SUMMARY
Gametes of \textit{C. reinhardi} lack the cell wall which vegetative cells possess. Just below the cell apex gametes form a fertilization tubule which is up to 2 \( \mu \) long and 0.2 \( \mu \) in diameter; its plasma membrane and that of the apex have slender tubular projections. At the base of the fertilization tubule regularly lies the choanoid body, a collar-shaped cytoplasmic organelle; the plasma membrane overlying the body appears as an electron-dense ring. Gametes possess two 'free' basal bodies in addition to the basal bodies of the two flagella.

In the initial stage of union the conjugating cells are connected by the fertilization tubule whose plasma membrane is continuous with that of both copulants. At one end of the tubule lies a conspicuous choanoid body, but at the other end is a small structure which possibly is a homologue of the choanoid body. Subsequently, the fertilization tubule shortens and widens until finally no tubule exists and the apical ends of the two protoplasts adjoin. The merging cells then bend like a jack-knife and lateral alignment of the protoplasts occurs. This four-flagellated zygote becomes motile at about the time when the flagellar bases of the former gametes seem to approach each other and when fibrillar elements of the flagellar roots come into contact. In the motile zygote the nuclei do not fuse but remain ensheathed in the cup-shaped plastids of the two gametes.

A mating of plus (+) and minus (−) strains cultured, respectively, for high and low starch content suggested that gametes of only the plus (+) mating type contain the choanoid body. Since it appears that the gamete containing the choanoid body also produces the fertilization tubule, it is inferred that gametes of only the plus (+) mating type produce the fertilization tubule. Should further investigation support this inference, it would be established that there is a structural basis for designating the plus (+) mating type as male and the minus (−) type as female.

Fertilization involves fusion of the gamete membranes through the mediation of a specialized structure (the fertilization tubule) and in this respect there are similarities to certain aspects of fertilization in animal phyla. The relation of the fertilization tubule to the protoplasmic bridge of other species of \textit{Chlamydomonas} is discussed.

INTRODUCTION
Fine-structural details of the fertilization process in algae are to a considerable extent still unknown. The process in the green alga \textit{Prasiola stipitata} has been studied in thin sections by Manton & Friedmann (1960). In this species the plasma membranes of the two gametes coalesce and then the internal cellular contents merge. In the beginning the membrane of one of the two spermatozoid flagella coalesces with the membrane of the egg, and the fibrillar core of that flagellum...
becomes the first part to be incorporated into the egg protoplast. However, the coming together of the gametes by means of such a flagellum is not characteristic of all types of algal fertilization. It was suggested (Friedmann, 1964) that fertilization in algae might follow two different patterns, the one being exemplified by *Prasiola* and the brown algae in which the gametes join by means of one of the male flagella, and the other by *Chlamydomonas* in which the junction involves rather the apical regions of the gametes. It is therefore of interest to investigate details of the pattern in *Chlamydomonas* by electron microscopy.

Among the *Chlamydomonas* species generally used in laboratory investigations, *C. moewusii* and *C. eugametos* have motile zygotes, termed 'vis-à-vis pairs' by Lewin (1954a), which seem to represent a type found only in a relatively small number of algae. In contrast, the four-flagellated motile zygote of *C. reinhardi* seems to be a characteristic type occurring in the great majority of isogamous green algae. This species, therefore, was selected for the present investigation.

In *C. eugametos*, early electron-microscopic studies by Lewin & Meinhart (1953) showed the protoplasmic bridge described by Moewus (1933) connecting the two gametes in the motile zygote (vis-à-vis pair) and Gibbs, Lewin & Philpott (1958) in a study of thin sections reported that the bridge, delimited by a membrane, is first filled with homogeneous cytoplasm, and that later an electron-dense core deriving from the blepharoplasts appears to grow across the bridge. Johnson (1964) reported having studied by electron microscopy the sexual reproduction of *C. moewusii*.

Fertilization in *Chlamydomonas reinhardi* was observed in the light microscope and recorded by phase-contrast cinemicrography and the results will be treated in a forthcoming publication. In the present study an attempt was made to match the stages in fusion of the protoplasts seen in thin sections with the stages seen in cinemicrographic records. A short preliminary report of the present study was published earlier (Friedmann, Colwin & Colwin, 1966).

