



Age-related prenatal maternal effects and postnatal breeding experience have different influences on nestling development in an altricial passerine

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Reproductive success and nestling performance are related to the age of parents across several vertebrate taxa. However, because breeding experience and prenatal maternal investment in reproduction often covary, the source of these age-related differences can be difficult to determine. In this study, we evaluated the influence of prenatal maternal effects and postnatal breeding experience on the performance of nestling tree swallows *Tachycineta bicolor* by conducting a carefully controlled partial cross-fostering experiment. We swapped half-broods of nestlings between the nest of a young first-time breeding female and the nest of a female known to have previously raised and fledged young. Our manipulation did not influence the within-brood nestling hierarchies, and controlled for the effects of egg laying order. We found that nestlings of older females were heavier just prior to fledging regardless of the breeding experience of the attending female. In addition, fledglings raised by experienced females grew their flight feathers faster, and had greater probability of fledging. Our study demonstrates that prenatal investment in reproduction by older females can have long-term consequences on nestling mass, and suggests limited potential for compensatory mass gains prior to fledging. Because our analyses controlled for feeding rates, our results also suggest that foraging quantity and quality are not the only benefits nestlings gain by being raised by an experienced female.

Reproductive success and offspring performance is age-related in a large number of vertebrates (Clutton-Brock 1988, Pusey 2012). Older females produce larger and heavier offspring (Kindsvater et al. 2012, Kindsvater and Otto 2014), which often have faster rates of growth, greater chances of survival (Blomqvist et al. 1997), and longer lifespans (Fox et al. 2003). These age-related effects are the result of differential prenatal maternal investment, prenatal breeding experience, postnatal breeding experience, and life-history strategies. In birds for example, older females lay larger eggs (Christians 2002) that produce larger nestlings at hatch (Bitton et al. 2006, Krist 2011). Furthermore, older individuals are better foragers in several species (reviewed by Marchetti and Price 1989) which can lead to greater rates of growth (McCarty 2001). The effects of maternal investment and breeding experience can persist into adulthood, therefore influencing offspring fitness (Giordano et al. 2014), and population dynamics (Benton et al. 2008). However, age-related traits of breeding adults often covary, making it difficult to disentangle their individual contribution to offspring development and success (Forslund and Pärt 1995, Bize et al. 2002). As a consequence, the independent effects of prenatal and postnatal maternal effects are poorly understood.

Prenatal investment in the eggs of birds is in the form of protein-rich albumin, fats and carotenoids in the yolk, and several hormones and metabolites such as corticosterone and testosterone (Sotherland and Rahn 1987, Schwabl 1993, Williams 1994, Hayward and Wingfield 2004), many of which have been found to influence nestling development (Saino et al. 2003, 2008, Bonisoli-Alquati et al. 2008, Love and Williams 2008, Ismail et al. 2013). Age-related maternal effects manifest themselves when females that are older and/or in better condition transfer larger amounts of beneficial resources to their eggs (Hargitai et al. 2006, Hasselquist and Nilsson 2009). However, these older females are also generally more experienced breeders, and are capable of investing more in eggs and incubation prior to hatch, and higher quality care to nestlings. Indeed, age-related improvements in foraging and breeding skills are also associated with greater nestling performance. For example, older birds often breed on higher quality sites than younger ones (Greenwood and Harvey 1982), a large contributing factor to reproductive success (Pärt 2001), and nestling survival can be influenced by age-related and condition-dependent incubation patterns (Bogdanova et al. 2007, Tombre et al. 2012).

The difficulties in separating the contribution of age-related effects on offspring performance results from the

multiple factors that can affect nestling development. These include nestling size hierarchies due to the laying schedule (1 egg a day in most passerines) and onset of incubation, sequential increases in egg mass throughout the laying sequence (Bitton et al. 2006), and variation in allocation of hormones and metabolites to eggs throughout the laying sequence (reviewed by Grootuis et al. 2005). To circumvent these issues, several studies have used cross-fostering designs to disentangle the source of variation; full broods of nestlings have been swapped between older and younger adults (Bize et al. 2002, Hegyi et al. 2006). These studies have been very successful at establishing relationships between performance and maternal effects, but some of the covariance between pre- and postnatal effects is not completely accounted for by this experimental design (details in Krist and Remeš 2004). Furthermore, first-time breeding females cannot always be confirmed with 100 percent certainty, making it difficult to efficiently pair nests of experienced and inexperienced females. In this study, we circumvented these issues by performing a highly controlled partial cross-fostering experiment using tree swallows *Tachycineta bicolor*.

