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Evolution

Asexual metazoans undergo senescence

(aging/germ line/soma/differentiation/Oligochaeta)

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ABSTRACT **August Weismann popularized the notion that metazoans have a potentially immortal germ line separated from a mortal soma, and evolutionary biologists regard senescence as an evolved characteristic of the soma. Many have claimed that metazoans that do not sequester their germ line have no clear distinction between germ line and soma, and consequently they should lack senescence. Here we present experimental evidence that senescence occurs in the asexually reproducing marine oligochaete *Paranais litoralis*. We also analyze data reported in Sonneborn's classical study and show that the rhabdocoel *Stenostomum incaudatum* undergoes senescence. We argue that the stability of commitment to somatic function and the fact that asexual metazoans form their germ cells from undifferentiated stem cells are sufficient to allow for senescence of the asexual metazoan's soma. Thus the evolution of somatic differentiation, and not germ-line sequestration, would be the necessary condition for the evolution of senescence.**

Senescence can be defined as the decline of fitness components of an individual with increasing age, owing to internal physiological deterioration (1). Evolutionary theory proposes that senescence of multicellular individuals has evolved owing to a decline in the force of natural selection on later age classes (2). This decline permits the persistence in the population of alleles with deleterious effects on postreproductive stages or alleles with positive effects early in life but with pleiotropic negative effects late in life.

August Weismann popularized the idea of a complete separation in metazoans between an immortal germ line and a soma that would serve to transfer the germ products to the next generation and then senesce (3). Research on senescence has therefore focused on the question of germ-line sequestration. Individuals in species that sequester a germ line should senesce, whereas those where the separation does not exist should not senesce at all, since the soma's fate is tied to that of the germ cells.

Those metazoans that appear not to sequester their germ lines are capable of both sexual and asexual reproduction. Studies of longevity and senescence in such asexual metazoans [e.g., *Hydra* (4–7) and planarians (8)] have provided conflicting results. Sonneborn's classical study (9) of budding forms of the rhabdocoel *Stenostomum incaudatum* demonstrated a decline in the fission rate with age and signs of decrepitude prior to death (e.g., production of offspring with structural abnormalities). Sonneborn's results have been interpreted (e.g., ref. 7, pp. 89–91; ref. 10, pp. 234–235) as evidence of senescence in clonal lineages, instead of individual senescence. Sonneborn set about to show that fission in asexually reproducing animals such as *Stenostomum* and fission in Protozoa were different processes. He called the two products of fission of *Stenostomum* "anterior" and "posterior." Sonneborn concluded (ref. 9, p. 81) that "a line of successive anteriors shows most of the characteristics of a single 'individual' that develops, grows old, and finally dies, in the meantime giving rise to new 'individuals' at the posterior end." Sonneborn's "anterior lines" were clearly single individuals followed throughout life, and not clonal lineages.

To our knowledge, the only evidence for the hypothesis that asexual metazoans may lack senescence comes from a study by Bell (11–13), who showed that survival was independent of age in two species that reproduce asexually, the aelosomatid annelid *Aelosoma tenebrarum* and the naidid oligochaete *Pristina aequiseta*.

Long-term survival of clonal lineages and colonial organisms seems sometimes to be taken as evidence of the lack of individual senescence in asexual metazoans (e.g., ref. 14, p. 77). Individual senescence is a decline in fitness components of an individual with increasing age, whereas clonal senescence may be defined as a decline in the average fitness of a clonal lineage with time (generations). The evolutionary theory of senescence explains the evolution of individual senescence but has no bearing on clonal senescence. The two processes can operate independently. Even if individuals undergo no senescence, clonal lineages could still suffer a reduction of average fitness through time by, for example, the accumulation of slightly deleterious mutations by Muller's Ratchet (15). Additionally, the evolutionary theory of senescence does not offer clear predictions on the presence of colony senescence. A colony may be considered as a clonal lineage where individual members of the clone (frequently called ramets) have remained connected. Thus, colony members may be subject to individual senescence while the colony as a whole may undergo clonal senescence. Cited cases of clonal tunicates (e.g., ref. 16) or corals (e.g., ref. 17) that have lived through long periods of time are frequently used as examples of the lack of individual senescence in those species. Although those records may provide information about clonal senescence, they do not provide evidence regarding individual senescence. A model developed to analyze the fate of individual members of a clone suggested that senescence may be prevented because "clonal reproduction alters the patterns of selective pressure on different stages in the life cycle" (18). This conclusion, however, is based on the restrictive assumption that asexually produced offspring are born at a greater age than those produced sexually.

