

The More the Merrier?

Entropy and Statistics of Asexual Reproduction in Freshwater Planarians

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Abstract The trade-off between traits in life-history strategies has been widely studied for sexual and parthenogenetic organisms, but relatively little is known about the reproduction strategies of asexual animals. Here, we investigate clonal reproduction in the freshwater planarian *Schmidtea mediterranea*, an important model organism for regeneration and stem cell research. We find that these flatworms adopt a randomized reproduction strategy that comprises both asymmetric binary fission and fragmentation (generation of multiple offspring during a reproduction cycle). Fragmentation in planarians has primarily been regarded as an abnormal behavior in the past; using a large-scale experimental approach, we now show that about one third of the reproduction events in *S. mediterranea* are fragmentations, implying that fragmentation is part of their normal reproductive behavior. Our analysis further suggests that certain characteristic aspects of the reproduction statistics can be explained in terms of a maximum relative entropy principle.

Keywords Life-history theory · Planarians · Asexual reproduction · Entropy · Optimization

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1 Introduction

A fundamental problem in evolutionary biology is to identify the principles that govern the reproduction strategies of living beings. According to traditional life-history theory, natural selection favors strategies that maximize benefit-to-cost ratios, i.e., optimize the trade-off between traits (e.g. clutch/litter size versus parental energy expenditure or offspring size), and thus result in optimized reproduction rates with maximal offspring and parental fitness [23–25, 27, 30]. Most organisms are subject to internal noise, such as fluctuation on the molecular or cellular level, and exposed to complex deterministic and stochastic changes (e.g., seasonal, climatic) in the environment. In such cases, deterministic reproduction strategies may be more costly and less “optimal” than randomized strategies. This raises the question of whether and to what extent, non-deterministic reproduction strategies can be understood in terms of information-theoretical concepts [11, 14, 31] that incorporate Darwinian principles.

Here, we examine this question experimentally and theoretically by studying the clonal reproduction strategy of the freshwater planarian *Schmidtea mediterranea*, an important model organism for stem cell and regeneration research [15, 19, 21]. The capability of planarians to regenerate an entire organism from a minuscule piece is facilitated by a large stem cell population that comprises ~25–30% of all cells [1–3, 15, 19, 20]. Due to this stem cell contingent, planarians can reproduce asexually by dividing spontaneously into a larger head and smaller tail. These pieces then rebuild the missing body parts, including a central nervous system, within about a week [8, 15, 19]. Because of the complexity of planarians, it comes as no surprise that their reproduction dynamics show great variability. Statistical methods are very useful in the analysis of planarian reproduction in order to distinguish intrinsic randomness from systematic effects due to genetic or environmental influences.

Over the course of the past 18 months, we recorded approximately 5000 asexual reproduction events of the planarian *S. mediterranea*. In a recent paper [8], two of us compared the dynamics of interacting and non-interacting asexual planarian populations. We found that the distribution of waiting times between consecutive reproduction events exhibits multimodal behavior reflecting memory effects in the regeneration/reproduction processes. The present work complements and extends the discussion in [8]. Specifically, we now compare the reproduction strategies of head and tail parents (Fig. 1E) in terms of binary fissions and fragmentations (multiple consecutive divisions within a short time span, see discussion below), and the resulting properties of the offspring that stem from these two types of reproductive events.

In the planarian literature, fragmentation has usually been regarded as an abnormality appearing primarily under unfavorable environmental conditions [5] and been largely neglected [6], possibly due to strain-related differences in the reproduction dynamics or limited data, or has been treated as a “fission crisis” with multiple offspring [32]. Peter et al. [17] discuss fission statistics in the asexually reproducing species *Dugesia tahitiensis* and mention the occurrence of fragments; interestingly in this system, fragments seem to originate from the tail piece [17], whereas in our strain they originate from the head. The only detailed study on fragmentation to our knowledge is in a related species, *Phagocata vivida*, by Kawakatsu [5, 12], who notes that the two processes are entirely different: Fission events occur in approximately regular time intervals, whereas fragmentation events happen suddenly and seem to differ biomechanically from fissions.

The results presented below demonstrate that, even under controlled laboratory conditions and without environmental challenges, planarians adopt a randomized reproduction strategy that includes about 30% fragmentation events. Thus, based on our large-scale experimental approach and statistical analysis, we show that fragmentations are part of the

normal reproductive behavior in *S. mediterranea*. In fact, our results suggest that fragmentations that produce medium-sized clutches are favorable in terms of parent and offspring survivorship. As an interesting theoretical result, we find that certain statistical characteristics of the asexual reproduction strategy can be understood in terms of a maximum relative entropy principle [11] with the reference measure related to offspring survival.