Tree swallows are one of only two species of North American passerines where females (as opposed to males) delay plumage maturation (Winkler et al. 2011, Coady and Dawson 2013). Females returning for the first time from the wintering grounds (second year: SY), have dull brown plumage with a limited number of iridescent feathers; older females (after second year: ASY) exhibit male-like iridescent plumage (Hussell 1983). In natural populations where tree cavity nest sites are limited, SY females have reduced opportunities to reproduce (Rosvall 2008). However, when nesting sites are abundant, SY females will often secure a mate and raise young. Females are the primary care giver at all stages of reproduction: only females incubate the eggs and brood the nestlings at night (Winkler et al. 2011), and even though males provision nestling throughout the day, female visitation rates are on average much higher (males: ~ 6.9 feeds per hour, females: ~ 8.3 feeds per hour; Lombardo 1991, McCarty 2002). In general, older female tree swallows invest more in reproduction and have greater reproductive success than younger females (Robertson and Rendell 2001). ASY females initiate nests earlier (De Steven 1978, Robertson and Rendell

2001), lay larger clutches (De Steven 1978, Robertson and Rendell 2001) with heavier eggs (De Steven 1978, Wiggins 1990, Ardia et al. 2006b), and have greater hatching and fledging success (De Steven 1978, Robertson and Rendell 2001, Bitton et al. 2006). For all these reasons, tree swallows are an excellent model to investigate age-related differences in reproductive success and nestling performance.

In this study, we conducted a cross-fostering experiment designed to disentangle prenatal maternal effects from postnatal breeding experience effects on nestling performance and probability of fledging in tree swallows. Half-broods from inexperienced SY females were swapped with half-broods of ASY females which were known to have previously raised young. We predicted, based on results from previous full-brood cross-fostering experiments (Wolf et al. 2011), that nestling size and mass prior to fledging would be influenced by prenatal maternal effects. In contrast, because they are often influenced by microhabitat conditions (Dawson et al. 2005, Pérez et al. 2008, Ardia et al. 2010), we predicted that growth rates of mass and size, as well as measures of immunocompetence and probability of fledging, would be more heavily influenced by the age of the attending mother.

Methods

This study was conducted on a population of tree swallows breeding in nest boxes near Prince George BC, Canada (53°45'N, 122°32'W). Adults breeding at this site have been captured and banded since 2000. During the breeding seasons of 2005 and 2006 the site held 125 nest boxes mounted on fence posts, placed approximately 20–30 m apart. We visited the nest boxes every day starting in early May, keeping track of the nest building and egg laying progress until all birds had completed egg laying. During the incubation period, we caught females on nests and determined their age as either SY or ASY based on plumage characteristics (Hussell 1983) or bands when available.

On the day after hatching was completed, we swapped half the nestlings between two nests, one attended by a SY female, the other by an ASY female (Fig. 1). When nesting cavities are limited, SY females may not get the opportunity to breed