EXPERIMENTS AND RESULTS

To test the hypothesis of absence of senescence in asexual metazoans, we investigated the life schedules of *Paranais litoralis*, an estuarine naidid oligochaete that usually reproduces asexually by fission; sexually mature individuals are rarely found in the field. Cocoons—i.e., capsules containing sexually produced eggs—were never observed in our laboratory cultures, although sexually mature individuals were found sporadically. The parental individual produces off-

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spring by regular growth posterior to a fission zone located at a constant number of segments from the parental head. The offspring contains no segment of the parent and is composed entirely of new tissue (19). By the time parent and offspring split, the offspring's head is already formed and an additional offspring is already being grown by the parent.

In May 1988 we collected *Paranais* from a population at Flax Pond, a salt marsh on the north shore of Long Island, NY. The worms were brought to the laboratory, where several mass cultures were established. Four experiments designed to test the presence or absence of senescence in *Paranais litoralis* were carried out with worms from these mass cultures. The experiments started in June 1988 (experiment 1), May 1989 (experiment 2), and July 1990 (experiments 3 and 4). In November 1990, additional cultures were established with worms collected from a population at Stony Brook Harbor, Long Island, NY. These worms were used in experiment 5 (started in May 1991) and experiment 6 (started in September 1991). All six experiments had the same design. Repetition allowed us to control for possible laboratory artifacts and permitted statistical analysis of the results. A total of 171 worms were followed from birth to death to determine individual longevity. At birth worms were placed in individual 20-ml dishes with food (Flax Pond surface mud sieved through a 1-mm mesh and frozen at −80°C) ad libitum. Dishes were inspected daily to remove the offspring produced, which were discarded. Water [collected from Stony Brook Harbor at high tide, filtered through glass microfiber GF/C filters (Whatman), and kept in an oven at 60°C overnight] and food were changed daily. Experiments were carried out at 22°C, except for experiment 4, which was carried out at 10°C. From the individual longevity data, the number of survivors (a_x) was determined for 10-day intervals in experiments 1, 2, 3, 5, and 6 and for 23-day intervals in experiment 4. Worms lived on average 2.3 times longer at 10°C than at 22°C, so that 23- and 10-day intervals are equivalent with respect to average longevity. Survival rates (P_x) from age x to age $x + 1$ were calculated as a_{x+1}/a_x and were plotted against age class x for each experiment (Fig. 1).

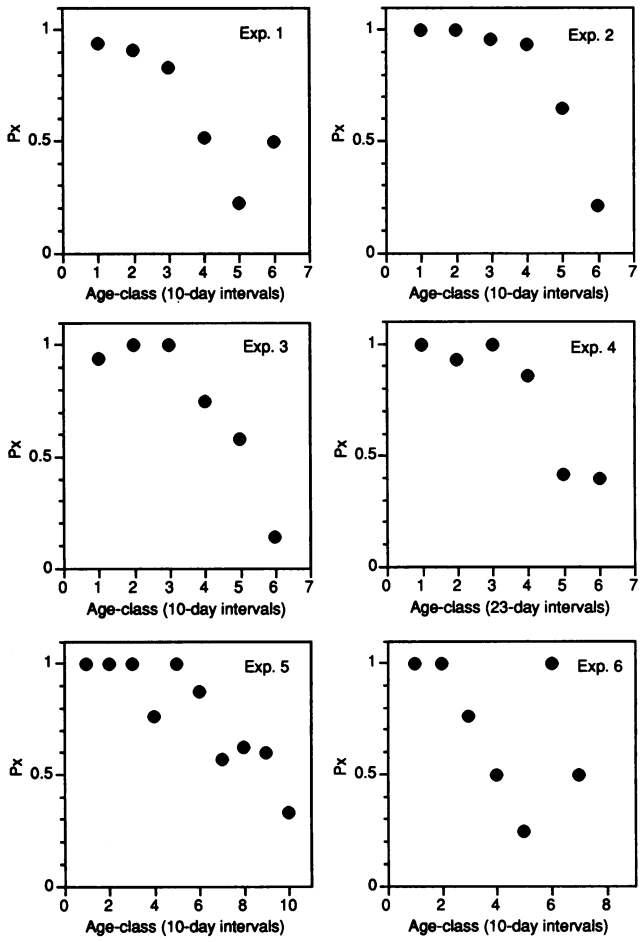

FIG. 1. Age-specific survival rates (P_x) versus age class (x) for *Paranais litoralis*.

