



MORTALITY PATTERNS SUGGEST LACK OF SENESCENCE IN HYDRA

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Abstract—Senescence, a deteriorative process that increases the probability of death of an organism with increasing chronological age, has been found in all metazoans where careful studies have been carried out. There has been much controversy, however, about the potential immortality of hydra, a solitary freshwater member of the phylum Cnidaria, one of the earliest diverging metazoan groups. Researchers have suggested that hydra is capable of escaping aging by constantly renewing the tissues of its body. But no data have been published to support this assertion. To test for the presence or absence of aging in hydra, mortality and reproductive rates for three hydra cohorts have been analyzed for a period of four years. The results provide no evidence for aging in hydra: mortality rates have remained extremely low and there are no apparent signs of decline in reproductive rates. Hydra may have indeed escaped senescence and may be potentially immortal. © 1998 Elsevier Science Inc.

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INTRODUCTION

THE POTENTIAL immortality of hydra has generated close to a hundred years of controversy. However, most studies report only anecdotal information that cannot be used as evidence for (Hertwig, 1906; Berninger, 1910; Boecker, 1914; David, 1925) or against (Goetsch, 1925; Schlottke, 1930; Brien, 1953) senescence. The only study in which actual data are presented (Hase, 1909) seems to support the presence of senescence in hydra (Martínez, 1993). However, the general consensus among modern hydra researchers seems to be that hydra lacks aging because it is capable of constant renewal of its body (Brien, 1953; Loomis and Lenhoff, 1956).

Hydra has a simple body plan. It is essentially a tube with a head at one end and a foot or basal disk at the other. The head consists of two parts: the apical hypostome, or mouth region, surrounded by the tentacle zone from which typically six tentacles emerge. The body wall is composed of two epithelial layers separated by an acellular basement membrane, the mesoglea. Hydra has only about 20 cell types that are organized into three cell lineages: two epithelial and

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one interstitial. Each lineage consists of a population of stem cells with indefinite self-renewal capacity, and several differentiation products. Interstitial cells reside in the interstices among the epithelial cells. The epithelial cells of the tentacles, the tip of the hypostome, and the foot are nondividing differentiation products of epithelial cells of the body column (Campbell, 1965; Campbell, 1967a). Neurons, nematocytes, secretory cells, and gametes are differentiation products of interstitial cells.

A striking characteristic of hydra is its tissue dynamics. The epithelial cells of the body column are continuously in the mitotic cycle. This continuous production of epithelial cells in the body column is balanced by a loss of cells: 85% of the epithelial cells are incorporated into developing buds while the rest are sloughed off at the tip of the tentacles, hypostome, and basal disk (Bosch and David, 1984). A result of these dynamics is that epithelial cells are constantly displaced either apically onto the head, or basally onto developing buds, or onto the foot.

A consequence of this dynamic behavior is that an individual cell does not exist long in a hydra body. Nondividing differentiated cells of all three lineages are lost by displacement from the body column within 20 days (Campbell, 1967b). Dividing stem cells of the interstitial lineage have a cell cycle time of 18–30 h, while stem cells of the epithelial lineages have a cell cycle time of three to four days (David and Campbell, 1972). Hence, cells are either constantly renewing by cell division, or they are lost from the animal in a relatively short period of time. This capacity for constant renewal is the main reason behind the claim that hydra is potentially immortal.

MATERIALS AND METHODS

To test for the presence or absence of senescence in hydra, the mortality patterns of four groups of individuals of *Hydra vulgaris* were analyzed. A group of 45 animals was collected from Swan Pond, a freshwater lake in Long Island, New York, on December 1991. These individuals (control) were maintained as controls even though their exact ages were unknown. Three cohorts derived from the control group by asexual reproduction were separated on December 1991 (group 1; 50 animals), January 1992 (group 2; 30 animals), and March 1993 (group 3; 20 animals). Animals were raised in an environmental chamber at constant temperature under an 8:16-h light:dark cycle. They were maintained individually in culture dishes and fed freshly hatched *Artemia* sp. (5 to 15 nauplii per hydra). Approximately 6 to 10 h after feeding, hydra were transferred into clean dishes with fresh medium. Offspring were counted and discarded at that time. Temperature, culture medium, and feeding frequency changed when hydra cultures were transferred from the State University of New York at Stony Brook (22°C; GF/C filtered Swan Pond water; fed every other day) to the University of California at Irvine [20°C; hydra medium (1 mM CaCl in Arrowhead spring water); fed three times a week].

