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A multigenerational effect of parental age on offspring size but not fitness in common duckweed (*Lemna minor*)

P. M. BARKS & R. A. LAIRD

Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada

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Abstract

Classic theories on the evolution of senescence make the simplifying assumption that all offspring are of equal quality, so that demographic senescence only manifests through declining rates of survival or fecundity. However, there is now evidence that, in addition to declining rates of survival and fecundity, many organisms are subject to age-related declines in the *quality* of offspring produced (i.e. parental age effects). Recent modelling approaches allow for the incorporation of parental age effects into classic demographic analyses, assuming that such effects are limited to a single generation. Does this 'single-generation' assumption hold? To find out, we conducted a laboratory study with the aquatic plant *Lemna minor*, a species for which parental age effects have been demonstrated previously. We compared the size and fitness of 423 laboratory-cultured plants (asexually derived ramets) representing various birth orders, and ancestral 'birth-order genealogies'. We found that offspring size and fitness both declined with increasing 'immediate' birth order (i.e. birth order with respect to the immediate parent), but only offspring size was affected by ancestral birth order. Thus, the assumption that parental age effects on offspring fitness are limited to a single generation does in fact hold for *L. minor*. This result will guide theorists aiming to refine and generalize modelling approaches that incorporate parental age effects into evolutionary theory on senescence.

Introduction

Age-related declines in physiological and demographic performance (known as ageing or senescence) seem inherently maladaptive, but occur nonetheless in many taxa (Jones *et al.*, 2014). Evolutionary theorists have proposed a variety of mechanisms to explain this apparent paradox (e.g. mutation accumulation, Medawar, 1946, 1952; antagonistic pleiotropy, Williams, 1957; disposable soma, Kirkwood, 1977; Kirkwood & Holliday, 1979; reliability theory, Gavrilov & Gavrilova, 2001; Laird & Sherratt, 2009, 2010), all centred around the realization that the force of natural selection tends to decline with increasing age (Hamilton, 1966). Simply put, natural selection discounts relatively old age classes because, assuming any nonzero level of mortality, fewer and fewer individuals survive to increasingly advanced ages.

One simplifying assumption implicit in the majority of theoretical work on senescence is that all offspring are of equal quality (e.g. Hamilton, 1966; Kirkwood & Rose, 1991; Vaupel *et al.*, 2004). Under this assumption, fitness and the force of natural selection depend on age trajectories of two fitness components – survival and fecundity. Thus, senescence, from an evolutionary perspective, is generally defined as a decline in the rate of survival or fecundity (or both) with increasing age. As others have pointed out (Kern *et al.*, 2001), this view of senescence omits age-related declines in offspring quality (i.e. parental age effects), for which there is evidence in a wide range of taxa (Priest *et al.*, 2002; Descamps *et al.*, 2008; Bouwhuis *et al.*, 2010; Gillespie *et al.*, 2013b; Barks & Laird, 2015).

Recent analyses suggest that, if offspring quality does in fact decline with increasing parental age (or

Correspondence: Patrick Barks, Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada, T1K 3M4.
Tel.: +1 403 332 4046; fax: +1 403 332 4039;
e-mail: patrick.barks@gmail.com

similarly, with increasing birth order), classic demographic methods that do not account for parental age effects may underestimate age-related declines in the force of selection (Pavard & Branger, 2012; Gillespie *et al.*, 2013a) (see also Appendix S1).

In a previous study (Barks & Laird, 2015), we demonstrated parental-age-related declines in offspring quality in *Lemna minor* L., a small aquatic plant in the subfamily Lemnoideae (the duckweeds). Specifically, we found that offspring (here referring to asexually produced ramets) produced late in their parent's life were smaller and had lower fitness than their earlier-produced siblings. To fully understand how variation in offspring quality influences the force of natural selection, we need to understand not only how offspring quality changes with parental age, but also whether effects of parental age carry over across multiple generations. For example, Hercus & Hoffmann (2000) found that, in *Drosophila serrata*, offspring fitness declined both with increasing maternal age and increasing grandmaternal age (the age of the grandmother at the mother's birth). Intuitively, natural selection should discount old age classes if individuals within those classes tend to produce offspring of relatively low quality. This discount should be especially large if the negative effects of old age carry over across multiple generations.