**MATERIALS AND METHODS**

Cultures of *C. reinhardi* were obtained from the Culture Collection of Algae at Indiana University (strains 89 and 90) and from Dr Ruth Sager, Columbia University, New York (strains 21 gr and 6145 c). These have been used with similar results. However, strain 21 gr, considered as plus (+), crosses with strain 89, also considered as plus (+); and conversely, strain 6145 c, considered as minus (−), crosses with 90, also considered as minus (−). These strains were subsequently tested against strains 11/32 a (+) and 11/32 b (−) obtained from the Culture Collection of Algae and Protozoa at Cambridge University; the latter strains represent the original isolates of Dr G. M. Smith sent to Cambridge in 1950. It was found that strains 21 gr and 90 are of the plus (+) mating type and 6145 c and 89 are of the minus (−) mating type. Cultures were grown on agar plates under standard culture conditions (14-h light cycle) using Bold's (1949) modified Bristol medium or nutrient medium I of Sager & Granick (1953). Gametes were harvested in distilled water and suspensions of mating gametes were fixed in 1% phosphate-buffered osmium tetroxide or in 4% phosphate-
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buffered glutaraldehyde at pH 7.2. After fixation for 2-2.5 h at room temperature, the preparations were washed, dehydrated and embedded in Epon according to the procedures of Luft (1961). Sections were cut with a DuPont diamond knife, stained with lead citrate (Venable & Coggeshall, 1965) and uranyl acetate (saturated aqueous solution diluted before use with an equal volume of absolute ethanol). For the present study the osmic-fixed material proved more suitable and all the electron micrographs show such material. Sections were examined with an RCA EMU 3 C electron microscope. Electron micrographs generally were taken at original magnifications of × 5500 to × 32,000; the final magnifications were obtained by photographic enlargement, and their approximate values are indicated with each figure.

OBSERVATIONS

Structure of the cell apex

Since initial stages of fertilization in Chlamydomonas involve the apical portion of the cell, some knowledge of the structure of this will facilitate the understanding of the cytological events to be presented. Ringo (1967) has recently described the structure of this region in some detail and only such additional material as is pertinent
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will be presented here. A diagram of the region is shown in Fig. 1; longitudinal sections are shown in Figs. 3–5, 8 and 10, and cross-sections in Figs. 28–32. Some of these figures are of vegetative cells or of unattached gametes and others are of fused cells, but the structures to be described are essentially the same in appearance whichever the state of the cell.

The two flagella arise from opposite sides of the base of a small apical papilla (Figs. 1, 3, 4). Their bases are connected by one larger distal striated cross-band (Figs. 3–5, 10, 30) and two smaller proximal striated bands. The three bands represent the ‘striated fibres’ of Ringo. Longitudinal sections of the larger band show, in addition to the conspicuous cross-striations, about 5 slender longitudinal fibres (Fig. 5). Beneath the larger cross-band originate four fibrous flagellar roots (‘microtubule bands’ of Ringo) which, when seen from the cell apex, first run crosswise (Fig. 31) and then turn towards the posterior end of the cell, each one running close to the cell surface within one of the four lateral ridges (Fig. 32) which occur in the apical portion. The flagellar roots are readily seen in many of the figures, such as Figs. 10, 12–14, 19, 20, 26, 27 and 33–35. There are tubular components and also finer fibres in these roots. In the cross-section of a root shown in Fig. 37, two of the finer fibres lie between one proximal and three distal tubular components.

One basal body is associated with the base of each flagellum but in addition to these, two other basal bodies which apparently are not associated with the flagella are present in each cell. The four basal bodies are situated in regular positions between the arms of the cross formed by the flagellar roots, as shown in cross-sections of an apical region in Figs. 31, 32. In this specimen the two ‘free’ basal bodies, shown in cross-section, had their long axes parallel to that of the body, whereas the long axes of the basal bodies of the flagella were oblique to these, and indeed, were somewhat oblique to each other. A similar situation was found in several cells. In some specimens, however, one of the free basal bodies lay perpendicular to ‘associated’ ones are shown in Figs. 33 and 36.

Additional features in the cell apex are the two contractile vacuoles (Figs. 8–14, 20) and the mitochondria. Each of the four lateral ridges of the cell apex shown in Fig. 32 contains a section of a mitochondrion. Probably, however, there are only two mitochondria in the apical region; thus the four sections in Fig. 32 do not represent four separate mitochondria.

Development of gametes from vegetative cells

The cell wall. Vegetative cells possess a cell wall, whereas gametes do not. This wall is thin and not very readily visible in the light microscope. In electron micrographs the most conspicuous part of the wall is at the anterior end of the cell (Fig. 3) where it forms a thick plate. The plate is traversed by two channels through which the flagella protrude.

In the process of becoming gametes, vegetative cells lose their cell walls. Figure 4 shows the anterior end of a gamete. It is uncertain whether the wall is dissolved in situ or first shed and subsequently dissolved. Generally, no traces of empty cell
walls can be found in gamete suspensions; however, sections of gametic cells with part of the cell wall still present occasionally were observed.