When P_x is plotted against age class x, a declining trend constitutes evidence of senescence. All six experiments showed a very consistent decline in age-specific survival with age. The pattern of decline did not seem to be affected by the large difference in the average longevity between individuals of experiment 4 and the others. The statistical significance of the decline of age-specific survival with age was tested by two different approaches, summarized in Table 1. The first approach combined Friedman's method for randomized blocks and Page's L test for ordered alternatives. By Friedman's method we tested the null hypothesis that age-specific survival rates did not differ. Experiments 1–4 were taken as blocks and P_x as the treatment effects. We rejected the null hypothesis and concluded that age-specific survival rates are significantly different. Page's L test demonstrated that there is a monotonic decline of age-specific survival rates with increasing age. The second approach consisted of using runs-up-and-down tests for each experiment to test the null hypothesis that the observed trends were random. The exact probabilities for the runs-up-and-down test were calculated from the cumulative probability distribution (20). By combining the independent probabilities calculated for each experiment, using Fisher's technique (21), we were able to reject the null hypothesis and demonstrate that there is a statistically significant decline of age-specific survival rate with age. Experiments 5 and 6 were not included in any of the statistical tests because worms used in these experiments were collected from a different population.

Although the main objective of Sonneborn's study (9) was not to investigate senescence but rather to demonstrate that the two products of fission in *Stenostomum* were not physiological and structurally equivalent, the study provided data that can be analyzed in a way similar to that of our experiments. Sonneborn followed four groups of 100 individuals from birth to death. From these data we calculated age-specific survival rates, P_x (Fig. 2). Plots showed a statistically significant (see Table 1) decline of age-specific survival rate with age, indicating that *S. incaudatum* undergoes senescence. This analysis confirms Sonneborn's qualitative impressions, cited above.

Table 1. Statistical analyses of age-specific survival rates

	Paranais litoralis	*Stenostomum incaudatum*
Friedman's method for randomized blocks		
Friedman test statistic	17.429	22.729
Degrees of freedom	5	7
Probability	0.004	0.002
Page's L test for ordered alternatives		
Page's L statistic	357	782
Probability	<0.001	<0.001
Runs-up-and-down test		
$P(r < r')$ Case 1	0.0833	0.0686
Case 2	0.0028	0.0076
Case 3	0.0833	0.1389
Case 4	0.3278	0.0957
Combined probability	<0.005	<0.005

In the runs-up-and-down tests, cases 1–4 correspond to experiments 1–4 for *Paranais* and groups 2A–5A for *Stenostomum*.

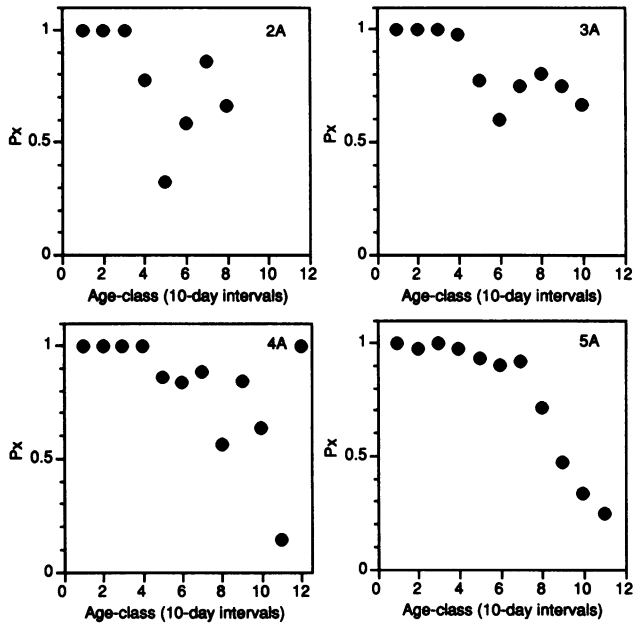

FIG. 2. Age-specific survival rates (P_x) versus age class (x) for *Stenostomum incaudatum* as calculated from Sonneborn's data (ref. 9, table 5, p. 83). Each plot corresponds to one of the four groups of individuals (2A, 3A, 4A, and 5A) followed by Sonneborn.

DISCUSSION

Our data on *Paranais* and the analysis of Sonneborn's data on *Stenostomum* indicate that metazoans capable of asexual reproduction by fission can undergo senescence. Furthermore, these results call into question the assertion that an unequivocal distinction between germ line and soma is a condition for the evolution of senescence. This claim is commonly made (e.g., refs. 1, 12, 22, 23) and appears to be an outgrowth of Williams' theory on the evolution of senescence that "regards senescence as an evolved characteristic of the soma" to be found "wherever a soma has been evolved, but not elsewhere" (24). The claim has persisted, despite Williams' prediction that "While asexual clones [protozoan clones] should not show senescence, asexually reproducing individuals may be regarded as having somas and they should, according to the theory, show senescence" (24).