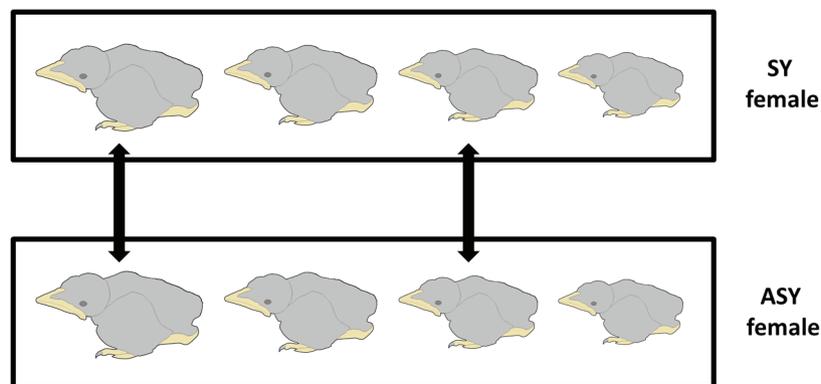


Figure 1. Experimental design for cross-fostering half of the offspring from the nest of a second year (SY) female tree swallow with those from the nest of an after second year (ASY) female. Illustrations depict nestlings in the order they hatched. Nests (denoted by boxes) and swapped nestlings were selected so that nest hierarchies were not modified. In paired nests that contained either six and/or seven nestlings three young were swapped, in paired nests with five and/or four nestlings two young were swapped. For simplicity, only a swap involving nests with four nestlings each is illustrated.

(Rosvall 2008); to avoid including in our experiment an ASY female that had no prior breeding experience, only ASY females that were known to have successfully raised young in a previous year were selected when matching nests. Furthermore, the nests of individual females were used only in one year of the study. The nests used for the exchange of nestlings were selected following several criteria. We chose nests that hatched on the same day and, when possible, had the same clutch size (population mean clutch size across both years: SY = 5.38 ± 0.86 SD, ASY = 6.12 ± 0.78 SD, $t_{2,105} = 4.25$, $p < 0.01$). We never paired nests that had clutches that differed by more than one egg. When more than one SY/ASY pair combination was possible (e.g. two ASY nests and one SY nest of same clutch size that all hatched on the same day), we paired the nests for which the average mass of the nestlings (measured using an electronic scale to the nearest 0.01 g) were most similar. In nine cases, paired nests had the same number of nestling; in paired nests that contained either six and/or seven nestlings we swapped three nestlings, in paired nests with five and/or four nestlings we swapped two. In four instances we paired a nest of five eggs with one of four eggs; in these cases, we swapped only two nestlings. To avoid the potential effects of laying order on our experiment (Schwabl 1993, Török et al. 2007), we exchanged, in broods with 6 or 7 nestlings, an early, middle and late hatched chick (based on mass; Clotfelter et al. 2000). In broods of 5 nestlings we exchanged an early and middle, middle and late, or early and late hatched chick. Furthermore, all swaps were conducted only if they did not influence the position of nestlings in the mass hierarchies of either nest. We succeeded in pairing 14 nests which contained 76 nestling in 2005, and 12 nests which contained 67 nestlings in 2006 for a total of 13 experimental pairs of broods.

All nestlings were weighed to the nearest 0.125 g using a spring scale every 2 d, from ages 2 to 16 d. The length of the ninth primary flight feather was measured to the nearest 0.5 mm with a ruler, from day 8 to day 16, as primary feathers of most nestlings do not begin growing until 6 d of age (Dawson and Bidwell 2005). For each nestling, we calculated growth rate constants for body mass and ninth primary using a logistic and linear model, respectively (details in Dawson et al. 2005). Nine days after hatch, we evaluated the T-cell mediated immune response (CMI) of nestlings. We injected 30 μ l of 2 mg ml⁻¹ phytohaemagglutinin-P (Sigma) in phosphate buffered saline (pH 7.4) under the epidermis of the right wing web using a 30-gauge needle and measured the thickness of the wing web (to the nearest 0.01 mm) with a pressure sensitive thickness gauge immediately prior to and 24 h \pm 15 min standard error (SE) after the injections. Because wing thickness may change naturally during the 24-h period, the left wing web was also measured. CMI was calculated as the difference in thickness of the wing web before and 24 h after the injection, minus the change in thickness in the control wing web (Smits et al. 1999). We quantified parental provisioning rates when nestlings were 10, 11, and 12 d old by observing each nest for 1 h between 06:30 and 12:30 h, providing accurate estimates of food delivery by adult tree swallows during the peak demand by nestlings (McCarty 2002). To account for potential effects of weather and time of day, two observers watched a grouped