The fertilization tubule and the choanoid body. A very distinctive feature of the gamete stage is the fertilization tubule, a cylindrical organelle up to 2 µ long and about 0.2 µ in diameter (Figs. 7-17). There is some probability (to be considered below) that this tubule characterizes only one of the mating types. The position of the tubule in the gamete is shown in Fig. 1. The four lateral ridges of the cell apex are separated from each other by grooves, of which there are also four. The two flagella arch over two of the grooves, and the fertilization tubule arises from one of the other grooves. The base of the tubule is situated slightly off the median of the groove, and lies approximately at the level of the flagellar bases. The site of the tubule in one of the grooves can be seen in the serial sections in Figs. 11-14, while Figs. 8 and 10 illustrate the position of the tubule in relation to the flagella. An early stage in the development of the tubule is shown in Fig. 7; presumably this specimen was an incipient gamete since the cell was still enclosed within a wall. The young tubule appears as a hemispherical protrusion of the surface of the cell and its plasma membrane appears as a very dense boundary. The base of the fertilization tubule is conical in all stages and around the base a ring-shaped segment of the plasma membrane sometimes appears to stain more heavily than elsewhere. Sections of this electron-dense ring may be seen in Figs. 7, 8. The tip of the tubule is rounded and appears in some sections to have a dense terminal cap (Fig. 17). It is uncertain, however, whether this terminal cap is always present.

The fertilization tubule carries a varying number of slender tubular projections on its surface (Figs. 1, 9-13, 15-17). These projections vary in length up to 0.5 µ and their diameter is about 25 µ; their terminal portions are at least in some instances dilated. Such projections occur also on the surface of the apical papilla, including the area around the base of the fertilization tubule (Figs. 1, 10). In longitudinal sections of the projections the plasma membrane often appears to be less dense than elsewhere; when the projections are seen in cross-section, however, as in Fig. 10, the membrane usually appears to be similar to the plasma membrane of other parts of the cell. Similar tubular projections occasionally are seen also on the surface of flagella of fusing gametes (Fig. 6). Possibly the projections of the fertilization tubule and of the flagella have some role in establishing surface contact between fusing gametes or in causing 'stickiness' of flagella during clumping (Wiese, 1965) or during pairing. These projections apparently resemble the surface papillae of earthworm spermatozoa described by Cameron & Fogal (1963); the function of the papillae is unknown, but their location in the acrosomal region suggests that these papillae, too, may play some role related to contact between the gametes at fertilization.

The cytoplasm of the fertilization tubule contains slender anastomosing electron-dense threads which form a reticulate pattern (Figs. 11, 12, 15).

Located in the conical base of the fertilization tubule lies a small but conspicuous ring-like organelle, here termed the choanoid body (Figs. 1, 7-10, 14-16, 22-24, 26-30, 33, 37, 38). The shape of this body is that of a lopsided collar or an obliquely truncated hollow cone; the broader end is in the proximal position. Thus in the
developing tubule (Fig. 7), as in older tubules, the broad end faces into the cell. It is just above the choanoid body that the plasma membrane at the base of the fertilization tubule forms the electron-dense ring mentioned above. Although the choanoid body is regularly associated with the fertilization tubule of the gamete, it remains present longer than the tubule and can be seen even after the protoplasts of the pairing gametes have extensively merged. It is beyond the province of this paper to trace the ultimate fate of the choanoid body.

The substructure of the choanoid body is not entirely clear. However, some sections give evidence that the body is built of tubular components which form an irregularly interwoven unilayered mat (for example, Figs. 15, 16, 37), and from certain sections it would seem that at least some of the tubular components run nearly parallel to the base of the cone (Figs. 16, 38). These components appear to be bound together by slender dense threads which in some sections radiate into the surrounding cytoplasm. The threads give the choanoid body a 'hairy' appearance (Figs. 37, 38), which is more pronounced in the four-flagellated zygote than in earlier stages.

**Union of the gametes**

For convenience, in the present description the process of gamete fusion is divided into three stages: (i) association by means of the fertilization tubule to form the initial stage of the zygote; (ii) lateral alignment of the merging protoplasts; and (iii) flagellar co-ordination in the young zygote. No sharp line of demarcation can be drawn between these stages as is evident from the micrographs, some of which might equally well be placed at the end of one stage or at the beginning of the next stage. The agglutination (clumping) of gametes, which occurs prior to their merging, and the late stages in the development of the zygotes, are not within the scope of the present study.

The gamete components of the zygotes shown in the micrographs are designated plus (+) or minus (−) on the basis of evidence to be presented below.

**Association by means of the fertilization tubule.** The earliest stage of gametic union observed in sections was that of conjugating cells connected by a fertilization tubule. The tubule joins the two conjugants at corresponding positions near their apices (Figs. 2, 21–23). The plasma membrane of the tubule is continuous with that of both copulants. Since both are covered by a common continuous membrane this stage represents an early zygote. Initially the diameter of the fertilization tubule which connects the gametes (Fig. 21) is similar to that in an unassociated gamete. Soon, however, the tubule shortens and its diameter increases until finally no tubule exists and the apical end of one protoplast immediately adjoins that of the other within their common membrane. Figures 18–20 and 22–24 represent successive stages in this process.