Here we ask, do parental-age-related declines in offspring quality in *L. minor* carry over across multiple generations? There is some evidence to suggest that they do, at least in terms of offspring size (one aspect of quality). Specifically, Wangermann & Ashby (1951) found that late-produced offspring (referring again to asexually produced ramets) in *L. minor* were much smaller than their earlier-produced siblings. Moreover, these small, late-produced plants themselves produced relatively small first offspring compared with earlier-produced plants, suggesting a grandparental age effect on offspring size. In the current study, we extend the work of Wangermann & Ashby (1951) by examining variation in a measure of offspring quality more closely related to fitness (the individual intrinsic rate of increase, r_{ind}), over a wider range of parental and ancestral ages. For the sake of clarity, we note that, although the phenomena we are studying (i.e. senescence, parental age effects) may occur at multiple levels of biological organization (i.e. at the level of ramets, genets, or both), the current study focuses on the ramet level only. We therefore use terms like 'individual', 'plant', 'parent', 'offspring' and 'age' in reference to asexually produced ramets. Although the combination of clonal and sexual reproduction adds a layer of complexity to classic evolutionary theory on senescence (e.g. Orive, 1995; Pedersen, 1995; Gardner & Mangel, 1997), the basic tenets of evolutionary theory on senescence (i.e. an expected decline in the force of selection with age) still apply at the level of ramets (Pedersen, 1995).

Materials and methods

Overview

To test whether parental age effects carry over across multiple generations, we sought to compare the fitness (measured as the intrinsic rate of increase) of 512 focal plants comprising 16 'birth-order genealogies' (Fig. 1). Birth order is a proxy for parental age reflecting the temporal order in which the offspring of a given parent are born. Specifically, an individual with birth order N is the Nth offspring born to its parent. In *L. minor*, parents have two meristematic pockets (right and left) from which offspring may detach, so we define N_P as the pocket-specific birth order where P can either be right ('R') or left ('L'). For example, a plant with birth order N_R is the N_Rth offspring to detach from the right meristematic pocket of its parent. Because, in *L. minor*, offspring develop alternately between the two meristematic pockets, a plant with birth order N_P will generally have an overall (pocket-independent) birth order of $N = 2 \times N_P$ or $N = 2 \times N_P - 1$, depending on which pocket produced the first offspring. To limit potential heterogeneity in our sample, we studied right-produced offspring only, with exceptions noted below.

In our study, the birth-order genealogy of each focal plant was captured by two variables: immediate birth order and ancestral birth order. Immediate birth order was the birth order of a focal plant with respect to its parent (target values in our study were $N_R = 1$, 3, 5, or 7), whereas ancestral birth order reflected birth order over the three preceding generations (target values were N_R-N_R-N_R = 1-1-1, 3-3-3, 5-5-5 or 7-7-7). Previous research documented declines in offspring size and fitness with increasing *immediate* birth order in *L. minor* (Wangermann & Ashby, 1950; Barks & Laird, 2015). If parental age effects carry over across multiple generations, then we expect frond size and fitness to decline with increasing *ancestral* birth order as well.

Study species

Lemna minor is a tiny aquatic angiosperm found in freshwater lakes and wetlands throughout the world (Landolt, 1986, pp. 275–282). Individual plants have a highly reduced shoot architecture and are therefore referred to as 'fronds' (Lemon & Posluszny, 2000). They float freely on the water's surface, often occurring in dense mats when conditions are favourable. Reproduction in *L. minor* is predominantly asexual, although flowering does occasionally occur in the wild (Landolt, 1986, pp. 167–169). During asexual reproduction, daughter fronds develop in alternating succession from one of two meristematic pockets located on either side of the parent frond (Lemon & Posluszny, 2000). The first daughter to develop from a given parent is initiated very early – while the parent is still developing within

