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Embryological Perspective of Sexual Somatic Development in Ciliated Protozoa: Implications on Immortality, Sexual Reproduction and Inheritance of Acquired Characters

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Phil. Trans. R. Soc. Lond. B 1990 **329**, 287-305
doi: 10.1098/rstb.1990.0171

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CONTENTS

		PAGE
1.	Introduction	288
2.	Embryological perspective of somatic development during sexual reproduction in *Paramecium* and in hypotrichous ciliates	288
3.	Immortality: unicells versus multicells	291
4.	Sexual reproduction: ontogeny versus multiplication	295
5.	Inheritance of acquired characters: asexual perpetuation versus embryological assessment	297
6.	Epilogue	302
	References	303

> An important and interesting question then arises as to the individuality of the Infusoria before and after conjugation. The destruction of the old somatic nucleus during conjugation is proved, but there is also evidence of a less satisfactory nature that the somatic cytoplasm undergoes regeneration after the act. If it be assumed that the old somatic cytoplasm is gradually replaced by the conjoint sexual cytoplasm of the two conjugates, then the individuality of the Infusorian before and after conjugation is not identical. It is clear that there is partial somatic death during conjugation; it is not clear, however, that there is complete somatic death. It is to the elucidation of this important question that we may look with confidence to future investigations.
>
> (Hickson 1903, pp. 395–396)

SUMMARY

This essay addresses somatic development during sexual reproduction of ciliated protozoa, which is interpreted as an embryological phenomenon resembling embryogenesis of multicellular organisms. The uniqueness of this somatic development, as distinct from asexual development, resides in its dependence on new information associated with the germ nucleus, and on its involvement of both maternal and postzygotic informational inputs. This understanding derives from experimental dissection of nuclear control of somatic development in *Paramecium*, and in several hypotrichous ciliates. The embryological perspective enables us to reorganize our thinking on several historical issues of development and evolution: whether protozoa are immortal, and whether mortality only arose together with multicellularity; whether their sexual process can be regarded as reproduction, equivalent to sexual reproduction of multicellular organisms; whether the inheritance of acquired cortical variations of non-genic origins in ciliates constitutes a threat to neo-Darwinism. Conceptual predicaments on these issues have often stemmed from unwarranted parallelism drawn between asexual propagation of protozoa and sexual reproduction of multicellular organisms. The embryological reply to these questions is that ciliated protozoa are mortal, since during fertilization the maternal soma perishes by resorption, and is replaced by a new one which develops *in situ* in the maternal soma. The consequence of their sexual process is the same as in sexually reproducing multicellular organisms, in that the post-fertilization protozoan is an ontogenetically new individual, equipped with a new soma unlike those generated during asexual propagation. On the basis of the characteristic *in situ* development of the embryonic soma during sexual reproduction, two evolutionary perceptions are formulated. First, the extensiveness of resorption of the maternal soma, and release of development of the embryonic soma from cytotactic constraints imposed by the maternal soma, constitute major themes of phylogenetic evolution. Second, the evolutionary outcome of acquired cortical variations has to be evaluated in terms of the fidelity of perpetuation of such variations through sexual reproduction, and their potential of being assimilated into the genomic programme of embryonic development. The evolutionary predictions accordingly may turn out to be radically different from those based on the inheritance of such variations during asexual propagation alone.

Phil. Trans. R. Soc. Lond. B (1990) **329**, 287–305
Printed in Great Britain

1. INTRODUCTION

Protozoa are difficult subjects conceptually. They appear not to fit easily into major themes of biology, and often constitute exceptions or threats, rather than falling in line with the common rules developed for other biological groups. This problem, arising from the unicellularity of these organisms, has a long history.

To begin with, Huxley noted that protozoa presented a dilemma to the cell-theory of Schleiden and Schwann soon after its proposal: '...the difficulty with regard to these organisms has been evaded by calling them "unicellular" – by supposing them to be merely enlarged and modified simple cells; but does not the phrase an "unicellular organism" involve a contradiction for the cell-theory?...the admission of the existence of unicellular organisms appears to us to be virtually giving up the cell-theory for these organisms' (Huxley 1853, p. 304). The problem of homology between protozoa and multicellular organisms has persisted for over a hundred years. It spurred rebellions against the Cell-Theory at the turn of the century (Whitman 1893; Dobell 1911, 1914). It remained a subject of debate in as late as the middle of this century (Baker 1948*a*, *b*; see also Grimstone (1961, pp. 132–134)). In the era of modern cell biology, a compromise is made by according protozoans a status equivalent to both metazoan cells and the whole metazoan organism in different contexts (Grimstone 1961). Ciliates are now addressed as 'both unicellular and compound organisms' (Jerka-Dziadosz & Beisson 1990), a designation that is gaining acceptance, though the problem that Huxley raised remains.

To Weismann, protozoa were a nuisance rather than a help in the formulation of his germ-plasm theory (Weismann 1885, 1893). He stated: '...it is not advisable at present to begin the study of heredity by a consideration of the simplest beings, and to ascend from the unicellular to the multicellular organisms' (Weismann 1893, p. 21). The excuse given was that not enough was then known of the mode of inheritance of the lower forms. Weismann obviously had difficulty with protozoa, which he bypassed in his theorization, and he dealt with them by regarding them as representing the stage of organic evolution before the demarcation of germ and soma, which had only arisen along with the origin of multicellularity. Although he recognized that in ciliated protozoa the germ nucleus (micronucleus) provided a clear-cut physical basis of perpetuation of the germ-plasm, he maintained that they were immortal, and failed to integrate these organisms into his germ-plasm theory (see Weismann 1891).

Paramecium genetics was closely associated with cytoplasmic inheritance around the middle of this century (see Sonneborn 1947; Beale 1954). The findings of Sonneborn's school on the inheritance of the killer trait, mating types and serotypes, with their Lamarckian reverberations, were disquieting to those who upheld the nucleus as the site whereby all heredity was to be interpreted (Nanney 1983, 1985; Harwood 1985). Reconciliation with mainstream genetics came when the inheritance of these characters was eventually understood to have their basis in the DNA molecule (Nanney 1986). Cytoplasmic inheritance in ciliates then took on another form, in the autonomous perpetuation of acquired cortical variations through asexual propagation (Sonneborn 1963, 1970*a*; see also Nanney (1980) and Frankel (1989)). This time, the involvement of nucleic acids, and genotypic difference, are almost certainly ruled out. The challenge posed by cortical inheritance of ciliates is both developmental and evolutionary, and it makes ciliates appear even more enigmatic as it takes issue with neo-Darwinism.

This quick survey of some of the puzzles presented by protozoa, taking leaps of 50 years of biological history, is tailored as an introduction to a number of erroneous notions commonly associated with protozoa. The errors are often not attributable to non-protozoologists, but instead are committed primarily within the protozoan circle. Some of these issues are no longer fashionable, and they are not being debated as frequently as they were decades ago. They have filtered down into common texts, and continue to influence our thinking on major biological problems. They will be taken up in turn in the following sections. I shall evaluate these notions in the light of a new way of thinking about somatic development during sexual reproduction of ciliated protozoa, an important area that has been largely overlooked by ciliatologists. I believe these misconceptions can be rectified, and approached from a new angle, by the recognition that during sexual reproduction the post-fertilization ciliate behaves as an embryo in developing an ontogenetically new soma, inasmuch as an embryo develops from the zygote in the case of multicellular organisms. This I refer to as the embryological perspective of ciliate sexual somatic development.

2. EMBRYOLOGICAL PERSPECTIVE OF SOMATIC DEVELOPMENT DURING SEXUAL REPRODUCTION IN *PARAMECIUM* AND IN HYPOTRICHOUS CILIATES

The embryological perspective stems from our experimental dissection of nuclear control of somatic development of *Paramecium* during sexual reproduction, in particular from the function of the germ nucleus (micronucleus) in the development of the oral apparatus.

There are two phases of the life cycle during which an oral apparatus develops. During asexual propagation by binary fission, a new oral apparatus is generated close to the old one. The new oral apparatus is passed on to the posterior daughter cell, while the anterior daughter cell receives the old one with some modifications. In contrast, during conjugation or autogamy (uniparental self-fertilization), the old oral apparatus is resorbed while a new one is being generated (Ng & Newman 1984*a*). During this time, the nuclear apparatus also reorganizes: the somatic nucleus (macronucleus) disintegrates, and the germ nucleus (micronucleus) undergoes meiosis to generate gametic nuclei from which the zygotic nucleus is derived; the zygotic nucleus in turn undergoes mitosis

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

twice to generate post-zygotic nuclei, two of which then become the new micronuclei and the other two develop into new macronuclei. Although oral morphogenesis during asexual and sexual reproduction proceeds in a similar manner (see figure 1 *a*, *b*), the processes in these two phases come under different nuclear controls and are thus dissimilar in their ontogeny.

Ciliates are well-known for their nuclear dimorphism (for a succinct account see Grell (1973, p. 96ff)). The germ nucleus is traditionally held to be somatically inert and to serve only to reorganize the nuclear apparatus during sexual reproduction, while the somatic nucleus controls somatic function. However, it is now clear that the germ nucleus does participate in oral development during asexual propagation by binary fission (Ng & Mikami 1981; Ng & Tam 1987; Chau & Ng 1988*d*; for review and other citations, see Ng (1986)). This is shown by removing the germ nuclei from the cell by microsurgery. Several fissions after the operation, the amicronucleate cell line enters a period of growth depression, characterized by the development of abnormal oral structures, such as fragmentation and abnormal alignment of oral membranelles, reduction of the buccal cavity especially the cytopharynx, and impairment of feeding (figure 1*c*). Although abnormal, an oral apparatus is invariably generated. This shows that the germ nucleus plays a role in maintaining the normality of oral development. However, the cell line can return to near-normal with subsequent propagation, showing that this role of the germ nucleus is replaceable.

