Constant mortality and fertility over age in *Hydra*

Ralf Schaible[a,1], Alexander Scheuerlein[a,1], Maciej J. Dańko[a], Jutta Gampe[a], Daniel E. Martínez[b,2], and James W. Vaupel[a,c,d,2]

[a]Max Planck Institute for Demographic Research, 18057 Rostock, Germany; [b]Department of Biology, Pomona College, Claremont, CA 91711; [c]Max-Planck Odense Center on the Biodemography of Aging, DK-5000 Odense, Denmark; and [d]Duke Population Research Institute, Duke University, Durham, NC 27708

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Senescence, the increase in mortality and decline in fertility with age after maturity, was thought to be inevitable for all multicellular species capable of repeated breeding. Recent theoretical advances and compilations of data suggest that mortality and fertility trajectories can go up or down, or remain constant with age, but the data are scanty and problematic. Here, we present compelling evidence for constant age-specific death and reproduction rates in *Hydra*, a basal metazoan, in a set of experiments comprising more than 3.9 million days of observations of individual *Hydra*. Our data show that 2,256 *Hydra* from two closely related species in two laboratories in 12 cohorts, with cohort age ranging from 0 to more than 41 y, have extremely low, constant rates of mortality. Fertility rates for *Hydra* did not systematically decline with advancing age. This falsifies the universality of the theories of the evolution of aging that posit that all species deteriorate with age after maturity. The nonsenescent life history of *Hydra* implies levels of maintenance and repair that are sufficient to prevent the accumulation of damage for at least decades after maturity, far longer than the short life expectancy of *Hydra* in the wild. A high proportion of stem cells, constant and rapid cell turnover, few cell types, a simple body plan, and the fact that the germ line is not segregated from the soma are characteristics of *Hydra* that may make nonsenescence feasible. Nonsenescence may be optimal because lifetime reproduction may be enhanced more by extending adult life spans than by increasing daily fertility.

nonsenescence | biodemography | aging | invertebrates | clonal reproduction

The classic genetic theories of the evolution of aging formulated by Medawar (1) and Williams (2), mathematically specified by Hamilton (3), and further explained by Charlesworth and Williamson (4) predict increasing mortality and decreasing fertility from maturity for iteroparous multicellular species. As Hamilton (3) put it, senescence starting at maturity is inevitable. Subsequent theoretical advances by Kirkwood (5, 6) and others (7–9) allow a more nuanced range of possibilities. Hamilton (3), however, continues to be widely cited, usually uncritically and as dogma.

Compilations of data (10, 11) suggest a variety of age trajectories of mortality and reproduction among organisms, including nonsenescence with constant age-specific death and fertility rates. Constant mortality after the age of reproductive maturity has been reported in field studies of some species of vertebrates (e.g., great tits, collared flycatchers) and nonvertebrates (e.g., hermit crabs, red abalone) (11), and may be common in plants (10, 11). These empirical studies, however, are problematic because sample sizes are small, especially at older ages to which few individuals in the wild survive (12–14). Even at ages just after maturity, sample sizes for species observed in the wild are too small to detect whether mortality and fertility are indeed constant or are changing gradually.

To conclusively demonstrate that senescence starting at maturity is not universal for all iteroparous multicellular species, i.e., to refute Hamilton's (3) canonical assertion, a study is needed that follows large numbers of individuals from maturity to advanced ages. If large populations are kept under benign conditions in laboratories or other protected environments, some individuals live to older ages, permitting detection of mortality increases and fertility decreases with age in, for example, nematode worms, *Drosophila*, medflies, and rodents (15, 16), as well as in humans. Hence, we tested the hypothesis that *Hydra* mortality and fertility are constant over age by following large populations under controlled conditions for extended periods that greatly exceed the life expectancy of *Hydra* in the wild.

Constant mortality in a population can be observed even if the risk of death rises with age for all surviving individuals in the population—if some individuals are frailer than others with a higher chance of death at any specific age. In this case, aging of the survivors will increase average mortality while the death of frailer individuals will lower average mortality for the surviving cohort: the two processes can balance each other (17, 18). To determine whether individuals are deteriorating with age, it is informative to study aging on the individual level. This cannot be done by observing deaths alone because individuals die only once. Individual aging can, however, be studied by observing repeated reproductive events. If an individual's fertility is constant or increasing with age, then this is strong evidence that the individual, on balance, is not deteriorating with age. Hence, we carefully studied fertility via asexual reproduction of individual *Hydra*. Deaths in *Hydra* under laboratory conditions turned out to be so rare that it is unlikely that compositional change could account for constant mortality over age, but evidence that fertility does not decline with age reinforces the conclusion that *Hydra* do not suffer senescence.