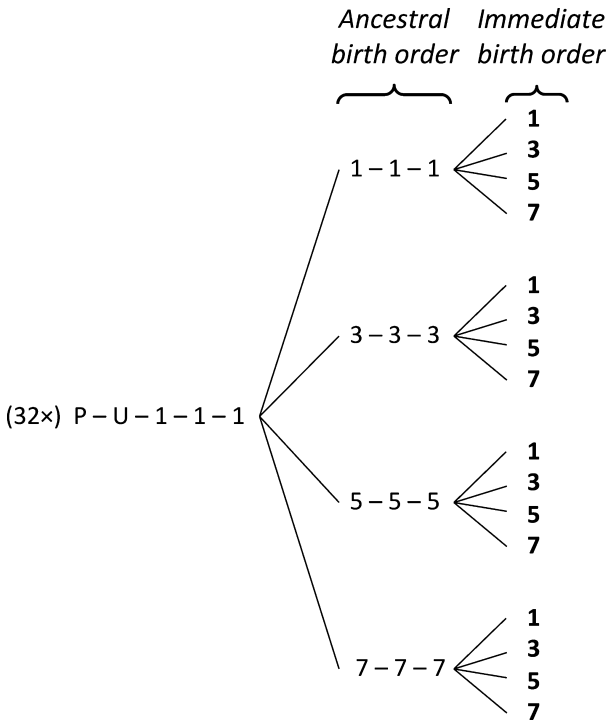

Fig. 1 Schematic of our 16 birth-order genealogies. The leftmost element represents the earliest-tracked ancestors and the rightmost elements represent focal fronds. Numbers represent the pocket-specific birth order (N_P) of a given frond with respect to its immediate parent. The 'P' at the far left of the schematic represents one of 32 progenitor fronds initially taken from a stock culture, and the adjacent 'U' represents the progenitor's first observed offspring, which is always of unknown birth order because the progenitor may have released offspring while still in the stock culture. The birth-order genealogy of each focal frond is captured by two variables: immediate birth order (birth order with respect to the immediate parent), and ancestral birth order (birth order over the three preceding generations).

its own parent – and successive daughters usually begin developing before the previous daughter has detached (Lemon & Posluszny, 2000). As daughter fronds develop, they remain joined to their parent via a structure called the 'stipe', which eventually severs once the daughter is mature (Landolt, 1986, pp. 66–67). Life-history traits in *L. minor* are generally quite plastic (Wangermann & Ashby, 1951), but under optimal conditions plants will live for about 25–30 days and produce roughly 10–15 offspring (Lemon *et al.*, 2001; Barks & Laird, 2015).

Plants and growth conditions

The single strain of *L. minor* used in this study was initially collected from a small wetland at the University of Lethbridge in Lethbridge, Alberta (49.6792°N, 112.8726°W). From these wild-collected plants, we created a sterile, single-genotype stock culture following the protocol described in Hillman (1961), as further detailed in Appendix S2. The stock was maintained in half-strength Schenk and Hildebrandt (S-H) growth medium (S6765, Sigma-Aldrich, St. Louis, MO, USA), which we supplemented with sucrose (6.7 g L^{-1}), yeast extract (0.067g L^{-1}) and tryptone (0.34 g L^{-1}) to make microorganism contamination more easily detectable.

Except for the stock culture, plants used in this study were grown individually in 60 × 10 mm Petri dishes containing 10.5 mL of S-H medium (supplemented as described above). Petri dishes were arranged on cookie-cooling racks and kept inside growth chambers at 24°C with a 15:9 photoperiod and photosynthetic photon flux density at plant height of approximately 400 μmol m^{-2} s^{-1}. To account for nutrient depletion and evaporation of the growth medium, every 4 days plants were aseptically transferred to new Petri dishes containing fresh growth medium.

Plant observation

To create our 16 genealogical sequences (4 immediate × 4 ancestral birth orders) and measure the fitness of focal fronds, we had to keep track of reproduction by individual plants on a daily basis. This daily tracking regime began with 32 progenitor fronds initially taken from the stock culture ('P' in Fig. 1), and continued until all focal fronds were deceased. During each daily observation period (i.e. census), we noted how many daughters detached from each meristematic pocket of each parent since the previous census, and updated a tally of the number of daughters detached from each meristematic pocket of each parent since birth. Detached daughters were aseptically removed from the Petri dish and discarded if they were not needed, or transferred to their own fresh Petri dish if they were of the requisite birth order to continue our planned genealogical sequence (see Fig. 1).

Measuring frond fitness and size

We estimated the fitness of focal fronds using the individual intrinsic rate of increase, r_{ind} (McGraw & Caswell, 1996), which tells us the expected rate of population increase (fronds per frond per day) in the lineage hypothetically descending from a particular focal frond, assuming that all descendants have the same lifespan and fecundity schedule as their focal frond ancestor. This metric is well suited for combining survival and fecundity schedules into a single value that can be used to compare relative contributions to future generations across different subsets of a population.