2 Materials and Methods

2.1 Planarian Maintenance

In our experiments, we used the asexual strain of *S. mediterranea*, held under the same conditions for non-interacting worm experiments previously described in [8]. Each worm was kept in its own petri dish (100 mm diameter \times 20 mm height) filled with 25 mL of planaria water at a constant temperature of 20°C, fed once a week and cleaned twice a week. When planarians divided, the head and tail pieces were separated into new dishes, which were labeled with barcodes containing information about their family history and division date. Reproduction events were recorded by counting the planarians several times per week corresponding to an effective time resolution of 2.5 ± 0.5 days.

2.2 Reproduction Statistics and Data Analysis

We recorded the exponentially growing family trees originating from five individual (clonal) planarians over the course of an 18 month period using a custom-built barcoding and database system [28], yielding a data set comprising approximately 5000 individuals. The database system was written in Java using NetBeans (Oracle, Redwood Shores, CA). Data analysis, including examination of relationships among reproductive waiting times, number of offspring, survival rates and worm areas, was conducted in Java and Matlab (MathWorks, Natick, MA, Version R2009b) using the Matlab Java interface.

2.3 Measurement of Planarian Area

From a macroscopic perspective, flatworms can be viewed as quasi-two-dimensional organisms, as their height is very small compared to their length and width. We therefore characterize their size in terms of area rather than volume. Planarians were imaged in brightfield with a LEICA MZ16FA stereo microscope (Leica Microsystems, Wetzlar, Germany), using a Basler CCD camera A601f (Basler AG, Ahrensburg, Germany) and Basler BCAM Viewer (Basler AG, Ahrensburg, Germany). Homogenous lighting was provided by a Schott Ace I halogen light source (Schott NA, Southbridge, MA). It is experimentally challenging to determine the area of a worm at the moment of division. Therefore, worm pieces were imaged right after asexual reproduction was recorded. Based on our time resolution, reproductive events can have happened between 0 and 3 days before recording. The newly regenerated tissue formed during this time, however, is unpigmented and negligible in size relative to the rest of the piece. The image analysis algorithm we use only picks up pigmented tissue, so the error in the estimated worm areas is small. The size of the parent worm can be inferred by summing the sizes of all fragments, and the clutch area is defined as the sum of the areas of all offspring pieces. Thus, the total clutch area is given by the size of the parent worm minus the size of the head piece. The image analysis and planarian area calculations were performed in Matlab using the image analysis toolbox.

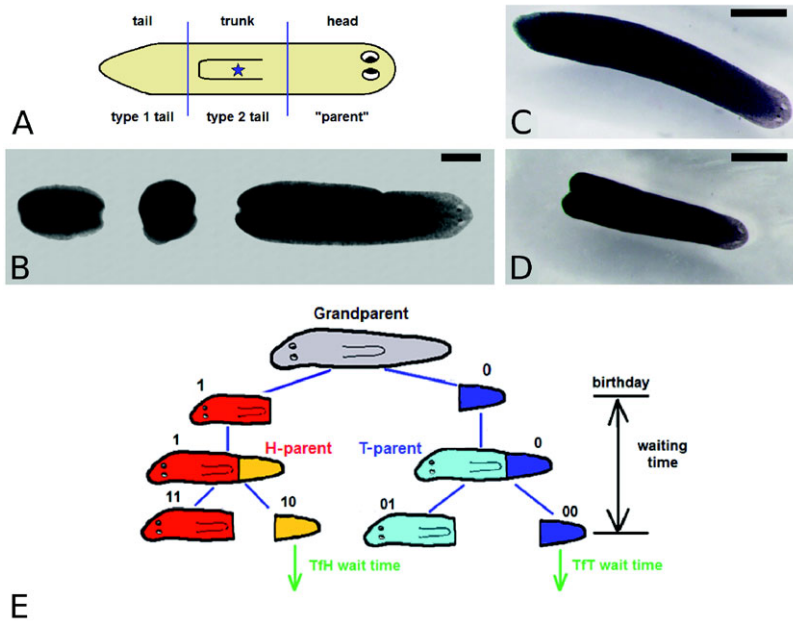


Fig. 1 (A) Sketch of a planarian to illustrate the terminology used throughout the paper. The division into head, trunk, and tail parts is determined by the division sequence rather than anatomical structure. The star indicates the pharynx, which serves as the planarian’s mouth and anus and is necessary for food intake and growth. The pharynx usually remains with the head piece after division, but frequently gets lost for fragmentations with $k \geq 3$. (B) Planarian imaged after fragmenting into three pieces. (C) Parent planarian after a regeneration period of 7 days following fission and (D) following fragmentation into three pieces. The regeneration of the tail is considerably slower after fragmentation. Scale bars correspond to 1 mm. (E) Illustration of H(ead)- and T(ail)-parents. For simplicity, only fissions are shown. The definition of waiting time (RWT) and parent-dependence for tail offspring is also shown