pair of nests (i.e. from which nestlings were cross-fostered) simultaneously. Observations never occurred during rainy periods and hourly provisioning rates over the three-day period were averaged for each nest prior to analyses. Soon after hatch, we captured the female and male attending the nestlings and measured mass to the nearest 0.125 g and length of their wings to the nearest 1.0 mm. To determine if female age was related to male age (which could influence parental care; Hegyi et al. 2006) we estimated the age of the attending male at each nest. Age of male tree swallows cannot be determined according to plumage characteristics but they have high nest-site fidelity (Winkler et al. 2011) and fewer than 5% change breeding sites throughout their lifetime (Winkler et al. 2004). Therefore, we considered all males banded in previous years as ASY, and males without bands as SY (following Bitton and Dawson 2008). We visited the nests regularly until all nestlings had fledged, and noted any individuals that died before leaving the nest.

We investigated the influence of maternal female age and breeding experience on nestling performance while controlling for feeding rates and year effects (independent fixed effect factors). We analyzed as dependent variables the rates of mass and feather growth because these attributes are often associated with female quality (Bitton et al. 2008), mass and feather length at day 16 and cell-mediated immunocompetence because these metrics are correlated with post-fledging success (Tella et al. 2000, Monrós et al. 2002). We also determined the influence of female age and experience on the probability that a nestling would fledge (fledged = 1, did not fledge = 0). We fit linear mixed models using restricted maximum likelihood – ‘lmer’ from the package ‘lme4’ (R Development Core Team, Bates et al. 2012) for continuous dependent variables (rate of mass gain, mass at day 16, primary feather growth rate, primary length at day 16, and cell-mediated immunocompetence), and used the ‘glmer’ function to fit a generalized linear mixed model to assess the influence of all the fixed effect factors on probability of fledging (binomial distribution with log-link function). In these models we included data available for all nestlings and set the nest of origin, nest raised, and an identifier of paired nests as random factors. Removal of the identifier of paired nest random factor did not influence any of the results and so was not included in the final models. To avoid the shortcomings of stepwise multiple regression (Whittingham et al. 2006 and references therein) we first analyzed complete models which included the interaction between maternal and brood-rearing female age; if the interaction term was not significant it was removed to investigate only the main effects. Full models which include non-significant terms are appropriate when the main effects investigated come from experimental manipulation, as the parameter estimates of the main effects should not be extensively influenced by the inclusion of other factors (Burnham and Anderson 2002, Whittingham et al. 2006). Variance explained by the fixed effects (marginal R²), and variance explained by the fixed effects and random variables (conditional R²) were calculated as described in Nakagawa and Schielzeth (2013). We estimated p-values for the fixed effects by iteratively comparing the full model to one reduced by each factor using likelihood ratio tests.

We compared the mass and size of attending adults between cross-fostered nests, and feeding rates, using paired t-tests and determined if females were paired with males of similar age using a McNemar's test with continuity correction. We report means \pm SE unless stated otherwise.

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.6q711>> (Bitton and Dawson 2016).

Results

We found an effect of female age on nestling mass at day 16, growth of the ninth primary feathers, and fledging success (Table 1). Nestlings hatched from eggs laid by ASY females ($21.25 \text{ g} \pm 0.27 \text{ g}$; Fig. 2) were heavier prior to fledging than those of SY females ($20.46 \text{ g} \pm 0.25 \text{ g}$). The mass of nestlings prior to fledging also decreased with later hatching dates, and varied between years, but was not influenced by the age of the attending female or feeding rates. Nestlings

attended by ASY females grew their ninth primary feather faster ($4.84 \pm 0.11 \text{ mm d}^{-1}$; Fig. 3A) than those attended by SY females ($4.53 \pm 0.11 \text{ mm d}^{-1}$). Feather growth rates also increased with feeding rates, differed among years, but were not influenced by the age of the maternal female or hatching date. Nestlings attended by ASY females had greater probability of fledging (0.99 ± 0.02 ; Fig. 3B) when compare to nestlings attended by SY females (0.89 ± 0.08). Fledging success also increased with feeding rate and differed among years, but was not influenced by maternal age or hatching date.