The Cnidaria (Hydrozoa), which include the freshwater *Hydra*, are classified at the root of multicellular animal life. A *Hydra* polyp consists of a small sack with two tissue layers, an endoderm

Significance

How an organism changes with age and why the pattern of change differs across species are questions that have intrigued biologists since Aristotle. Patterns of change can be described by trajectories of birth and death rates over age. For humans and many other mammals, mortality increases and fertility declines with age among adults. For other species, however, a remarkable variety of patterns has been observed. Although roughly constant mortality and fertility trajectories have been reported for some species, the data are problematic because sample sizes are small, especially at older ages. Here, we present compelling evidence for constant mortality and reproduction of *Hydra* using data from careful, large-scale studies over 8 y with 2,256 individuals.

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[1]R.S. and A.S. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: jwv@demogr.mpg.de or dem04747@pomona.edu.

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and ectoderm, tentacles and a mouth at the top, and a foot at the bottom (19). Three distinct stem cell lineages are present: epithelial stem cells (endoderm and ectoderm lineages), which produce epidermal and digestive cells, and interstitial stem cells, which are the precursors of all of the remaining somatic and germ cell types. Although there is some evidence that interstitial stem cells have a tendency to function as precursors of germ cells (20), these specific cell lineages can be generated de novo from multipotent interstitial stem cells throughout the life span of an individual. Hence, *Hydra*, like plants, do not have a clear distinction between a germ line and a soma (21).

The adult size of individual *Hydra* can vary depending on the environment (e.g., food and temperature), but growth is always determinate: *Hydra* reach adult size when lateral bud formation starts (22). Although sexual reproduction has been observed, *Hydra* generally reproduce clonally via forming buds from somatic cells of all three lineages that initiate new individuals (23). After detachment from their mother, these buds are independent, self-maintaining, reproductive individuals termed ramets that all share the same genome (22, 23). The collective population of ramets that share the same genome is termed a genet (24). A new genet is created by sexual reproduction. Genet age may substantially exceed the age of the individual ramets that are alive at a given moment. From the perspective of evolutionary theories of senescence, the genet is subject to natural selection and hence is of fundamental interest (25).

In the pioneering study of aging in *Hydra* (26), the only major study to date, individuals were collected from the field, yielding an assortment of genets of unknown and most likely different ages. A total of 145 individuals was followed. Weekly fertility (from asexual reproduction) varied erratically and differed substantially among cohorts. The fluctuations arose, in part, from the small initial cohort sizes of 20–50 polyps. The small sample sizes were further reduced by high early mortality in two of the four cohorts and by accidental deaths.

Here, we report results from a study using 2,256 individuals observed in 12 cohorts in two different laboratories, Pomona (P) and Rostock (R), over a period of 2,925 d (more than 3.9 million *Hydra* days). Individuals from three *Hydra* strains (*Hydra magnipapillata* strain 105, nine cohorts; *Hydra vulgaris* strain AEP, two cohorts; and *Hydra vulgaris* strain ARG45a, one cohort) were cultured individually under controlled laboratory conditions with constant food supply. Genet age of individuals when the experiments were initiated in the nine strain-105 cohorts was at least 33 y, whereas the genet age of individuals in one of the strain-AEP cohorts was a year or less, because the population was produced from eggs. Genet age of individuals in the other two *Hydra vulgaris* strains is unknown.