In early stages of union there is a conspicuous choanoid body at the plus (+) end of the tubule but at the minus (−) end there is only a small slender structure (Figs. 18–21). The origin of this small structure is unknown but in some ways the structure resembles a much reduced choanoid body and possibly it is a homologue of this. The plasma membrane overlying the small structure has an electron-dense ring (Figs. 18,
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21, 22) similar to that which overlies the choanoid body. Throughout the stages during which the tubule shortens and widens, the choanoid body persists in undiminished size, while the small structure at the minus (−) end of the tubule seems to disappear. This structure is barely visible in Fig. 23 and seems to be absent in Fig. 24. The cytoplasm surrounding the choanoid body in the zygote is distinctly hyaline and appears to be free of ribosomes or other plasmatic particles (Figs. 24, 26, 27, 29, 30, 33, 37).

At first the slender tubular projections of the membrane of the fertilization tubule persist (Figs. 18–21), but later they mostly disappear (Figs. 22, 23). A few projections, however, may still be seen as late as the stage illustrated in Figs. 28–32.

Fig. 2. Diagrammatic representation of the two possible positions of paired gametes connected by a fertilization tubule. The outline represents the plasma membrane and the dark discs represent flagellar bases which appear in cross-section. The upper pair is in the cis position and the lower pair in the trans position.

The members of a pair initially may occupy either of two positions in relation to each other. The fertilization tubule joins both gametes at sites lateral to the cell apex, and it is possible for the gametes to be connected with their apices either on the same side or on the opposite sides of the tubule (Fig. 2). These two patterns differ by a torsion of 180° in the relative position of the gametes. The two positions might be likened to the cis and trans position of organic molecules. Both positions occur in C. reinhardi. The pair shown in Figs. 22 and 23 is in the cis position and those shown in Figs. 18–21 and 25 are in the trans position.

Lateral alignment. Figs. 24 and 25 represent the end of the first stage in the merging of the gamete proplasts: the fertilization tubule is no longer discernible and the apical ends of the two gametes are in direct apposition within their common plasma membrane. The nucleus of each gamete still lies in the cavity of a cup-shaped plastid (Fig. 25). During the next stage the merging cells bend as a jack-knife and the region of cytoplasmic confluence widens, extending toward the posterior end. A pair of merging gametes in the jack-knifing phase is represented in Figs. 28–32; the member
shown on the left appears in cross-section, the one on the right in longitudinal section. A later stage is represented in Figs. 26, 27 and 33–35; by this stage the region of confluence has extended all the way to the posterior end but a lateral furrow still persists on the cell surface, and this furrow permits one still to distinguish the two component protoplasts. The furrow or groove is apparent in Fig. 34, while Fig. 33, which represents a section closer to the median of the same zygote, shows it only as a small interruption. Within the zygote membrane, then, the shapes of the two gamete protoplasts can for some time be distinguished (Fig. 27).

Stage of flagellar co-ordination. The lateral alignment of the gamete protoplasts within the common plasma membrane does not indicate that the zygote is already motile. Observations of living cells at the stage of lateral alignment show that the four flagella beat haphazardly and no locomotion takes place. Only later, after the movements of the pairs of flagella from the two gametes become co-ordinated, does the four-flagellated zygote start to swim. The living zygote by this time has assumed a more regularly rounded shape than hitherto and sections showing zygotes of this shape (Fig. 36) are assumed to show specimens at this stage. The site of continuity between the merging gametes is no longer marked by the furrow or groove seen in earlier stages. As the zygote becomes more rounded the bases of the two flagellar pairs seem to approach each other more closely than before. The fibrous components of the flagellar roots of the two former gametes come into contact over some length and there is some indication that these fibres may be connected by periodically arranged slender filamentous strands. Figures 34 and 35 show the fibrous components during an early stage of their meeting. It is unknown whether some connexion between the flagellar roots is causally related to flagellar co-ordination, but in any case, it seems as though it is only after the above-mentioned changes take place that the zygote becomes motile. The two cup-shaped plastids, each embracing a gamete nucleus, persist during the motile stage of the zygote.

As mentioned previously, gametes contain, besides the basal bodies associated with the two flagella, two additional, unattached basal bodies. Zygotes have four flagellar and four free basal bodies. Figures 31 and 32 show the flagellar and the free basal bodies in one member of a jack-knifing pair, while Figs. 26, 33 and 36 show some of the free basal bodies in zygotes in later stages. In Figs. 26, 27, 33 two free basal bodies may be seen lying between the four basal bodies of the flagella while in Fig. 36 one of the free basal bodies is situated outside those related to the flagella.