To calculate individual intrinsic rates of increase, we created a $\omega \times \omega$ Leslie matrix for each focal frond, where ω was the frond's reproductive lifespan in days. Each matrix was populated with age-specific fertilities

(F_i) across the top row (number of daughters released while in age class i), age-specific survival probabilities (P_i) on the subdiagonal (survival was set to 1 for each age class through which the focal frond survived), and all other elements were set to zero. Individual intrinsic rates of increase were then calculated as the natural logarithm of the dominant eigenvalue of each Leslie matrix.

One difficulty associated with the individual intrinsic rate of increase is its sensitivity to the length of time between when offspring are born and when they are counted (Brommer *et al.*, 2002). For example, if a frond is first observed to have detached from its parent at census b (the birth census), we only know that it detached sometime between censuses $b - 1$ and b. If the frond detached immediately after census $b - 1$, then its first age class is best defined as the period between censuses $b - 1$ and b (definition #1; post-breeding census). In contrast, if the frond detached immediately before census b, then its first age class is best defined as the period between censuses b and $b + 1$ (definition #2; prebreeding census). In our study, we could never be sure which definition of the first age class was more appropriate for any given focal frond (this uncertainty applies to all demographic studies on organisms that do not reproduce in uniformly spaced pulses). We incorporated this uncertainty into our analysis using multiple imputation, as described in the Data Analysis section.

Frond surface area was measured in ImageJ v. 1.43u (Rasband, 2012) based on images captured with a microscope-mounted digital camera. When a frond has daughters attached, it can be difficult to delineate that frond's perimeter. We therefore captured images for surface area measurement late in each focal frond's life when it had no attached daughters.

Sample loss and skipped censuses

In creating our 16 birth-order genealogies, offspring with birth order $N_R = 7$ were sometimes difficult to obtain because fronds of relatively high birth order occasionally develop in a 'folded', deformed manner (Lemon & Posluszny, 2000; Barks & Laird, 2015), which can make it difficult to track the birth order and total number of their offspring (i.e. it can be difficult to distinguish left from right daughters, or daughters from granddaughters). Additionally, parents do not always produce ≥ 7 offspring from each meristematic pocket (although this was relatively rare in our study compared with the 'folding' described above). If a required $N_R = 7$ was not produced or appeared too deformed to reliably track, we attempted to retain its $N_R = 6$, $N_L = 7$, or $N_L = 6$ sibling instead (with preference given in that order). In a few cases where a required $N_R = 5$ was too deformed to reliably track, we retained its $N_L = 5$ sibling instead. Such swaps were not possible when the relevant siblings had already been discarded by the time it was realized that the target frond could not be reliably tracked. Thus, if we could not track a frond's reproduction with certainty and a swap was not possible, the lineage was discontinued resulting in sample loss. Although we aimed for 512 focal plants, we were only able to successfully track 423 focal fronds to their death. As expected, sample loss increased with both immediate and ancestral birth order (Fig. 2).

Over an 8-day period towards the end of our study, extraneous circumstances resulted in focal fronds being observed every second or third day instead of daily. Because our fitness metric was derived from the complete reproduction schedule of each focal frond, the skipped observation periods add a small degree of uncertainty to fitness estimates for those focal fronds that were still alive during the 8-day period in question (96 of the 423 focal fronds were affected). We deal with this uncertainty using multiple imputation, as described below.

Data analysis

All analyses were conducted in R v. 3.1.1 (R Core Team, 2014). Our raw data and R scripts are archived at Dryad (Barks & Laird, 2016).