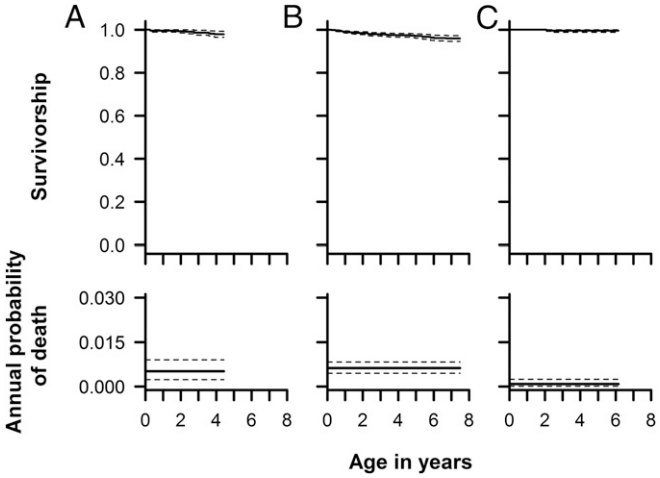

Fig. 1. Demographic trajectories. Survivorship and mortality of *Hydra* by age. Survivorship (Kaplan–Meier estimator; *Upper*) and annual probability of death (*Lower*) in 12 *Hydra* cohorts. (*A*) Combined data of the three Pomona cohorts. Cohort P1: *H. vulgaris* strain AEP first generation, 22 individuals derived from fertilized eggs, observed after they were fully grown at 30 d of age; combined with 98 individuals that were raised from buds of the first generation. Cohort P2: 150 *H. vulgaris* strain ARG45a. Cohort P3: 150 *H. vulgaris* buds from strain AEP. (*B*) Combined data of a subset of Rostock cohorts that had the same annual probability of death as the Pomona cohorts. R1–R3 and R6–R9 (1,428 individuals, *H. magnipapillata* strain 105). (*C*) Rostock cohorts R4 and R5 (408 individuals, *H. magnipapillata* strain 105) that differed from the other Rostock and all Pomona cohorts.

Results

Death rates in all cohorts were constant (Fig. 1 and Table 1) and very low. Independent of their laboratory or strain origin, 10 of the 12 cohorts had the same annual probability of death of 0.006 with an average of one annual death in 167 individuals (Fig. 1 *A* and *B*, and Table 1). Two cohorts of the at least 33-y-old genet

Table 1. General statistics for all cohorts in the study

Laboratory and strain	Cohort name	Cohort size at start	Observation period, days	Exposures, *Hydra* days	Natural deaths	Catastrophic deaths	Estimate minimum genet age, years	Annual probability of death	Estimate age at 5% of *Hydra* alive	Average monthly bud production per *Hydra*
Rostock	R1	204	2,926	423,251	4	30	~33.00	0.0034	868	0.60
Hydra	R2	204	2,711	498,637	11	19	~33.50	0.0080	372	0.66
magnipapillata	R3	204	2,529	453,880	9	24	~34.00	0.0072	414	0.78
strain 105	R4	204	2,245	435,219	1	13	~34.50	0.0008	3,572	0.48
	R5	204	2,010	387,478	1	16	~35.00	0.0009	3,180	0.51
	R6	204	1,829	331,608	9	19	~35.50	0.0099	302	0.90
	R7	204	1,516	288,919	3	13	~36.00	0.0038	790	0.72
	R8	204	1,219	232,737	4	19	~36.50	0.0063	478	0.60
	R9	204	1,068	205,854	2	11	~37.00	0.0035	845	0.93
Pomona	P1	120	1,709	176,013	6	5	~0.25	0.0124	241	2.40
Hydra vulgaris	P2	150	1,661	230,690	1	4	Unknown	0.0016	1,893	4.17
(Pallas 1766)	P3	150	1,636	227,396	2	0	Unknown	0.0032	933	3.33

Annual probability of death varied among the cohorts, and between the Rostock and Pomona location, but were not significantly different. Mortality was high initially in the first Rostock cohort, but then declined to lower levels over time, probably due to accommodation of the individuals to laboratory conditions. We therefore discarded the first 400 d of R1. Death due to catastrophes was more common in the Rostock cohorts, presumably because handling procedures were different. Also, budding rates varied among the cohorts and differed between the Rostock and Pomona location due to different feeding protocols.

strain had an even lower constant annual probability of death of 0.0009, which differed statistically from all of the other populations (Fig. 1*C* and Fig. S1). It is remarkable that death rates were independent of genet age. The Rostock cohorts, which were assayed as successive generations of a founder cohort, maintained constant low death rates, indicating that mother's age did not matter (Table 1, cohorts R1–R9, and Fig. S1). Moreover, death rates of the Rostock cohorts with genet age of 41 y were also indistinguishable from death rates in a Pomona cohort with genet age of a year or less (Table 1, cohort P1, and Fig. S1). Assuming that the mortality rates we measured over ages from 0 to 41 y are representative, such low levels of mortality imply that 5% of individuals still would be alive after between 494 and 3,376 y, depending on the cohort (Table 1).

Fertility was assessed as monthly averages of the daily number of buds produced by asexual reproduction. Because mortality was so low, changes in the composition of the cohorts were negligible. Levels of fertility were higher and fluctuated more in the three Pomona cohorts (P1–P3; Fig. 2), which were kept at higher feeding rates than the nine *H. magnipapillata* strain 105 cohorts in Rostock (Fig. 3). Although fertility increases with age for the individuals studied were somewhat more frequent than decreases (Table 2), reproduction rates for most individual *Hydra* (80%; Table 2) were constant, at least when assessed over several years. This is particularly apparent in the sequentially initiated Rostock cohorts, for which it was possible to separate the effects of age from fluctuations over calendar time in laboratory environments. These fluctuations are driven by some environmental (laboratory) conditions and can be substantial. This also appears to be the case in the Pomona cohorts, although for these cohorts we are not able to separate laboratory effects from cohort effects.

