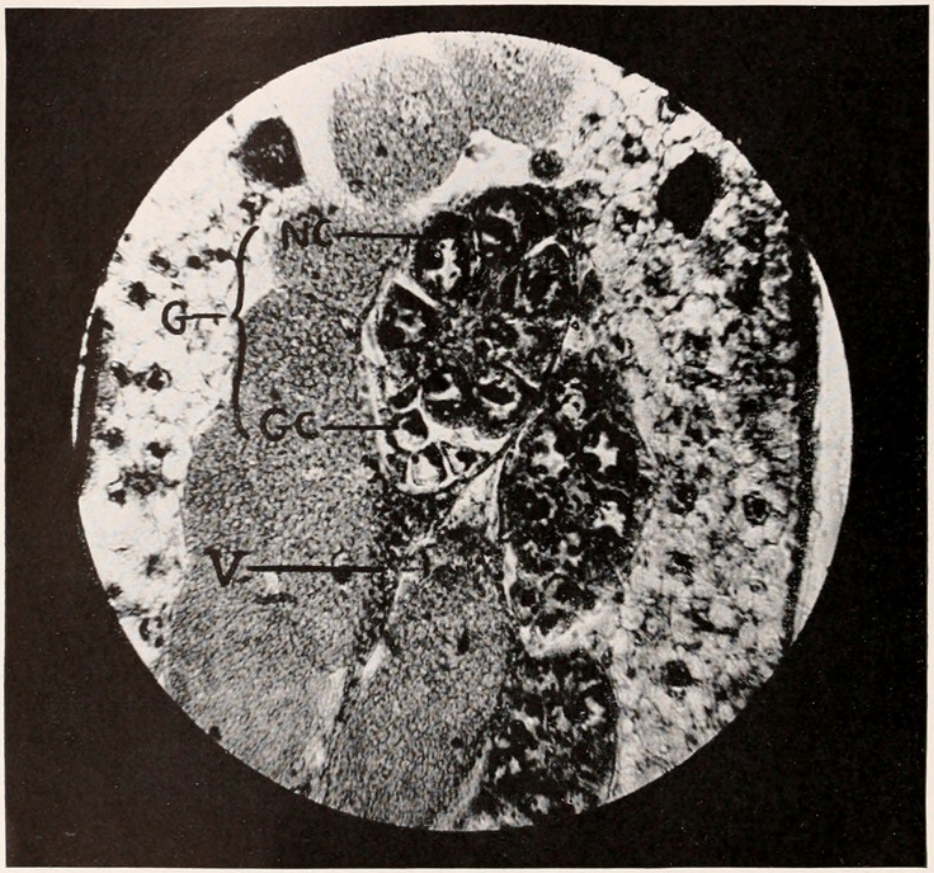

FIG. 2. Photomicrograph of an embryonic ovariole of a gamic female aphid. *G*, germarium; *GC*, germ cell; *NC*, nurse cell; *V*, vitellarium.

ovariole. This is similar to the adult condition although the cells seem to be more loosely packed and the entire germarium is more elongated than in the adult. The center of the ball of nurse cells contains yolk, but there is no yolk stream. Germ cells occupy the region of the germarium posterior to the nurse cells. The ovariole tube surrounds the germarium as a simple squamous epithelium and continues posteriad as a small tubular vitellarium with a narrow lumen. The vitellarium contains no eggs or oöcytes.

In parthenogenetic female embryos of the same degree of develop-

ment the ovariole consists of a terminal filament, a germarium, a vitellarium, and parthenogenetically developing germ cells (Fig. 3). Each germarium consists of nurse cells and germ cells. The nurse cells form a ball of cells at the tip of the ovariole tube within which is found a small amount of yolk. A yolk stream extends from the center of the ball of nurse cells through the neck of the ovariole into the youngest growing oöcyte. The germ cells lie just behind the nurse cells. Each germarium is surrounded by the closed end of the ovariole tube which