Implicit in these ideas is the assumption that germ-line sequestration is the process that ensures an unequivocal distinction between germ line and soma. If somatic and germ-line cells do not segregate, then it would follow that any cell can become part of the germ line and senescence should not evolve. This is the basis for the prediction of lack of senescence in asexual metazoans. Two lines of evidence suggest that we cannot characterize even asexual invertebrates as having interchangeable germ and soma: (*i*) cell commitment to somatic function is fairly stable and (*ii*) germ-cell formation in taxa that do not appear to sequester a germ line generally results from undifferentiated stem cells.

Cell determination and differentiation is usually a step-by-step process driven by some intrinsic property of the cell or by external signals. Some cells become terminally differentiated—that is, acquire an extreme specialization and no longer divide—but cells from most tissues are capable of dividing. Moreover, nuclei from both differentiated and terminally differentiated cells may remain genetically totipotent—that is, capable of supporting embryonic development. Thus, if somatic cells were capable of abandoning their specific function, they might be able to become germ-line cells. However, changes in cell commitment, either via dedifferentiation/redifferentiation or via transdifferentiation, are possible but rare, and, in general, differentiated cells are fairly stable and may maintain their state through several cycles of growth and division. The stability of cell commitment is probably an evolved feature that is maintained by natural selection because cells abandoning their specific function may have highly disruptive effects at the individual level (25, 26).

Germ-line formation in metazoans follows one of three patterns: preformistic, intermediate, and epigenetic (27). In metazoans with preformistic mode (e.g., trematodes, nematodes, holometabolous insects, anuran amphibians), primordial germ cells segregate from the somatic cells very early during embryonic development, and they often seem to include germ-cell-specific germ plasm already distinguishable in the oocyte or the zygote. In metazoans with intermediate mode (e.g., molluscs, echinoderms, urodele amphibians), primordial germ cells appear rather late during embryonic development by induction of undifferentiated (usually mesodermal) cells. Asexual metazoans (e.g., coelenterates, turbellarians, some oligochaetes, bryozoans) form germ cells epigenetically by maintaining populations of stem cells capable of differentiating into various somatic cell types or detouring into a pathway of germ-cell formation throughout adult life. Moreover, germ-cell formation may take place after several asexual generations. Some fissile metazoans have mechanisms that resemble germ-line sequestration, at least in the sense that they limit the access of any cell to the germ line. Recent studies (28, 29) have shown that *Hydra oligactis* has a population of multipotent stem cells that give rise to somatic cells (i.e., nerve cells, nematocytes, and gland cells), and two additional subpopulations of germ-line-restricted stem cells whose only function is to produce sperm and eggs. Similar mechanisms may exist in other asexual metazoans but may have been overlooked due to our limited knowledge of development in those groups. In any case, germ cells in asexual metazoans are formed from undifferentiated stem cells and not from any somatic cell.

There does not seem to be justification for the claim that metazoans without germ-line sequestration should not undergo senescence because *any* cell can become a germ cell. The stability of commitment to somatic function and the pattern of germ-cell formation in asexual metazoans indicate that processes other than germ-line sequestration may prevent somatic cells from entering the germ line. We believe that the evolution of somatic differentiation and hence of an integrated multicellular soma, and not of germ-line sequestration, was the necessary condition for the evolution of senescence. The transition from multicellularity in colonial protozoans to cellular differentiation some 500–1000 million years ago marked a crucial point for the evolution of the metazoan soma. Some cells lost their capacity to produce a new individual; they became committed to functions other than reproduction. Free from the need for immortality, the metazoan soma became liable to undergo senescence. Genes with negative effects on that soma will be differentially selected depending on their timing of expression. Selection will be stronger against genes with negative effects in the prereproductive and reproductive periods. Once the task of passing totipotential cells, either sexually or asexually, to the next generation has been accomplished, the soma may suffer internal physiological deterioration without fitness consequences for the individual. As Weismann put it, "Upon differentiation between germinal and somatic cells, natural selection was, speaking metaphorically, trained to bear on the immortality of the germ-cells, but on quite other qualities in the somatic cells . . ." (30). Thus, ". . . the body, or soma appears in a certain sense as a secondary appendage of the real bearer of life, the reproductive cells" (31).

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