On the other hand, the role of the germ nucleus in oral development during sexual reproduction is markedly different, and is indispensable. Unlike oral development during binary fission, the initial stage of assembly of the oral membranelles in the oral primordium (field of basal bodies) defines a crucial developmental hurdle unique to sexual oral development (stage 2, figure 1*b*). The germ nucleus is indispensable for crossing this hurdle. Amicronucleates induced to undergo autogamy or conjugation invariably become arrested at stage 2 (Ng & Mikami 1981; Ng & Newman 1984*b*; Chau & Ng 1988*d*; Jurand & Ng 1988). The pre-existing maternal macronucleus and oral apparatus disintegrate as usual, leaving an astomatous post-sexual cell destined to perish (figure 1*d*). Specifically, stage 2 of oral development occurs soon after the pre-gametic nucleus (a post-meiotic derivative of the germ nucleus) has moved into the vicinity of the oral field; there it undergoes mitosis to form two gametic nuclei. The gametic nuclei (and perhaps the pre-gametic nucleus also) are intimately involved in providing the crucial morphogenetic information for the initiation of oral membranelle assembly. This is shown by experiments with cell lines possessing defective micronuclei that may fail to generate any gametic nuclei with meiosis (Tam & Ng 1986; Chau & Ng 1988*a*, *c*, 1989), and also through rescue experiments whereby a gametic nucleus is reintroduced into an amicronucleate cell during conjugation (Chau & Ng 1988*b*). The function of the germ nucleus in oral development during sexual reproduction is thus decisive, as without its par-

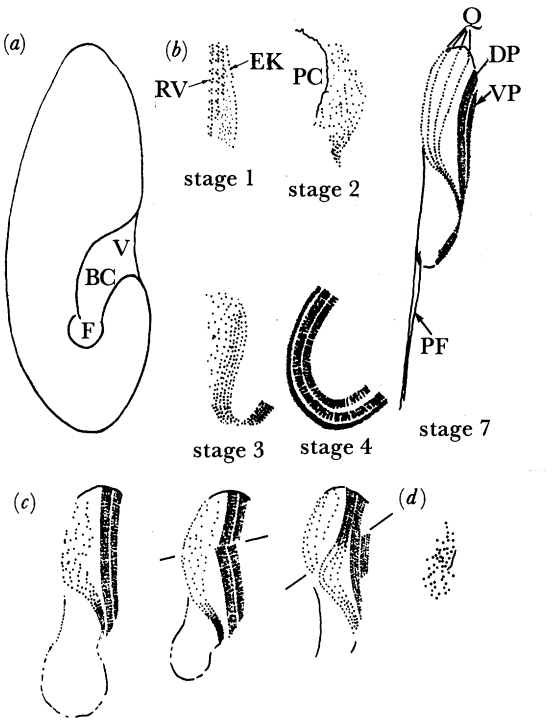

Figure 1. The oral apparatus of *Paramecium tetraurelia* and its development (from Ng 1990). (*a*) Right view of a cell showing the buccal cavity (BC) wherein lies the oral membranelles. V, vestibulum; F, food-vacuole-forming region. (*b*) Stages 1, 2, 3, 4 and 7 of oral development during conjugation, in right-ventral views. In stage 1, the basal bodies of the oral primordium arise from an area between the right wall of the vestibulum (RV) and two special rows of basal bodies (the endoral kineties, EK). In stage 2 – the crucial developmental hurdle – the basal bodies of the oral primordium begin to align into longitudinal rows which eventually develop into the oral membranelles. The paroral cone (PC) of the mating partner is also shown; this is where the pregametic nucleus (a post-meiotic derivative) resides to give rise to two gametic nuclei, and where exchange of gametic nuclei and formation of the zygotic nucleus take place. Alignment of basal bodies soon gives rise to a 6-row hook-shaped primordium (stage 3). Addition of basal body rows gives a 12-row C-shaped primordium; the 12 rows are demarcated into the three pro-membranelles each of which consists of four compact rows of basal bodies (stage 4). Further development proceeds with the development of the buccal cavity (stage 5, not shown), differentiation of the right-most oral membranelle (quadrulus) and the right side of the buccal cavity (ribbed-wall) (stage 6, not shown), and eventually the postoral microtubules (PF) (stage 7). For the three oral membranelles, the basal bodies of the quadrulus (Q) spread out in the anterior region, whereas those of the dorsal and ventral peniculi (DP, VP) remain compact. The cell does not feed until a slightly later stage (stage 8). (*c*) Defective oral morphologies typical of the early stage of vegetative propagation of amicronucleate cell lines, showing misalignment of oral membranellar basal bodies, their fragmentation and lateral displacement along a 'fault line', and a reduction of the posterior spiral of the quadrulus and dorsal peniculus and the length of the buccal cavity (cf. stage 7 in (*b*)). (*d*) Result of arrest of oral development at stage 2 in amicronucleates in sexual reproduction. The post-sexual cell becomes astomatous, possessing only a few dozen basal bodies lying in a shallow oral depression.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

ticipation oral development aborts early at stage 2. This differs from the situation in binary fission during which an oral apparatus, although abnormal, is invariably produced in the absence of the germ nucleus. This unique role of the germ nucleus highlights specific requirements of oral development that are exclusive to sexual reproduction.

Additional experiments reveal other unique controls of sexual oral development after the initiation of oral membranelle assembly in stage 2. As oral development proceeds through stage 3 to completion (figure 1*b*), the zygotic nucleus undergoes two mitotic divisions. The post-zygotic nuclei mediate oral development after the initiation of oral membranelle assembly. This has been implicated in the analyses of micronucleate-defective cell lines in autogamy (Tam & Ng 1986; Chau & Ng 1988*a*, *c*, 1989), and also shown by surgical removal of the zygotic nucleus, or the first post-zygotic nuclei (Ng & Fujishima 1989); under both circumstances, abnormal oral apparatuses are produced. The function of the germ nuclear derivatives during sexual reproduction is thus pervasive, providing the crucial signal for initiating oral membranelle assembly at stage 2, as well as affecting the subsequent morphogenetic steps.

In addition to its dependence on derivatives of the germ nucleus, oral development during sexual stages also makes use of the pre-existing maternal macronucleus (of the vegetative cell). The best demonstration of this is through the construction of heterokaryotic chimeras, with micro- and macronuclei of different genotypes residing in a common cytoplasm. This is done with a temperature-sensitive mutant, *short-1*, which produces shorter oral apparatuses (Tam & Ng 1987*a*, *b*). When sexual reproduction of the heterokaryon takes place at a non-permissive temperature, it is the genotype of the maternal macronucleus, and not that of the micronucleus, which determines oral length, irrespective of allelic dominance (Tam & Ng 1987*c*). Supportive evidence is also available from studies of interspecific heterokaryons (Chau & Ng 1988*e*).

The fact that oral development during sexual reproduction differs from that in binary fission in terms of nuclear control is significant. It shows that oral development during sexual reproduction is unique, and resembles the embryonic development of multicellular organisms, in requiring information from the germ-line nucleus, and involving two sets of information. The first set is maternal, released by the post-meiotic derivatives (the pre-gametic and gametic nuclei) of the germ nucleus, together with inputs from the maternal macronucleus. The second set is strictly post-zygotic, furnished by the immediate mitotic derivatives of the zygotic nucleus. This parallels the early embryogenesis of multicellular organisms, including insects, echinoderms, amphibians and mammals, which also relies on maternal messages accumulated in oocytes, as well as the information provided by the early post-zygotic nuclei (reviewed in Johnson 1981; Sherman & Schindler 1983; Johnson *et al.* 1984; Browder 1985; Davidson 1986; Schultz 1986). This parallelism between ciliate and multicellular development furnishes a conceptual framework for regarding oral development in sexual reproduction in *Paramecium* as an embryological phenomenon (Ng & Fujishima 1989; Ng 1990).

Within this embryological framework, one begins to understand why sexual and asexual oral development differ in terms of control. Most ciliates do not generate heteromorphic gametes or sexually reproducing individuals. Instead, the vegetative cell, when induced to enter the sexual cycle, acts at successive stages as the multicellular equivalents of gamete, zygote and embryo. The sexual events of gametogenesis, fertilization and embryogenesis are temporally and spatially compacted, proceeding uninterrupted, and all within the confines of a single cytoplasmic entity. The restriction to a single cytoplasmic entity imposes a stipulation on the development of the new soma, and the elimination of the old one, during sexual reproduction. Unlike multicellular organisms where embryonic development is spatially separated from the maternal soma, the new soma of ciliates generated during sexual reproduction arises *in situ*, in the maternal cytoplasm, by replacing old structures with new ones. It is this very aspect of sexual development in ciliates that has been largely overlooked, and this negligence has become the source of confusion concerning issues like mortality, reproduction and the inheritance of acquired characters, as will be explored in subsequent sections.

As the new soma has unique morphogenetic requirements for its development, and is 'embryonic' in ontogeny, it is likely to be endowed with new potentials unavailable with asexual propagation. The post-sexual ciliate begins the clonal cycle with a new soma and a reorganized (genetically recombined) nuclear apparatus, and in this sense the conception of the clonal cycle is analogous to the embryological beginning of multicellular organisms. This embryological conceptualization of sexual development in *Paramecium* also provides a rationale as to why the germ nucleus is endowed with the important somatic function of sexual morphogenesis. This, contrary to the traditional belief of somatic inertness of the germ nucleus of ciliates, becomes comprehensible, because of the similar requirement for new and vital information from the germ-line nucleus for embryonic development in multicellular organisms.

Can the embryological perspective of sexual somatic development in *Paramecium* be generalized? One group of ciliates, the hypotrichs, has furnished a test case. In hypotrichs, reorganization of the cortical ciliature during sexual reproduction is more extensive. All, or nearly all, of the pre-existing ciliature is resorbed and replaced by a new set. Sexual cortical development of hypotrichs presents an interesting puzzle that can be rationalized also within the embryological framework, once the dependence of sexual somatic development on the germ nucleus has been experimentally dissected. This analysis, and its developmental and evolutionary implications have been presented in detail elsewhere (Ng 1990). It suffices here to recall the salient features.

The puzzle with hypotrichs is that during conjugation they characteristically undergo repetitive, apparently redundant, rounds of cortical reorganization. During each round the pre-existing ciliature is mostly, if not entirely, resorbed and replaced with a

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

new set. There are two rounds of such reorganization in *Euplotes*, and three rounds in others like *Stylonychia*, *Pseudourostyla* and *Paraurostyla*. The nature of these cortical reorganizations has been dissected through the study of amicronucleate cell lines generated by microsurgery. When amicronucleate ciliates conjugate, the first round of reorganization can proceed without the germ nucleus; the second round on the other hand strictly depends on the presence of the germ nucleus (*Pseudourostyla*, *Stylonychia*). In particular, the immature somatic nucleus (macronuclear anlage) derived from a post-zygotic nucleus is responsible for initiating the second round of reorganization. Similar conclusions have also been reached for *Euplotes*, by interfering with the development of the macronuclear anlage chemically, or with ultraviolet (UV) microbeam irradiation (Kloetzel 1981, 1983). Moreover, in *Euplotes*, it has been shown by UV irradiation of the maternal somatic nucleus that it is also involved in the second round of cortical reorganization (Fidler *et al.* 1985).