We did not find any effect of female age on nestling mass growth rates, ninth primary feather length at day 16, cell-mediated immune response, or recruitment (Table 1). Nestling mass growth rates were different between years, but were not influenced either by maternal age, attending female age, hatching date, or feeding rates. Similarly, the cell-mediated immune responses of nestlings was different between years but were not influenced by maternal age, attending female age, hatch date, or feeding rate. None of the independent

Table 1. Summary results from generalized linear mixed models investigating the effect of age-related maternal investment and breeding experience on the attributes of nestling tree swallows. Parameter estimates of age-related factors (maternal and attending female age) are presented as after second year in relation to second year females. Factors in bold are significant in likelihood ratio tests.

Nestling attribute (n)	Marginal R^2	Conditional R^2	Parameters	Estimate \pm SE	t	p
Growth mass (127)	0.152	0.786	Maternal age	-0.006 ± 0.011	-0.499	0.615
			Attending age	0.009 ± 0.029	0.308	0.735
			Feeding rate	-0.142 ± 0.114	-1.244	0.169
			Hatch date	0.001 ± 0.004	0.167	0.853
			Year	0.057 ± 0.031	1.824	0.048
Mass day 16 (125)	0.236	0.435	Maternal age	0.788 ± 0.336	2.346	0.019
			Attending age	0.320 ± 0.269	1.191	0.205
			Feeding rate	0.299 ± 1.046	0.286	0.734
			Hatch date	-0.104 ± 0.049	-2.118	0.025
			Year	-0.827 ± 0.385	-2.148	0.023
Growth 9th primary (127)	0.320	0.700	Maternal age	0.039 ± 0.055	0.712	0.460
			Attending age	0.306 ± 0.156	1.954	0.035
			Feeding rate	1.127 ± 0.608	1.853	0.044
			Hatch date	-0.011 ± 0.020	-0.561	0.525
			Year	0.434 ± 0.160	2.718	0.005
9th primary day 16 (125)	0.142	0.678	Maternal age	-0.841 ± 0.618	-1.360	0.178
			Attending age	2.427 ± 1.861	1.304	0.152
			Feeding rate	-0.016 ± 7.226	-0.002	0.999
			Hatch date	-0.333 ± 0.242	-1.379	0.126
			Year	3.308 ± 1.911	1.731	0.059
Cell-mediated immunoresponse (131)	0.235	0.293	Maternal age	-0.007 ± 0.038	-0.194	0.840
			Attending age	0.049 ± 0.045	1.074	0.241
			Feeding rate	0.051 ± 0.178	0.289	0.760
			Hatch date	0.007 ± 0.006	1.156	0.207
			Year	-0.246 ± 0.047	-5.280	<0.001
Fledged* (133)	0.434	0.629	Maternal age	0.449 ± 0.661	0.680	0.469
			Attending age	2.475 ± 1.216	2.035	0.022
			Feeding rate	11.335 ± 5.375	2.109	0.046
			Hatch date	0.072 ± 0.193	0.370	0.797
			Year	2.024 ± 1.129	1.792	0.039

* logistic regression presents z scores, not t scores.

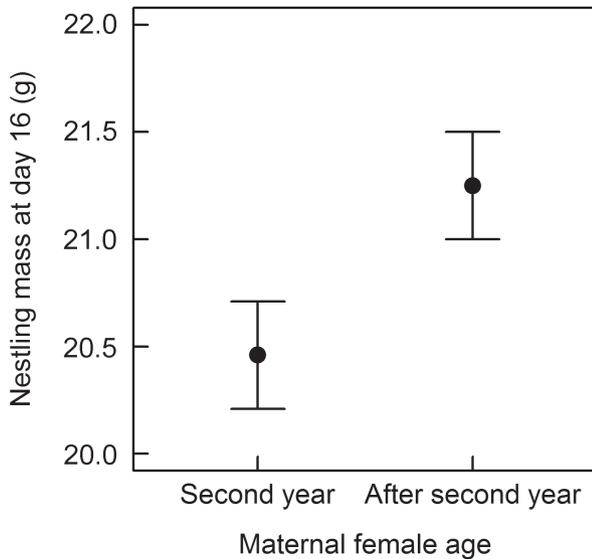


Figure 2. Nestling tree swallows hatched from eggs laid by after second year females were heavier at 16 d of age (just prior to fledging) than those hatched from eggs laid by second year females. Means and standard errors were estimated after controlling for other independent factors in a linear mixed model (see Methods for details) which included nest of origin and nest raised in as random factors.

