

An experimental test for age-related improvements in reproductive performance in a frog that cares for its young

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Abstract Reproductive performance often increases with age in long-lived iteroparous organisms, a pattern that can result from within-individual increases in effort and/or competence. In free-living populations, it is typically difficult to distinguish these mechanisms or to isolate particular features of reproduction-influencing outcomes. In captive *Oophaga pumilio*, a frog in which mothers provide extended offspring provisioning via trophic eggs, we experimentally manipulated the age at which females started breeding and then monitored them across repeated reproductive events. This experiment allowed us to decouple age and experience and isolate maternal care as the proximate source of any differences in performance. Younger first-time mothers produced larger broods than older first-time mothers, but did not rear more offspring to independence. Across repeated reproductive events, maternal age was unassociated with any metric of performance. At later reproductive events, however, mothers produced fewer metamorphs, and a lower proportion of individuals in their broods reached independence. These patterns suggest that performance does not improve with age or breeding experience in this frog, and that eventual declines in performance are driven by reproductive activity, not age per se. Broadly, age-specific

patterns of reproductive performance may depend on the proximate mechanism by which parents influence offspring fitness and how sensitive these are to effort and competence.

Keywords Breeding experience · Life history · Parental care · Reproductive success · Senescence

Introduction

Age and reproductive performance are often positively associated in iteroparous animals, especially early in life (Forslund and Pärt 1995). In the wild, this pattern can arise from the non-random appearance or disappearance of phenotypes in the breeding population; higher quality individuals may, for example, live longer and be better breeders (Cam and Monnat 2000). Positive associations between age and reproductive performance can also result from within-individual processes. Age-related increases in performance can stem from increased effort at older ages, when residual reproductive value is lower (the “restraint hypothesis”: Martin and Festa-Bianchet 2011). Older individuals might also perform better because they are more competent (the “constraint hypothesis”). Parental care often requires traits that individuals have never before expressed, and so care quality seems especially likely to improve with experience, particularly across early reproductive events (Daunt et al. 1999; Balbontin et al. 2007; Limmer and Becker 2009). Disentangling the effects of age and experience is difficult in nature because relatively experienced individuals are typically also relatively old (Forslund and Pärt 1995). Even when experience is clearly important, the mechanisms underlying improvements in competence often remain unclear (e.g., more skilful foraging vs. better access to prey via social dominance).

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We used a captive breeding experiment to test for age-related improvements in the reproductive performance of a frog (*Oophaga pumilio*) in which mothers provision developing tadpoles with frequent deliveries of trophic eggs (Brust 1990; Maple 2002). By manipulating the age at which females were first allowed to breed, we were able to decouple the effects of age and experience on three metrics of performance at each reproductive event: (i) initial brood size (tadpole number), (ii) reproductive success (metamorph number), and (iii) care efficiency (proportion of a brood that completed metamorphosis). We began by testing the prediction, unique to the restraint hypothesis, that older first-time mothers would produce and rear more offspring than younger first-time mothers (Cam and Monnat 2000). We then monitored female reproduction across multiple reproductive events, the wide range of initial pairing ages allowing us to test both the restraint and constraint hypotheses by comparing females of similar age but different experience (and vice versa). Furthermore, by studying frogs in captive conditions and thus removing the confounds of resource limitation and mate quality, we could isolate the quality of maternal care as the likely source of any age- or experience-related changes in performance.

Materials and methods

O. pumilio is a small terrestrial frog native to Central America. Females lay clutches of approximately five eggs in the leaf litter, where they are tended by males (Pr hl 2005). Most clutches fail to hatch or hatch incompletely, and tadpoles from successful clutches are moved individually to rearing sites (e.g., leaf axils) by mothers (Pr hl 2005). Females regularly revisit these sites during the ~45-day tadpole development period, providing the trophic eggs upon which tadpoles depend (Brust 1990; Maple 2002). From April, 2011, to May, 2014, we monitored the reproduction of captive-bred female *O. pumilio* in a colony at Tulane University (LA, USA), initially established with wild-caught frogs from four populations. Female reproductive performance does not differ among populations and does not depend on whether her mate is from the same or a different population (Dugas and Richards-Zawacki 2015). Details on captive breeding are available in Dugas and Richards-Zawacki (2015). Briefly, one male and one female were held together and provided with four tadpole deposition sites (the maximum reported brood size in the wild: Brust 1990). Frogs were fed with live *Drosophila melanogaster*, and food was not limiting. We monitored tadpole deposition and development with twice-weekly censuses, moving any new metamorphs to separate rearing tanks. Captive-bred females were moved from their juvenile rearing tanks into the breeding population when breeding females died and/or other research priorities necessitated the establishment of new pairs, and so they were first paired with a mate at

haphazard ages. The ranges of initial pairing age (86–968 days post-metamorphosis) and age at first tadpole production (253–1264 days) span most of the expected lifespan in the wild (Dugas et al. 2015).