As previously mentioned, fitness estimates for some of our focal fronds were subject to uncertainty due to skipped censuses, and fitness estimates for all fronds were subject to uncertainty regarding the most appropriate definition of the first age class. We explicitly accounted for both sources of uncertainty using multiple imputation – generating multiple simulated data

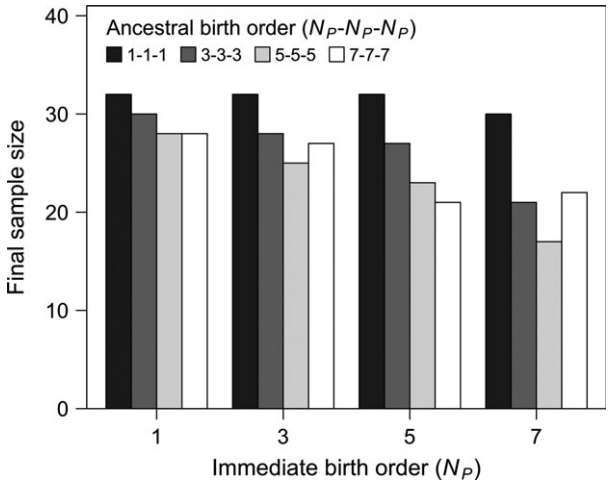

Fig. 2 Final sample size (number of focal fronds) for each of the 16 birth-order genealogies. Samples were lost when a frond failed to produce a daughter of high enough birth order to continue the planned genealogical sequence, or when a frond's reproduction could not be tracked with certainty (due to the frond developing in a folded manner, as described in the text).

sets where missing values are stochastically replaced with plausible values (outlined in Schafer, 1999; Nakagawa & Freckleton, 2008). Each imputed data set is analysed using standard methods (general linear models in our case), and parameter estimates are then 'pooled' to account for the variance both within and among data sets. Hypothesis testing on pooled parameter estimates can be accomplished with a Wald-type test statistic D_m, as described in Meng & Rubin (1992). We generated $m = 10$ simulated data sets (the generally recommended range for m is 3–10; Rubin, 1987; Nakagawa & Freckleton, 2008) using our own imputation algorithm (described below) and used the *pool* and *pool.compare* functions within the R package MICE (van Buuren & Groothuis-Oudshoorn, 2011) to pool parameter estimates and obtain test statistics and *P*-values. We used the above-described protocol for our main hypothesis test on fitness vs. immediate and ancestral birth order, and also for *post hoc* contrasts following from the main test. Diagnostic plots generated for a subset of imputed data sets suggested that parametric assumptions were consistently violated (residuals were positively skewed), so intrinsic rates of increase were natural-log-transformed, which consistently improved the normality of residuals. We applied the Bonferroni correction during *post hoc* testing to limit Type I error rates.

The two sources of uncertainty in our analysis were constrained in that 'missing' entries logically could only take on one of two or three possible values. Specifically, we considered only two possible definitions of the first age class (prebreeding or post-breeding census), and we never skipped more than two sequential censuses for a given focal frond (so the range of uncertainty in an offspring's date of birth was at most 3 days). In each imputation, for each focal frond, we randomly and with equal probability assigned one of the two possible definitions of the first age class. Likewise, in each imputation, for each daughter of a focal frond observed to have detached during a census immediately following one or more skipped censuses, we randomly assigned the daughter to one of the two or three possible parental age classes, selected with equal probability (see example in Table S1). Note that our imputation step did not directly generate intrinsic rates of increase *per se*, but rather stochastically generated a portion of the information used to subsequently calculate a focal frond's individual intrinsic rate of increase.

Testing the effect of birth order on frond size did not require imputation as skipped observation periods did not add any uncertainty to our estimates of frond size. Thus, we assessed the effect of immediate and ancestral birth order on frond size using analysis of variance (ANOVA) and *post hoc* Tukey's tests. We again used standard diagnostic plots to confirm that parametric assumptions were met.

Results

Offspring size was significantly affected by both immediate ($F_{3,413} = 99.9$, $P < 0.001$) and ancestral ($F_{3,413} = 43.5$, $P < 0.001$) birth order, whereas offspring fitness was affected by immediate birth order ($D_{m3,345} = 14.3$, $P < 0.001$) but not ancestral birth order ($D_{m3,170} = 0.4$, $P = 0.8$). Offspring size and fitness both peaked at an immediate birth order of $N_P = 3$ and declined with increasing immediate birth order thereafter (Figs 3 and 4). Similarly, offspring size peaked at ancestral birth order N_P-N_P-N_P = 3-3-3 and declined thereafter (Fig. 4).

Uncertainty in fitness estimates due to the differing age-class definitions and skipped censuses (i.e. variation among imputations; Fig. 3 bottom) was small compared with variation in fitness within imputations (Fig. 3 top).

