

PROTOZOA

Weismann's germ-plasm theory was based on the differentiation of germinal and somatic cell lines in many-celled organisms. In unicellular forms there is evidently no such cellular differentiation, and Weismann surmised that these organisms are potentially immortal—a view already expressed by Ehrenberg in 1838. This conclusion was questioned as a result of the growing knowledge of the occurrence and nature of conjugation—first noted in the ciliate Infusoria.

Conjugation was observed by Leeuwenhoek (1695) and by O. F. Müller (1786) but was usually misinterpreted until the work of Bütschli (1873 and later) and Engelmann (1876). The ciliates have two types of nuclei—macronucleus and micronucleus—which were earlier interpreted as ovary and testis, respectively. Bütschli and Engelmann made out their nature and found that the macronucleus was lost at the time of conjugation. They developed the view that these animals are not potentially immortal but have a definite life cycle that requires conjugation to renew the vitality of the line (which becomes senescent and dies out in the absence of conjugation). This speculation was based on observation of the structural changes.

The cytological details of conjugation were worked out independently by Maupas and by R. Hertwig in 1889. They described the degeneration of the macronucleus, the exchange of micronuclei with the fusion of those from the two individuals, and the reconstitution of the new macronucleus from a division product of the biparentally derived new micronucleus. With these results, it became possible to relate the process of conjugation to the better-known sexual reproduction of higher forms.

Maupas had already (1888) published his elaborate studies on the relation of conjugation to senescence and rejuvenescence and had fur-

nished an extensive experimental basis for the ideas suggested by Bütschli and Engelmann. There followed a long series of studies (by Hertwig, Calkins, Woodruff, Jennings, and others) on this general subject (see summary by Jennings, 1929).

Jennings understood that sexual reproduction should lead to segregation, recombination, and increased variability; and he set out to study these. The material was difficult, especially because conjugation was erratic and uncontrollable in its incidence. This difficulty was overcome by the discovery of mating types in *Paramecium* (Sonneborn, 1937), leading to the study of the ciliates by Mendelian methods.

It was shown by Blakeslee (1904 and later) that morphologically similar strains of the fungus *Mucor* fall into two types (called "plus" and "minus"). Sexual reproduction occurs only between the two types, never between individuals of the same type. The similarity to those forms having distinct male and female gametes was obvious, and such examples (soon found in many lower organisms) were often discussed as extreme examples of sexual differences in which the structural differences of eggs and sperm were absent.

Sonneborn found that similar relations occur in *Paramecium aurelia*, the different asexually produced lines falling into plus and minus series, between which, under appropriate conditions, conjugation occurs quickly and often in a striking *en masse* way, when plus and minus strains are mixed. But the analogy with sex is somewhat strained by the fact that each member of a pair contributes a micronucleus to the other, so that the natural comparison is that of this migrant nucleus with the sperm of higher organisms; in other words, *Paramecium* may be thought of as hermaphroditic, yet mating types occur. The comparison to sexual types was still further strained when Jennings (1938 and later) found that, in *P. bursaria*, there are eight mating types, of such a nature that each will conjugate with any one of the other seven. Eight sexes?

The comparison of mating types to sexual differences had already been complicated by the earlier work on "relative sexuality" in algae. This development begins with the work of Hartmann (1925) on the brown alga *Ectocarpus*. Here the gametes are structurally different, one type (comparable to sperm) being an active swimming body, and the other (comparable to an egg) nonmotile. These gametes are produced only when two compatible plants are in contact. Hartmann found that each plant gave consistent results when it was repeatedly tested against another one; but it was not possible to recognize male and female plants. Instead, there seemed to be a graded series, from extreme male to extreme female. Plants between these extremes could function as females

when paired with extreme males, and as males when paired with extreme females.

Similar systems were found in other algae and were studied in detail by Hartmann and his students. One of the most striking cases, that of *Chlamydomonas*, was studied by Moewus, who developed an elaborate biochemical and genetic interpretation. This work at one time figured largely in the literature of genetics, though there were doubters almost from the beginning. It has now been very generally concluded that the experimental results of Moewus are wholly unreliable, and that the data were largely made up to fit his theories rather than based on observations. This is, in fact, now considered to be one of the very few scientific scandals in biology (see Ryan, 1955, *Science*, **122**:470).

There is no doubt about the reality of the phenomenon of relative sexuality, and it is clearly something that needs study and analysis; but the papers of Moewus have unfortunately given it unpleasant associations.

There is one special process that occurs in *Paramecium aurelia*—and rarely or not at all even in other species of the genus—that has played a large part in the genetical study of the ciliates. This process, called endomixis (or, now, autogamy) was described by Woodruff and Erdmann in 1914; their account of the cytological details was corrected and brought into better agreement with later genetic results by Diller in 1934. In this process, the macronucleus degenerates and is replaced by a division product of the micronucleus. The micronucleus itself undergoes meiosis, and one of the resulting haploid nuclei divides to give two identical nuclei—a process similar to what happens at conjugation. But at conjugation one of these two genetically identical nuclei migrates to the other individual, and there fuses with the nonmigratory product in that individual, thus producing in each ex-conjugant a diploid biparental micronucleus, from which the whole nuclear complement of its descendants arises by division. In autogamy, the two identical sister micronuclei fuse, and again the whole nuclear complement of the descendants arises by division from this uniparental diploid nucleus. It follows from this that, after autogamy has occurred, a line of *P. aurelia* individuals is genetically homozygous until conjugation (or mutation) occurs.

One result of the more recent studies is the demonstration that the phenotype of a ciliate is dependent on its macronucleus, which was derived from a micronucleus and does not survive conjugation or autogamy. The germ-plasm theory is valid here; the macronucleus is somatic, the micronucleus is germinal.

The unusual properties of the ciliates have been exploited by Sonneborn and his co-workers (Kimball, Preer, Beale, Siegel, and others—see Beale, 1954, for a summary) to produce a coherent body of knowledge about the genetics of the ciliates that is important in many current developments but is beyond the scope of this book.