Relation of choanoid body to mating type

As indicated above, during the earlier stages of the zygote, when the two protoplasts are connected by a fertilization tube, a conspicuous choanoid body is found at only one end of the tube. When the tube shortens and widens as the protoplasts increasingly merge, the position and orientation of the choanoid body remains stable. The broad end of the body, which in the gamete is the proximal end, in the zygote continues to face toward that 'gamete' from which the body originated. Thus the orientation of the choanoid body can serve to indicate, even in the zygote, which of the two gametes produced the body (compare Fig. 7 with Figs. 23, 24).
An attempt was made to determine whether gametes of one or of both mating types produce the choanoid body. A culture of the plus (+) strain, 21 gr, was labelled by high starch content of the plastids and then mated with a starchless culture of the minus (−) strain 6145c. This labelling permitted the strains of the two components of the zygote to be distinguished in sections. In each of seven pairs which were examined, the component which contained a conspicuous choanoid body belonged to the high starch strain. In addition, three unattached gametes which had a choanoid body were also of that strain. From these findings it was concluded that only one of the mating types produces the choanoid body. Since the high starch strain was of the plus (+) mating type, it was concluded that it is this type which produces the choanoid body. The evidence, however, derives from only a single labelled mating experiment, so that additional evidence is needed before the choanoid body can be related conclusively to the plus (+) mating type. Nevertheless, throughout this paper, the position and orientation of the choanoid body are used as the basis for designating the merging gametes as plus (+) or minus (−).

**DISCUSSION**

*The fertilization tubule*

The fertilization tubule of *Chlamydomonas reinhardtii* as revealed by electron microscopy may represent a structure reported in light-microscopical studies of various green algae. Earlier investigators of isogamous species repeatedly mentioned a fine cytoplasmic connexion between two pairing gametes, and the dimensions of the fertilization tubule are such as to place it within the range of light-microscopical visibility. The first record stems from Ghorozhankin (1875), who illustrated in figure 29 of his paper a fine connexion between fusing gametes of *C. reinhardtii* (*C. pulvisculus* Ehrb.). Other investigators (including Wille, 1878; Dangeard, 1901; Korschikoff, 1927; Strehlow, 1929; Lerche, 1937; Pringsheim & Ondraček, 1939; Bold, 1949; Herndon, 1958; Stein, 1958; and Gerloff, 1962) have described or illustrated similar structures in a number of other isogamous species. It seems possible that a structure corresponding to the fertilization tubule may occur also in oogamous species. A 'perforatorium' was described, for example, in an oogamous species of *Chlamydomonas* (Geitler, 1954), and Hoffman (1961), writing of fertilization in the oogamous *Oedogonium cardiacum*, states that 'a delicate cytoplasmic connexion is established between the egg and the tip of the sperm' and indicates that subsequently this connexion, or strand, rapidly thickens. In fungi, as well as in algae, there is evidence of a structure which may also prove to be comparable to the fertilization tubule. Thus, in the chytridiomycete *Blastocladiella cystogena* the anterior ends of the two uniflagellate isogametes are connected at the initial stage of fertilization by a thin 'pseudopodium' (Whiffen, 1942).

In *C. reinhardtii* the narrow fertilization tubule establishes continuity between the plasma membranes of the two copulants, bringing the contents of the cells into continuity, and thus the zygote has its inception. This circumstance brings to mind
that which prevails in a few isogamous species of *Chlamydomonas*, such as *C. eugametos* and *C. moewusii*. In these species the two gametes become continuous at their apical ends to form a motile entity, the *vis-a-vis* pair of Lewin (1954a), in which the two protoplasts are connected by a protoplasmic bridge (Moewus, 1933; Gerloff, 1940; Mitra, 1951; Lewin, 1950, 1952, 1954a, b, 1957a, b; Lewin & Meinhart, 1953; Tsubo, 1956, 1957, 1961). The relation of the fertilization tubule of *C. reinhardtii* to the protoplasmic bridge needs clarification.

The condition of motility during the period when the tubule or the bridge, respectively, is present may have some significance for evaluating the relationship. *Vis-a-vis* pairs, in which the bridge occurs, exhibit the locomotor activity of a single entity, that is, the motile zygote; and this motility ceases as soon as the two protoplasts merge into one spherical body. In contrast, the zygotes of *C. reinhardtii* become motile only at a stage later than that of the fertilization tubule, indeed, only after the protoplasts have nearly finished merging. The tubule of *C. reinhardtii*, then, characterizes the pre-motile stage of the zygote, whereas the bridge of the other species characterizes the motile stage.

The fine structure of the bridge (Lewin & Meinhart, 1953; Gibbs *et al.* 1958) is difficult to equate with that of the tubule as described in the present paper. For *vis-a-vis* pairs Gibbs *et al.* referred to ‘tenuous extensions’ which project from the apical papillae of the cells. These extensions were reported from stages before ‘complete protoplasmic bridges were found’, and in at least one case such extensions ‘were seen to have met’. It seems probable, therefore, that the various stages of the fertilization tubule of *C. reinhardtii* would correspond to these tenuous extensions rather than to the protoplasmic bridge into which presumably the extensions develop.