Several different approaches have been used to determine the presence or absence of senescence in metazoans. A classical method is to plot the logarithm of a cohort survival probabilities (l_x) vs. age, and visually inspect the shape of the curve. An approximately “diagonal” (sensu Deevey, 1947) curve would indicate constant mortality rates and no senescence. The decision of whether or not a line is sufficiently diagonal is inevitably subjective, so that this approach is bound to generate controversy. The important parameter is not the fraction of the original population that reaches a given age x , that is l_x , but rather the probability of surviving from age x to age $x + 1$, that is, age-specific survival P_x (or its inverse age-specific mortality Q_x). If senescence is present, we expect to see an increase in age-specific mortality with increasing age. For birth-flow populations (sensu Caswell, 1989) in which reproduction is

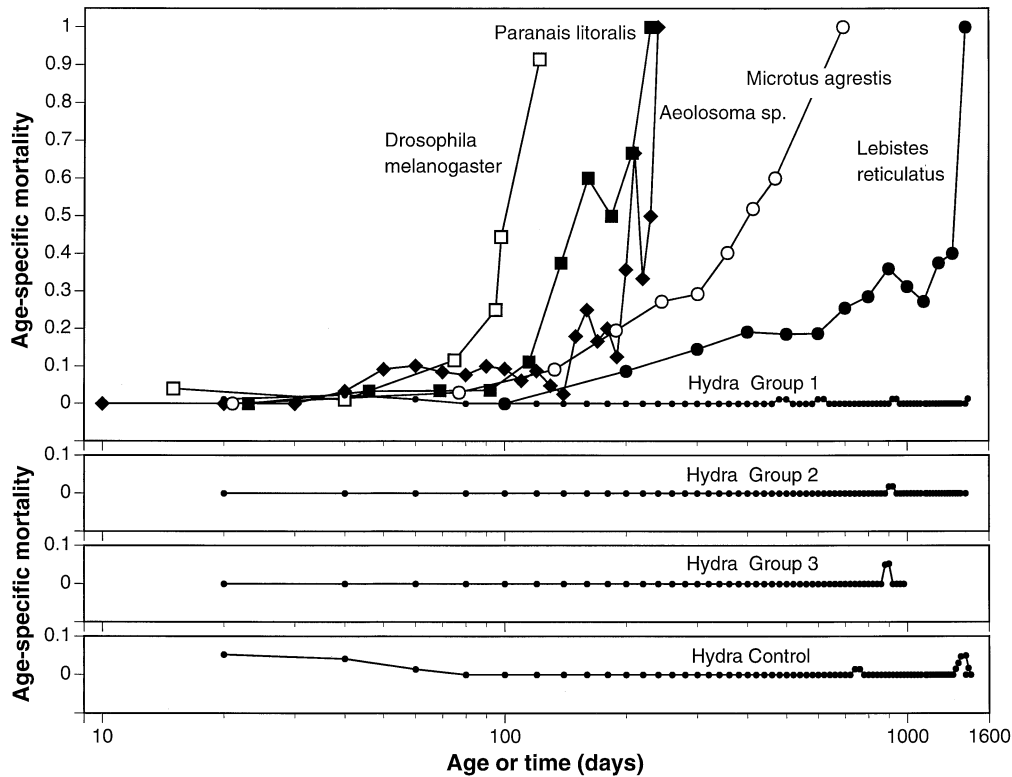


FIG. 1. Age-specific mortality rates. Abscissa (logarithmic scale) represents age (groups 1, 2, and 3) or time (control). Animals that were accidentally killed during handling were excluded (control: three; group 1: five; Group 2: two). Data on *Drosophila melanogaster*: Miquel, *et al.*, 1976; *Microtus agrestis*: Leslie and Ranson, 1940; *Lebistes reticulatus*: Comfort, 1961; *Paranaïs litoralis* and *Aeolosoma sp.*: my own experiments.

more or less continuous over a time interval, age-specific mortality rates (Q_x) can be calculated as $1 - [(a_x + a_{x+1}) / (a_{x-1} + a_x)]$, where a_x is the number of survivors to age x .