3 Results and Discussion

3.1 Fission vs. Fragmentation

In the course of a basic reproductive event, a planarian splits asymmetrically into a larger “head” and a smaller “tail” piece. Sometimes, several such divisions occur consecutively within a short time span (Figs. 1A and B). We refer to a reproductive event as a “Teilung” F_k , with k denoting the number of offspring produced. From the biological point of view, it is necessary to distinguish a “fission” event, defined as an isolated division F_1 , from a “fragmentation” event $F_{k \geq 2}$, with multiple divisions within a short time span, as they lead to different types of offspring.

In the case of a simple fission, a single offspring is produced. This daughter worm has to regenerate the anterior body part and will be called a “type-1” tail. By contrast, a fragmentation F_k with $k \geq 2$ gives rise to two different types of offspring: The first division again produces a type-1 tail, whereas subsequent divisions produce so-called “trunks” or “type-2” tails that need to regenerate both anterior and posterior sections (Figs. 1A and B). Throughout, the term “offspring” will be used jointly for type-1 and type-2 tails.

The criterion that defines the end of a fragmentation process F_k is specified as follows: Since planarians require approximately one week after a fission to regenerate a fully pig-

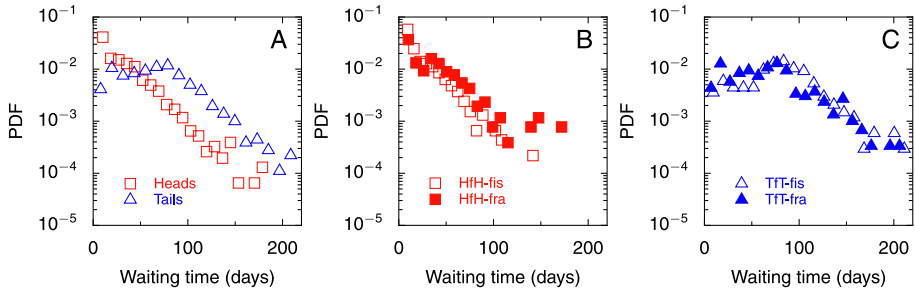


Fig. 2 (A) Reproduction waiting time distributions (RWTDs) of heads (*squares*) and tails (*triangles*). Multiple divisions as part of a fragmentation event are only counted as one reproductive event; total number of reproductive events of H-parents $N_H = 1822$ and T-parents $N_T = 1527$. Tails typically need a longer regeneration period than heads before they are able to generate offspring. (B) RWTDs for heads stemming from heads that fissioned (HfH-fis; $N = 691$) and heads stemming from heads that fragmented (HfH-fra; $N = 319$). For heads, the recovery period after fragmentation is typically longer than after fission. (C) RWTDs for tails from tails that fissioned (TfT-fis; $N = 319$) and tails from tails that fragmented (TfT-fra; $N = 299$)

mented tail (Fig. 1C), two successive divisions belong to the same fragmentation F_k if they took place within a period of 5 days, which is consistent with the time resolution in our experiments. Adopting this criterion, we observed fragmentations F_k with up to $k = 6$ offspring; however, the case $k = 6$ is extremely rare ($\leq 0.1\%$ of all divisions) and will mostly be neglected in our subsequent data analysis. Finally, we will assume throughout that aging effects in *S. mediterranea* are negligible [15].

3.2 Reproduction Waiting Time Distributions

After a reproduction cycle has ended, planarians undergo a regeneration period during which missing body parts are rebuilt. The duration of this phase is approximately one week, but the subsequent growth phase to when the next reproduction cycle can ensue, can vary significantly depending on various (largely unknown) biological and physical factors. Figure 2A shows the reproduction waiting time distributions (RWTDs) of heads and tails, respectively. As noted in [8], and also evident from Fig. 2A, the RWTDs of planarians exhibit a multimodal fine-structure that reflects memory of the parent type (head or tail). This memory could be due to local differences in the stem cell population and thus regeneration potency, since it has been recently shown that there exists an anterior-posterior gradient of an irradiation-sensitive (X2) stem cell subpopulation [16]. The memory effect implies that it is advisable to distinguish “H-parents”, corresponding to regenerated former head pieces, from “T-parents” regenerated former tail pieces (Fig. 1E). It is important to stress that in contrast to the RWTDs given in [8], which show the waiting times between elementary division events, the diagrams in Fig. 2 depict the waiting times between successive fissions or fragmentations F_k , i.e., the times intervals between divisions that belong to the same fragmentation F_k are not counted in Fig. 2.

In order to understand the structural properties of the waiting time PDFs in Fig. 2A, it is useful to separate waiting periods after simple fissions F_1 from those after fragmentations $F_{k \geq 2}$. The corresponding contributions are shown in Figs. 2B and C. Generally, heads from heads that fragmented (HfH-fra) require a longer recovery period due to a decreased head size (Figs. 1D and 5D below) and delayed tail regeneration when compared to heads stemming from H-parents that fissioned (HfH-fis) (Figs. 1C, D). Therefore, the RWTD of

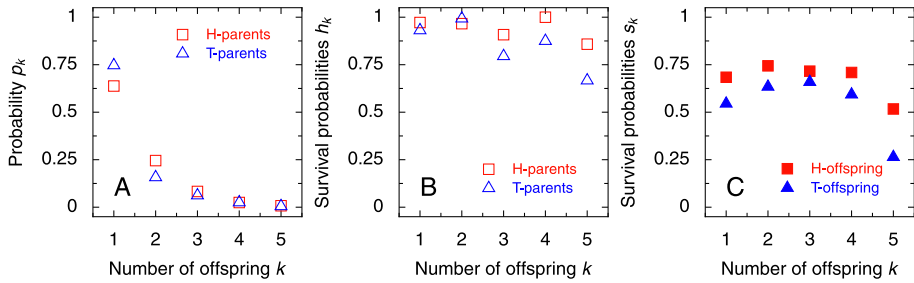


Fig. 3 (A) Experimentally measured probability distribution of the number of offspring produced per Teilung by H(ead)-parents and T(ail)-parents; see also Fig. 4 for a logarithmic representation and maximum entropy fits. (B) Survival probability h_k of parent worms after a Teilung with k offspring. Total sample size $N_H = 1046$, $N_T = 864$; sample size $N_{T/H}(k)$ per data point: $N_H(1) = 711$, $N_H(2) = 231$, $N_H(3) = 76$, $N_H(4) = 21$, $N_H(5) = 7$ and $N_T(1) = 693$, $N_T(2) = 126$, $N_T(3) = 34$, $N_T(4) = 8$, $N_T(5) = 3$. (C) Survival probability s_k of offspring coming from H-parents or T-parents; sample size $N_H = 1282$, $N_T = 1063$. We note that the survival probability of offspring from T-parents is considerably smaller than that of offspring from H-parents. This suggests that the history of the parent worm affects the survival of offspring

the HfH-fra pieces is shifted to longer times (Fig. 2B). Moreover, fragmentations $F_{k \geq 2}$ may occasionally cause the loss of the pharynx (eating tube), resulting in even longer recovery periods of parent worms, as the head must first rebuild the pharynx before it can intake nutrients and grow again [5, 19]. During pharynx regeneration, the worm shrinks as its energy expenditure exceeds its energy input (which is zero during that phase).

The more pronounced bimodality in the RWTDs of tail pieces, can be partially attributed to memory of the parent-type [8], but also reflects dependence on the division mode of the parent worm (Fig. 2C), similar to our observations for heads. Generally, planarians have to exceed a minimal size before they can reproduce again [5]. The smallest planarian we recorded reproducing had an area of 1.5 mm^2 , while the second smallest was observed to divide at 2.03 mm^2 . On average, both H-parents and T-parents were significantly larger before dividing, but we did not find a well-defined critical size, which, once reached, spontaneously leads to division into smaller pieces as an energetically favorable state (Fig. 5A below).

3.3 Offspring Number Distribution and Survival Probabilities

From our data we can determine the probability p_k that a parent worm produces k offspring when undergoing asexual reproduction. The resulting distributions for H-parents and T-parents are shown in Fig. 3A. For either parent type, we find an approximately exponential decay of the probabilities p_k with offspring number k , with a maximal weight on simple fissions F_1 .

Figures 3B and C show the survival probability for offspring and parents as a function of the offspring number k per Teilung. From a naive (purely “Darwinian”) point of view, one would expect that the planarians have adopted a reproductive strategy that maximizes the number of living animals at a given time. Thus, our initial expectation was to find that the survival probabilities become maximal at $k = 1$, since simple fissions F_1 occur most frequently according to the results in Fig. 3A. Interestingly, this is not the case, as evident from the location of the maxima at $k = 2, 3$ in Figs. 3B and C. This suggests that either the reproduction of planarians is random and does not following a deterministic optimized reproduction strategy or, alternatively, that offspring survival is not directly proportional

to offspring reproductive success; the latter is the important parameter determining fitness according to natural selection [25, 26]. Since the recovery periods for tails are very long, the presently available data do not yet allow us to evaluate the latter hypothesis. Therefore, we now focus on the possibility of quasi-random reproductive behavior, and we will see that a maximum relative entropy approach can capture certain aspects of the data quite well.

3.4 Maximum Relative Entropy Principle

In this next section, we will try to establish a connection between the offspring number probability p_k and the survival probabilities of parents and offspring, h_k and s_k , respectively, by means of a maximum relative entropy principle [11, 31] that combines an information-theoretic approach with “Darwinian” preferences related to offspring/parent survival.

The approximately exponential decay of the division number distributions in Fig. 3A suggests that the experimentally determined distributions p_k can be derived from a suitably formulated maximum entropy principle. To summarize the mathematical framework, consider the problem of maximizing the relative entropy (negative Kullback-Leibler divergence [13])

$$S = - \sum_{k=1}^n p_k \ln \frac{p_k}{q_k} \tag{1a}$$

under the normalization and mean value constraints

$$1 = \sum_{k=1}^n p_k, \quad m = \sum_{k=1}^n k p_k. \tag{1b}$$

This optimization problem formalizes the idea that a planarian adopts a random reproduction strategy, due to intrinsic randomness and incomplete information about its environment, while trying to match the constraint that only a certain mean number m of offspring can be produced per reproduction period (e.g., due to physiological or genetic constraints). The non-negative quantities $\{q_k\}$ in (1a) define a reference measure¹ on the set of possible reproduction outcomes $k = 1, 2, \dots$. Biologically plausible candidates for $\{q_k\}$ will be considered in detail below. The above optimization problem is equivalent to finding the distribution $\{p_k\}$ that satisfies the condition

$$0 \stackrel{!}{=} \frac{\partial}{\partial p_j} \left[S + \alpha \left(1 - \sum_{k=1}^n p_k \right) + \beta \left(m - \sum_{k=1}^n k p_k \right) \right] \tag{2}$$

with Lagrange multipliers α, β . Solving (2) for p_k and using the normalization constraint (1b) to eliminate α , one finds that the optimal distribution takes the weighted Boltzmann-type form²

$$p_k = \frac{q_k \exp(-\beta k)}{\sum_{k=1}^n q_k \exp(-\beta k)}. \tag{3}$$

¹The reference measure $\{q_k\}$ can be thought of as assigning *a priori* weights to the different fission/fragmentation states $k = 1, 2, \dots, n$.

²The Boltzmann-type probability distribution (3) is well-known in physics, e.g., in quantum mechanics with k playing the role of the energy level and q_k denoting the degeneracy of the level.

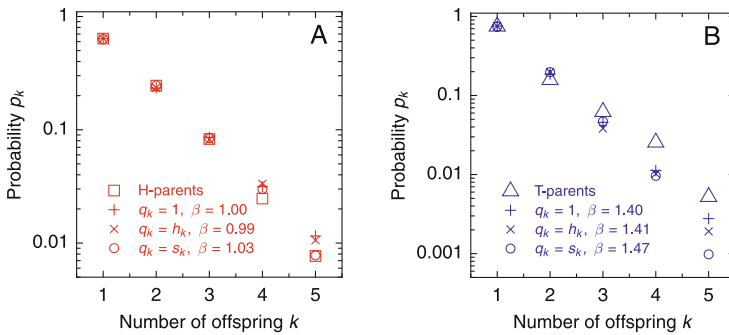


Fig. 4 Experimentally measured probability distributions of the number of offspring produced per fission/fragmentation, and corresponding maximum entropy fits based on the three different reference measures described in the text using $n = 5$. (A) For H-parents the best fit to the experimental data is obtained for the choice $q_k = s_k$ corresponding to the offspring survival preference. (B) For T-parents the best (but not very good) fit to the experimental data is obtained for the $q_k = 1$

The distribution (3) features three ‘inputs’: the reference measure $\{q_k\}_{k=1,\dots,n}$, the maximum number of offspring n , and the Lagrange multiplier β . The ‘temperature’ β can be viewed as a fit parameter that quantifies the intrinsic randomness of the division process. The upper offspring bound n can be estimated from the fact that the total volume of offspring during a division cannot exceed the volume of the parent worm. From our experiments we obtain $n \simeq 5$ for both T-parents and H-parents.

If one assumes that (3) presents a viable model for the fragment number distribution then the most interesting question, from a biological perspective, relates to the choice of the values $\{q_k\}$ adopted by the planarians. Intuitively, the quantities $\{q_k\}$ can be interpreted as an intrinsic reference measure, applied by the organism to increase the likelihood of those reproduction processes that enhance the fitness of the family, e.g., by increasing the survival probability of offspring and/or parents. We next discuss possible candidates for the q_k and compare the resulting distributions with our experimental data.

Uniform Reference Measure From a biological point of view, the uniform reference measure $q_k = 1$, for all $k = 1, 2, \dots$, reflects the assumption that none of the Teilung events is intrinsically preferable. In this case, the entropy (3) reduces to the standard Shannon-Gibbs-Boltzmann entropy, and we have the following relation between (m, β, n)

$$m = \frac{1}{1 - e^{-\beta}} + \frac{n}{1 - e^{-\beta n}}, \quad (4)$$

where the range of β -values is restricted due to $1 \leq m \leq n$. The resulting fits for $q_k = 1$ are shown as (+)-symbols in Figs. 4A and B, respectively. The only remaining fit parameter β was determined by minimizing the sum of squared residuals.

Preference of Offspring Survival Another plausible possibility is the hypothesis that the reference measure is linked to the survival probabilities of the offspring, s_k (Fig. 3C). Fixing $q_k = s_k$ and fitting β yields the (o)-symbols in Fig. 4.

Preference of Parent Survival As a third possibility, we consider that the reference measure q_k is linked to the survival probability of the parent worms after a division. We denote by h_k the probability that a parent worm, which has produced k offspring, survives and

undergoes another division. The estimates for these probabilities from our experimental data are shown in Fig. 3B. Assuming that $q_k = h_k$ and using β as free fit parameter, we obtain the probabilities indicated by (\times)-symbols in Figs. 4A and B.

The comparison of the three different fits with the experimental data suggests that the reproduction statistics of H-parents is generally well described by the maximum entropy hypothesis. The best agreement is obtained when adopting $q_k = s_k$, corresponding to a preference of offspring survival, see (\circ)-symbols in Fig. 4A. By contrast, one observes noticeable differences between the experimentally determined offspring distribution for T-parents and the maximum entropy fits for each of three reference measures (Fig. 4B). For T-parents, the choice $q_k = 1$ yields the smallest relative deviation from the data, but the fit is not great for $k \geq 3$. We also tested several other candidates for q_k , such as the mean number of surviving offspring $q_k = ks_k$, but none of those led to a better agreement.

From a biological perspective, there exist a number of reasons why the reproduction statistics of T-parents could be qualitatively different from that of H-parents: Tail pieces undergo a more complex regeneration process as they have to rebuild their eating tube and other internal organs, in particular the brain, before being able to produce offspring. A tail's behavior may even be considerably influenced through memory of its ancestral history as indicated by the reproduction waiting time distributions, which differ significantly depending on whether the tails originate from a T-parent or H-parent [8].

In summary, we find that the maximum entropy approach captures the main features of the observed reproduction statistics for H-parents quite well and future data will allow us to test this approach in detail against Darwinian models that take into account the reproductive success of future generations.

In the remainder, we will try to get first insights into the alternative possibility that the planarians do follow a deterministic optimized reproduction strategy based on optimizing future reproductive success instead of survival probabilities. To this end, we examine the relationships between worm size, waiting time, and number of offspring produced, in an attempt to explain why simple fissions F_1 occur most frequently despite the higher survivorship observed in fragmentations $F_{k=2,3}$.

3.5 Correlations Between Parent Size, Offspring Number and Recovery Times

We investigate whether the interplay between offspring size, number of siblings (offspring) and parent recovery times favors simple fissions. The inverse relationship between offspring number and invested resources per offspring has been suggested to affect offspring maturation time and offspring and parental fitness in other systems [25, 29].

Our imaging data shows that, typically, planarians grow bigger the longer their recovery period (Fig. 5A). For both H-parents and T-parents we observe a monotonous but nonlinear correlation between average size before fission/fragmentation and waiting time τ (T-parent area increases between 40 and 180 days from 2.8 mm² to 4.0 mm²). This can be well described by a fractional increase $\propto \tau^{1/2}$. We hope that future investigations will allow us to identify the biophysical mechanisms underlying this power-law behavior. The relationship between parent size and number of offspring is shown in Fig. 5B. The origin of the unexpected nonlinearity in the H-parent area vs. number of offspring correlation is unclear at present; for T-parents we have at present no data for $k = 3, 4$, but the relationship appears to be linear. For both parent types we find, however, linear relations between mean reproduction waiting time and offspring number (Fig. 5C).

Interestingly, T-parents are generally smaller than H-parents when they divide, despite their considerably longer recovery times. Moreover, T-parents wait significantly longer be-

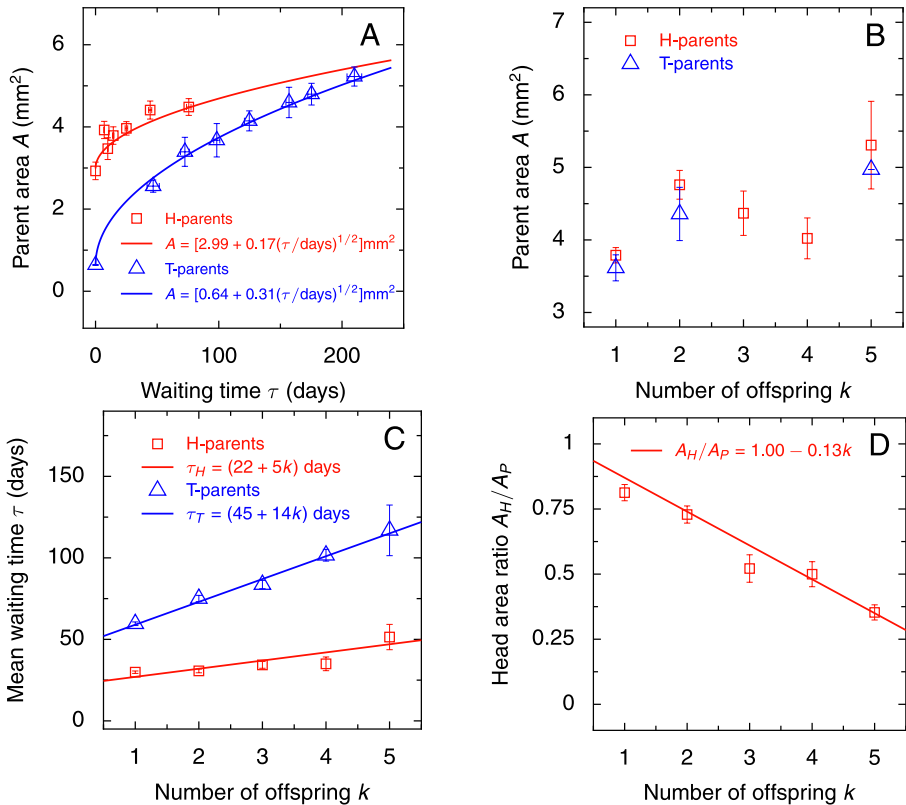


Fig. 5 (A) The average area A of H-parents and T-parents before Teilung increases with the average reproduction waiting time. Lines show best power-law fits to the raw data; sample size $N_H = 184$, $N_T = 56$. (B) Parent area prior to Teilung vs. number of offspring. For T-parents the relationship appears to be roughly linear but is not yet conclusive due to the limited sample size of tail imaging data. For H-parents we observe a non-monotonous dependence; sample size $N_H = 148$, $N_T = 45$. (C) Linear relationship between parent waiting time and number of offspring produced. Fits are to the raw data; sample sizes $N_H = 1822$, $N_T = 1527$. (D) The relative head area A_H after fission/fragmentation (normalized by the parent area A_P before Teilung) decreases linearly with the number of offspring. Linear fit is again based on the full raw data set; sample size $N = 193$

fore fragmenting into multiple pieces (Fig. 5C), whereas for H-parents divisions occur generally within 50 days or less, independent of the number of offspring produced.

Thus, roughly, the results in Figs. 5A–C can be summarized as follows: The longer a planarian waits to divide, the larger it grows on average, and the more pieces it is likely to shed off. Figure 5D shows that the head size decreases to $\leq 50\%$ of the original parent size for fragmentations with $k \geq 3$. In contrast, shedding off one or two pieces leaves the worm with 70–90% of its initial body size. Similar results for simple fissions were reported by Sheiman et al. [22] who quantified the areas of heads and tails after fission in a small population of *Dugesia tigrina*.

Naively, one would expect that the average offspring size decreases with the number of siblings, since similar trade-offs have been frequently reported for sexual organisms [23, 25]. Remarkably, we find that this is not the case for asexual planarians. Our data shows that the average offspring size does not change much with increasing sibling number

(Fig. 5D). Typically, only bigger parent worms produce a large number of offspring, and offspring size is roughly kept constant at the expense of a parent's head size. This finding may explain why the survival probability of the offspring is not affected by the clutch size (Fig. 3C).

Another possible resource-allocation-based trade-off, which could explain the observed probability distribution of offspring produced per Teilung (Figs. 3A and 4), is the trade-off between the reproduction frequency and the number of offspring produced. For head parents, this trade-off does not seem to play a role: We do not observe a strong dependence of the reproduction frequency on the number of offspring produced (Fig. 5C; shedding 5 pieces at once is an exception). For T-parents, we do observe that fragmentations occur at a slower rate than fissions, but this decrease in reproduction frequency is outweighed by the additional number of offspring produced (Fig. 5C). Thus, while this trade-off cannot explain the observed reproductive behavior dominated by simple fission events either, it may be a dominant factor determining the reproductive strategy in the natural planarian habitat. In the wild, a prolonged waiting time causes an increase of the probability to get killed by predators before the next reproductive event and, therefore, the different reproduction modes clearly influence the future reproductive potential of the parent. It may well be that the planarians have not yet adapted their reproductive strategy to the constant environmental conditions in the laboratory, and future data could reveal a change in the reproduction probability distributions.

In summary, with regard to the offspring's reproductive ability, our data thus far indicates no trade-off between offspring size and number, which could negatively influence offspring fitness as seen in other systems [7, 24, 30]. However, the various kinds of type-1 and type-2 offspring are not identical as they originate from different parts of the parent's body (Figs. 1A and B); this difference could have an impact on their future reproductive success and, therefore, it may be worth studying individual optimization strategies [4, 18]. Forthcoming data on the reproduction dynamics from our ongoing experiments, combined with an analysis of the molecular composition of the different offspring types, will allow us to address this problem in the future.

In this context, it would also be interesting to investigate how amputation and wounding affect the reproductive ability of planarians. The location of an amputation site determines the size of the remaining planarian body and, thus, will strongly influence its regeneration and subsequent waiting time. Depending on the position of the cut relative to the anterior pole, amputation could be thought to resemble either fragmentation with multiple offspring (if amputation is performed closer to the head) or simple fission (if the cut is made more posteriorly) but there also exist important differences: Amputation occurs as a sudden externally induced event, whereas self-induced fission or fragmentation is preceded and initiated by a series of structural changes [9, 17]. It is therefore possible that details of the regeneration dynamics after amputation and wounding could be rather different from those after natural reproduction [9, 10]. A quantitative spatial mapping of amputation sites and their effect on reproduction waiting times will be required to answer this question and presents an interesting avenue for future research.

4 Conclusion

Our investigation of clonal reproduction in the asexual strain of the freshwater planarian *S. mediterranea* shows that these multicellular organisms adopt a mixed strategy that comprises both asymmetric binary fission events (~70%) and fragmentation events (~30%)

producing multiple offspring. Our results further show that the probability distribution of the number of offspring produced during Teilung can, at least approximately, be derived from a suitably formulated maximum relative entropy principle. Since the worms were kept under constant laboratory conditions in our experiment, the latter observation seems to suggest that planarians adopt a random reproduction strategy rather than adapting to the homogenous environment with a deterministic strategy that maximizes offspring and/or parent survival. It is, however, also possible that planarians' reproductive strategy is optimized in the "Darwinian" sense with respect to environmental fluctuations, and that this behavior is still present in our laboratory planarian population since the time for adaptation to constant lab conditions may not have been sufficient. Therefore, we plan to continue and extend our experimental study by exposing selected worm families to varying environmental conditions. We hope that future data will allow us to identify whether randomized reproduction in planarians is caused by intrinsic noise or whether it is an optimized evolutionary response to environmental fluctuations.

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