The unique nuclear requirement of the second reorganization parallels the situation in *Paramecium*. These observations of nuclear control of cortical reorganization, plus other morphogenetic considerations, reveal that the second reorganization is the true sexual somatic development, whereas the first round is essentially an asexual event akin to cortical development of the incipient posterior daughter cell during binary fission. (The nature and origin of the first and third rounds of cortical reorganization is discussed at length in Ng (1990).) Furthermore, the same embryological argument advanced for *Paramecium* also applies to hypotrichs. Sexual somatic development (the second cortical reorganization) depends on informational input from a derivative of the germ line nucleus, in hypotrichs the post-zygotic macronuclear anlage, as well as from the maternal somatic nucleus. In view of this, sexual development in hypotrichs is an ontogenetically unique event, and it can also be rationalized within an embryological framework.

At present, one cannot confidently extend the embryological perspective to all groups of ciliates, as *Paramecium* and the several hypotrichs are the only ones that have been experimentally dissected regarding nuclear control of somatic development during sexual reproduction. Indeed, the traditional focus on asexual processes of ciliate development has left us with little description of somatic development during sexual reproduction. Even for the hymenostome *Tetrahymena*, which is among the most-studied ciliates, the resorption of the pre-existing oral apparatus and its replacement by a new one during conjugation have been described in some detail only recently (Tsunemoto *et al.* 1988). A brief note reports that the micronucleus is important in oral development during conjugation of *Tetrahymena thermophila* (Kaney 1989). As to flagellated protozoa, somatic reorganization of the complex extranuclear organelles during sexual reproduction, such as flagella and axostyles, has been documented for a number of symbiotic polymastigotes and hypermastigotes (see brief summaries in Wenrich (1954); Cleveland (1956); Grell (1967)); experimental dissection of the nature of such reorganization, particularly in terms of nuclear control is needed. Accordingly, it would be premature to generalize the embryological conceptualization among ciliates or protozoans. Despite this, generalization is likely to be acceptable for ciliates, because, of the few examples available, two rather different hymenostomes, *Paramecium* and *Tetrahymena*, both renew their soma by replacing their oral apparatus during conjugation, and in both the micronucleus plays a crucial role in this process. Furthermore, *Euplotes* is considered to be evolutionarily remote from the other hypotrichs investigated in this context, and yet sexual development in all of them can be considered within the same embryological framework developed for *Paramecium*. In any case, biological themes are commonly first founded on a few chosen experimental systems, and generalization comes only later. It will therefore be useful to begin with the perception based on *Paramecium* and the few hypotrichs, to see how some of the common beliefs are challenged by the embryological perspective of sexual somatic development of ciliates.

3. IMMORTALITY: UNICELLS VERSUS MULTICELLS

Protozoa are deemed to be immortal. The first formulation of this view is attributable to Ehrenberg in the early part of the last century (see Bell (1988) for a historical account). Debates on this issue have all but subsided and it is not easy to assess how widespread this notion currently is. But as such the notion of immortality of protozoa has become a convenient, and sometimes casual, starting point in speculations on the origin of sexuality or multicellularity. One can find this in recent writings. For example, protozoa were regarded as basically immortal, but mortality arose when sexuality evolved (Takagi 1988, p. 132). Again, in depicting the evolutionary origin of multicellular organisms from unicells, it has been surmised that 'death...was the price of multicellularity' (Raff & Kaufman 1983, p. 350). This remark was introduced at the point when the segregation between germ line and soma appeared on the scene. This is perhaps not coincidental, for the roots of the dichotomy between immortal unicells and mortal multicells can be traced to August Weismann (Weismann 1883*a*, *b*, 1886, 1890, 1891, 1893).

Weismann based his notion of immortality of unicells on three interwoven premises. First, he adopted a narrow view of reproduction, by accepting asexual cell division as the only form of reproduction in the unicells. Because each individual divides into two identical parts, '...it is impossible to decide whether one of them is younger or older than the other. Hence, in a certain sense these organisms possess immortality. ...Each individual of any such unicellular species living on the earth today is far older than mankind, and is almost as old as life itself' (Weismann 1883*a*, pp. 72–73; see also Weismann 1883*b*, p. 111). Weismann then went on to contrast this situation with mortality encountered in multicellular organisms, where there is a clear-cut separation between the germ cells and the soma.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Second, the notion of mortality of unicells was in direct conflict with the Weismannian view of immortality of reproductive cells, bearers of the germ. 'Among unicellular organisms natural death was impossible, because the reproductive cell and the individual were one and the same: among multicellular animals it was possible, and we see that it has arisen' (Weismann 1883*b*, p. 112). The unicellular protozoa posed a dilemma for Weismann. Bound by the fact that the reproductive cell and the individual protozoan were one and the same entity, and that reproductive cells must be immortal, he was forced to presume that immortality was a more primitive condition of life, and suggested that multicellular organisms have originated from unicells when there is a demarcation between germ cells and somatic cells, and along with this the diversification of the latter, and the inevitable mortality of the soma. Thus '...the somatic cells must have perished after a certain time, while the reproductive cells alone retained the immortality inherited from the Protozoa' (Weismann 1883*a*, p. 76). Again, the germ cells '...could not lose their immortality, if indeed the Metazoa are derived from the immortal Protozoa, for from the very nature of that immortality it cannot be lost' (Weismann 1883*b*, p. 141; see also Weismann 1890). He considered death as an adaptation prompted by natural selection, as worn-out individuals were valueless and even harmful to the species (Weismann 1883*b*, p. 136; for an appraisal of Weismann's views on the evolution of ageing, see Kirkwood & Cremer (1982)).

To Weismann, the death of the soma of multicellular organisms is certified by the leaving behind of a corpse (Weismann 1883*b*, pp. 145–146). Nothing of this sort, however, is seen naturally during protozoan propagation. He felt he could distinguish '...between the division of an Infusorian into two daughter-cells, and the death of a Metazoon, which leaves offspring behind it, by calling attention to the absence of a dead body in the process of fission among Infusoria' (Weismann 1883*b*, p. 115). Thus Weismann was comparing asexual reproduction of protozoa with sexual reproduction of multicellular organisms.

Third, contrary to prevalent beliefs, Weismann did not think that fertilization had a vitalizing effect. He doubted if protozoa ever needed to be rejuvenated, and he thought that they could propagate indefinitely by cell division: 'I could only consent to adopt the hypothesis of rejuvenescence, if it were rendered absolutely certain that reproduction by division could never under any circumstances persist indefinitely' (Weismann 1886, p. 292). In addition, he was not satisfied that conjugation had any connection with rejuvenation (Weismann 1886, pp. 289–294; 1890). These notions formed a sequel to an earlier reply to his contemporary, Götte, who believed that protozoa were mortal (see Weismann 1883*b*). However, Götte was in the unfortunate position of supporting his case by citing encystment as a process of rejuvanation: when the protozoan encysts it undergoes transformation equivalent to death, but then the encysted mass rebuilds anew, divides, and the progeny excyst as new individuals. Götte's view on the origin of mortality was in diametric opposition to that of Weismann. Said Götte: 'The phenomena of death were transmitted by heredity from the unicellular forms to the Metazoa when they arose. Death does not therefore appear for the first time in Metazoa, but it is an extremely ancient process which goes back to the first origin of organic beings' (quoted in Weismann (1883*b*, p. 112)). I believe this conclusion of Götte is essentially correct, although he has founded his case on the wrong premise, and it was not difficult for Weismann to demolish this base, a job which he took great pains to accomplish.

In a later essay, Weismann (1891) refuted the assertion of Maupas that conjugation brought about rejuvenation. His 'theory of mingling' emphasized the mixing of the germs of two individuals during fertilization as an essential process to create variations for selection to act upon, and that this is the sole significance of fertilization. He drew attention to the close parallel between the nuclear divisions during fertilization of protozoa and multicellular organisms, and on this basis sought to explain away the observation that certain protozoans would perish if prevented from conjugating. He regarded conjugating ciliates as being equivalent to the sex cells of multicellular organisms, which, if they did not participate in fertilization were doomed to perish and this presumably was a necessary consequence of the preparatory process of fertilization: 'But, just as eggs, in which these internal changes have once been carried out, cannot remain indefinitely thus prepared, but very soon change so that they are no longer adapted for fertilization, and finally decay, – so it is with an Infusorian which has passed the time for conjugation; it becomes incapable of conjugating, and finally, of living' (Weismann 1891, p. 205). To him, this did not indicate the natural death of protozoa: '...natural death cannot be admitted to obtain among Infusoria *in general*, inasmuch as it only occurs in *those animals which are abnormal in not attaining to conjugation*' (Weismann 1891, p. 207).

Weismann was not alone in asserting the immortality of protozoa. Lankester, a contemporary of Weismann, shared the same view for similar reasons: '...the reproductive cell being itself and alone the individual Protozoon, there is nothing to die, nothing to be cast off by the reproductive cell when entering on a new career of fission' (Lankester 1885, p. 837). Dobell believed that 'It has been shown beyond all reasonable doubt that under suitable conditions ciliates are able to live and multiply, in their own fashion, for an unlimited time...' (Dobell 1914, p. 180). He was also committed to the view that '...conjugation in the ciliates does not result in rejuvenation...' (Dobell 1914, p. 181). On the other hand, Hickson spoke of 'partial somatic death during conjugation', and that 'the individuality of the Infusorian before and after conjugation is not identical' (Hickson 1903, pp. 394–395). The state of knowledge on sexual somatic development, however, did not allow Hickson to go any further. To draw a parallel with the embryonic development of multicellular organisms from germ cells, he had to rely on the postulation of a specialized cytoplasmic region ('sexual cytoplasm')

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

surrounding the germ nucleus of ciliates, from which the post-fertilization soma was derived. He was obviously trying to fit within the Weismannian framework of demarcation between the germ and the soma.

Imperfect as Weismann's arguments on the immortality of unicellular organisms may seem to be in light of present day knowledge, their essential features have surfaced now and then when the issue of senescence and death in unicells has been raised. Chatton & Lwoff (1936) discussed immortality of ciliates based on perpetuation of cellular organelles during asexual reproduction. Jennings posed the questions: 'Are senescence and death phenomena that have taken origin only as organisms became multicellular and differentiated? Are they the consequence of division of labour, specialization, and interdependence among the cells of a multicellular organism, as many have held?' (Jennings 1942, p. 29).