factors investigated influenced the length of ninth primary feather at day 16.

Within each pair of nests, ASY females were heavier ($20.00 \text{ g} \pm 0.45 \text{ g}$) than SY females ($18.98 \text{ g} \pm 0.22 \text{ g}$, paired t -test: $n = 13$, $t = -2.67$, $p = 0.021$), but did not differ in length of wing (ASY: $115.31 \text{ mm} \pm 0.92 \text{ mm}$, SY: $114.12 \text{ mm} \pm 0.79 \text{ mm}$, paired t -test: $n = 13$, $t = -1.07$, $p = 0.301$). Similarly, the social mates of ASY females were heavier ($20.17 \text{ g} \pm 0.24 \text{ g}$) than males paired with SY females ($19.44 \text{ g} \pm 0.26 \text{ g}$, paired t -test: $n = 13$, $t = -2.66$, $p = 0.021$), but had comparable lengths of wings (males paired with ASY: $119.77 \text{ mm} \pm 0.61 \text{ mm}$, males paired with SY: $119.85 \text{ mm} \pm 0.79 \text{ mm}$; paired t -test: $n = 13$, $t = 0.08$, $p = 0.936$). Feeding rate at nests of SY female were no different than those at nests of ASY females ($t = -0.93$, $DF = 11$, $p = 0.37$). Even though SY females were generally paired with males apparently breeding at the site for the first time while ASY females were paired with recaptured individuals, the trend was not statistically significant (McNemar's test: $n = 13$, $\chi^2 = 3.125$, $p = 0.077$).

Discussion

Maternal effects regulate a number of offspring traits throughout the nestling period in birds (Mousseau and Fox 1998). However, covariance between prenatal investment and postnatal care has limited our understanding of the independent contribution of age-related effects on offspring development. By performing a partial cross-fostering experiment that controlled for hatching date, nestling mass hierarchies, and laying order (Fig. 1), we partly disentangled the influence of age-related breeding investment and age-related breeding experience on nestling performance in tree