RESULTS

Mortality rates

Data on mortality were collected for a period of four years. Figure 1 shows age-specific mortality rates for the three cohorts of hydra and for the control hydra of unknown age. Mortality curves for the marine oligochaete *Paranaïs litoralis*, the aeolosomatid annelid *Aeolosoma sp.*, the fruit fly *Drosophila melanogaster*, the fish *Lebistes reticulatus*, and the vole *Microtus agrestis*, have been included for comparison. These species were chosen because they are known to undergo senescence and because owing to their longevities they could be plotted in the same graph. Hydra mortality rates have remained close to zero for four years. In contrast, all the other species show the increase in mortality rates with increasing age, which is typical of species that undergo aging. One possible explanation for the apparent independence of

mortality from age in hydra is that hydra does not age. An alternative explanation is that hydra lives much longer than four years and we are looking at the mortality pattern of “young” hydra. Distinguishing between these two hypotheses requires a prediction of the potential longevity of hydra (see Discussion).

Reproductive rates

Figure 2 shows mean age-specific budding rates. Plots for the Stony Brook and the Irvine periods have been drawn separately for each group. A reduction in budding rates is expected for the Irvine period because at lower temperature and feeding frequency hydra grow less, and consequently, bud less (Otto and Campbell, 1977).

DISCUSSION

Reproductive rates

The general deterioration of an individual associated with senescence is sometimes, but not always, reflected by a decline in reproduction. All hydra included in these experiments reproduced both sexually and asexually. In hydra, production of eggs or sperm and budding can occur concomitantly, as it was the case for the animals in these experiments. The actual sexual reproductive output of individual hydra could not be estimated because animals remained isolated so no offspring were produced. Furthermore, given the experimental design egg or sperm production were difficult to estimate. Notwithstanding, age does not seem to have an effect on the potential capacity to reproduce sexually: after four years males were still carrying numerous testes and females regularly produced eggs.

Similarly, after four years of observation, hydra continued to reproduce asexually. Over this period an individual hydra has produced on average 448 asexual offspring (group 1; $n = 39$; mean \pm SD: 448 ± 63). Figure 2 suggests that there has been a slight decline in budding rates since the beginning of the experiment. It is tempting to conclude that this decline represents an indication of aging. However, causes other than age could be responsible for it. For example, the slight decline in budding rates for groups 1 and 2 over the first year could represent the physiological adaptation of hydra cells to a novel environment with a unique food source and constant temperature and light conditions. Furthermore, the pronounced dips observed at Irvine correspond to temporary reductions in feeding frequencies. In fact, the general shape of the curves in Fig. 2 suggests that environment rather than age seems to be responsible for the overall fluctuation in budding rates in these experiments. Notice that for both periods, all four groups show almost identical patterns of fluctuations independently of their age. To corroborate this observation, correlations between the budding rates of the different hydra cohorts were calculated (Table 1). The data values were paired according to the age of the animals or the date of data collection. In all cases correlations are much higher when the values were paired by date than by age. This result indicates that budding rates seem to be responding more to culture conditions than to age. Furthermore, if senescence were to result in a decline in budding rates, one would expect the budding rates of group 3 to be similar to, instead of lower than, those of groups 1 and 2 at equivalent ages. We cannot rule out the senescence may indeed be regulating budding rates, but the apparent decline observed in Fig. 2 seems to be environmentally driven. The effect of aging in budding rates, if any, is difficult to elucidate from the collected data.

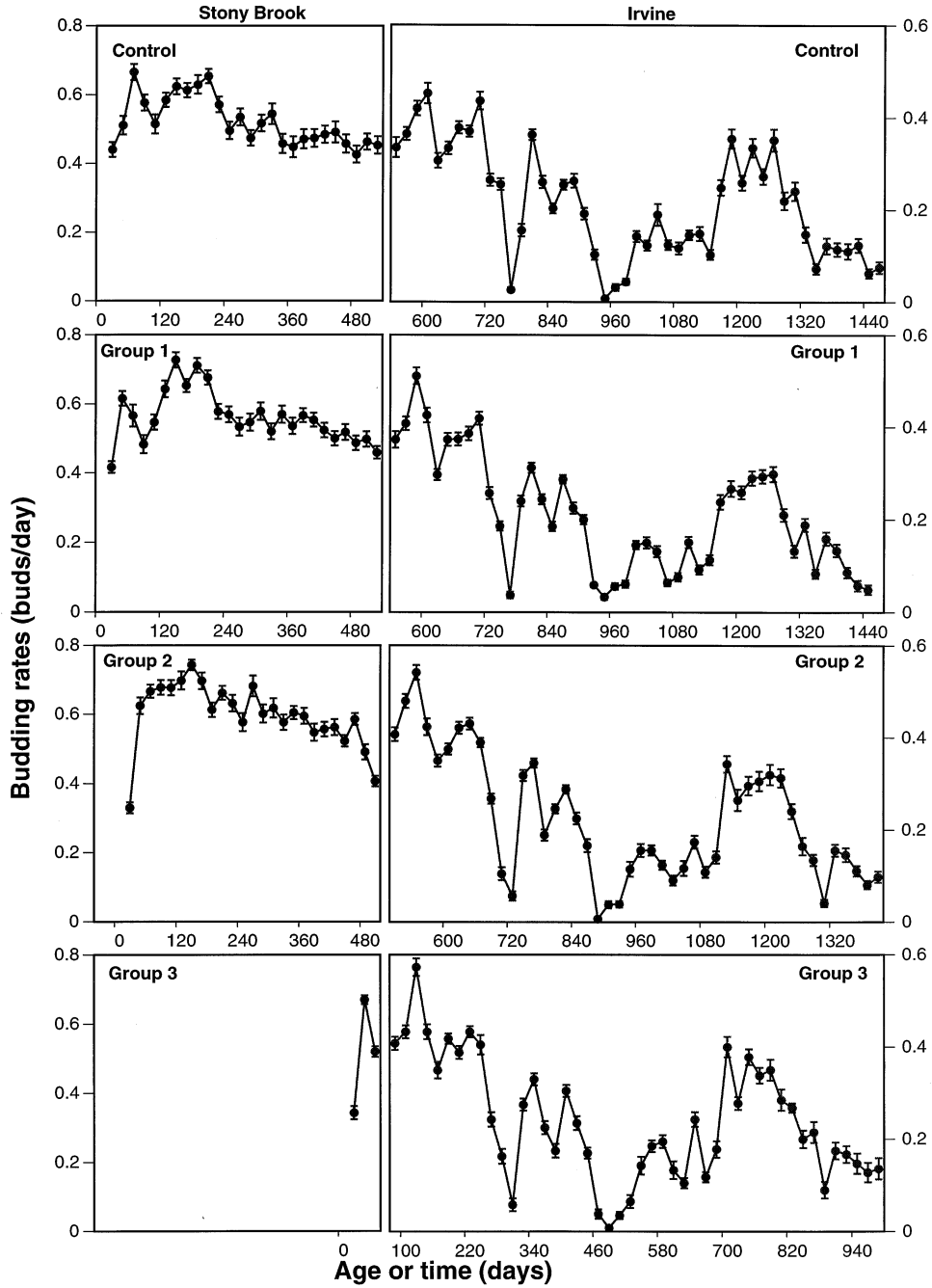


FIG. 2. Mean age-specific budding rates and standard errors. Calculated for each individual over 20-day periods as number of offspring produced in that period divided by 20. Ages for individuals of the control group were unknown so rates were calculated every 20 days starting at the beginning of the experiment.

TABLE 1. PEARSON PRODUCT-MOMENT CORRELATIONS BETWEEN THE REPRODUCTIVE RATES OF DIFFERENT GROUPS PAIRED ACCORDING TO DATE (ABOVE THE DIAGONAL) OR AGE (BELOW THE DIAGONAL). ALL COEFFICIENTS ARE SIGNIFICANTLY DIFFERENT FROM ZERO ($P < 0.01$).

| | <i>Groups</i> | | | | |
|---|---------------|----------|----------|----------|------|
| | <i>1</i> | <i>2</i> | <i>3</i> | <i>C</i> | |
| 1 | | 0.92 | 0.94 | (0.97) | Date |
| 2 | 0.88 | | 0.95 | (0.83) | |
| 3 | 0.37 | 0.37 | | (0.90) | |
| | Age | | | | |

Mortality rates

The shape of the age-specific mortality rates for hydra provide strong evidence for the lack of senescence in hydra. Over a period of four years mortality rates have remained extremely low. It could be argued that four years is not long enough, and that if hydra were maintained for, say, 10 more years, one might witness senescence. However, theoretical and comparative considerations suggest that four years is an adequate period of observation.

The evolutionary theory of senescence states that organism-level senescence has evolved because the strength of natural selection declines with increasing age (Medawar, 1952; Williams, 1957; Hamilton, 1966; Charlesworth and Williamson, 1975; Charlesworth, 1980). Thus, selection may be unable to remove from a population mutations with deleterious effects if they affect individuals after a certain age. The price (paid in fitness) of having a gene with deleterious effects early in life is higher than the price of having a gene with deleterious effects later in life. Genes acting during the prereproductive period will be selected equally with maximal strength. After the onset of reproduction the force of selection will decline monotonically with time, and the probability of fixation of genes with deleterious effect on fitness will consequently increase. Senescence would thus be the result of the accumulation of such genes acting relatively late in relation to the age of first reproduction. Consequently, evolutionary theory predicts a positive correlation between age of first reproduction and maximum lifespan, so that animals that start reproducing soon after birth have shorter lifespans. Figure 3 confirms such a relationship for animals. To maintain the orthogonality of the axes, the relationship age of first reproduction vs. longevity after age of first reproduction was estimated ($n = 61$, Pearson's correlation coefficient: 0.91). The average or maximum lifespan of hydra are not known. Thus, hydra is represented by a point indicating the age reached by individuals of group 1. Notice that the point for hydra lies outside of the 99% confidence region. For a species with a prereproductive period of only 5 to 10 days, hydra clearly reaches ages that exceed the expectation from the age of first reproduction-longevity after age of first reproduction plot. A linear fit (intercept: 0.85; slope: 1.04; r^2 : 0.83) to these data (log-transformed) predicts that hydra should live between 1.2 to 2.6 months. The observed (minimum) lifespan for hydra is already at least 20 times as long. Other animals with similar onset of reproduction live between 1 to 8 months. Hydra has already survived at least five times as long with no apparent signs of deterioration. I suggest that four years is sufficient time for hydra to exhibit signs of senescence (e.g., increase in mortality) and

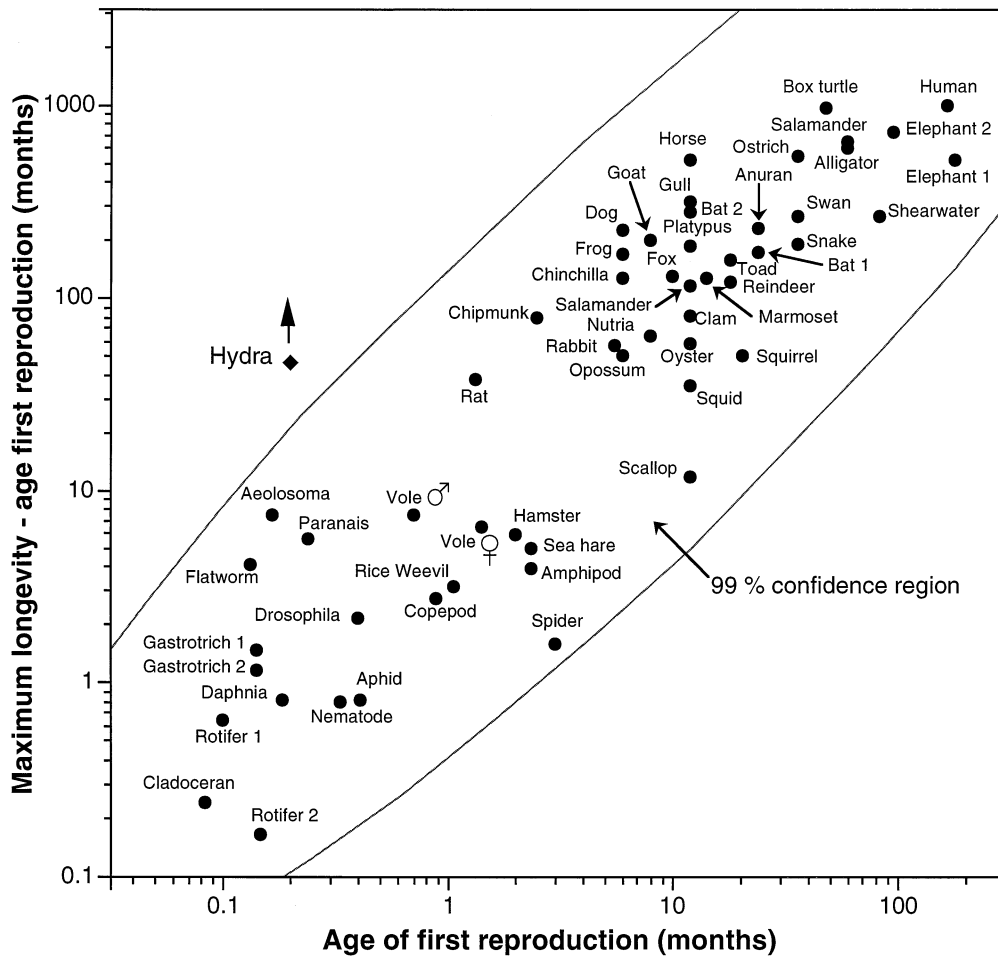


FIG. 3. Age of first reproduction vs. maximum longevity minus age of first reproduction in animals. When maximum longevities were not available, average longevities were used (six cases). Data from the literature: Winsor and Winsor, 1935; Birch, 1948; Spector, 1956; Marshall, 1962; Herried, 1964; Volkmann-Rocco and Fava, 1969; Altman and Dittmer, 1972; Service and Lenski, 1982; Verdones-Smith and Enesco, 1982; Johnson, 1984; Barrows *et al.*, 1985; Luckinbill and Clare, 1985; Duellman and Trueb, 1986; Balsamo and Todaro, 1988; Austad, 1989; Bacon and Andersen-Harild, 1989; Mills, 1989; Wooller *et al.*, 1989; Heller, 1990; Luo and Fox, 1990; Promislow and Harvey, 1990; Skadsheim, 1990; Wong and Wong, 1990; Zug, 1993.

that the absence of such signs indicates that hydra does not undergo senescence and is potentially immortal.

The lack of senescence of hydra does not characterize all cnidarians. In fact, individual hydranths of some colonial cnidarians undergo senescence (Brock and Strehler, 1963; Hughes, 1987). Neither can hydra's potential immortality be restricted only to cnidarians. There has been much discussion, for example, about the potential immortality of planarians (Child, 1915; Haranghy and Balázs, 1980), turbellarians that have high rates of somatic cell turnover and seem

to have long lifespans (Balázs and Burg, 1962; Haranghy and Balázs, 1964). Escaping senescence, however, might be restricted to animals with simpler, dynamic bodies that can be constantly renewed from populations of stem cells. Given the tissue dynamics of hydra, over a period of four years somatic epithelial cells have divided on average 300 times and the whole hydra body may have been fully replaced at least 60 times. The evolution of more complex bodies with tissues and organs with a higher degree of specialization might have resulted in, or perhaps required, a loss of the capacity of renewal and thus permitted the evolution senescence.

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