The early protozoan studies did not allow an unequivocal decision as to whether they can propagate indefinitely by cell division (see Jennings 1929). There was also the uncertainty as to whether death of certain species in laboratory cultures might not be due to external factors, and not reflecting the intrinsic limit of a natural life-span. But it soon became clear that at least some, though it may not be all, protozoa were potentially mortal, for if not rejuvenated by the sexual process of conjugation these would decline in vigour, and subsequently perish within a certain number of cell generations characteristic of the stock or species (see Jennings 1942; Bell 1988). Thus Jennings (1942) came to the conclusion that in the course of evolution ageing and death appeared before the advent of multicellularity, a view shared later by Sonneborn (1978).

The early investigators of ciliates (Bütschli, Engelmann, Hertwig, Gruber, Maupas) were well aware of the phenomenon of rejuvenation of cell lines after conjugation (see Calkins 1901; Wilson 1925). Rigorous demonstration of this was due to the work of Calkins, and Woodruff & Spencer (see Jennings 1929). But why do protozoans age, and what is the basis of their rejuvenation by the sexual processes of conjugation or self-fertilization (autogamy)? On these two interrelated questions, speculations have historically centred around the nucleus. Thus Bütschli (1876) thought that rejuvenation involved the replacement of a worn-out somatic nucleus, and Hertwig (1903) also spoke of correction of the nucleo-cytoplasmic ratio by adjustment of the size of the somatic nucleus after its replacement (see Jennings 1929; Sonneborn 1954). Jennings (1929) pointed out the need to replace the somatic nucleus. Kofoid, with emphasis on the zygote as the pivotal phase of the life cycle, thought that the disintegration of the old somatic nucleus represented '... the death of the soma of the conjugant, the future of which is henceforth under a new genetic control' (Kofoid 1941, p. 580). It is of interest to note, in this connection, that even Weismann recognized the need for the replacement of the somatic nucleus of ciliates, and he even went as far as relating this to the somatic death of multicellular organisms: 'In the Metazoa the whole cellular structure of the body – the soma – is worn out by the processes of life, and suffers natural death: in just the same way the Infusorian macronucleus cannot continue its functions for unlimited generations, but must be renewed from time to time' (Weismann 1891, p. 182). Weismann probably would have changed his views on immortality of protozoa, if he had come to grips with the fact that conjugation of ciliates involved not only renewal of the somatic nucleus, but also of the non-nucleated part of the soma, the ciliature.

Ironically, even to early protozoologists it was clear that the body of certain hypotrichous ciliates underwent structural deterioration with age (see, for example, Maupas 1888; see also Calkins 1901, p. 241; Bell 1988). More recent examples are offered by ageing *Paramecium*, where the position of the oral apparatus in the cell becomes abnormal, leading to disturbances of separation of the daughter cells during binary fission (Sonneborn & Dippell 1960); abnormal oral structures may also develop. In ageing *Euplotes*, the average number of somatic ciliary rows also declines, and exhibits greater variability, and lower fidelity of perpetuation (Frankel 1973). To Engelmann (1876), and others, the observation of somatic deterioration during asexual reproduction, together with somatic reorganization during sexual reproduction, were sufficient to furnish a rationale for the necessity of conjugation. Jennings (1929), working with problems of inheritance of protozoa, was well aware of the endurance and perpetuation of acquired physiological and structural modifications ('dauermodifications') during asexual propagation in a variety of protozoa. Prompted by the observation that such modifications usually disappeared during conjugation, he later reasoned that cumulative degenerative changes of form and structure were to be eliminated through the sexual process, during which the cell underwent a '... profound making over of the cytoplasm, ... producing rejuvenation' (Jennings 1941, p. 731; see also Jennings 1942). He was perplexed, however, by the fact that it was the changes brought to the nucleus that constituted the most prominent feature during conjugation, and yet the seat for the disappearance of the dauermodifications appeared to be in the cytoplasm.

This lead was taken up by Nanney (1974), who considered plausible the idea that somatic deterioration arose by cumulative defects in organellar assemblies incurred with age. In simpler terms Sonneborn (1978) spoke of wear and tear of the body through use. These notions are particularly relevant to ciliates, in view of their mode of inheritance of cellular structures during asexual reproduction. Ciliates propagate asexually by binary fission, and the successive fission products can be considered as individual units constituting a long assemblage of 'clonal cylinder' (Frankel & Nelsen 1981; Frankel 1989). This unique feature accounts in part for the significant role played by pre-existing cortical structures in the determination of development and patterning of new structures (Sonneborn 1963). With asexual propagation of the pre-existing soma, the developmental blueprints that it carries for the generation of new structures are also perpetuated, and hence the form and pattern of the new structures will

tend to conform to those of the pre-existing ones. Nanney (1980) referred to this phenomenon as 'structural inertia'. It can be envisaged that with wear and tear the pre-existing structures, together with their developmental blueprints sooner or later become defective (Ng & Newman 1984*a*; Ng & Tam 1987). Consequently, the soma of the individuals in the later part of the clonal cycle will become progressively more and more defective.

In support of this notion, both Nanney and Sonneborn cited the study of Siegel (1970). This concerns the propagation of anterior and posterior daughter cell lines derived by binary fission, in relation to the development and inheritance of the oral apparatus of *Paramecium*. Siegel showed that cell lines of successive isolates of anterior daughters were inferior in growth compared with the corresponding lines of posterior daughters. During binary fission, the old oral apparatus is left with the anterior daughter while the newly assembled one is passed on to the posterior daughter. Presumably the old oral apparatuses inherited by the anterior daughter lines become structurally more defective compared with those inherited by the posterior daughter lines. This situation finds a close parallel in the flatworm *Stenostomum*, which also undergoes asexual reproduction by an analogous transverse fission of the body, and exhibits a similar differential survival between anterior and posterior daughter lines (see Sonneborn 1978). Nanney came to the conclusion: 'Perhaps only through the extensive reconstruction process associated with sexual reorganization can a complete renewal of cellular structures be accomplished' (Nanney 1974, p. 94).

In the 1960s, a secluded group of Chinese researchers, reporting on the repetitive cortical reorganization during conjugation of *Stylonychia*, disagreed with the Weismannian notion of immortality of protozoa, thinking that in each round of reorganization part of the soma of the parental generation was eliminated as 'carcasses' (Tchang *et al.* 1965). However, the nature of these reorganizations was unclear and could not be correctly interpreted without experimental dissection of the nuclear control of somatic development during conjugation (see previous section).

The notion that a complete renewal of the soma may be possible only through reconstruction of cellular structures during sexual reproduction adds a new dimension to the appreciation of the cause and consequence of sexual reproduction in ciliates. The goal of fertilization is not simply to replace a defective somatic nucleus, or to purify the genome via elimination of hidden recessive mutations, or to effect genetical recombination to generate diversity, but also to equip the post-fertilization individual with a new soma. This position can be reinforced, and further extended by reformulating our ideas about sexual development of ciliates within an embryological framework, in particular according to the uniqueness of nuclear control of construction of the new soma.

As set forth in the previous section, during fertilization of ciliates a new soma is constructed, whose nature I regard as embryonic. Here two unique features of development of the new soma need to be reiterated. First, it develops *in situ*. In the same cytoplasmic entity of the maternal soma the structures constituting the new soma arise, while those of the old soma are resorbed. Second, the control of sexual somatic development differs from that occurring during asexual propagation, in that it requires information from the germ line nucleus, and it parallels the situation of embryonic development in multicellular organisms. This conceptualization of ciliate sexual development resolves the dilemma Weismann faced, that in protozoa the reproductive cell and the individual are one, and it removes the necessity to postulate that protozoans are immortal. Ciliates as species do not perpetuate by maintaining an immortal soma as a vehicle for the germ. Rather, during sexual reproduction the old soma is resorbed, and in its place a new soma develops to carry the germ nuclei. There is no 'casting off of the corpse', and no need for this, to mark the mortal state of the old soma as in multicellular organisms. In this manner, ciliates have concealed their somatic mortality from generations of biologists. It is nevertheless evident that the old soma degenerates, but that this phenomenon becomes hidden because in the same cytoplasmic entity the new soma arises. This replacement of the moribund soma during fertilization, as opposed to its casting off, may be general among unicells that lack a demarcation of the germ and somatic nuclei into different (cellular) compartments. The developmental and evolutionary strategies of unicells are likely to be elaborated on this premise.

At present, we do not know to what extent cumulative somatic deterioration may contribute to clonal senescence. Moreover, there are examples to show that cortical variations acquired by the old soma are transmissible through sexual development (see later section). Although such examples certainly indicate that the old, maternal soma still exerts an influence on the development of the new, embryonic soma, the extent of such influence remains to be quantitated. None the less, the important developmental lesson is that the new soma develops while the old one is being resorbed, and this suggests that the influence of the old soma on the development of the new one during this time might be attenuated. It is perhaps not even necessary for the defective determinative blueprints of the old soma to be eliminated totally with one round of sexual reproduction, but instead such elimination may take successive rounds of sexual reproduction to accomplish. Sexual reproduction thus provides a unique opportunity for embryonic somatic structures to be constructed as the developmental constraints of the old soma become relaxed, or even abolished. This, together with the fact that there is new informational input from the germ nucleus for the development of the new soma, suggest that the developmental outcome of the new cell structure is likely to be different from the ones generated during binary fission. This view of ciliate sexual development takes account of our understanding of the phenomenon of rejuvenation after sexual reproduction. The clone resulting from fertilization and embryonic development represents a new generation. It begins not only with a new set of nuclear

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

apparatus, but in addition, a new soma that is largely if not entirely free of the blemishes incurred and perpetuated in the old soma of the previous generation. Because of the ontogenetic uniqueness of the new soma, it is likely to be endowed with new potentials that are not available to those generated during binary fission, inasmuch as the development of a multicellular embryo generates an entirely new individual.