Controlling for fluctuations in the laboratory age-specific budding rates for the Rostock cohorts eventually reached a cohort-specific constant level. However, the shape of the trajectories was different among cohorts and three groups could be identified (Fig. 3). Group A (cohorts R1–R3 and R7–R9) had a "burn-in" phase during which monthly budding rates gradually increased over 2 y to reach a constant level that differed among cohorts. This burn-in phase was extended in group B (cohorts R4 and R5): fertility remained low during the first 2.5 y and then rose to a fertility rate similar to the other Rostock cohorts of group A with around 0.6 buds per month. Both cohorts in group B differed from all of the others with respect to their significantly lower death rates (Fig. 1*C*). Finally, group C (cohort R6) had high fertility just after establishment that subsequently declined to a low and constant value. Mortality and asexual reproduction remained low and constant regardless of whether eggs or sperm were produced (as in all Pomona cohorts) or not (as in all Rostock cohorts).

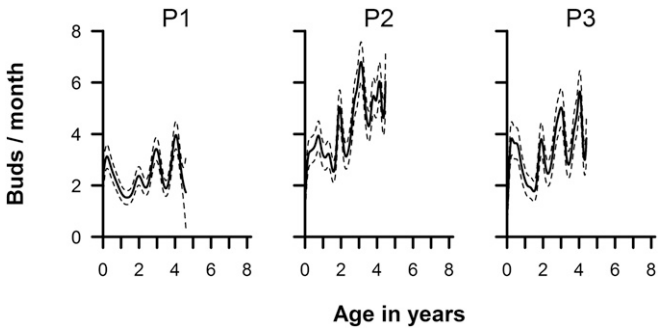

Fig. 2. Demographic trajectories. Fertility of *Hydra* by age in three Pomona cohorts. Fertility expressed as smoothed daily budding rates. Cohort P1: 120 buds of *H. vulgaris* strain AEP; cohort P2: 150 buds of *H. vulgaris* strain ARG45a; cohort P3: 150 *H. vulgaris* buds from strain AEP.

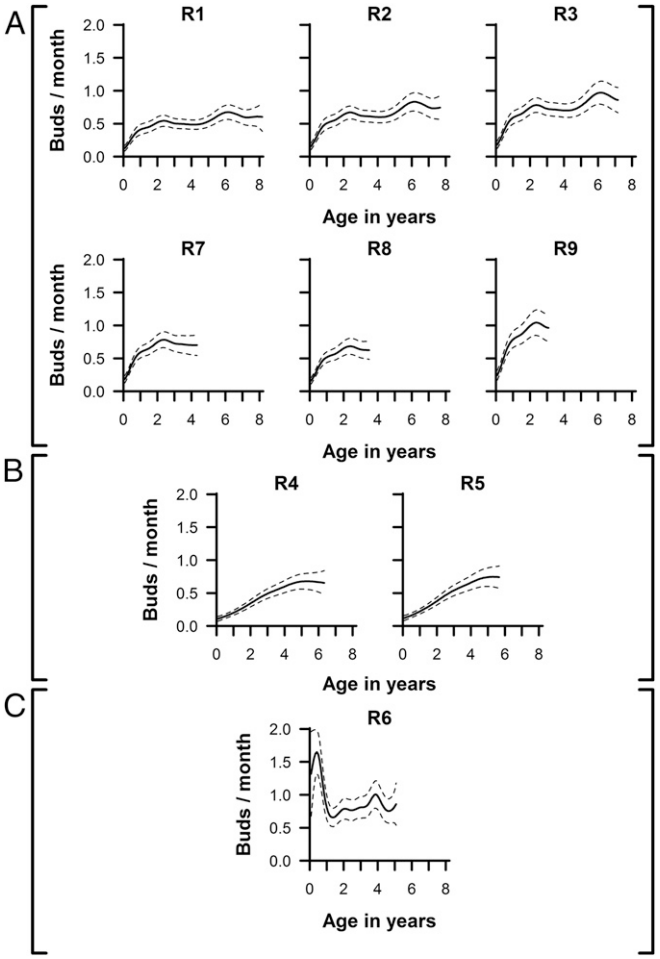

Fig. 3. Demographic trajectories. Fertility of *Hydra* by age in nine Rostock cohorts. Smoothed monthly budding rates of Rostock cohorts controlled for environmental effects. (*A*) Cohorts R1, R2, R3, R7, R8, and R9 had identical shapes of the budding curve, but differed in the level of monthly budding. (*B*) Cohorts R4 and R5. Both cohorts had an identical shape but also differed in the monthly budding. (*C*) Cohort R6. The shapes of the cohorts listed in *B* and *C* were significantly different from *A*.