To test for the predicted increase in performance with age and experience, we assessed three metrics of performance at each reproductive event: (i) initial brood size, (ii) number of metamorphs produced, and (iii) efficiency, or the proportion of the brood that survived through metamorphosis (metamorphs/brood size). We began by asking whether maternal age at first reproduction was associated with each response variable using generalized linear models. For all three models, we initially included both linear and quadratic effects of female age and the linear effect of “days paired prior to first tadpole production.” The latter effect accounts for age at first reproduction being shaped both by age at pairing and subsequent female mating decisions.

We then tested the prediction that across repeated reproductive events, maternal age and reproductive event number (i.e., experience) would be positively associated with our three metrics of performance. We used generalized linear mixed models with random intercepts fitted for each female; 6 of 22 females mated with 2 males during the study period, and so we also fit random intercepts for male nested within female. We initially included both the linear and quadratic effects of age and reproductive event number in all models, as age- or experience-related changes in reproductive performance may plateau or even reverse direction (Forslund and Pärt 1995). When non-significant, we removed quadratic terms and re-tested linear effects. We also included the covariate of “time since last reproductive event” to account for any influence of recovery on performance (Cam and Monnat 2000); this required the exclusion of first reproductive events in this analysis. In models of efficiency, we examined the linear and quadratic effects of brood size because larger broods might be more difficult to rear; neither effect was ever significant (all $p > 0.380$), and so, we removed it from models presented in the “Results” section.

We used PROC GENMOD and GLIMMIX in SAS (v9.2) for all analyses. For brood size and metamorph number, we specified a Poisson error distribution, and we used the events/trial syntax with a binomial error distribution for models of metamorphs/brood size. Because of scaling differences, we Z-transformed all predictor variables except “reproductive event number” prior to analysis.

Results

We observed the first reproductive event of 26 captive-bred females. The quadratic effect of age on performance was never significant (all $\chi^2_1 < 0.4$, $p > 0.531$), and so we removed it from all models. Older first-time mothers produced smaller

broods at their first reproductive event ($\chi^2_1=4.5, p=0.034$; Fig. 1), but female age was not associated with the number of metamorphs produced ($\chi^2_1<0.1, p=0.895$; Fig. 1) or metamorphs/brood size ($\chi^2_1=1.2, p=0.278$; Fig. 1) at this event. Time between pairing and first reproduction was non-significant in all models ($\chi^2_1<2.4, p>0.124$).

We examined the effects of age and reproductive event number (i.e., experience) across ≥ 3 reproductive events in 22 captive-bred females (mean \pm SD: 8.0 \pm 4.0 events; range: 3–17 events). The quadratic effect of age was never a significant predictor of performance ($\chi^2_1<1.0, p>0.314$), and so we removed it from all models presented below. Initial brood size was not associated with the linear effect of age ($\chi^2_1=0.2,$

$p=0.653$; see Fig. S1 in the electronic supplementary material) or the linear and quadratic effects of reproductive event number (linear: $\chi^2_1<0.1, p=0.869$; quadratic: $\chi^2_1=0.2, p=0.647$; linear alone: $\chi^2_1=1.3, p=0.261$; Fig. 2). The number of metamorphs produced was not associated with maternal age at the reproductive event ($\chi^2_1<0.1, p=0.949$; Fig. S1) but was marginally associated with both a negative linear and positive quadratic effect of reproductive event number (linear: $\chi^2_1=3.8, p=0.052$; quadratic: $\chi^2_1=3.4, p=0.064$; Fig. 2). Metamorphs/brood size was similarly unrelated to maternal age ($\chi^2_1>0.1, p=0.774$; Fig. S1) but was associated with both