The electron-dense rings in the plasma membrane where the fertilization tubule of *C. reinhardtii* arises in one gamete and where it joins the other gamete recall the annular thickening of the plasma membrane of intercellular bridges described by Fawcett and his associates (see Fawcett, 1961), but in the present case the intercellular connexion is clearly between two formerly separate cells.

The choanoid body described in the present study does not seem to have been observed by previous investigators; the electron micrographs of sections of *vis-a-vis* pairs by Gibbs *et al.* (1958) do not permit any conclusions to be drawn regarding the presence or absence of such a body.

**Comparison with fertilization of other groups**

A comparison of fertilization in *C. reinhardtii* with that in other algae and in certain animal groups elicits some interesting points of similarity. So far as is known, in all these groups syngamy is attained by fusion of the gamete membranes through the mediation of a specialized structure or region of the male gamete. Thus in the oogamous green alga *Prasiola stipitata* (Manton & Friedmann, 1960; Friedmann, 1962) the mediating organ is one of the flagella of the male gamete. Such a structure has been reported also from light-microscopical studies in *P. stipitata* (Friedmann, 1960) and *P. meridionalis* (Cole & Akintobi, 1963; Bravo, 1965), as well as in brown algae (Friedmann, 1961). In all these cases this special flagellum exists prior to
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gametic association. In *C. reinhardi* the mediating structure is the fertilization tubule, which is formed only in relation to fertilization, and this special structure functions to achieve syngamy by contact, and then fusion, with the plasma membrane of the other gamete. In some ways this resembles certain of the events of fertilization in such animal phyla for which information from electron microscopy is available. In these animal groups a special segment of sperm plasma membrane, deriving from the acrosome, develops into a special organelle, the acrosomal tubule, and it is this tubule which makes contact and fuses with the egg plasma membrane (reviewed by Colwin & Colwin, 1964, 1967b). Not only is the acrosomal tubule the structure which normally participates in this fusion but there is evidence that this tubule is the only segment of sperm membrane capable of fusing with the egg plasma membrane (Colwin & Colwin, 1967a). Even though these algal and animal groups are phylogenetically widely separated, all achieve syngamy by gamete membrane fusion, and the participating segment of membrane of the male gamete is that of a special structure.

The joining of two cells to form a new single cellular entity by means of fusion of their plasmalomenmas was first demonstrated in gametes (Colwin & Colwin, 1960, 1961, 1963a, b; Manton & Friedmann, 1960; Szollosi & Ris, 1961) and has since been demonstrated between gametic and somatic cells (Colwin & Colwin, 1967a); the same means of junction is implicit also in fusion between somatic cells (see, for example, Okada, 1962; Harris, Watkins, Ford & Schoeff, 1966).

**Relation of mating type to sex**

The nature of the gametes involved in isogamous fertilization requires some comment. According to the accepted definition, isogametes are morphologically identical, and for this reason they are usually designated as plus or minus rather than as male or female. There is evidence, however, which seems to indicate that the plus (+) and minus (−) mating types of isogamous organisms are at least physiologically and biochemically different. Thus, in isogamous species of *Chlamydomonas* with zygotes in the *vis-à-vis* pair stage, one member of the pair retains its motility while the other becomes paralysed (Lewin, 1950, 1952, 1954a); and in *C. reinhardi* and *C. eugametos*, both the structure and action of the sex substances are different in the two mating types (Förster & Wiese, 1955; Förster, Wiese & Braunitzer, 1956).

Now it seems that in *C. reinhardi* there may be some structural basis for deciding which mating type would represent which sex. As described above, only one gamete of a pair appears to form a conspicuous choanoid body. Since the choanoid body in the gamete is always associated with the fertilization tubule, it is inferred that the fertilization tubule too is formed only by this gamete. Although the other gamete seems to form a structure which is a possible homologue of the choanoid body, this structure is much smaller and less conspicuous than the choanoid body and it seems possible that this gamete, conversely, does not form a fertilization tubule. Since the labelling experiment suggested that all gametes which form the choanoid body belong only to the plus (+) mating type, there is some basis for inferring that the fertilization
tubule is formed only by gametes of the plus (+) mating type. Should further investigation demonstrate what is here inferred, then the plus (+) mating type, whose gametes produce the fertilization tubule, could be designated as the male sex and the minus (−) mating type as the female sex.