Discussion

The mortality profile of *Hydra*—as shown in this 8-y study of various strains kept in two different laboratories—is remarkable. Death rates that are low and constant irrespective of ramet and genet age (which ranged from 0 to 41 y), are features that no other species across the tree of life, up until now, has been conclusively shown to have achieved. Our mortality data are consistent with the results of the shorter and much smaller, pioneering study by Martínez (26). His laboratory-kept strains of *Hydra* (145 individuals) showed no detectable increase in mortality with age over an observational period of 4 y. Jones et al. (11) reported constant rates of mortality in species other than *Hydra* (e.g., red abalone and the great tit) based on much less conclusive data and at a much higher level than the rates for *Hydra* that are presented here. There is no a priori reason, however, to suspect that other species are not capable of achieving life histories of low and constant mortality. Possible candidates are organisms with long recorded life spans, such as sponges, corals, ascidians, and some plants (14). Available data, however, are too sparse to provide the statistical power necessary to conclusively demonstrate constant low mortality.

Table 2. Individual fertility

Category	First: second quarter, %	Second: third quarter, %	Third: fourth quarter, %
Decreasing fertility	8	2	4
Constant fertility	80	76	89
Increasing fertility	12	22	7
	100	100	100

We compared, for all cohorts combined, the number of buds produced by an individual between consecutive quarters of the observation period. Using a X^2 test, we tested for equality and established for how many individuals budding significantly (at the 95% level) increased or decreased.

Reproduction rates of *Hydra* in our study were constant after an initial phase of increasing or decreasing fertility. Feeding regime affected fertility but did not affect mortality. In a previous study of *H. magnipapillata*, Schaible et al. (27) found that budding increased linearly with food intake, whereas survival without food (a proxy for the investment in somatic maintenance) was unaffected by previous feeding conditions. Hence, *Hydra* may prioritize the investment of available resources to levels of maintenance that ensure very low mortality and then use excess resources to produce buds.

Although the isogenic Rostock cohorts were kept under constant feeding conditions, there was variation in the level of fertility. A significant part of these differences may be attributable to chance variation in the physiology of successive isogenic generations (28). This has been documented in other species kept in a constant environment (28–30).

Life history traits of *Hydra* in this study did not vary independently of each other. The two cohorts with significantly lower death rates showed decreased fertility in the first 2.5 y (Figs. 1*C* and 3, group *B*). This implies that some individuals under constant conditions are able, at least temporarily, to boost their investment in maintenance at the expense of reduced fertility.

The key finding of our study is that *Hydra* appear to be able to maintain themselves without accumulating damage and mutations, such that constant (and very low) mortality and approximately constant fertility levels persist over extended periods of time under laboratory conditions—up to 8 y for the individuals (ramets) that we observed and up to at least 41 y in the genets. These durations are much longer than the life expectancy of an adult polyp in the wild. We have not been able to observe survival of individual polyps, but we have been able to roughly estimate population size. Some field studies (31–33) and results of our own observations in a pond we studied in Northern Germany show a considerable seasonal fluctuation in *Hydra* abundance. Early in the season (March, April), population density increases with an abundance peak in June; during this period, buds and immature *Hydra* are numerous. The population crashes in July and August: no or very few individuals can be observed. At the end of the season in October, the *Hydra* populations somewhat recover. During winter, *Hydra* abundance is constant at a low level depending on the duration of the low temperature period and the thickness of the ice cover. Hence, it seems likely that nearly all *Hydra* born over the course of a year—mostly born in March through June and, to a lesser extent, October—live a few months or less. We conclude that, although there may be some polyps in the wild that are many years old, the average individual at maturity faces a short life numbered in weeks rather than in years.

How and why can *Hydra* in the laboratory achieve a life history without senescence for an extended period of years? We cannot rule out senescence starting at an age beyond the period we could observe. Because the canonical genetic theories of the evolution of aging—mutation accumulation and antagonistic pleiotropy—predict that senescence should start at maturity and should increase as survivorship declines, we can, however, conclude that these theories do not hold for all species. Theories that permit senescence to start at ages later than maturity cannot be ruled out, although an explanation of why senescence could be delayed so long in *Hydra* is called for. Because the germ line is not segregated from the soma in *Hydra,* the disposable soma theory, which allows late-onset senescence, is not directly applicable, but some version of it might hold for *Hydra*. Sophisticated recent research on the mathematics of the process of mutation accumulation (9, 34) may provide such an explanation. Instead of a delay in eventual senescence to an age vastly higher than average life expectancy, the alternative and simpler hypothesis would be that *Hydra* are able to maintain themselves for indefinitely long durations.

Nonsenescence may be feasible because of the simplicity of the *Hydra* body plan and cellular processes, at least compared with more complex animals. Most *Hydra* cells are continually proliferating stem cells that are never silenced (35). The natural turnover of cellular material in *Hydra*, which is complete after 3–4 wk (36), is a potent way of preventing the accumulation of damage such as metabolic wastes that cannot be transported out of the cell. Dańko et al. (37) show in a theoretical model that cell turnover, high fraction of stem cells, together with damage-dependent cell selection are capable of preventing senescence in *Hydra*. In addition to this high cellular turnover, *Hydra* has developed a high level of emergency repair sensu Kirkwood (6) and can completely regenerate even after most body and tissue structures are destroyed (38). Furthermore, *Hydra* must be able to repair more routine damage, maintain the integrity of their telomeres, and sustain an efficient and robust immune system (23). These traits carry immediate selective advantages in an environment where dangers due to stochastic environmental hazards, predation, and infection are severe (23).

Nonsenescence for an extended period after maturity may be a stable evolutionary strategy because in *Hydra* the germ line is not segregated from the soma (21). Mutations in *Hydra* cells may be transmitted via budding to subsequent generations. Consequently, and similarly to the protection of the germ line in organisms that segregate the germ line from the soma, maintenance and repair of cells is critical to prevent the accumulation of damage (2, 5). If clonal reproduction is initiated by soma cells (instead of parthenogenesis that proceeds from an egg through the full morphogenetic course), this imperative may require prolonged high maintenance over many consecutive generations, as seen in our *Hydra* dataset. Despite high levels of maintenance, the integrity of the stem cell lineages might slowly undergo deterioration through the accumulation of damage (39). This damage might be negligible or neutral in the environment at the time but might be detrimental over a long time period or if the environment changes. At an extended timescale spanning decades or perhaps hundreds or thousands of years, however, the quality of the gametes might deteriorate, as demonstrated by Ally et al. (40) in stands of poplar clones. The life span of a *Hydra* genet therefore might be limited by mutation accumulation over sufficiently long timescales. Under these circumstances, occasional sexual reproduction might counteract the deterioration and restore viability of the gametes.

How *Hydra* maintain constant mortality over the course of life is an important question. Equally important is: why is it evolutionarily optimal for *Hydra* to do so—and at such a low level of mortality? As noted above, because many of the cells of a *Hydra* may be involved in reproduction, high levels of maintenance and repair are favored by evolution—consistent with Kirkwood's disposable soma theory (14, 41). If long life in the laboratory is a byproduct of the exigencies of reproduction by asexual budding and of the need to regenerate damaged or lost body parts, then many such species that do not sequester the germ line may experience low and constant mortality under protected conditions.

It is known, however, that some of these species show a decline in age-specific survival with age—they senesce. This is well documented for the asexual metazoans *Paranais litoralis* (Oligochaeta) and *Stenostomum incaudatum* (Turbellaria) (42) and some species of plants (43, 44). In addition, unrepaired damage has been shown to accumulate over consecutive generations in unicellular species, such as *Escherichia coli* (45, 46) and fission (47) and budding yeast (48).

Thus, the fact that *Hydra* does not sequester the germ line may not be sufficient to explain the high levels of maintenance and repair that are prerequisites for nonsenescence. Additional factors may be required. Nonsenescence might have evolved as an adaptive strategy if a few fortunate *Hydra* are sheltered in rare niches and it is these individuals that ensure the continuity of the population (49, 50). Although no data are available, it is possible that some *Hydra* in the wild survive for many years and these *Hydra* are crucial for the species' evolutionary fitness.

In sum, evolution pressures that favor high levels of maintenance and repair in *Hydra* together with a capacity for regeneration and for preventing deterioration may have jointly favored the *Hydra*'s life history of nonsenescence. Our experiments provide compelling evidence that death and reproduction rates for *Hydra*, under laboratory conditions, are constant over age. Why remains an enigma, but glimmers of explanation beckon.

The title of Martínez's pioneering study (26) is "Mortality Patterns Suggest Lack of Senescence in *Hydra*"; in contrast, the title of this article is "Constant Mortality and Fertility over Age in *Hydra*." The earlier study was suggestive; this study is conclusive over the period of observation. The earlier study focused on mortality; this study shows that both mortality and fertility are constant. Researchers could dismiss the earlier study as small, incomplete, and inconclusive—and could continue to assert, citing Hamilton (3), that for all multicellular organisms with repeated reproduction mortality inevitably rises with age starting at maturity and fertility inevitably falls. This view is no longer tenable.

Materials and Methods

Empirical Data. Mortality and fertility data are from assays of nine cohorts of *Hydra magnipapillata* strain 105 at the Max Planck Institute for Demographic Research (Rostock, Germany), and three cohorts with individuals of *Hydra vulgaris* strains AEP (two cohorts) and ARG45a, at Pomona College (Claremont, CA), with 2,256 individuals started since 2006. Each individual was cultured in isolation in plastic multiwell culture plates under identical laboratory conditions. Deaths because of catastrophic or natural death (see below) and asexual reproduction via the production of new buds were assessed daily.

Rostock cohorts (R1–R9; 1,836 individuals; 3.26 million *Hydra* days) were initiated sequentially with an interval of about 6 mo, producing cohorts of different ramet ages at a given time. Genet age for the Rostock cohorts was more than 33 y at the start of the study. Pomona cohorts (P1–P3; 420 individuals; 0.63 million *Hydra* days) were initiated at the same time and ramets had the same age. The genet age of individuals in one of the strain-AEP cohorts was a year or less, because the population was produced from eggs. Genet age of individuals in the other two *Hydra vulgaris* strains is unknown. See *SI Materials and Methods* for further details.

Definitions of Death. In the course of the experiment, we observed two types of death, "natural death" and "catastrophic death." Natural deaths occurred in individuals that died in the absence of extrinsic forces. This happened over the course of four stages: (*i*) *Hydra* were less mobile and took longer to capture the three shrimp that were provided as food; (*ii*) after 2–5 d, tentacles shortened and took the shape of clubs; the polyps got more transparent; (*iii*) after an additional 2–5 d, the whole polyps shortened in length and completely ceased locomotion; and (*iv*) after an additional 2–5 d, the polyps were completely dissolved in the buffer solution. In contrast, catastrophic deaths occurred suddenly and with human impact, for example, when individuals became attached to the lid of the culture dish and dried out subsequently or when individuals were lost during buffer change or by accidentally dropping the culture dish.

Mortality Analysis for All Cohorts. For each of the cohorts (nine in Rostock, three in Pomona), the following models for the age-specific hazard of death $\mu(x)$, where x is age (in days), were fitted:

$$\text{Constant hazard (exponential distribution)}: \mu(x) = \lambda,$$

$$\text{Weibull–Makeham hazard}: \mu(x) = a\,x^b + c,$$

$$\text{Gompertz–Makeham hazard}: \mu(x) = a\,e^{bx} + c.$$

Parameters were estimated by maximizing the log-likelihood function. Catastrophic deaths were treated as right-censored observations (at the respective age at death), and individuals alive at the end of study were right-censored at their age at the end of the observation period.

The log-likelihood function for the unknown parameter(s) θ is given by $\ln L(\theta) = \sum_{i=1}^{n} [\delta_i \ln \mu(x_i;\theta) - H(x_i;\theta)]$, where n is the sample size, x_i is the age at death or right-censoring of individual i, the parameter vector θ is either $\theta = \lambda$ (for the exponential), $\theta = (a, b)$ (for Weibull and Gompertz), or $\theta = (a, b, c)$ (for Weibull–Makeham and Gompertz–Makeham). The event indicator δ_i is =1 for deaths, and =0 for right-censored observations. $H(x_i, \theta)$ is the integrated hazard of the model (51).

All multiparameter models contain the constant-hazard model as a special case; that is, the exponential model is nested in the multiparameter models. Whether the exponential distribution is appropriate or not was assessed by a likelihood ratio test (52). The exponential model was rejected (at the significance level $\alpha = 0.05$) only for the first Rostock cohort (R1). We left-truncated all individuals at age 400 d (only in this first cohort) and analyzed mortality beyond this age (Table S1). Consequently, the log-likelihood was adjusted for left truncation at u_i (=400) as $\ln L(\theta) = \sum_{i=1}^{n} [\delta_i \ln \mu(x_i;\theta) - H(x_i;\theta) + H(u_i;\theta)]$.

The maximum-likelihood estimates for an exponential distribution can be derived analytically (number of deaths divided by total time at risk) (51); however, for the multiparameter models, maximization of the log-likelihood has to be performed numerically. All log-likelihood functions were optimized using Matlab (53) (Nelder–Mead simplex method).