In evolutionary terms, ciliates perpetuate the species in a manner similar to multicellular organisms, despite their lack of cellular separation between germ and soma. In both, the soma serves as a vehicle for the perpetuation of the germ, but it is doomed to perish. As the soma deteriorates, it is simply discarded and formed anew from germ cells in multicellular organisms, but replaced *in situ* in ciliates. The evolutionary benefit of multicellularity is thus the ease with which deteriorating soma can be abandoned, and the release of embryonic development from its constraints. As to ciliates, and maybe other protozoans as well, the extent and mode of replacement of the soma during sexual reproduction is likely to be a predominant evolutionary theme during the emergence of higher taxa. This is because the long-term survival of a species will depend on how well the germ is preserved, and that in turn will be determined by the efficacy of the post-fertilization soma. The latter constitutes a major problem for the unicellular ciliates where the embryonic soma has to develop within the cytoplasmic entity of the maternal soma. It is imperative for these organisms to devise solutions to overcome the hegemony of the old soma in the construction of the new one, so that the new one may become efficacious in survival and propagation. This goal is achieved basically during sexual reproduction by resorption of the old soma, together with its developmental blueprints. This perception suggests that the extent of somatic renewal, and the developmental solutions adopted by various groups, may serve as plesiomorphic characters for the evaluation of phylogenetic relationships amongst the higher taxa. Somatic renewal during sexual reproduction certainly varies in different groups of ciliates. As we have seen, this is quite extensive in hypotrichs, involving the entire cortex, but is apparently restricted to the oral apparatus in hymenostomes like *Paramecium* and *Tetrahymena*. Aside from its obvious physiological significance, the oral apparatus is the most important organelle of the ciliate in terms of development, acting for example as an epicentre from which successive morphogenetic waves originate during cell division (Iftode *et al.* 1989). It is thus not surprising that if anything needs replacing it will be the oral apparatus. Analysis of somatic renewal during sexual reproduction at the ultrastructural and molecular levels, and also in diversified groups of ciliates, will throw light on the extent of renewal, and also on the nature of the new soma.

4. SEXUAL REPRODUCTION: ONTOGENY VERSUS MULTIPLICATION

Weismann regarded conjugation of protozoa as similar to the sexual reproduction of multicellular organisms insofar as the 'mingling of the hereditary substances of two individuals', or 'mingling of individuals or of their germs', a process of significance which he named 'amphimixis' (Weismann 1891, p. 180; Weismann 1893, p. 20). However, he distinguished between conjugation and reproduction. To him, and many others, reproduction works strictly with numbers, and it qualifies only if there is a multiplication of individuals: 'I have been accustomed for many years to urge, in my lectures, that conjugation is not reproduction, but rather its opposite; for reproduction implies an increase of at least one in the number of individuals, while conjugation leads to a decrease, two individuals fusing into one' (Weismann 1891, p. 207). Thus he treated binary fission during asexual propagation as the only form of reproduction in protozoa, and compared this to procreation in multicellular organisms by sexual act.

Weismann was obviously in the company of early protozoologists, who shared the same premise that reproduction of protozoa was fulfilled by an increase in number with division (see Calkins 1901, p. 243). Said Calkins: '...I believe with Bütschli, Engelmann, Maupas, Hertwig, and many others, that it ("conjugation") cannot in itself be regarded as primarily a reproductive act' (Calkins 1901, p. 213). By 'conjugation', Calkins was referring not only to the sexual process typified by ciliates, but also the union of specialized individuals or gametes of other groups including the flagellates (Calkins 1901, p. 214). The reluctance to strike a parallel between the sexual process of protozoa and sexual reproduction of multicellular organisms was, in part, a reaction against earlier erroneous analogies drawn between protozoan organelles and sexual organs of multicellular organisms: the contractile vacuole as a spermatic reservoir (Ehrenberg); the macronucleus as the ovary and the micronucleus as the testis (Balbiani) (see Kent 1880–1881, pp. 91–99; Calkins 1901, pp. 13–14). This situation was also attributable to the ignorance about the underlying cause of rejuvenation by conjugation, in particular the lack of appreciation of somatic reconstruction during the process; Kent in fact came close to such understanding and recognized conjugation as sexual reproduction, but he was hampered by insufficient information on the details of sexual somatic development (Kent 1880–1881, pp. 97–98).

This state persisted with little change over the following half century of protozoan studies, despite the accumulation of many details of fertilization in diversified groups of protozoa during this period. Minchin distinguished the sexual process from reproduction, regarding the latter as a matter of multiplication achieved with cell fission (Minchin 1912, pp. 130–133). Wilson put it bluntly: '...in the Protista sexuality and reproduction appear as quite distinct, and in some respects opposite processes. The unicellular protistan has but one mode of multiplication, cell-division, in itself a purely asexual process; and the immediate effect of syngamy, obviously, is not to increase but to decrease the number of cells' (Wilson 1925, p. 580). It is less problematic to apply the term 'sexual reproduction' to flagellates,

where specialized gametic cells are involved in the sexual process, and where the zygote may undergo a number of fissions to generate multiple individuals. But ciliates exhibit neither of these phenomena during fertilization. Turner remarked: 'It (conjugation of ciliates) is a sexual process, differing from ordinary union in that it is not directly related to reproduction. Two organisms enter into the relationship; no third party – no progeny – has come into being' (Turner 1941, p. 617). Likewise, Cleveland remarked that the polymastigote flagellate *Urinympha* did not undergo sexual reproduction, despite the occurrence of a sexual process resembling autogamy of *Paramecium*, as 'The same cell that begins the cycle ends it, and without undergoing division' (Cleveland 1956, p. 174). Anderson recently noted: '...upon separation the conjugants do not immediately undergo reproductive fission. Binary fission typically occurs sometime later. Hence, in these species sexual processes do not coincide with organismic reproduction' (Anderson 1988, p. 375). The influence of these views obviously extends beyond the circle of protozoologists: 'In ciliates,...sex is completely decoupled from reproduction: two cells enter sexual conjugation, and two cells emerge from it' (Bell 1988). Others, nevertheless, have applied the term 'sexual reproduction' to protozoa in general (see, for example, Hyman 1940; Jennings 1941; Wenrich 1954; Grell 1967; Manwell 1968; Kudo 1971). On one occasion, after giving a talk in a Ciliate Meeting in Shanghai, China, in 1986, I was queried by an eminent protozoologist as to whether autogamy in *Paramecium* should be addressed as sexual reproduction.

What about numbers? The reply is simple. If the entire world's human population adheres to the rule of producing two offspring per family, then the population is not going to increase in number. Under this circumstance, obviously we still would talk about sexual reproduction in human beings. The crux of the problem is ontogeny, rather than multiplication. Sexual reproduction is to be understood as the creation of ontogenetically new individual(s), whereas the consideration of increase in number is peripheral. This is precisely where early protozoologists had failed, and their focus on number had even generated some extreme views about sexual reproduction of multicellular organisms (see Calkins 1901, pp. 243–244). In the human example there will be a transient increase in family size when the parents reproduce, but then the parents eventually perish, and thus the number of family members is reduced to the original two. The same argument applies even when each family produces only one child, leading to a dwindling of the population. Hence, to sexually reproduce or not is not a matter of numbers, as commonly associated with reproduction of our own and other species. Rather, from a developmental perspective it is a matter of generation of new individuals. In protozoa, if we accept that the soma developed during fertilization is ontogenetically different from the maternal soma, then clearly we are dealing with two distinct individuals, the parent (conjugant) that possesses the old soma, and the progeny (exconjugant) that is equipped with the new soma. The parent conjugant perishes as its soma is resorbed, and at the same time the progeny develops *in situ*. The sexual progeny is thus a new individual, since it has a new set of nuclear apparatus and a new soma.

A very similar focus on ontogeny has been expressed by Manwell (1968, p. 207): 'It is also true, but often overlooked, that reproduction does not always result in increase in numbers. Literally, reproduction means no more than the creation of a new organism, and hence does not necessarily mean multiplication; thus the word should apply equally to the making over of an old one. In the ciliates conjugation, hemixis, autogamy, cytogamy, and sometimes even encystment...do not result in multiplication – they simply give rise to profound modifications of the organisms concerned.' The embryological perspective consolidates this view by directing our attention to the uniqueness of sexual somatic development, now that we understand such 'profound modifications' or 'making over of an old one' should involve the construction of an embryonic soma. This construction, proceeding *in situ* in the maternal soma, is concealed, but significantly it provides an ontogenetically sharp distinction between the parent and the progeny.

My concern is not over semantics. One might as well use 'sexual reorganization', as opposed to 'asexual multiplication', to designate sexual processes. The confusion over whether conjugation of ciliates should be regarded as sexual reproduction is closely tied up with a conceptual flaw. It stemmed from the impressive ability of protozoa to duplicate themselves as individuals, and thus multiply in number, by cell division. Moreover, over the past century protozoologists interested in the study of fertilization were fascinated by the variety of nuclear reorganization in diverse groups of protozoa, whereas somatic development during the sexual process has received little attention. The embryological perspective of ciliate sexual development originates from the perception that the maternal soma is resorbed during fertilization, and that the new soma constructed at the same time is ontogenetically unique. The latter rests on the understanding of the control of construction of the new soma by the information residing in the germ nucleus. Sexual reproduction in ciliates involves genetic reorganization of the nucleus, as well as development of the embryonic soma of the new individual. This I believe is a more complete description of the outcome of their sexual process. In this sense, protozoa undergo true sexual reproduction, as do multicellular organisms.

Stripped of sexual reproduction, protozoa will be left only with asexual propagation (such as binary fission) as the only means of reproduction. This is unfortunate, because when parallels between reproduction of protozoa and multicellular organisms are drawn, it is frequently between asexual propagation of protozoa and sexual reproduction of multicellular organisms, and developmental and evolutionary implications have been derived on this basis. We have seen how Weismann, and others, had erred on this account in his assertion of immortality of protozoa, failing to recognize that the pre-sexual soma and the post-sexual soma represent distinct individuals. Weismann was well aware of the similarities of fertilization in protozoa and

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

in multicellular organisms, but he failed to draw a parallel between the two in somatic development associated with fertilization, and this has contributed to the difficulty in integrating the protozoa into his germ-plasm theory. In the next section I consider yet another pitfall arising from the focus on asexual propagation of protozoa in drawing a parallel with sexual reproduction of multicellular organisms.

5. INHERITANCE OF ACQUIRED CHARACTERS: ASEXUAL PERPETUATION VERSUS EMBRYOLOGICAL ASSESSMENT

When cortical inheritance of ciliates is discussed in the controversial context of inheritance of acquired characters, the problem is unfortunately compounded by the lack of appreciation of sexual somatic development of ciliates. The autonomy of the cortex in directing regeneration and perpetuation of structures during asexual reproduction has led Lwoff to conclude that '...the organization (of the cortex) is not commanded by the genome but behaves as an acquired hereditary character' (Lwoff 1990, p. 109). However, as long as attention is restricted only to asexual reproduction, the genetical and evolutionary implications of inheritance of acquired cortical variations of ciliates will remain ill-defined.