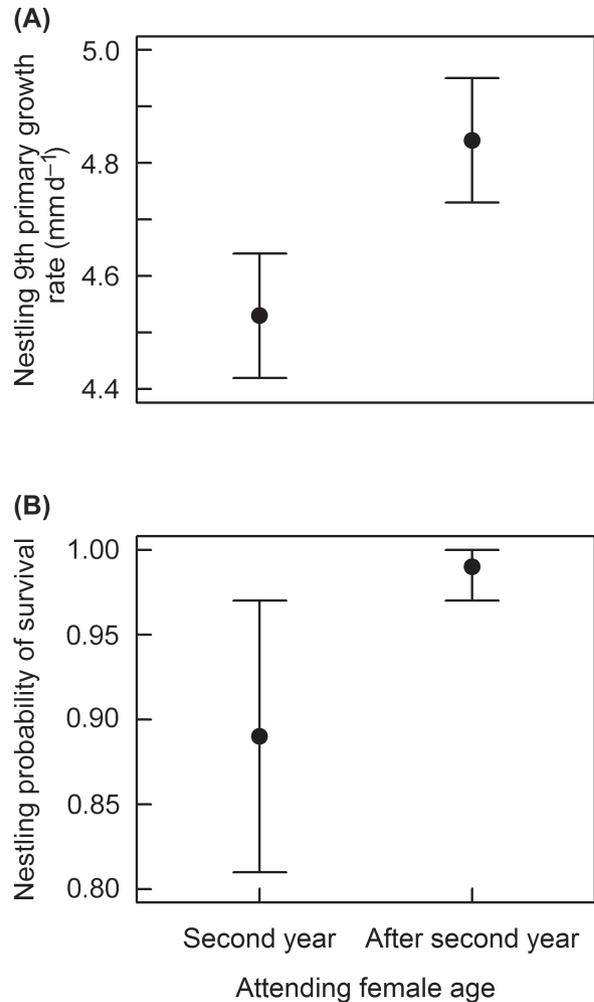


Figure 3. Nestling tree swallows raised by experienced after second year females (A) grew their ninth primary flight feathers faster, and (B) had greater probability of fledging than those raised by inexperienced, second year females. Means and standard errors were estimated after controlling for other independent factors in a linear mixed model (see Methods for details) which included nest of origin and nest raised in as random factors.

swallows. Nestlings produced by older females were heavier just prior to fledging, and nestlings raised by older females had greater growth rates of flight feathers, and marginally greater probability of fledging. This study demonstrates that differences in prenatal investment and postnatal breeding experience have long-term consequences on nestlings, independently of one another.

Mass just prior to fledging, which has been associated with the probability of post-fledging survival (Monrós et al. 2002), was influenced by maternal female age (Fig. 2), not the age of the female that raised the brood. As predicted, nestlings of ASY females were on average heavier than nestlings of SY females. It is possible that ASY females laid larger eggs, data we did not collect, which correlate with nestling mass early in the brood rearing period in swallows (Bitton et al. 2006). Other studies have found a link between egg and nestling mass in cross-fostering experiments as well (Styrsky et al. 1999), but even if larger eggs are often laid by older females in a number of bird species, the effects of large

eggs are not often detectable just prior to fledging (Krist 2011), as is the case in tree swallows (Bitton et al. 2006). It is probable then that older females have greater capacity for investing metabolites and/or hormones in the eggs such that nestlings can use these compounds to grow larger (Bogdanova et al. 2006), independently of feeding rates and brood care received. Alternatively, it is also possible that the ASY cohort is composed of individuals that are better locally adapted than the SY cohort (Brooks and Kemp 2001) but our results are in agreement with others which have shown that maternal effects, particularly prenatal investment, seem more important than genetics in determining mass of offspring during brood rearing (Wolf et al. 2011). The relationship between nestling mass and investment in the egg seems strong. In one study for example, the mass of nestlings that received supplemental feeding until they fledged was no heavier than nestlings that did not receive supplemental feeding but whose mothers had received extra food prior to the egg laying period (Giordano et al. 2014).

Nestlings raised by more experienced breeders had greater feather growth rates (Fig. 3A) and greater probability of survival to fledging, even after controlling for feeding rates (Fig. 3B). This suggests that other aspects of parental care, such as better management of daily activity budgets (Røskaft et al. 1983) and/or quality and size of the food bolus delivered could account for these differences. Similar results were obtained in an experiment on collared flycatchers *Ficedula albicollis* where females of the same age but with different breeding experience produced nestlings with different performance; inexperienced female produced smaller and lighter nestlings (Cichoń 2003). Furthermore, full brood cross-fostering experiments have demonstrated that quality parental care provided by experienced breeders can offset the effects of small eggs (Bogdanova et al. 2006). Even though older females have been found to feed nestlings with equal frequency as younger females in one study of tree swallows (Lozano and Handford 1995), older individuals are better foragers in several species (reviewed by Marchetti and Price 1989). In addition, nestling survival can be influenced by age-related incubation patterns (Bogdanova et al. 2007), and could be influenced by time spent brooding (Bogdanova et al. 2006). As a consequence, it is uncertain in tree swallows and other species which aspect of breeding experience is most beneficial to nestlings; future studies should determine how breeding experience alone influences nestling performance.