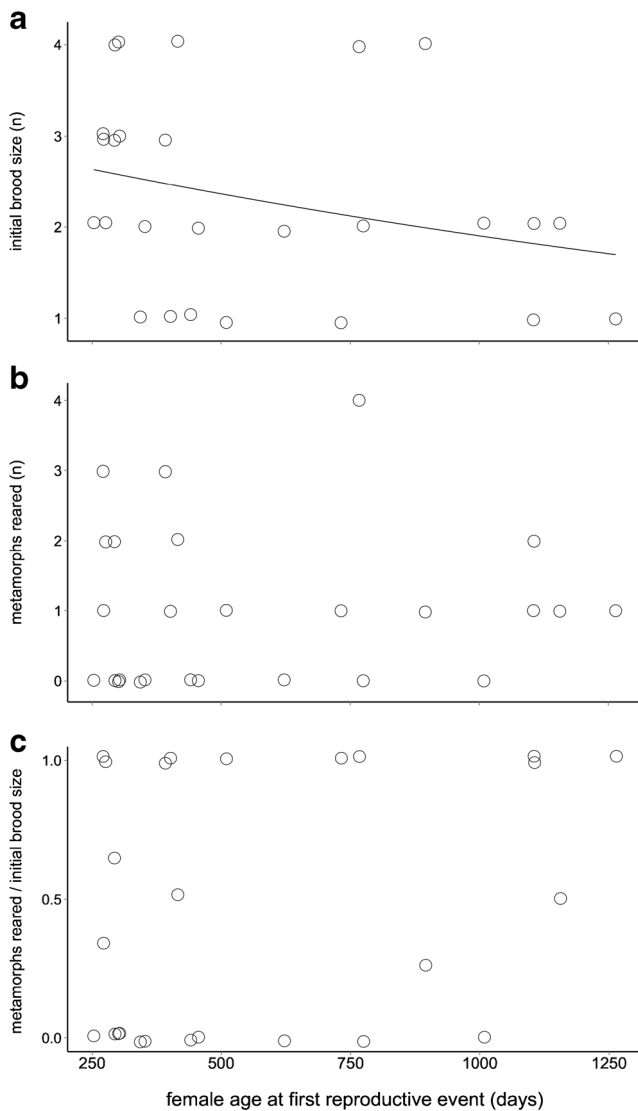


Fig. 1 The relationships between captive *O. pumilio* females' age at first reproduction and initial brood size (a), number of metamorphs a female reared at this first reproductive event (b), and the ratio of these two parameters (c) at this first reproductive event. Each data point represents a single female's first reproductive event, and regression lines indicate significant ($p<0.05$) relationships. Data are jittered