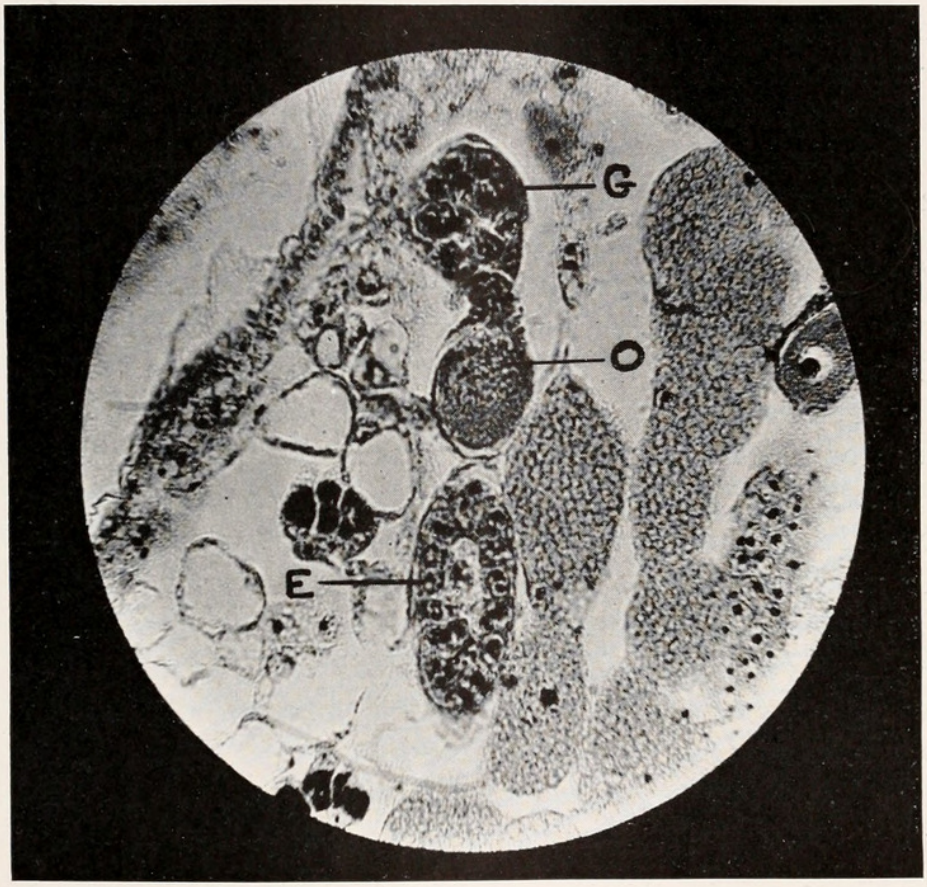

FIG. 3. Photomicrograph of an embryonic ovariole of a parthenogenetic female aphid. *E*, egg undergoing cleavage; *G*, germarium; *O*, oöcyte.

consists of a thin squamous epithelium. The ovariole tube continues posteriad of the germarium as a thin-walled vitellarium which invariably contains a growing oöcyte just behind the germarium and an egg undergoing cleavage behind the oöcyte.

To insure that embryos identified as gamic females were actually gamic, parthenogenetic winged females were fixed when they were producing gamic females only. The parthenogenetic female embryos were studied only in wingless parthenogenetic females which seldom produce gamic females. The offspring of both types of parents produced before fixation were reared and examined.

Do Germaria Control Differentiation of Ovarioles?

The possibility that germaria play a significant rôle in the differentiation of ovarioles is indicated by three observations. (1) Adult ovariole differences are due primarily to the type of egg development that occurs within the ovariole and to the size of the germarium. (2) Germaria contain and produce the germ cells that develop within the ovarioles. (3) Germaria are the first reproductive structures to be differentiated in the embryo (Lawson, 1939).

Determination of an embryo into either a gamic or parthenogenetic female must occur sometime during parthenogenetic development between the growth of the oöcyte and the differentiation of the germaria. This determination may affect the embryo in two ways. (1) It may include the entire embryo in its effect so that the differentiation of the germaria would simply represent the first differential reaction of a general condition throughout the embryo. This would exclude the germaria from any significance in future development. (2) It may occur at the time that germaria are developing and affect them only. According to this assumption the embryo would be potentially capable of developing into either a gamic or a parthenogenetic female prior to determination of the germaria. After this event the aphid (more specifically the ovarioles) would become gamic or parthenogenetic depending on the nature of the germaria.

The study of normal gamic and parthenogenetic female aphids offers no choice between the two ways in which determination may occur. However, aphids intermediate between gamic and parthenogenetic are occasionally produced and an analysis of these intermediates gives us a choice.