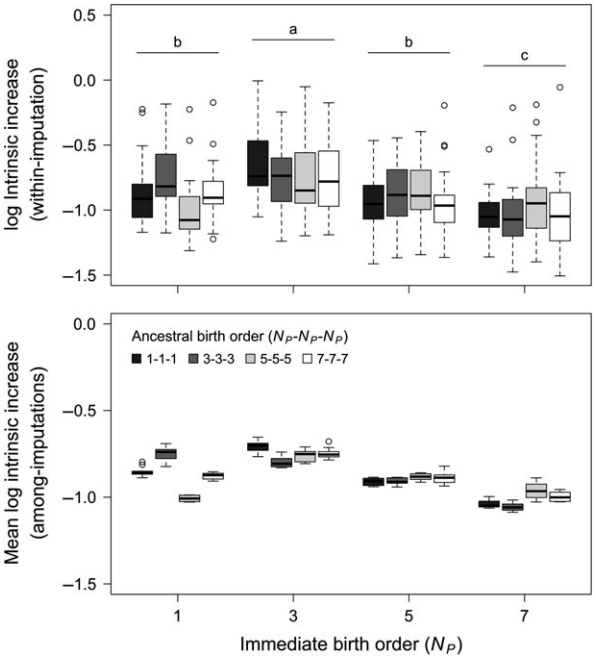

Fig. 3 Natural-log-transformed individual intrinsic rates of increase (ln r_{ind}) by immediate and ancestral birth order. The top panel depicts intrinsic rates of increase for one of the 10 imputed data sets, whereas the bottom panel depicts variation in mean intrinsic rates of increase *among* the 10 imputed data sets. Note that the range of the *y*-axis is smaller in the bottom panel (for greater clarity), and even so, variation within imputations (top panel) is much greater than variation among imputations (bottom panel). Letters above the boxplots indicate significant differences among immediate birth orders based on Bonferroni-corrected *post hoc* contrasts. There was no significant effect of ancestral birth order on intrinsic rates of increase. Boxes depict the median and first and third quartiles, and whiskers extend to the lowest and highest data points within 1.5 times the interquartile range of the first and third quartile, respectively.

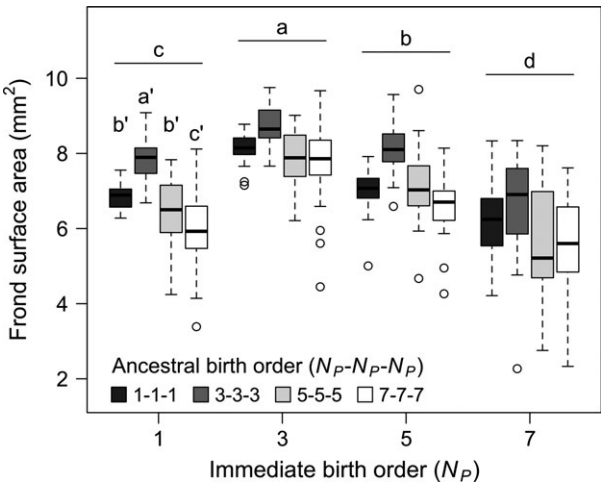

Fig. 4 Frond surface area by immediate and ancestral birth order. Letters above the boxplots indicate significant differences among birth orders based on Tukey's *post hoc* tests. For graphical clarity, *post hoc* differences among ancestral birth orders are depicted only for the first level of immediate birth order, but actually apply to ancestral birth order independent of immediate birth order (as we did not model an interaction). Boxes depict the median and first and third quartiles, and whiskers extend to the lowest and highest data points within 1.5 times the interquartile range of the first and third quartile, respectively.

Discussion

Our results suggest that, in *L. minor*, parental age effects on offspring size carry over across multiple generations, but parental age effects on offspring fitness (measured as the individual intrinsic rate of increase, r_{ind}) do not. Specifically, despite offspring fitness declining with increasing immediate birth order (recall that birth order was a proxy for parental age), the fitness of focal fronds was unrelated to the birth order of their three closest ancestors. At least in *L. minor*, parental age effects on offspring fitness seem to 'reset' at each new generation.