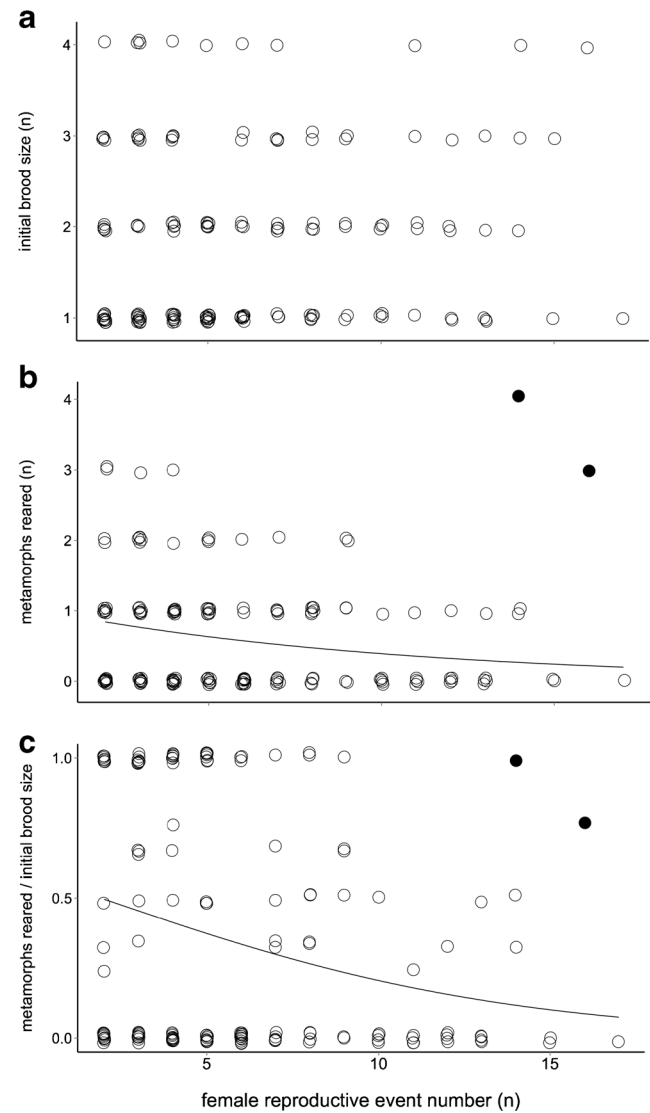


Fig. 2 Reproductive performance of captive *O. pumilio* females across repeated reproductive events. We considered three metrics of performance: initial brood size (a), number of metamorphs a female reared from a brood (b), and the ratio of these two parameters (c). Filled circles denote extreme observations that drove quadratic effects in initial models. Each data point represents a single reproductive event, and regression lines indicate significant ($p<0.05$) linear relationships with outliers removed. Data are jittered

a negative linear and positive quadratic effect of reproductive event number (linear: $\chi^2_1=4.9$, $p=0.027$; quadratic: $\chi^2_1=4.1$, $p=0.044$; Fig. 2). Recovery time was never significantly associated with any metric of reproductive performance (all $\chi^2_1 < 1.3$, $p > 0.240$).

Two unusually successful reproductive events by experienced mothers (Fig. 2) drove the quadratic relationships between reproductive event number and metamorph production and efficiency. After removal of these observations (and the then non-significant quadratic effects: both $\chi^2_1 < 0.1$, $p > 0.908$), linear associations between reproductive event number and metamorph production and efficiency remained negative and significant (metamorphs: $\chi^2_1=7.3$, $p=0.007$, Fig. 2; metamorphs/brood size: $\chi^2_1=7.4$, $p=0.006$, Fig. 2).

Discussion

By experimentally manipulating age at first reproduction across much of the expected lifespan, we were able to assess the independent effects of age and experience/reproductive activity on maternal performance in a frog that cares for its young. While age-related improvements in performance are predicted to arise via numerous mechanisms, reproductive performance instead declined with age and experience in captive *O. pumilio* mothers. Contrary to the predictions of the restraint hypothesis, younger first-time mothers produced larger, not smaller, broods. Younger first-time mothers did not, however, differ from older ones in the number of offspring reared to independence. Despite these patterns, the age of first-time mothers was not significantly related to the efficiency of care, likely because so many broods failed to produce any metamorphs (Figs. 1 and 2). Across repeated reproductive events, we found no evidence of the positive effect of maternal age on performance predicted by the restraint hypothesis or for the eventual decline in performance expected with age-dependent senescence (Martin and Festa-Bianchet 2011). While the constraint hypothesis predicts a positive association between experience and performance, more experienced *O. pumilio* mothers instead reared a smaller proportion of each brood to independence and produced fewer independent offspring.

Given benign captive conditions in this experiment, variation in the quantity and/or quality of maternal care seems the most likely proximate source of variation in reproductive performance. In the wild, both the size and frequency of trophic egg meals are important to successful *O. pumilio* development (Maple 2002). A decline in egg quality seems a good proximate candidate to mediate the negative associations between performance and age at first reproduction and subsequent breeding experience (Moore and Harris 2003; Beamonte-Barrientos et al. 2010), as egg quality could shape hatching success, initial tadpole size, and later development (Maple

2002). Overall, these patterns suggest that the physiological underpinnings of reproductive success in *O. pumilio* are constrained by age-independent (but not age-dependent) senescence, and they highlight the importance of this often-overlooked mechanism (Martin and Festa-Bianchet 2011). Generally, post-zygotic care can buffer differences in other aspects of performance (e.g., physiological declines; Beamonte-Barrientos et al., 2010). In the taxonomically diverse animals that provision young with trophic eggs, however, morphological or physiological constraints on oogenesis, along with associated physiological costs (Dugas et al. 2015), might limit mothers' ability to adjust post-zygotic investment facultatively.

Explaining among-individual variation in reproductive success is central to understanding and predicting ecological and evolutionary dynamics in natural populations. Although age is associated with reproductive performance across taxonomic groups, the causes and consequences of this pattern might differ among groups. It remains unclear whether a comprehensive explanation for this pattern exists, much less what this explanation might be (Forslund and Pärt 1995; Moore and Harris 2003; Martin and Festa-Bianchet 2011). Ultimately, selection on aging and longevity depends critically on whether and how performance is sensitive to age-related physiological and behavioral changes. Our work underscores the importance of broad taxonomic sampling when testing life-history theory and suggests that further refinement of this theory will require predictions and explanations that accommodate the diverse ways parents influence the fitness of their offspring.

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