Flagellar co-ordination

In vis-à-vis pairs, as described by Lewin (1954a, b) locomotion of the paired copulants is achieved only when the two flagella of one mate become paralysed, after which the flagella of the other mate propel the pair, and it has been suggested that some physiological action might be responsible for communicating this effect. In *C. reinhardi*, however, the flagella of both mates become co-ordinated and together propel the zygote. The evidence presented in the present paper shows that the flagellar roots deriving from the different gametes come into contact and thus it seems possible that some interconnexion between these may be responsible for the flagellar co-ordination. It is of interest to note that in each case motility is achieved at a stage before the gamete nuclei have fused; the same is true, also, for example, in the uniflagellate motile zygote of *Prasiola* (Friedmann, 1960).

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Fig. 3. Apical region of a vegetative cell in longitudinal section. Each flagellum (f) rising from the base of the apical papilla (a) protrudes through a channel in the thick apical plate which is part of the cell wall (w). A striated cross-band, shown at higher magnification in Fig. 5, connects the two flagellar bases. × 39,000.

Fig. 4. Apical region of a gamete in longitudinal section. Note that the cell wall is absent. × 39,000.

Fig. 5. Enlarged portion of Fig. 3. The larger cross-band (t) which connects the two flagellar bases shows cross-striations and about five slender longitudinal fibres. × 100,000.

Fig. 6. A nearly longitudinal section of a flagellum of a gamete. Slender tubular projections (p) of the plasma membrane resemble those present on the fertilization tubule. × 54,000.

Fig. 7. Early stage in the development of the fertilization tubule (t) in a specimen (possibly an incipient gamete) in which the cell wall (w) is still present. Two parts of a well-developed choanoid body (c) appear in the base of the longitudinally sectioned tubule. The plasma membrane of the tubule appears as a very dense layer except just above the choanoid body, where it appears as a thinner electron-dense ring (arrows). × 39,000.

Fig. 8. Longitudinal section of an apical region showing the position of the fertilization tubule (t) in relation to one of the flagella (f). Note the two contractile vacuoles (v). × 39,000.
Figs. 9-14. Various aspects of the fertilization tubule and nearby structures.  
× 47000.

Fig. 9. A grazing longitudinal section of the fertilization tubule (t) with the choanoid body (c) at its base. Note the slender tubular projections (p) of the plasma membrane of the tubule. (v, contractile vacuole.)

Fig. 10. Longitudinal section through the apical region of a gamete, showing the relative position of the base of the fertilization tubule (t) containing the choanoid body (c), and parts of the flagellar apparatus, including a flagellar root (r), the larger cross-band (s) seen in cross-section, and part of a flagellar base (b). Both the fertilization tubule and cell apex bear slender tubular projections (p), some of which appear in cross-section in the upper right corner of the figure.

Figs. 11-14. Part of a series of sections showing the base of a fertilization tubule in cross-section (two sections between Figs. 11 and 12 are omitted). In Figs. 12-14 the tubular components of the flagellar roots (r) appear in oblique section. (c, choanoid body; v, contractile vacuole.)
Figs. 15, 16. Consecutive sections showing a fertilization tubule (t) which bears slender tubular projections (p) and contains cytoplasm of reticulate appearance. The choanoid body (c) is shown in grazing section in Fig. 15 and some of the tubular components of this body are shown in cross-section in Fig. 16. × 57,000.

Fig. 17. Distal part of a fertilization tubule (t) in grazing longitudinal section showing part of the terminal cap. × 57,000.

Figs. 18—20. Serial sections of a zygote at an early stage of fertilization (a still earlier stage is shown in Fig. 21). The fertilization tubule connects the members of the pair. Note that through the mediation of this tubule the plasmalemmas of the two original gametes now constitute one continuous plasma membrane. This pair is in the trans position. In the gamete on the left, at the end of the fertilization tubule lies a small and delicate structure resembling a choanoid body, overlying which is an electron-dense ring in the plasma membrane (arrows); the gamete on the right shows a well-developed choanoid body (c) lying beneath an electron-dense ring in the plasma membrane. In the gamete on the left, two free basal bodies (x) are visible; one is in cross-section and the other in longitudinal section, hence their long axes lie at right angles to each other. A free basal body (x) is also visible in the gamete on the right. Gamete of plus (+) mating type is shown on right. (b, basal body of flagellum; r, flagellar root.) × 33,000
Fig. 21. The apical ends of two gametes connected by a narrow fertilization tubule at a very early stage of gametic union. The pair is in the trans position. In the gamete on the right side, at the end of the fertilization tubule, lies the edge of a well-developed choanoid body (c). In the gamete on the left, at the end of the tubule, lies a structure which resembles a choanoid body but is less dense and much smaller; the small electron-dense ring in the plasma membrane overlying this structure is indicated by the arrows. (b, basal body; f, flagellum; n, nucleus; v, contractile vacuole.) Gamete of plus (+) mating type is shown on right. × 33,000. The inset shows at higher magnification the fertilization tubule in continuity with both gametes. × 65,000.