J. Maynard-Smith, in upholding the Weismannian and gene-centred view of evolution as opposed to Lamarckism, has raised the concern: '...to an evolutionist, the interesting question about, for example, cytoplasmic localization is not whether such localization is essential for proper development, but whether, if the localizations are changed, the result will be an adult which produces eggs with similarly altered localizations.... In general, the answer to such questions is no. There are a few well-established exceptions, of which the phenomenon of "cortical inheritance" in ciliates is perhaps the most important. Neo-Darwinists should not be allowed to forget these cases, because they constitute the only significant experimental threat to our views' (Maynard-Smith 1983, p. 39; see also Maynard-Smith (1986, pp. 24–25), and discussion by Nanney (1984, 1985)).

Early examples of cortical inheritance of ciliates have been documented by Jennings (1929). More recent examples are reviewed by Aufderheide *et al.* (1980) and Frankel (1989). The most illustrative case is furnished by the inheritance of 180°-rotated somatic ciliary rows, which run in opposite orientation to that of normal ciliary rows (figure 2). This finding was first reported in *Paramecium* (Beisson & Sonneborn 1965), repeated in *Tetrahymena* (Ng & Frankel 1977), and also noted for marginal cirral rows in two hypotrichs, *Stylonychia* (Grimes *et al.* 1981) and *Paraurostyla* (Jerka-Dziadosz 1985). The origin of rotated ciliary rows is semi-surgical, via heteropolar juxtaposition of two conjugants (*Paramecium*), or of two daughter cells in an abortive binary fission (*Tetrahymena*). Once generated, the rotated ciliary rows can propagate, in the rotated orientation, during asexual multiplication of the cell line for hundreds of cell generations. The fact that ciliary rows can propagate in the rotated configuration

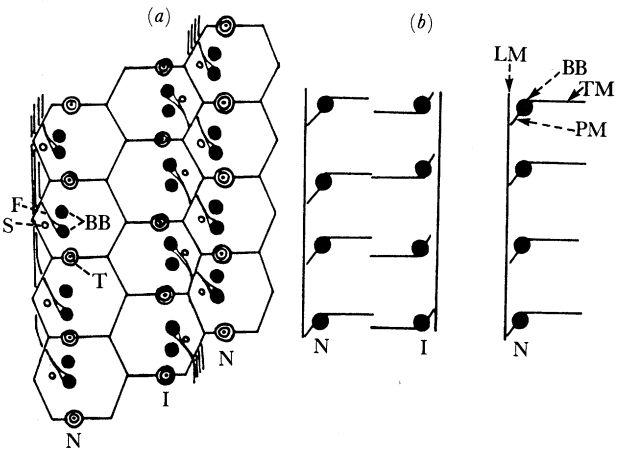

Figure 2. Normal and 180°-rotated ('inverted') somatic ciliary rows in the cortex of (*a*) *Paramecium* (after Sonneborn 1970*a*) and (*b*) *Tetrahymena* (after Ng & Frankel 1977). In both cases, the cell is oriented with its anterior end towards the top of the page. N, normal row; I, inverted row; BB, basal body; F, kinetodesmal fibre extending from the anterior corner of the basal body towards the anterior end of the cell in normal rows; S, parasomal sac; T, tip of trichocyst; LM, longitudinal microtubular band running from pole to pole on one side of the basal body row; PM, post-ciliary microtubular band; TM, transverse microtubular band. When new basal bodies proliferate in ciliary rows, they arise anterior to pre-existing basal bodies in normal rows, but posterior to pre-existing ones in rotated rows. The other structures associating with the newly formed basal bodies along the rotated rows are likewise inverted.

shows clearly that the structural asymmetry of the cortical unit, comprising the basal body and associating structures, dictates the pattern of new units that are to be formed within the row. New basal bodies, for example, arise immediately anterior to old ones along normal ciliary rows, but posterior to old ones in the rotated rows, thus revealing the presence of localized cortical determinants within the microgeography of the cortical units defining the precise site of origin of new basal bodies. Some cells inevitably lose the rotated rows with propagation and selection for others still possessing them is frequently necessary for perpetuation. Sonneborn (1970*a*, *b*) noted briefly that inverted ciliary rows of *Paramecium* persisted through repeated rounds of fertilization also.

The second example relevant to our discussion is the inheritance of the doublet biotype of *Paramecium* (Sonneborn 1963, 1970*a*). Doublets possess two complete sets of cortical structures, including two oral apparatuses, deployed on opposite surfaces of the cell. These have been derived through anomalies of conjugation, such as the failure of conjugants to separate from each other, or natural graft of a piece of cortex from the vicinity of the oral apparatus of one conjugant onto its mate (Sonneborn 1963), or graft of an entire oral apparatus (Ng 1987). Again, once generated the doublet biotype can perpetuate during asexual propagation, and even after conjugating with singlets. Conversely, one of the two oral apparatuses of a doublet can be eliminated following damage with ultraviolet microbeam irradiation (Hanson 1962; Hanson & Ungerleider 1963), or laser microbeam (S.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

F. Ng, unpublished data). The loss of the irradiated oral apparatus is followed by the gradual disappearance of one set of cortical structures, and subsequently this leads to reversion to the singlet biotype. Again, the inheritance of the doublet biotype shows that cortical variations can propagate rather autonomously during asexual reproduction. Sonneborn (1963) documented the effort to rule out the possibility of genomic alterations as contributive to the origin of the doublet biotype, by crossing doublets with singlets to show that the doublet and singlet forms were inherited directly from their cytoplasmic parents.

I do not think that cortical inheritance of ciliates, as it now stands, constitutes a threat to the Weismannian foundation upon which neo-Darwinists build. In the first place, I should emphasize that the hereditary and evolutionary significance of cortical inheritance resides not so much in the high degree of fidelity of transmission of acquired cortical variations during asexual propagation. It would be wrong to compare asexual propagation of ciliates with embryogenesis of multicellular organisms. Although it is of heuristic value to point out that the egg of multicellular organisms may possess localized developmental determinants as does the cortex of *Paramecium*, it is inappropriate to foster a parallelism between the two systems regarding the (self-)perpetuation and long-term maintenance of such determinants (see Sonneborn 1970*a*, p. 364). The lesson from cortical inheritance of ciliates is a meaningful one with its bearings on development, and ciliates have provided useful experimental models for the dissection of epigenetic mechanisms of determination of form and pattern (see Frankel 1989). But cortical inheritance of ciliates will need to be established on a firmer foundation before any evolutionary lesson on the problem of inheritance of acquired characters can be extracted. This is because the transmission of cortical variations through asexual propagation is essentially 'somatic' in nature, which is more akin to the multiplication of somatic cells in multicellular organisms, though the analogy is understandably not exact. The long-term stability and maintenance of acquired cortical variations should be evaluated first in the context of development of the ciliate's embryonic soma during sexual reproduction. Until this is done, it is improper to compare cortical inheritance of ciliates with embryogenesis of multicellular organisms in an evolutionary context, for one is forced to rely on the perpetuation of cortical variations during asexual propagation of ciliates to draw a parallel (see Lwoff 1990).

Before exploring further along this line, let me reformulate the question as to how ciliate cortical inheritance may come into possible conflict with the Weismannian framework. It should be stated first that the physical separation between the germ nucleus and somatic nucleus of ciliates is as clear-cut as the separation of germ cells and somatic cells of multicellular organisms, and hence ciliates comfortably fall within the Weismannian framework of strict demarcation of germ and soma: '...the micronucleus brings about the continuity of the germ-plasm' (Weismann 1891, p. 181). Furthermore, the intimate involvement of the germ nucleus in the development of the embryonic soma during sexual reproduction of ciliates is also in line with the Weismannian view of germ-directed embryogenesis. On the other hand, the *in situ* development of the embryonic soma of ciliates presents an additional feature outside the Weismannian framework of germ-directed embryogenesis. Because the embryonic soma develops not in spatial separation from the maternal soma, the determinative blueprints of the maternal soma become directly relevant to the development of the embryonic soma. It is not difficult to appreciate this, as the assembly of embryonic structures does not take place in a vacuum, but rather in the arena provided by the maternal soma. It is in this setting that acquired cortical variations such as rotated ciliary rows and the doublet biotype become inheritable in the short term through sexual reproduction, and passed onto the soma of the new individual. An evaluation of long-term cortical inheritance will have to take this into consideration also. However, as set forth below, if one admits the uniqueness of the ciliate's embryonic soma, and the participation of germ-line genomic information in its development during sexual reproduction, then predictions on the evolutionary outcome of acquired cortical variations might be formulated from a Weismannian standpoint.

One may begin by asking the question: how do maternal somatic blueprints operate during development of the embryonic soma of ciliates? Three variables come into mind. The first is the nature of such blueprints, defined in terms of the structure with which they are associated. The second is the extent of renewal of the maternal soma, in terms of resorption of maternal structures, during sexual reproduction. The third is the scope of involvement of the genome, especially the germ nucleus or the post-zygotic nuclei, in the development of the embryonic soma. Let us first take up apparently simple cases like the rotated ciliary rows. In *Paramecium* and *Tetrahymena*, the pre-existing (maternal) ciliary rows are not known to be resorbed and generated anew during sexual reproduction. Thus it is easy to see that the rotated rows perpetuating in the maternal soma may simply persist through sexual reproduction, and in this way the rotated pattern is directly inherited by the post-fertilization individual.

However, the propagation of ciliary rows depends also on other provisions extrinsic to the rows. A ciliary row may be lost as a result of failure of proliferation of basal bodies along the row, eventually leading to disappearance of the row in subsequent cell generations. Proliferation of new basal bodies occurs in the later part of the cell cycle, and this is largely restricted to a zone in the middle third of the cell. This shows that the decision of basal body proliferation does not reside with the ciliary rows alone, even though the precise location of origin of a new basal body within the cortical unit is dictated by determinative blueprints vested with the units. Instead, proliferation of basal bodies along ciliary rows is prescribed by some 'external instructions': transcellular signals possibly in the form of morphogenetic waves (Iftode *et al.* 1989). Evidently, for the perpetuation of row asymmetry, be

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

it normal or rotated, the primary condition is the proliferation of basal bodies along the row, which is a result of interaction between the global external instructions and the local determinative blueprints of the cortical units. At present, the mechanistic basis of such interaction is not understood. But studies on *Tetrahymena* and also on the dorsal ciliary rows of *Euplotes* indicate that the genome plays a role in monitoring the total number of rows a cell possesses, and this would directly or indirectly affect basal body proliferation along the rows.