In the next step, we estimated the cohort-specific hazard levels and calculated 95% confidence intervals. The confidence interval for each λ is based on the likelihood ratio statistic. It is preferred over confidence intervals based on large-sample normal approximations if the log-likelihood function shows a lack of symmetry, which is the case here. The confidence interval is given by those values of λ for which the statistic $\ln R(\lambda) = 2 \cdot [\ln L(\hat{\lambda}) - \ln L(\lambda)]$ is smaller than the $(1-\alpha)$-quantile of the χ^2 distribution with df = 1. For $(1-\alpha) = 0.95$, this critical value is $C = 3.8415$. The value $\hat{\lambda}$ is the maximum-likelihood estimate for each sample. The limits for the confidence intervals were determined numerically by using the function uniroot in R (54). The results are shown in Fig. S1.

We tested whether different cohorts, within each laboratory, share the same constant level of mortality. This was done via a likelihood ratio test (LRT) and confirmed by a Kolmogorov–Smirnov test (Table S2). Identical hazard levels were rejected for the Rostock cohorts (LRT statistic: 19.5862; *P* value: 0.0120; df = 8) and for the Pomona cohorts (LRT statistic: 6.2277; df = 2; *P* value: 0.0444). Rejection of a common hazard level for the Rostock cohorts is due to a comparatively low level of mortality in cohorts R4 and R5 (one death for 435,219 and 387,478 d of exposure, respectively) (Fig. S1). In Pomona, cohort P1 shows higher mortality than cohorts P2 and P3.

If cohorts R4 and R5 are excluded, a common hazard level across the remaining 10 cohorts (3 in Pomona, 7 in Rostock) is not rejected (LRT statistic: 12.4170; df = 9; *P* value: 0.1908). The common level of mortality is estimated as $\hat{\lambda} = 1.6618\text{e-}05$ (per day), with 95% confidence interval (1.2464e-05; 2.1605e-05). This level of mortality corresponds to an annual probability of death $q = q_{x,\,x+365} = 1 - e^{-365\,\hat{\lambda}} = 0.006047$, with a 95% confidence interval (0.004539; 0.007855). For the two cohorts R4 and R5, a common level of mortality can be assumed (LRT statistic: 0.0067; df = 1; *P* value: 0.9345), and the estimated hazard is $\hat{\lambda} = 2.4310\text{e-}06$ (per day) with a 95% confidence interval (4.0423e-07; 7.5066e-06). It corresponds to an annual probability of death $q = 0.000887$, 95% confidence interval (0.000148; 0.002736).

Fertility Analysis for Rostock Cohorts. Unlike the Pomona cohorts, which were initiated at the same time and had the same age, the Rostock cohorts were initiated sequentially with an interval of about 6 mo, producing cohorts of different ages at a given time. We were therefore able to separate the effect of age (denoted by x) and calendar time (denoted by t). For each of the Rostock cohorts (1–9), we calculated the monthly number of buds (1 mo = 30 d) and the total time of exposure in each month. The budding rate was estimated by a Poisson model making the following assumptions: If $y(c,x,t)$ denotes the observed number of buds in cohort c at age x and time t, then the $y(c,x,t)$ are viewed as realizations from Poisson variables with expected value $m(c,x,t)$, which is given by the product of the budding rate $\lambda(c,x,t)$ and the exposure time $e(c,x,t)$ of the cohort at age x and time t: $m(c,x,t) = E(y(c,x,t)) = \lambda(c,x,t) \cdot e(c,x,t)$.

How the budding rates $\lambda(c,x,t)$ change with age x and potentially differ by cohort c is the core of the analysis. To allow for overdispersion that might be introduced by within-cohort heterogeneity, we allow the scale parameter ϕ of the Poisson distribution to be >1 and hence the variance to be larger than the mean of the Poisson variates. The dependence of the rates on age is assumed to be smooth, and the smooth terms are represented by penalized regression splines. Modeling was done on a log scale for the rates, as usual for Poisson regression. The potential shared effects of calendar time t were modeled as a random (intercept) effect b_t for $\ln \lambda(c,x)$, normally distributed with variance σ_t^2: $\ln \lambda(c,x,t) = \ln \lambda(c,x) + b_t$.

Several models for the cohort- and age-specific rates $\lambda(c,x)$ were considered, ranging from a single age-specific budding rate $\lambda(x)$ for all cohorts to a model with separate smooth rates $\lambda(c,x)$ for each single cohort. All resulting generalized additive mixed models (55) were estimated using the function gamm in the library mgcv of the statistical package R (54). Model comparisons were made based on the resulting Akaike information criterion (AIC). The model with the lowest AIC was chosen as the final model. This model results in three groups of cohorts, and within each group the shape of the age-specific budding rate is the same, however, possibly at different intensity [$\lambda(c,x) = \gamma_c \lambda_g(x)$].

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