Maternal effects did not contribute to the cell-mediated immunocompetence of nestlings measured with the PHA test. This is in contrast to findings from similar studies where heritable and/or early maternal effects explained part of the variance in the strength of nestling immune responses to external pathogens (full cross-fostering: Morrison et al. 2009; partial cross-fostering: Drobniak et al. 2015; but see Saino et al. 2008, Rutkowska et al. 2012). Investment of anti-oxidants into eggs, such as carotenoids (Berthouly et al. 2008, De Neve et al. 2008; but see Sutherland et al. 2012) and immune factors (Pihlaja et al. 2006), have been experimentally demonstrated to influence nestling immunocompetence, we expected a maternal age effect on this trait. Similarly, since nest microclimate and daytime temperature during the brood-rearing period correlate with

immune-health in various bird taxa (Garvin et al. 2006, Ardia et al. 2010), we might also have expected that older females, which arrive earlier to the breeding grounds and have access to more thermally favorable nest sites (Ardia et al. 2006a), to raise nestling with greater immunocompetence. It is possible we did not detect an effect because our paired design (half-broods cross-fostered with clutches hatched on the same day) controlled for hatching date and climate during the rearing period. Indeed, the only significant influence on nestling CMI were annual effects, suggesting climate plays a greater role in CMI than age-related maternal effects. It is also possible that the PHA test was not sensitive enough to detect actual differences between the groups, and a more sensitive assay or one measuring other aspects of nestling immune strength (e.g. immunoglobulins) might reveal age-related maternal effects.

Even though our design attempted to take into account as many confounding factors as possible, we were unable to control for a few potentially important contributors to nestling development. First, we did not determine sex of nestlings, which is associated with differential prenatal investment in several bird species (Gilby et al. 2012 and references therein). For example, female zebra finches *Taeniopygia guttata* in better condition are known to invest more testosterone in the yolk of eggs that produce females (Gilbert et al. 2005). Our results may have been different if our design had unintentionally generated broods with skewed sex ratios. However, there is no evidence for a laying sequence influence on nestling sex in tree swallows (Whittingham and Dunn 2000), and because our experiment involved selecting nestlings across the size hierarchy, and maintaining the hierarchy, we doubt that our manipulation had any effect on sex ratios among broods. Furthermore, we cannot completely rule out the effect of male parents on prenatal investment by females. In some species, females paired with attractive males are known to invest more in their nestlings than females paired with low-quality males (Gil et al. 1999), and an experiment similar to ours found differences in nestling performance in relation to male age in collared flycatchers (Hegyi et al. 2006). Thus, there could be an additive effect of female and male quality that was not accounted for when assessing prenatal maternal effects on nestling performance. A carefully controlled experiment similar to ours, but which would also control for male age and experience, is needed to determine these effects. Finally, our pairing of females with equal clutch size likely led us to include relatively high-quality SY females and relatively low-quality ASY females because ASY females laid on average more eggs than SY females. While this selection process was necessary to reduce other confounding factors (effects of size hierarchies and number of nestlings), it could have reduced our ability to detect significant age-related effects (increase type II errors), but made our analyses more conservative.

Even though our experimental design could not fully separate the effects of pre- and post-hatch female experience (nest site location and quality during incubation), overall our results suggest that both age-related maternal effects and post-hatch breeding experience can have influences on the performance of offspring while in the nest. Prenatal maternal effects only influenced mass at day 16 but this trait is important because it is a predictor of post-fledging

survival in many species (Monrós et al. 2002). Furthermore, our results also convincingly demonstrate that prenatal effects can be long-lasting regardless of post-hatching care, and explain much of the variance in reproductive success between first-time breeding and experienced females. Even after controlling for feeding rates, maternal care influenced nestling probability of fledging and wing growth. This suggests that other benefits to nestlings are provided by breeding experience. Through the use of a robust experimental design, this study helps resolve the individual contributions of pre- and postnatal maternal effects on nestling development in passerine birds. We recommend that future similar studies follow a similar protocol and further manipulate pre- and postnatal conditions to determine specific aspects of egg investment and brooding care that maximize nestling performance. Because life-history strategies can also influence post-hatch age-related investment in nestlings independently of breeding experience, our design could also be used in a study investigating only experienced breeders.

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