These intermediates, described by Shull (1930*b*), show the intermediacy in several structures of which the ovarioles only are to be considered here. The intermediacy is expressed in the ovarioles in a very irregular fashion so that no two aphids with intermediate ovarioles are necessarily identical. An intermediate aphid may be a mosaic with respect to the ovarioles, in that one or more of the ovarioles are strictly gamic while the others are strictly parthenogenetic. Any one ovariole may be intermediate in that the germaria are smaller than normal gamic germaria, but larger than normal parthenogenetic germaria. The contents of the vitellarium may be intermediate in three different ways. (1) Eggs may vary from gamic in being less opaque than strictly gamic eggs. (2) Gamic eggs may occur in greater numbers than is normal for a gamic ovariole. (3) Embryos characteristic of parthenogenetic ovarioles may be abnormal. According to Shull's description, it is possible to have any combination of the above conditions in one aphid.

The first type of intermediate mentioned here in which one individual contains both gamic and parthenogenetic female ovarioles could not be produced unless the ovarioles are able to develop within the aphid independently of one another. As the germaria appear in the embryo prior to other ovariole structures, it follows that the germaria also must develop and be determined independently of one another.

The second type of intermediate in which the germaria are intermediate in size between gamic female and parthenogenetic female germaria indicates that the mechanism of determination is such that intermediate germaria are determined and differentiated within the embryo as well as germaria that are strictly gamic or parthenogenetic.

The mechanism of determination suggested by Shull (1930*a*), in which a high level of some substance within the embryo produces one type while a low level of the same substance produces the opposite type, could very easily account for intermediate germaria. These intermediate germaria could result from a condition in which determination of the germaria occurred when the level of concentration of the determining substance was intermediate between the high and low extremes.

The remaining types of intermediate aphids show the intermediacy in the ovariole contents. All of these can be explained by assuming that the germarium attached to the end of each intermediate ovariole is intermediate. As gamic female germaria produce much yolk while parthenogenetic female germaria produce little yolk, it would be expected that intermediate germaria would produce an amount of yolk intermediate between the gamic and parthenogenetic extremes.

One of the types of intermediate ovarioles described by Shull had eggs that were gamic but less opaque than strictly gamic eggs. Such eggs could be produced by a germarium that was gamic with respect to the type of eggs produced but intermediate with respect to yolk production. The decreased amount of yolk in the eggs might make them less opaque than normal gamic eggs.

Another type of intermediate ovariole had gamic eggs in greater numbers than the typical one or two of strictly gamic ovarioles. One characteristic of gamic germaria is that they produce no more than two eggs while parthenogenetic germaria produce many more than two. The above intermediate ovariole could have resulted from a germarium that was intermediate with respect to the number of eggs produced, while at the same time it was gamic with respect to the type of eggs produced.

The last type of intermediate described by Shull contained par-

thenogenetic ovarioles in which the embryos were abnormal. The details of the abnormality were not described but it is possible that the abnormality could have been due to an intermediate germarium in which parthenogenetic oöcytes or eggs were produced plus a quantity of yolk greater than is normal for a parthenogenetic germarium. This abnormal amount of yolk very likely would interfere with normal embryonic development and produce abnormal embryos.

The theory is proposed that germaria are determined independently of one another and also of the rest of the aphid embryo, that a single germarium may be caused to develop into a gamic female type, a parthenogenetic female type or a type intermediate between the gamic and parthenogenetic types. It is proposed, further, that the germarium once determined, controls the differentiation of the ovariole to which it is attached and thus controls, in part, the development of the adult aphid type.

Summary

Winged parthenogenetic female aphids produce both parthenogenetic female and gamic female aphids.

The ovarioles of adult gamic female and parthenogenetic female aphids differ primarily in the nature of the eggs developing within them and secondarily in the size and secretory activity of the germaria. Gamic female germaria are large and secrete much yolk; parthenogenetic female germaria are small and secrete little yolk.

The ovariole differences apparent in the adult aphids are also evident in the embryos. In parthenogenetic female embryos of a late stage of development the embryonic ovarioles already contain oöcytes and eggs undergoing parthenogenetic development while in the gamic female embryos of the same stage of development the germ cells have not yet entered the vitellarium. The germaria of parthenogenetic female embryos are smaller than the germaria of the gamic embryos of the same stage of development.

In both gamic and parthenogenetic female embryos the germaria are the first reproductive structures to develop.

The theory is proposed that determination of the ovariole type (either gamic female or parthenogenetic female) affects the germaria only. Each germarium, thereafter, controls the development of the ovariole to which it is attached.

Aphids intermediate between gamic female and parthenogenetic female aphids with respect to the ovarioles are described and analyzed to support the above theory.

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