Evolutionary consequences of parental age effects

Intuitively, a parental age effect that is limited to a single generation should be much simpler to model than one that carries over or accumulates across generations. For instance, to incorporate single-generation parental age effects into classic population projection analyses (e.g. van Groenendael *et al.*, 1988), we should only need to track one additional variable: parental age at birth. In other words, instead of examining population-averaged age trajectories of survival and fecundity, we could separate age trajectories of survival and fecundity by parental age. This was the general approach used by Pavard and colleagues (Pavard *et al.*, 2007a,b; Pavard & Branger, 2012) to examine the effect of maternal care on the evolution of human life-history traits. In their models, offspring survival to maturity depended on maternal survival, the probability of which declined with increasing maternal age. In general, Pavard and colleagues found that incorporating the above-described maternal effect into population projection analyses resulted in an increased force of selection on adult (maternal) survival, and an increased rate of decline in the force of selection on maternal fecundity, compared with what was expected if maternal effects were ignored. In principle, it should be possible to extend this approach to examine parental age effects on adult traits (both survival and fecundity), like the effects we observed in *L. minor*. Because, in *L. minor*, offspring fitness depends on parental age but not parental survival *per se* (as it does in humans and other animals with parental care), we predict that the incorporation of parental age effects into demographic models for *L. minor* should generally lead to a relatively steeper decline in the age-specific force of selection on both adult survival and fecundity. There will be little selection to survive and reproduce at advanced ages if offspring produced at those ages are inherently of low quality.

Proximate causes of parental age effects

Although our study was not primarily concerned with the proximate cause of parental age effects, two of our findings potentially relate to proximate causation and warrant some discussion.

In general, proximate explanations for parental age effects can be grouped into three broad hypotheses: [1] mutation accumulation in parental reproductive tissues (Crow, 1997), [2] the accumulation and somatic transfer of deleterious compounds from parents to offspring (Ashby & Wangermann, 1951) or [3] declines in the quality of the environment in which offspring develop (e.g. declines in parental care or provisioning; Fox, 1993). We suggest that hypothesis 1 necessarily entails multigenerational effects, whereas hypotheses 2 and 3 do not (although they do not necessarily preclude them). Because we did not observe multigenerational effects of parental age on offspring fitness in our study, we suggest that parental age effects on offspring fitness in *L. minor* are best explained by some mechanism relating to hypothesis 2 or 3. As for parental age effects on offspring size in *L. minor*, we can again rule out hypothesis 1 because previous research demonstrated that, starting with small offspring produced late in their parents' life, successive generations of early-produced offspring consistently increase in size until the maximum size is attained (Wangermann & Ashby, 1951). This 'recovery' from parental age effects is inconsistent with mutation accumulation (hypothesis 1).

The remaining hypotheses (2 and 3 above) concern the accumulation of deleterious compounds and changes in the developmental environment, respectively. We have previously proposed (Barks & Laird,

2015) a putative mechanism for parental age effects specific to *L. minor* that falls within the scope of hypothesis 3. Specifically, in *L. minor*, each time a daughter frond detaches from its parent, a small amount of stipe tissue (a structure connecting the parent and developing offspring) is left behind in the parent's meristematic pocket (Lemon & Posluszny, 2000). We have therefore suggested that the accumulation of stipe tissue within a parent's meristematic pocket might increasingly constrict or modify the growth environment experienced by successive daughters, which may explain the decline in offspring size with increasing parental age. However, the stipe-accumulation hypothesis does not obviously entail multigenerational effects, making it inconsistent with results from the current study (at least with respect to frond size). That said, we can easily imagine auxiliary hypotheses that would lead to a multigenerational effect: for example, if independent of stipe accumulation, there exists a correlation between parent and offspring size (i.e. late-produced offspring will be small because they developed in a constricted environment due to stipe accumulation, and *their* offspring will be small simply because the parent was small). Studies that examine parental-age-related variation in both demographic and physiological traits will likely be needed to test the above-described hypotheses.

A second result from our study that potentially bears on the proximate cause of parental age effects is our finding that frond size and fitness both peaked at an immediate birth order of $N_P = 3$ (and for frond size, an ancestral birth order of N_P-N_P-N_P = 3-3-3). This pattern of size or fitness initially increasing with birth order has been documented previously: Claus (1972) found that frond size in *Lemna. perpusilla* peaked at a parental age of about 5 days and then progressively declined, and Barks & Laird (2015) found that the fitness of right-produced offspring in *L. minor* peaked at birth order $N_R = 2$ and declined thereafter. However, in the latter study, the fitness of left-produced offspring peaked at $N_L = 1$, and offspring size peaked at $N_P = 1$ for both right- and left-produced fronds (Fig. S1). Likewise, in Wangermann & Ashby (1951), offspring size in *L. minor* peaked at birth order $N_P = 1$ and declined thereafter. These conflicting results suggest that whether there is an initial increase in frond size or fitness with increasing birth order is strain or environment dependent.

What could be the proximate cause of an initial increase in offspring quality with increasing birth order? Hypotheses 1 and 2 for parental age effects, and the stipe-accumulation hypothesis (all described above) are unlikely candidates because mutations, somatic damage and stipe tissue would only ever accumulate over time (at least on average), so the resultant decline in offspring quality should be monotonic under these hypotheses. We therefore suggest that the initial increase in offspring quality with birth order likely relates to hypothesis 3 (excluding stipe accumulation) – some unique aspect of the environment in which first offspring develop. As noted previously, first offspring ($N_P = 1$) of *L. minor* are initiated very early in their parent's life – while the parent is still developing within its own parent. Thus, first offspring do in fact experience a different growth environment than subsequent offspring, which develop within a fully matured parent frond. We note also that we have consistently observed – in many strains of *L. minor* – a morphological difference between first offspring ($N_P = 1$) and all subsequent offspring. Specifically, in our experience, first offspring are never bilaterally symmetrical (their distal end is angled), whereas all subsequent offspring are symmetrical (their distal end is rounded) (Fig. S2). Whether this observation relates to the parental age effects on offspring size or fitness is unclear, but it again points to first and subsequent offspring experiencing somewhat different developmental environments, corresponding to hypothesis 3 above.

Caveats

There was a relatively high rate of missing data in our study (we aimed for 512 focal plants but only successfully tracked 423 to their death), and the rate of missingness increased with both immediate and ancestral birth order (Fig. 2). Could this pattern of missing data have significantly biased our results? We think it unlikely. In the current study, samples were primarily lost when fronds (generally of high birth order) developed in a folded manner and could not be reliably tracked. If, for a given birth order, fronds that are folded consistently have higher (lower) fitness than nonfolded fronds, then our study may have underestimated (overestimated) the decline in offspring fitness with increasing birth order. As far as we can tell, whether or not a frond is folded has little bearing on its fitness. In a previous study (Barks & Laird, 2015), we were able to track the reproduction of folded fronds over the duration of their lives (in that study we used a different genetic strain, and all fronds had ancestral birth order N_P-N_P-N_P-N_P = 1-1-1-1). Data from that study indicate that, for a given parental age, folded and nonfolded fronds have similar fitness (Fig. S3).

Conclusions

A recently developed modelling approach (Pavard *et al.*, 2007a,b; Pavard & Branger, 2012) allows for the incorporation of parental age effects into classic population projection analyses, assuming that the parental age effects are limited to a single generation. Our results suggest that this assumption holds in *L. minor*, at least with respect to a composite measure of offspring fitness – the individual intrinsic rate of increase. Whereas Pavard and colleagues' work was based on maternal

age effects on juvenile traits, the parental age effect we observed in *L. minor* affected adult traits (there is no juvenile period in *L. minor*) and thus may modify the force of selection in ways that have yet to be described. Following Kern *et al.* (2001), we suggest that an increased incorporation of parental age effects into evolutionary theory on senescence will further our understanding of the selective forces that have led to the remarkable diversity in patterns of senescence that exists in nature.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Intrinsic rate of increase and frond surface area by pocket-specific birth order (data from Barks & Laird, 2015).

Figure S2 Photographs depicting morphological differences between first ($N_P = 1$) and subsequent ($N_P > 1$) offspring.

Figure S3 Intrinsic rate of increase by parental age for 'folded' and 'normal' fronds (data from Barks & Laird, 2015).

Table S1 Example demonstrating our imputation method for skipped censuses.

Appendix S1 Parental age effects.

Appendix S2 Method for creating a sterile stock culture.

Data deposited at Dryad: doi:10.5061/dryad.m7n13

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