Figs. 22, 23. Consecutive sections showing a stage in gamete association later than the stages shown in Figs. 18–20 and 21. This merging pair is in the cis position. The fertilization tubule is shorter and wider than in earlier stages. It is evident that one continuous plasma membrane bounds the two original gametes. In the member on the left, at the end of the fertilization tubule, is a well-developed choanoid body (c), but in the member on the right at the end of this tubule the delicate choanoid-body-like structure is barely visible. The arrows point to electron-dense rings in the plasma membrane lying above the choanoid body and above the choanoid-body-like structure. (b, basal body of flagellum.) Gamete of plus (+) mating type is shown on left. × 33,000.

Fig. 24. A stage later than that shown in Figs. 22, 23; the fertilization tubule is no longer present as such. The former gametes lie closer to each other and are in communication over a greater area than during earlier stages, when the fertilization tubule is well defined. The choanoid body (c) lies in the plane of junction. The protoplast which the broad basal end of the choanoid body faces is considered to be that of the gamete from which the body derives. The delicate choanoid-body-like structure, earlier characterizing the other gamete, now seems to be absent. One continuous plasma membrane bounds the two original gametes. Gamete of plus (+) mating type is shown on left. × 33,000.
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Fig. 25. Early zygote in stage just before the beginning of jack-knifing. The fertilization tubule is no longer evident. This pair is in the trans position; note two of the flagellar bases (b). (l, plastid; n, nucleus.) x 19000.

Figs. 26, 27. Zygotes in the early stage of flagellar co-ordination. The basal bodies (b) of the flagellar pairs deriving from the two gametes are still relatively far apart. Within the common zygotic plasma membrane the region of protoplastic confluence is extensive, but both gamete protoplasts retain their original identities and, as seen in Fig. 27, the cup-shaped plastid (l) of each gamete, carrying starch granules (g) and a pyrenoid (y), still surrounds the nucleus (n) of that gamete. Note the hyaline cytoplasm around the choanoid body (c). (r, flagellar root.) Fig. 26, cross-section; Fig. 27, longitudinal section. In both figures the gamete of the plus (+) mating type is on the right. x 19000.
Figs. 28–32. Serial sections of a pair of merging gametes during the jack-knifing phase, at an earlier stage than that shown in Figs. 26, 27. The member on the left side is in cross-section, that on the right side in longitudinal section. In Fig. 28, the cell apex of the member on the left, shown in grazing section, still carries some slender tubular projections (p) of the plasma membrane; the four lateral ridges of the apex of this member (see Fig. 1) are shown in Figs. 29–32. The choanoid body (c), appears in Figs. 28–30; its broad basal end faces the member on the left. In Fig. 30 the member on the left is sectioned through the larger cross-band(s) which connects the flagellar bases. The basal bodies (6) of the two flagella plus the two free basal bodies (x) of this member appear in Figs. 31, 32; near the basal bodies the flagellar roots (r) run cross-wise (Fig. 31) and then bend toward the posterior end (Fig. 32). (m, mitochondrion.) The member of the plus (+) mating type is shown on the left. × 33000.
Figs. 33, 34. Two nearly adjacent sections of a zygote in the same early stage of flagellar co-ordination as that shown in Figs. 26, 27 (later than that shown in Figs. 28–32). The discontinuity (arrow) between the two gamete components in Fig. 34 represents a surface groove, since it is apparent in Fig. 33 that the cells are fused beyond that point (arrow). In Fig. 33 the basal bodies of two of the four flagella appear in cross-section, and those of the other two in longitudinal section; near the latter bodies can be seen several of the flagellar roots. Between the two pairs of bases lie two free basal bodies (x) and the choanoid body (c). Fig. 34 shows fibres of the flagellar roots (r) of the two conjugants in contact with each other. (v, contractile vacuole.) Portion of zygote on right side derives from the plus (+) mating type. x 25 000.

Fig. 35. A portion of Fig. 34 shown at higher magnification. In the region of contact between the flagellar roots, their fibres appear to be interconnected by slender, periodically arranged strands. x 85 000.

Fig. 36. Motile, zygote. The nuclei (n) of the two former gametes are still separate. Note parts of the two cup-shaped plastids (l). A free basal body (x) is visible near the flagellar basal body on the right. x 25 000.

Figs. 37, 38. Sections of region containing the choanoid body (c) in two zygotes. Fig. 37 shows fibrillar and tubular structural components of this body, many of them in cross-section. Fig. 38 illustrates the ring-like nature of the body; the plane of section is parallel to the base of this structure, which has the form of a truncated cone. Note the slender threads interconnecting with possible tubular elements. A cross-section of a root (r) of a flagellum is shown in Fig. 37; in addition to the tubular components there are electron-dense fibrillar components. x 78 000.