In both *Tetrahymena* and *Euplotes*, the number of somatic ciliary rows (corticotype) possessed by a strain or clone falls within limits ('stability range') prescribed by the genome (Nanney 1966; Heckmann & Frankel 1968; Frankel 1970, 1973; Laloë 1979; see also Frankel (1989) for a succinct account). *Tetrahymena thermophila*, for example, usually possesses 18–20 rows. Variations falling outside such limits, such as 25 rows or 15 rows, can be maintained through asexual propagation for up to hundreds of cell generations. However, such variations are sooner or later eliminated and the corticotype reverts back to the stability range. If strains of different corticotypes are crossed, the corticotype of the sexual progeny conforms in the short term to that of the cytoplasmic parent. However, even at an early stage of observation (about two dozen cell generations after conjugation), in some cases the clone exhibits a tendency to modulate towards the corticotype of its mate, or the two clones from a pair of conjugants converge towards a common distribution of row number (see Nanney 1966, pp. 961–963; Frankel 1973). These observations signify the action of the genome in defining row number. The important questions are: when exactly does the genome act, and whether it is the embryonic soma that constitutes the primary target of this action? An assessment of the situation of the exconjugants during the first few post-sexual divisions may reveal significant changes shortly after conjugation. It will be of interest to compare in detail the stability of corticotype before and after conjugation, to allow an assessment of the effect of conjugation per se on the fidelity of maintenance of row number. There are some indications that after conjugation corticotypes falling outside the stability range revert faster, but more data are required to decide whether the effect is introduced by conjugation (Nanney 1966). In addition, in several ciliates genes have been identified which affect the development of basal bodies and their positioning along ciliary rows (see Jerka-Dziadosz & Beisson 1990). It will be interesting to test the expression of these genes during sexual reproduction.

What one is looking for in *Euplotes* and in *Tetrahymena* might well be furnished by some observations on the conjugation of the ciliate *Chilodonella cucullulus* (Janus 1972). In asexually propagating cultures, the number of ciliary rows, the number of contractile vacuole pores and also their distribution, increase in variability. However, during the first post-conjugational division such variations are reduced. The documentation of this observation is brief, and a thorough understanding of this phenomenon awaits further investigation.

My view is that genomic mechanisms regulating the propagation of ciliary rows may be operative during sexual reproduction when the soma is subjected to renewal. The ciliary units of the conjugants may not exhibit any overt sign of change, such as resorption of basal bodies or their associating cytoskeletal elements. But the determinative blueprints associated with the units may be modulated by the genome during this time, and those that do not quite conform to the rule would be attenuated. One can envisage that during sexual somatic renewal, the genome defines a fixed number of ciliary rows by a hypothetical means of enumeration, and only augments the determinative blueprints along those rows thus defined. We do not yet have any solid proof of tampering of developmental blueprints of the cortical units by genomic intervention during sexual reproduction. But we should be aware of this possibility and start probing in this direction, as we do understand that information from the germ nucleus does intervene in a decisive manner in oral development during sexual reproduction.

In contrast to the perpetuation of somatic ciliary rows, the maternal oral apparatus is resorbed during sexual reproduction. In the case of *Paramecium* doublets of non-genic origin, despite the destruction of the maternal oral apparatuses two new (embryonic) oral apparatuses are generated, and the doublet biotype thus persists through sexual reproduction. This indicates that the blueprints associated with the two maternal oral apparatuses destined to perish nevertheless continue to function during sexual development, to specify the generation of two new oral apparatuses. On the other hand, new information associated with the germ-line nucleus is involved in the development of the new oral apparatus during sexual reproduction (§2). In particular, the gametic nucleus plays an indispensable role in the decision as to whether a new oral apparatus is to be generated along the oral meridian. The post-zygotic nuclei in addition play an important role in oral morphogenesis subsequent to the initiation of development. Thus, both cortical and germ-line determinants are required for the generation of the new oral apparatus.

To the ciliate, the dissolution of maternal structures is as important as the development of the new soma, because the embryo is developing *in situ*, and resorption of maternal structures will provide an opportunity whereby the developing soma is released, partly at least, from the constraints of the old and probably defective blueprints vested with the maternal structures. Along with this, germ-line information is involved throughout the development of the embryonic oral apparatus. As a result, the embryonic oral apparatus is ontogenetically unique, and very likely the determinative blueprints that it carries have been newly specified, for directing oral development during binary fission in the new clonal cycle. Such respecification of somatic determinative blueprints is expected to be rather complete in the case of the oral apparatus, because it is built entirely anew while the maternal oral structures are disintegrating. On the other hand, in the case of the ciliary rows that persist in sexual reproduction, any genomic tampering of the deter-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

minative blueprints of the cortical units will only result in their attenuation or augmentation, rather than a wholesale respecification. This will account for the 'structural inertia' exhibited in the propagation of ciliary rows (Nanney 1980). Thus the ontogenetic state of the embryonic soma varies with the type of organelle or structure, according to the interaction between germ-line information and the maternal somatic blueprints, the latter in turn depending on how extensively and how fast the maternal structure is destroyed.

From the foregoing discussion, it becomes clear that the evolutionary significance of acquired cortical variations rests not merely with their maintenance through asexual propagation of the cell line. An evolutionary evaluation would not be complete without assessing how faithfully these variations can be inherited by the new individual developed during sexual reproduction. Without doubt some of them, such as rotated ciliary rows and the doublet biotype, can indeed stand a chance of persisting through sexual reproduction. The conjugation experiments with the doublet biotype, based on crosses between a doublet and a singlet, were intended as rigorous tests to rule out the remote possibility that the acquisition and maintenance of this type of cortical variation are due to genomic alterations (Sonneborn 1963). It must be made clear, however, that none of the published accounts have provided a clue as to the degree of fidelity of inheritance of such characters through sexual reproduction. This is a central issue. The pertinent question will be whether the probability of loss of such acquired characters increases, or decreases, with each round of embryonic reorganization of the soma during sexual reproduction. This question is meaningful because of the ontogenetic uniqueness of the embryonic soma. The respecification of developmental information by the germ nucleus, and the possibility of release from the constraints of the maternal somatic blueprints, together provide a special condition for embryonic somatic development that is fundamentally different from asexual somatic development during binary fission. This will affect the likelihood of perpetuation of maternally acquired cortical variations subsequent to sexual reproduction. A rigorous examination, by detailed quantitation, of the fidelity of maintenance of acquired cortical variations before and also after sexual reproduction would be revealing.

The issue of inheritance of acquired characters can be approached from another angle, by asking how acquired cortical variations might become a stabilized feature of the species in evolutionary terms. Here we face two diametrically opposite philosophies on the relation between the cortex and the genome, regarding the direction of evolution of the cortex. Sonneborn was impressed by the reliability and stability of pre-existing molecular assemblies of the cortex in determining the placement and orientation of new structures. He thought that the cortex is gradually moving away from the control of the genome: 'If in an earlier stage of evolution the genome controlled development of cortical pattern, as it probably did, and then DNA-directed assemblies anchored in the viscous cortex had accidental effects in favouring initiating, localizing and orienting new assemblies at cell division, these initially accidental effects could have provided the beginning of an evolutionary development by natural selection leading eventually to complete dependence on existing cortical structure, with loss of genic control except for production of the molecular building blocks' (Sonneborn 1970*a*, p. 362).

Frankel (1983), on the other hand, drew attention to the transiency of acquired cortical variations in evolutionary terms, and thought that these might serve as a first step in evolutionary change if they became stabilized by subsequent gene mutations. Thus cortical variations of non-genic origin will move towards submission to genomic control in the process of stabilization. These two views are not necessarily mutually exclusive, and their applicability may depend on the type of cortical structure in question, and their stage of evolution. At present, at least in the case of oral development during sexual reproduction we are certain that the morphogenetic determinants do not reside entirely within the cortex; instead, important signals deciding whether or not an oral apparatus is to be generated are associated with the germ-line nucleus and its derivatives. What needs to be emphasized is that somatic development during sexual reproduction, involving disintegration of the maternal soma and new informational input from the germ-line nucleus, will provide a suitable occasion for the species to assess whether or not the acquired characters may be assimilated into the developmental programme of the genome. Disintegration of the maternal soma, while releasing the embryonic soma from some of the developmental constraints vested with the maternal soma, poses the potential hazard of losing the acquired character. On the other hand, new developmental input from the genome of the germ line may, depending on the circumstance, potentially favour the permanent retention of the acquired character. This evaluation of acquired cortical variations by the ciliate, during development of the embryonic soma under genomic intervention, particularly with information from the germ nucleus, may be referred to as 'embryological assessment'. For multicellular organisms, this task is clearly germ-centred and predictably straightforward, because any acquired somatic variations will be discarded together with the maternal soma, which is spatially demarcated from the embryonic soma. For ciliates, as we have seen, the *in situ* development of the embryonic soma introduces an additional factor, as it necessitates that the constraints of the maternal developmental blueprints be taken into consideration during this time.

I have thus far redirected the focus on sexual somatic development in formulating views on the evolution of acquired cortical variations. Concerning the stability of such variations through asexual propagation, the significance of this might be restricted to the likelihood of maintenance of such variations until their destiny is assessed during sexual reproduction of the ciliate. That is, stability during asexual reproduction alone is not sufficient to guarantee long-term maintenance, and it may even be unimportant in evolutionary terms.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Conceivably, even though a certain non-genically acquired cortical variation may be rather unstable, this can still stand a good chance of becoming part of the species if the embryological assessment is favourable. The outcome of this assessment will depend on the nature of the acquired cortical variation, the extent to which the genome is involved in modulating its development during sexual reproduction, and the disruption this may engender to the normal sexual developmental programme. These factors will together decide the evolutionary destiny of the acquired variation, whether it can be readily assimilated, or is to be rejected. An interplay between these parameters renders prediction complex. In the following, some speculations are offered on the evolutionary outcome of acquired cortical variations, by exploiting the situation of the rotated ciliary rows and the doublet biotype. I argue that the probability of an acquired cortical variation becoming a permanent feature of the species might have little to do with its stability of perpetuation.

To begin with, one may ask how likely would rotated ciliary rows become stabilized in evolution? Ciliary rows exhibit no sign of replacement during sexual reproduction. In addition, the control of the structural asymmetry of ciliary rows is epigenetic, i.e. under the jurisdiction of the microgeography of the ciliary units, and remote from the direct control of the genome. These two features enable ciliary rows to enjoy greater autonomy, and hence relative stability, in the perpetuation of the acquired rotated pattern through asexual and sexual reproduction. However, because of the epigenetic nature of control of cortical unit asymmetry, it is difficult to envisage that a simple switch in gene action can result in the integration of the rotated asymmetry into the developmental programme. The difficulty is compounded by the fact that we are considering the stabilization of the inversion between normally orientated ciliary rows. Given that the potential pathways of phenotypic transformation in biological systems are finite, and constrained by the make-up of the genetic and epigenetic system, certain morphological configurations will be prohibited (Alberch 1982). The juxtaposition of two ciliary rows of opposite polarity may come into conflict with global epigenetic rules specifying the spatial coordination of somatic ciliary pattern. Some preliminary observations in *Tetrahymena* suggest that the probability of loss of a rotated row juxtaposed with a normal row is higher, compared to a rotated row that is found between two similarly rotated rows (S. F. Ng, unpublished data). At present, there is no hypothetical mechanism as to how such topological juxtapositions may become assimilated, genetically or epigenetically, into the developmental programme. Nor is there an example of a species exhibiting similar inversions as a permanent feature, to show that stabilization of these is at all possible. Therefore, despite the high degree of autonomy of perpetuation of the rotated asymmetry, the embryological assessment of the rotated ciliary rows during sexual reproduction is bound to be negative in the long run.

As elaborated in previous paragraphs, although the genome has little to do with prescribing the asymmetry of cortical units, it does play a role in monitoring the proliferation of basal bodies within the rows. The latter may set the stage for genomic modulation during sexual reproduction. Hypothetically, if such modulation has a vector property, for example in the form of a signal emanating from a fixed source such as the anterior end of the cell, then the opposite orientation of the cortical units along rotated rows may pose a problem when the signal is encountered from one direction. A likely consequence of this assessment will then be attenuation or deterioration of the determinative blueprints in the rotated cortical units, or of their capability to respond to the global external instructions specifying basal body proliferation. This will lead to the loss of the rotated rows in subsequent clonal propagation. There are some indications that 'faults' in basal body proliferation are more common in rotated ciliary rows of *Tetrahymena*, which frequently exhibit wider gaps devoid of basal bodies along the rows (S. F. Ng, unpublished data). The evolutionary outcome is thus restoration of normality, rather than assimilation of the acquired novelty.

In contrast, the doublet biotype might stand a better chance of assimilation into the developmental programme. The oral apparatus is replaced during sexual reproduction. During this time there is a risk of losing the developmental blueprints associated with the pre-existing oral apparatus that is undergoing disintegration. Thus every time the doublet biotype sexually reproduces it faces a crisis in the perpetuation of this acquired variation. The system is hence potentially less stable than the perpetuation of rotated ciliary rows, though we still lack definite proof of this. In addition, because the generation of the new oral apparatus during sexual reproduction requires new informational input from the germ nucleus in a decisive manner, the perpetuation of the oral apparatus comes directly under genomic control at this time. In view of this, I think the doublet biotype stands a better chance of becoming integrated into the developmental programme, because oral development is closely monitored by the germ-line genome in every round of sexual reproduction, and the nature of the variation is not incompatible with the developmental programme. There is no reason to suspect that the genomic signals for oral development along the two oral meridians are not the same. The position values around the circumference of doublets are in a continuous sequence, and thus the kind of doublets under consideration is topologically balanced (see Frankel 1989, chapter 10). The limiting factor for perpetuation of the doublet biotype could well be maintenance of topological balance between the two somatic domains, through an epigenetic interaction between the two oral meridians (apparatuses). An alteration of gene action may promote oral development along both pre-existing oral meridians, and also favour the co-existence of the two domains, to attain some form of stable 'circumferential metamerism'. As noted by Frankel (1983), there are recognized ciliate and flagellate species, especially from the order Diplomonadida, possessing two identical sets of structures, and these might have originated from other species by stabilization of an acquired doublet

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

body form. Other modes of origin may be conceived, such as 'polymerization' of structures, or 'fixation' of the asexual phase of binary fission (Kirby 1949; Raabe 1971). None the less, irrespective of their mode of origin (genic versus non-genic) and the process leading to their stabilization, such examples clearly show that the doublet body form can be a topologically stable and permanent feature of the species.

In this connection, an observation in the experiments on damaging one of the two oral apparatuses of doublets by ultraviolet irradiation may be instructive (Hanson & Ungerleider 1973). After the loss of an oral apparatus induced by irradiation, if the incomplete doublet underwent autogamy within four fissions an oral apparatus could be (re)generated along the irradiated oral meridian. Thus during sexual reproduction the genome had acted to promote oral development along the irradiated oral meridian, despite the deterioration of cortical determinants for oral development following irradiation. Apparently, the genomic programme is capable of operating according to only a very minimal amount of residual cortical information left with the damaged oral meridian, and intervenes during sexual reproduction to rescue the development of the oral apparatus. This suggests, in corroboration with the conclusion from the study of amicronucleates, that the genome is playing a major role in the initiation of oral development, and in turn the perpetuation of the doublet biotype, during sexual somatic reorganization. This state perhaps is not far from the extreme (ultimate?) situation of full integration of development of the doublet biotype into the normal developmental programme, when the genome is able to direct the development of the doublet biotype even without the collaboration of any cortical information.

These considerations leave us with the paradoxical corollary that the chance of evolutionary assimilation of acquired cortical variations depends not so much on their stability of perpetuation through asexual propagation, but rather on how closely such structures are monitored by the genome during development of the embryonic soma in sexual reproduction, and also how compatible these variations are with the existing genetic and epigenetic programmes. The above contrast between rotated ciliary rows and the doublet biotype is purely hypothetical, and serves only to illustrate the situation that evolutionary assimilation of acquired variations might turn out to be inversely related to their stability of perpetuation in asexual propagation. That is, in evolutionary terms it is seemingly more meaningful to talk about those acquired cortical variations that are unstable, if there are reasons to believe that such are monitored closely by the genome during sexual reproduction, rather than the stable ones the genomic action on which is at best indirect. It is too early to judge how valid the above speculations might turn out to be. It will be interesting to look in this direction, and to formulate our questions and predictions based on the embryological perspective of ciliate sexual development. The speculative trip just taken accords much importance to the development of the embryonic soma under the intervention of germ line information during sexual reproduction of ciliates. This Weismannian bias is deliberate, intended to prompt a serious look at the stability of acquired cortical variations through sexual reproduction. The outcome of such undertaking might affect our evolutionary perspective on cortical inheritance of ciliates, and also on the inheritance of acquired characters in general.

6. EPILOGUE

The embryological perspective of ciliate sexual development allows us to reformulate our perceptions in several areas of protozoology. These issues have a long history, but they continue to influence our views on current issues, such as ageing, sexual development, and the inheritance of non-genically acquired characters. The nature of such inquiries are diversified, but the perplexities generated share a common basis, traceable to the preoccupation with the study of asexual development in ciliates, and the general negligence of morphogenesis during sexual development (see, for example, Weisz 1954; Hanson 1967; Tartar 1967). The most conspicuous feature of ciliate development during conjugation or self-fertilization is the reorganization of the nuclear apparatus, after meiosis of the germ nucleus and formation of the zygotic nucleus. The resemblance between protozoa and multicellular organisms in these nuclear events is close enough for investigators to address conjugants as gametes as they exhibit meiosis, and after fertilization as zygotes (see Wilson 1925; Turner 1941; Grell 1967), even though transformation from a vegetative cell to a gamete, upon conjugation, is not accompanied by conspicuous morphological changes in many groups. The usage of the terms 'gametogenesis' and 'fertilization' is common in the protozoan literature. The parallelism between ciliates and multicellular organisms, however, has stopped at the zygote, largely because little attention has been paid to replacement of the soma during sexual reproduction. The subtlety of resorption of the maternal soma, and the generation of a new soma for the sexual progeny have only been reported in restricted groups of ciliates and flagellates. Even for those that have been studied, little developmental significance has been accorded to the renewal of the soma.

This situation should change when one realizes that the development of a new soma during sexual reproduction constitutes an important phase of the life cycle, and that this development is unique, different from the situation in asexual propagation, but resembling the control of embryonic development of multicellular organisms. Protozoa are unusual in many ways, but they are basically the same life form as multicellular organisms, sharing by and large the same genetic code and cytoskeletal elements. I believe it is in the commonalities shared with multicellular organisms that their unusual features, such as cortical inheritance, are to be interpreted and understood. The embryological perspective directs our attention to one such common feature concerning the life cycle, namely the reconstruction of the soma after fertilization, and shows

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

how some of the misconceptions about protozoa may be rectified, and re-evaluated within the framework of embryonic development of an organism.

The formulation of the embryological perspective is at present grossly incomplete. First, we are still ignorant of the nature, origin and mode of action of the developmental determinants associated with the germ nucleus in ciliates. A hunt for genes expressing in the germ nucleus in *Paramecium* has not yet yielded any positive results (Tam & Ng 1987). The possibility remains that the determinants originate outside the germ nucleus, and come to be associated with the germ nucleus; during meiosis the nuclear envelope does not breakdown and one of the postmeiotic nuclei approaching the oral primordium acts as a vehicle for deliverance of such determinants at a critical stage of oral development. On the other hand, the germ nucleus of ciliates during meiotic prophase I goes through a phase reminiscent of the amphibian meiotic prophase lampbrush chromosome stage (see Raikov 1982); RNA labels have been shown to be associated with the nucleus in this stage but their significance awaits investigation (Sugai & Hiwatashi 1974; Martindale *et al.* 1985).

The second shortcoming relates to the question of generality. This has been alluded to in §2. Most probably the embryological perspective applies to ciliates in general, and will be vindicated as more ciliates are studied in this respect. Extension of this perspective to other protozoa, such as flagellates, faces the question of the absence of nuclear dimorphism, or demarcation between the germ and somatic nuclei, in these protozoa. There is also the problem posed by the lack of sexuality in some protozoa, or in some strains of certain genera like *Tetrahymena*, which are apparently able to propagate indefinitely by cell division. Do these protozoans possess alternative means of periodic somatic renewal during asexual propagation (like replacement of the oral apparatus in *Tetrahymena*) in lieu of sexual somatic development (see Kirby 1944)? Presuming that asexuality has arisen secondarily following abandonment of the sexual process, and independently in different groups of protozoa (Hawes 1963), somatic renewal without sex could have evolved before the loss of sexuality. New conceptual inputs into the embryological perspective are expected, and this perception will form the basis of inquiry of sexual somatic development in different groups of protozoa.

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(*Received 2 April 1990*; *Accepted 18 May 1990*)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES