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ORIGINAL ARTICLE

Reproduction is affected by individual breeding experience but not pair longevity in a socially monogamous bird

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Abstract

In socially monogamous animals, maintaining stable mating pairs across years has been hypothesized to result in increased reproductive success. However, previous individual breeding experience may independently affect reproductive success, regardless of pair stability. We examined associations between pair composition based on previous breeding experience, pair longevity, and reproduction in socially monogamous mountain chickadees, *Poecile gambeli*, in the Sierra Nevada. There were no significant differences in any reproductive parameters between pairs of experienced breeders that had bred together in previous years and those that were newly formed. Pairs in which both partners were experienced had heavier nestlings than both first-year breeders and mixed experienced–inexperienced pairs. Experienced females started laying eggs earlier and laid larger clutches regardless of their social mate's previous breeding experience. As all experienced birds were older than inexperienced breeders, it remains possible that observed differences between these groups were due to age rather than breeding experience. Overall, our data did not support the hypothesis that pair longevity drives reproductive investment.

Significance statement

We showed that pair longevity in birds with previous experience had no significant association with any reproductive parameters. Instead, we detected that individual-level characteristics related to either previous breeding experience or age (first-year breeders vs. older, experienced breeders) were associated with earlier breeding, larger clutch size, and heavier nestlings. While it remains unclear whether age, breeding experience, or both influence the observed differences in reproductive investment, our results do suggest that pair longevity may not be a critical determinant of reproductive investment in a relatively short-lived, socially monogamous species.

Keywords Breeding experience · Reproductive investment · Social monogamy · Pair longevity · Birds · Chickadee

Introduction

Animals may improve performance with experience, translating to increases in individual fitness (Dukas 2019). In the context of breeding, animals with more reproductive experience have been shown to have greater reproductive success than inexperienced first-year breeders (e.g., Wooller et al. 1990; Komdeur 1996; Woodard and Murphy 1999; Peralta-Sánchez et al. 2020). In socially monogamous taxa, experience can be classified by either pair composition or pair longevity. That is, experience and its expected benefits may be based on the experience of the individuals in each pair (pair composition) or based on a pair's experience breeding together (pair longevity).

The reproductive benefits of pair longevity have been well documented (van de Pol et al. 2006; Sánchez-Macouzet et al. 2014; Wiley and Ridley 2018). Such work suggests that not only is individual-level experience important, but pairs that gain experience breeding together year after year have been shown to have greater reproductive success than those that separate and breed with new mates (due to either mate mortality

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or divorce) (van de Pol et al. 2006; Sánchez-Macouzet et al. 2014). Assuming that stable pairs benefit from gaining experience breeding together, newly formed pairs composed of experienced individuals would be expected to have lower reproductive success than intact, experienced pairs. There are, however, numerous processes that may lead to divorce, some of which may be strategic, with different expected fitness outcomes for each partner (Lindén 1991; McNamara et al. 1999; Ramsay et al. 2000; Heg et al. 2003).

While pair longevity may be crucial in reproductive success, pair composition may also be a key factor involved in reproductive investment and success (e.g., Wiley and Ridley 2018) as pairs composed of experienced breeders may be expected to outperform pairs of inexperienced breeders (Dukas 2019; Peralta-Sánchez et al. 2020). For example, experienced individuals may be more efficient foragers or have better access to quality breeding territories, than inexperienced individuals (Fowler 1995; Woodard and Murphy 1999). However, given differences in sex roles in many socially monogamous systems with biparental care, matching of breeding experience between mates might not be necessary for successful reproduction (Fowler 1995; Komdeur 1996; Woodard and Murphy 1999; Peralta-Sánchez et al. 2020). Similarly, there are numerous aspects of reproduction which may be expected to correlate with the experience of one sex but not necessarily with that of its social mate or of the pair (e.g., female birds may determine aspects of egg laying behavior) (Fowler 1995; Woodard and Murphy 1999; Robertson and Rendell 2001; Caro et al. 2009). At the same time, indirect effects of the opposite sex on such reproductive metrics cannot be ruled out entirely (Brommer et al. 2015; Germain et al. 2016; Evans et al. 2020).

Previous work has focused primarily on either the role of individual breeding experience in reproduction or that of pair longevity in reproduction but not necessarily both together. However, gaining breeding experience may be classified as breeding with the same partner year after year or as the individual experience of each member of the pair, warranting exploration of these two aspects. Notably, previous work has addressed the importance of distinguishing between age and reproductive experience, which are frequently confounded because animals gain experience as they age (Wooller et al. 1990; Forslund and Pärt 1995; Fowler 1995; Komdeur 1996; Robertson and Rendell 2001). Here we investigated these questions in a system of resident mountain chickadees, *Poecile gambeli* (Kozlovsky et al. 2018; Branch et al. 2019). Mountain chickadees are socially monogamous secondary cavity nesters that readily use nest boxes (McCallum et al. 1999). In our long-term study system, the majority of chickadees are uniquely tagged, so pair longevity, pair composition (i.e., breeding experience of each mate), and reproductive investment can be easily monitored from year to year. Mountain chickadees are relatively short-lived with high overwinter adult mortality (Benedict et al. 2020) and are expected to breed every year possible, beginning in their first year of life. Time available for breeding and fledgling development is extremely limited at our montane sites, which regularly have snow cover at the initiation of breeding (Kozlovsky et al. 2018), and second broods are rare in this system.

Specifically, we tested whether pair longevity is associated with reproductive investment as we could unambiguously identify pairs of experienced breeders that bred together for more than one season and those that subsequently bred with new partners. We expected that pairs of experienced breeders that have bred together previously would have greater breeding investment than newly formed pairs of experienced breeders.

While we expected new pairs of experienced breeders to primarily form from mate loss given the high overwinter adult mortality in our system (Benedict et al. 2020), we also subset new adult pairs based on whether they appeared to have formed from mate loss or divorce. Previous work in closely related black-capped chickadees, *Poecile atricapillus*, suggests that females in newly formed pairs resulting from divorce have similar reproductive output as those that stayed with their partners (Ramsay et al. 2000). Mate loss, however, is not an active choice, and we predicted that new pairs of experienced breeders formed from mate loss would have lower reproductive investment than those formed from divorce and pairs that bred together previously.

We also tested whether pair composition, in terms of breeding experience, is associated with reproductive investment by comparing pairs of experienced breeders; pairs of inexperienced, first-year breeders; and pairs with one experienced and one inexperienced breeder. We predicted that older, experienced pairs would have greater reproductive investment than pairs in which one or both partners were inexperienced, first-year breeders.

Lastly, because certain reproductive investment metrics may be more readily mediated by one sex than the other and investment in certain aspects of reproduction may be related to the experience of one sex but not necessarily the other, we tested whether the reproductive experience of each sex was associated with select reproductive metrics, irrespective of their mate's breeding experience. We predicted that older, experienced females would initiate egg laying earlier and lay larger clutches than first-year breeding females, regardless of her social partner's breeding experience. Our prediction that experienced females would initiate egg laying earlier was based on the expectation that relatively earlier laying is most beneficial in our system where time available for breeding is time limited. However, the aspects of previous breeding experience that may influence the timing of clutch initiation and clutch size in female Parids (e.g., success of previous timing of laying or size of previous clutches) may be more complex (reviewed in Ramsay and Otter 2007).

Previous work on mountain chickadee reproductive investment found that males, but not females, adjusted their effort to provision nestlings more when brood sizes were experimentally increased (Grundel 1987). Thus, we considered nestling mass a potentially male-mediated metric and predicted that male breeding experience had the potential to drive observed differences in nestling mass between types of breeding pairs should they arise. Under the general hypothesis that breeding experience is positively associated with reproductive investment, we expected that older, experienced males would raise heavier nestlings than first-year breeding males, regardless of the age or breeding experience of his partner.

In this study, we were unable to definitively separate the effects of experience from age as all inexperienced breeders were first-year breeders. Thus, any differences detected between experienced and inexperienced breeders could potentially be due to age, experience, or both (Wooller et al. 1990; Forslund and Pärt 1995; Fowler 1995; Komdeur 1996; Robertson and Rendell 2001).

Methods

Study system

We collected all data by regularly monitoring ca. 350 nest boxes at our long-term study sites in US Forest Service Sagehen Experimental Forest (Sagehen Creek Field Station, University of California, Berkeley) near Truckee, CA, USA (Kozlovsky et al. 2018; Branch et al. 2019) during the 2016–2018 breeding seasons. Here, mountain chickadee breeding season may span from mid-April to mid-July at lower elevation sites (ca. 1900 m) and from early June to late July at higher elevation sites (ca. 2400 m).

We have been banding mountain chickadees at these sites since August 2014 (during either the breeding or nonbreeding seasons); birds are banded with uniquely colored leg band combinations, including a passive integrated transponder (PIT)-tag (Eccel Technology Ltd.) encased in a colored plastic leg band, so individuals can be identified both visually and by PIT-tag ID.

Reproductive metrics

We used four reproductive metrics: initiation of egg laying, clutch size, brood size, and mean nestling mass (see Kozlovsky et al. 2018; Branch et al. 2019). All nest boxes, including seemingly inactive ones, were regularly checked at least once a week for the first 2 months of the breeding season, beginning ca. 2 weeks before egg laying began. Boxes were checked more frequently once they were found to have eggs so we could determine when incubation began and estimate expected hatch dates. Initiation of egg laying was measured as the day of the year (where 1 January is day 1 of the year) when the first egg was laid. Clutch size, which varies greatly (typically 6–8 eggs but may range from 3–10 eggs) (Kozlovsky et al. 2018), was measured as the number of eggs present in the nest once incubation began. Brood size was measured on day 16 (15 d post-hatch), when all nestlings were weighed and fitted with unique US Geological Survey-issued metal leg bands. We chose to take these measurements on day 16 so as not to force fledge nestlings (nestlings fledge day 19–22) (McCallum et al. 1999); we consider these values to be good estimators of future fledgling success. The initiation of egg laying and clutch size were considered mainly female-mediated metrics (Caro et al. 2009). While it is uncommon for mountain chickadees to rear more than one brood in a breeding season (only observed at low elevation in some years), we only used metrics from the first breeding attempts each year.

Determining pair longevity and breeding experience

We identified previously PIT-tagged, breeding individuals either visually based on unique leg band combinations or in hand with a radiofrequency identification (RFID) scanner. We trapped and banded birds lacking PIT-tags (at nest boxes), including those with metal leg bands (previously banded as nestlings with known age, ca. $<3\%$ of the breeding population). Because mountain chickadees are resident, have relatively short dispersal distances, remain in the same area following post-fledging dispersal (McCallum et al. 1999), and we band extensively during the nonbreeding seasons, we considered chickadees banded during a given breeding season, aside from birds with metal leg bands, to be inexperienced, first-year breeders (including new birds recorded as “unbanded”). For birds initially PIT-tagged during the nonbreeding season, we recorded each bird’s approximate age in terms of whether or not it appeared to be in its first year of life using feather wear and morphology when possible (e.g., molt, shape of rectrices) (Pyle 1997). This allowed us to determine if a PIT-tagged bird was older than 1 year during a given breeding season, and any PIT-tagged bird lacking age estimates in these records was considered older than 1 year if it had been at least 1 year since time of initial capture. For each breeding season, we determined breeding experience for PIT-tagged and metal banded birds based on age recorded at time of initial capture: birds in their first year of life were considered inexperienced as they have not had an opportunity to breed previously, and those older than one year and those observed breeding in our nest boxes in previous years were considered experienced breeders as they have had at least one opportunity to reproduce (Branch et al. 2019). We consider this a rather conservative approach for this study as we are not estimating age in years but rather sexual maturity and potential reproductive experience. This does, however, prevent us from disentangling effects of age from breeding experience on reproductive investment. We sexed birds in hand by the presence of either a brood patch (females) or cloacal protuberance (males). We only used pairs for which identity was known for both male and female in these analyses.

We categorized pair experience type based on the experience of both the male and female: both experienced, both inexperienced, or mixed-experience (one is experienced, while the other is inexperienced). We determined pair longevity for all pairs of experienced individuals based on whether or not they had the same social mate as the previous year (pair identities from the 2015 breeding season were also used here). This could only be determined for pairs in which at least one bird was observed in two or more breeding seasons. Pairs of experienced birds that bred together previously were categorized as an "old pair" and those that had not were categorized as a "new pair." These new pairs of experienced birds could form from mate loss or divorce and were thus classified into these two categories. Previous mates were determined to be alive if they were either observed during subsequent breeding seasons or recorded visiting RFID enabled bird feeders (see Croston et al. 2017) during the nonbreeding season (fall 2016, spring 2019). Due to high overwinter mortality (~50%) (McCallum et al. 1999; Branch et al. 2019; Benedict et al. 2020), only RFID data collected after the following breeding season were used (e.g., automated RFID data starting after the 2017 breeding season were used for a previous mate from the 2016 breeding season). The formation of new pairs of experienced birds was categorized as a result of mate loss if their previous mate was determined to have died, while those with previous mates determined to be alive were categorized as a result of divorce. Because mountain chickadees at these sites may use natural cavities, we were unable to identify every breeding pair in the area. This prevented us from quantifying breeding experience and pair longevity in terms of the number of breeding seasons in which an individual participated and the number of successive years a pair bred together.

Statistical analyses

We used "lm" in the stats R package (R Core Team 2019) and type III ANOVAs ("Anova," car R package) (Fox and Weisberg 2019) to run and analyze linear models. All four reproductive metrics were used as dependent variables in models of pair composition (independent variable) and pair longevity (independent variable). Pair longevity was assessed in two series of models, one in which all newly formed pairs of experienced breeders were in the same category and one in which we separated these pairs based on whether newly formed pairs were a result of mate loss or divorce. Additional models on the two female-mediated metrics, egg laying initiation date and clutch size (dependent variables), were run using only female experience (independent variable). We also ran a model to assess the role of male breeding experience (independent variable) on mean nestling mass (dependent variable), as previous work has suggested that male provisioning may be important in this species (Grundel 1987). To control for potential differences between elevations and among years (Kozlovsky et al. 2018), we used elevation and year as independent fixed effects, allowing us to test for potential effects of experience independently of any variation across elevations and years. Because experience was the main effect of interest, we ran Tukey pairwise comparisons (least squares means (lsmeans), standard error, and P-values are reported here, emmeans (Lenth 2019; R Core Team 2019), and calculated adjusted R^2 values only for models with significant results for experience or pair longevity-related effects. All figures were made in R (R Core Team 2019) using the ggplot2 (Wickham 2016), ggforce (Pedersen 2019), and ggpubr (Kassambara 2020) packages.

Due to the nature of our study, a fully blind experimental design was not possible as the identities of almost all birds were known prior to breeding. However, we have used all possible nests detected for our analyses, and our measurements (clutch initiation date, clutch size, brood size, mean nestling mass) were likely unaffected by knowing the bird identity.

Results

Over the 3 years, we sampled a total of 242 nests and had 158 males and 161 females that were uniquely identifiable. Sample sizes differed across reproductive metrics due to outside factors such as nest predation at each stage in reproduction and limitations on tracking pairs year to year (Table 1). There were significant differences between elevations in all four reproductive metrics used, and as elevation comparisons are similar across all models, the directionality of these differences was the same. Birds at low elevation initiated egg laying significantly earlier, laid larger clutches, and raised larger broods than those at high elevation. However, chickadees at high elevation raised significantly heavier nestlings than those at low elevation. These elevation-level comparisons were consistent with previous findings in this system (Kozlovsky et al. 2018) and are not discussed here further as they were not the comparison of interest, but elevation-level statistics from each model are presented below. See Kozlovsky et al. (2018) for detailed between-elevation comparisons in reproduction in this population of mountain chickadees. See Table 1 for sample sizes and supporting statistics for all models.

Table 1 Sample sizes, F-statistics, P-values ($\alpha = 0.05$), and variables used for each model for each analysis. Sample sizes are presented in parentheses after each category for the main independent variable of interest. Note that elevation and year were independent variables used in all models but were not the main effect of interest. Adjusted R^2 values are only reported for models in which the main independent variable of interest was significant (significant P-values are in bold)

Dependent variable	Main independent variable of interest		Elevation	Year	R^2
Pair longevity, pt. I					
Initiation of egg laying	Old pair (43) vs New pair (32)	$F_{1, 70}=1.14$, $P=0.289$	$F_{1, 70}=101.34$, $P<0.001$	$F_{2, 70}=11.63$, $P<0.001$	–
Clutch size	Old pair (41) vs New pair (32)	$F_{1, 68}=0.24$, $P=0.624$	$F_{1, 68}=9.91$, $P=0.002$	$F_{2, 68}=0.90$, $P=0.4111$	–
Brood size	Old pair (39) vs New pair (28)	$F_{1, 62}=0.00$, $P=0.98$	$F_{1, 62}=9.24$, $P=0.003$	$F_{2, 62}=3.64$, $P=0.032$	–
Mean nestling mass	Old pair (39) vs New pair (28)	$F_{1, 62}=0.04$, $P=0.852$	$F_{1, 62}=3.45$, $P=0.068$	$F_{2, 62}=9.56$, $P<0.001$	–
Pair longevity, pt. II					
Initiation of egg laying	Old pair (43) vs New pair: mate loss (21) vs New pair: divorce (11)	$F_{2, 69}=0.57$, $P=0.57$	$F_{1, 69}=93.36$, $P<0.001$	$F_{2, 69}=11.44$, $P<0.001$	–
Clutch size	Old pair (41) vs New pair: mate loss (21) vs New pair: divorce (11)	$F_{2, 67}=0.17$, $P=0.841$	$F_{1, 67}=9.66$, $P=0.003$	$F_{2, 67}=0.93$, $P=0.398$	–
Brood size	Old pair (39) vs New pair: mate loss (17) vs New pair: divorce (11)	$F_{2, 61}=0.08$, $P=0.923$	$F_{1, 61}=8.34$, $P=0.005$	$F_{2, 61}=3.18$, $P=0.049$	–
Mean nestling mass	Old pair (39) vs New pair: mate loss (17) vs New pair: divorce (11)	$F_{2, 61}=0.13$, $P=0.877$	$F_{1, 61}=2.96$, $P=0.090$	$F_{2, 61}=9.46$, $P<0.001$	–
Breeding pair experience					
Initiation of egg laying	Experienced pairs (82) vs Inexperienced pairs (66) vs Mixed-experience pairs (94)	$F_{2, 236}=3.70$, **$P=0.026$**	$F_{1, 236}=334.08$, $P<0.001$	$F_{2, 236}=27.86$, $P<0.001$	0.620
Clutch size	Experienced pairs (80) vs Inexperienced pairs (62) vs Mixed-experience pairs (92)	$F_{2, 228}=1.91$, $P=0.149$	$F_{1, 228}=22.80$, $P<0.001$	$F_{2, 228}=7.82$, $P<0.001$	–
Brood size	Experienced pairs (74) vs Inexperienced pairs (55) vs Mixed-experience pairs (75)	$F_{2, 198}=0.44$, $P=0.643$	$F_{1, 198}=5.15$, $P=0.024$	$F_{2, 198}=6.24$, $P=0.002$	–
Mean nestling mass	Experienced pairs (74) vs Inexperienced pairs (55) vs Mixed-experience pairs (75)	$F_{2, 198}=5.39$, **$P=0.005$**	$F_{1, 198}=9.49$, $P=0.002$	$F_{2, 198}=10.40$, $P<0.001$	0.166
Female experience					
Initiation of egg laying	Experienced females (116) vs Inexperienced females (126)	$F_{1, 237}=11.30$, **$P=0.001$**	$F_{1, 237}=348.84$, $P<0.001$	$F_{2, 237}=29.62$, $P<0.001$	0.628
Clutch size	Experienced females (114) vs Inexperienced females (120)	$F_{1, 229}=5.26$, **$P=0.023$**	$F_{1, 229}=22.62$, $P<0.001$	$F_{2, 229}=7.73$, $P=0.001$	0.133
Male experience					
Mean nestling mass	Experienced males (121) vs Inexperienced males (83)	$F_{1, 199}=2.18$, $P=0.141$	$F_{1, 199}=8.12$, $P=0.005$	$F_{2, 199}=8.67$, $P<0.001$	–

Reproductive investment and pair longevity

Old vs. newly formed experienced pairs

There were no significant differences in the first egg date between old and new pairs of experienced breeders (Fig. 1a). Clutch size also did not differ significantly between experienced pair types (Fig. 1b). Brood sizes were statistically similar across experienced pair types (Fig. 1c). Similarly, nestling mass did not differ significantly with pair longevity (Fig. 1d).

Old vs. newly formed experienced pairs from either mate loss or divorce

Initiation of egg laying did not differ significantly between the three types of pairs composed of experienced breeders:

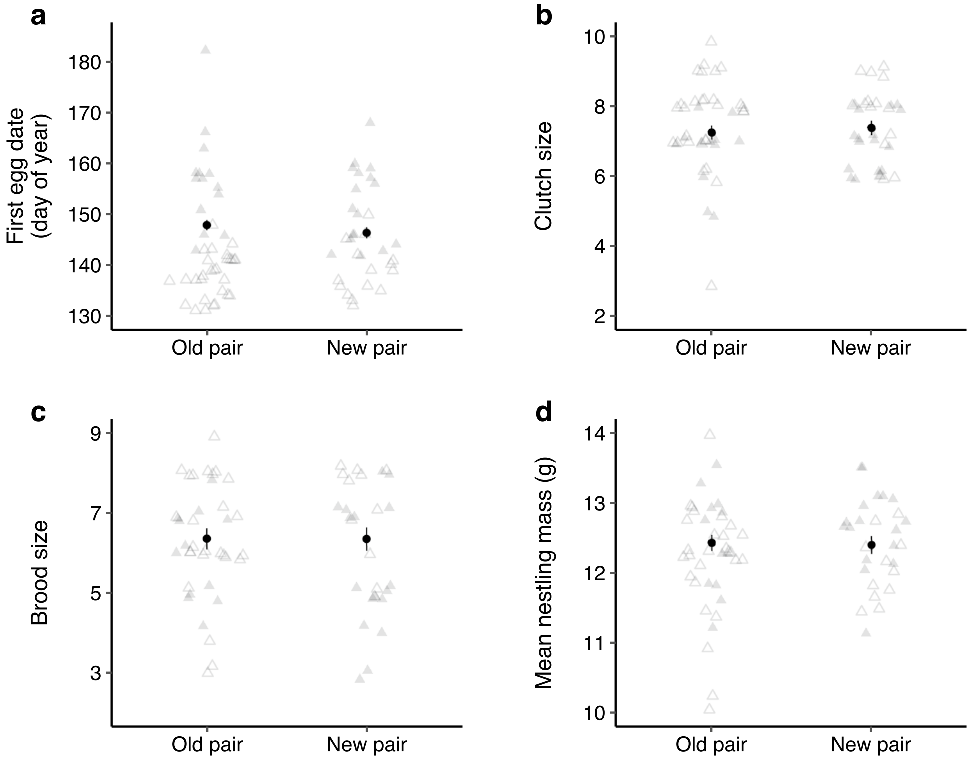

Fig. 1 Comparing reproductive metrics between old and new experienced pairs — "old pair" indicates pairs that have bred together in at least one previous breeding season; "new pair" indicates newly formed pairs of experienced breeders. Filled circles indicate the least squares mean within each experienced pair type across both elevation sites, and error bars show standard error. Raw data points are jittered across the x-axis reflecting the shape of the distribution (sina plot; ggforce R package (Pedersen 2019)). Open triangles indicate pairs from the low elevation site, and filled triangles indicate pairs from the high elevation site. **a** Initiation of egg laying measured by the day of year (1 January is day 1) when the first egg of the clutch was laid; **b** clutch size as the total number of eggs present in the nest at the initiation of incubation; **c** brood size measured as the total number of surviving nestlings present 15 d post-hatch; **d** mean nestling mass of each brood measured 15 d post-hatch

old pairs, new pair from mate loss, and new pair from divorce. Neither clutch sizes nor brood sizes nor mean nestling mass differed significantly across experienced pair types.

Reproductive investment and breeding pair experience

There were significant differences in the initiation of egg laying between pair experience types (Fig. 2a). In particular, experienced pairs (lsmean, 147 ± 0.64 d) initiated egg laying significantly earlier than inexperienced pairs (lsmean, 149 ± 0.72 d) (Tukey, P = 0.046). Experienced pairs also began laying earlier than mixed-experience pairs (lsmean, 149 ± 0.58 d), but these differences were not significant (Tukey, P = 0.062). There were no significant differences between inexperienced and mixed-experience pairs in the initiation of egg laying (Tukey, P = 0.933). There were no significant differences in clutch sizes between pair experience types (Fig. 2b). Similarly, there were no significant differences in brood sizes between pair experience types (Fig. 2c). Mean nestling mass differed significantly between pair experience types (Fig. 2d). Particularly, mean nestling mass in broods from experienced pairs (lsmean, 12.4 ± 0.09 g) tended to be greater than nestling mass in inexperienced pairs (lsmean, 12.1 ± 0.11 g) (Tukey, P = 0.069) and was significantly greater than nestling mass in mixed-experience pairs (lsmean, 12.0 ± 0.09 g) (Tukey, P = 0.005). There were no significant differences in mean nestling mass between inexperienced and mixed-experience pairs (Tukey, P = 0.815).

Reproductive investment and female breeding experience

Experienced females initiated egg laying significantly earlier than inexperienced females regardless of previous experience of males (Fig. 3a). Experienced females also laid significantly larger clutch sizes than inexperienced females (Fig. 3b).

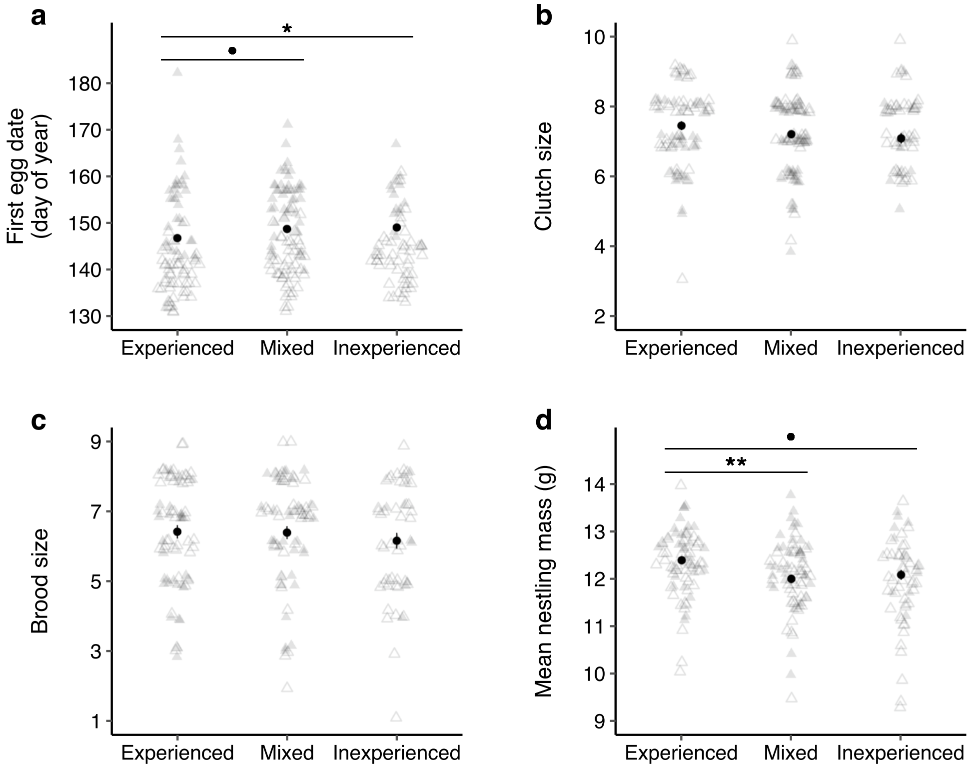

Fig. 2 Comparing reproductive metrics between experienced, mixed-experience, and inexperienced pairs. Filled circles indicate the least squares mean within each pair experience type across both elevation sites, and vertical bars show standard error. Raw data points are jittered across the x-axis reflecting the shape of the distribution (sina plot; ggforce R package (Pedersen 2019)). Open triangles indicate pairs from the low elevation site, and filled triangles indicate pairs from the high elevation site. Significance or near significance of pairwise comparisons are indicated with dots or asterisks (• $P \leq 0.1$; * $P \leq 0.05$; ** $P \leq 0.01$). **a** Initiation of egg laying measured by the day of the year (1 January is day 1) when the first egg of the clutch was laid; **b** clutch size as the total number of eggs present in the nest at the initiation of incubation; **c** brood size measured as the total number of surviving nestlings present 15 d post-hatch; **d** mean nestling mass of each brood measured 15 d post-hatch

Reproductive investment and male breeding experience

There were no significant differences in the mean nestling mass between broods raised by experienced males and those raised by inexperienced, first-year breeding males when pairs with both experienced and inexperienced females were combined.

Discussion

Contrary to our predictions, we found no significant differences in reproductive metrics between experienced pairs that bred together previously and newly formed experienced pairs, suggesting that pair longevity was not a significant predictor of reproductive investment. However, consistent with previous work on breeding experience and reproductive investment, we found differences in reproductive metrics between older, experienced and younger, inexperienced breeders (Wooller et al. 1990; Forslund and Pärt 1995; Komdeur 1996; Woodard and Murphy 1999; Peralta-Sánchez et al. 2020). Experienced breeding pairs initiated egg laying earlier and raised heavier chicks than inexperienced pairs. Critically, experienced females initiated egg laying earlier in the season and laid larger clutches than inexperienced, first-year breeding females.

Although we found that experienced pairs initiated egg laying earlier than other pair types, this finding appears to be driven mainly by differences in egg laying initiation between experienced and inexperienced, first-year females. Arguably, both initiation of egg laying and clutch size, primarily female-mediated metrics (Caro et al. 2009; but see Brommer et al. 2015; Germain et al. 2016; Evans et al. 2020), appear to be related to female breeding experience or age. This suggests that individual female experience may drive these female-mediated reproductive metrics, which has also been reported in other Passerines (Orell et al. 1994; Woodard and Murphy 1999; Robertson and Rendell 2001). Egg laying date has been related to hatch date, fledge date, and offspring recruitment (e.g., Nilsson and Smith 1988; Monrós et al. 2002), and larger clutches may potentially lead to larger

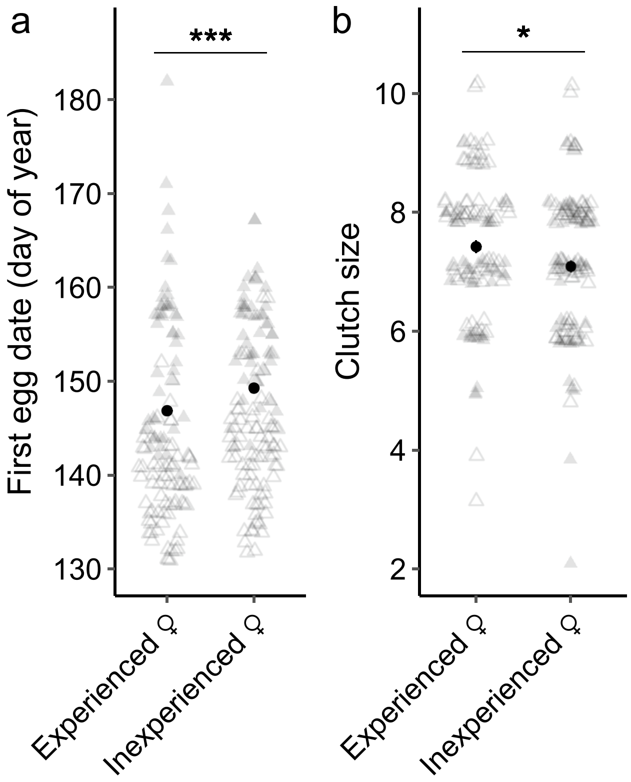

Fig. 3 Comparing reproductive metrics between experienced and inexperienced females. Filled circles indicate the least squares mean within each experienced female type across both elevation sites, and error bars show standard error. Raw data points are jittered across the x-axis reflecting the shape of the distribution (sina plot; ggforce R package (Pedersen 2019)). Open triangles indicate females from the low elevation site, and filled triangles indicate females from the high elevation site. Significance of pairwise comparisons are indicated with asterisks (* P ≤ 0.05; *** P ≤ 0.001). **a** Initiation of egg laying measured by the day of year (1 January is day 1) when the first egg of the clutch was laid; **b** clutch size as the total number of eggs present in the nest at the initiation of incubation

brood sizes and fledgling success (Fowler 1995). While in some systems optimal lay date differs between years (Monrós et al. 2002), time available for breeding and nestling development at our montane sites is limited, potentially favoring earlier reproduction.

Interestingly, we did not find significant experience-related differences in brood sizes. While larger clutch sizes may be expected to result in larger brood sizes, brood size may be influenced by many factors (e.g., provisioning, predation, weather, parasitism, etc.). As we measured brood size 15 days post-hatch, some nests had reduction in brood size during this time, while others did not. Because we do not have explicit data to determine causes of nestling mortality, we are unable to attribute any reductions in brood size to parent age or breeding experience. Even though brood sizes were statistically similar across pairs, we found that pairs of older, experienced breeders raised heavier nestlings than inexperienced or mixed-experience pairs. Previous work on mountain chickadees suggests that males may contribute more to nestling feeding than do females (Grundel 1987), but we did not detect significant differences between first-year breeding males and older, experienced males in nestling mass. Together, these results may highlight the importance of an experienced pair in raising heavier nestlings together and perhaps the success of the nest during later stages of reproduction. While observed differences in mean nestling mass seem rather small, even miniscule differences may equate to a substantial percentage of body weight in small Passerines. We thus interpret these effects to be biologically relevant. Greater nestling mass, measured a few days before fledging as in our study, has been shown to relate to fledging recruitment (e.g., Monrós et al. 2002). Taken together, these results suggest that older, experienced pairs may fledge higher quality offspring than other pair types and may ultimately have greater reproductive success.

Notably, we did not find differences between pairs of older, experienced individuals that bred together over multiple years and those that were newly formed. We interpret this to mean that individual-level characteristics, but not necessarily pair longevity, have an important role in reproductive investment, though there may be more complex factors we were unable to detect. We were unable to determine the causes of divorce in each of our observed cases, limiting our ability to make informed predictions regarding the related fitness consequences (Orell et al. 1994; McNamara et al. 1999; Heg et al. 2003). While some studies in closely related species suggest that divorce is more likely following a low output breeding season (*Parus major*, Lindén 1991), others have suggested low costs of mating with a new, experienced partner as a reason for divorce (*Poecile montanus*, Orell et al. 1994; *P. atricapillus*, Ramsay et al. 2000). The latter findings may explain our observed lack of differences in reproductive metrics between old pairs and newly formed experienced pairs.

Because chickadees at our site use natural cavities in addition to our nest boxes, we cannot detect all breeding pairs from year to year, limiting the number of observed old and newly formed experienced pairs. In addition, we had substantial differences in sample sizes between old and newly formed pairs of experienced breeders and particularly few newly formed pairs of experienced individuals resulting from divorce (Table 1). While the small sample size for newly formed experienced pairs resulting from divorce resulted in larger variance, we feel that our interpretations of these results are reasonable given the lack of statistically significant differences between old pairs and new pairs resulting from mate loss.

Mountain chickadees form pairs at the beginning of the nonbreeding season and maintain pair bonds with their social mate throughout the winter. This time provides an opportunity for newly formed social pairs to gain information about one another (discussed in *P. atricapillus*, Ramsay et al. 2000) and perhaps diminish the costs of breeding with a different partner. Overwinter mortality is estimated to be high in this population (Branch et al. 2019; Benedict et al. 2020), which may also limit the opportunities for pairs to remain intact over years. As such, forming a pair with a different mate might not disrupt reproductive output, unlike what has commonly been found in longer-lived avian species (van de Pol et al. 2006; Sánchez-Macouzet et al. 2014; Wiley and Ridley 2018).

As a socially monogamous species, mountain chickadees rear extra-pair young (Branch et al. unpublished), making it difficult to assess male reproductive fitness based solely on brood sizes and nestling masses without paternity analyses. However, given previous work in mountain chickadees that showed males increased feeding effort in response to artificial increases in clutch and brood sizes (Grundel 1987), we do not think males treat extra-pair young differently.

Unfortunately, we do not have exact ages of many individuals in our study population, and because of high annual adult mortality (Benedict et al. 2020), the majority of birds in our breeding population are ca. 1–2 years of age. Thus, we are currently unable to examine more fine-scale age-based differences in reproduction (e.g., Nol and Smith 1987; Wooller et al. 1990; Robertson and Rendell 2001) but will be able to address these ideas in future years as we accumulate more data. Some chickadees also use naturally available cavities, limiting us to categorizing individuals as either having or lacking reproductive experience rather than more continuous, finite quantifications of reproductive experience (e.g., Nol and Smith 1987; Fowler 1995; Robertson and Rendell 2001). Reproductive experience may differ across individuals of the same age, and while age and reproductive experience are not necessarily equivalent, they are strongly, positively correlated (Fowler 1995; Robertson and Rendell 2001). In this study, we classified all first-year breeders as inexperienced and those older than their first year as experienced breeders, making it impossible to fully separate the effects of age and experience on reproductive investment. Therefore, any differences between inexperienced and experienced breeders could be attributed to either age, breeding experience, or both. As short-lived animals with ca. 50–65% adult survival rates (Benedict et al. 2020), we expect that mountain chickadees should attempt to breed every year possible and that skipping reproduction is uncommon in any given year, especially for females. However, younger and lower ranking males have been shown to have lower success at obtaining a mate in closely related species (*P. atricapillus,* Schubert et al. 2007). It is possible that some males older than their first year of life that we initially banded outside of the breeding season and have categorized as experienced breeders were actually unable to secure a mate during their first breeding attempt and are actually inexperienced breeders. If a substantial amount of males were improperly categorized in terms of breeding experience, we would likely not have found any differences between experienced and inexperienced breeding pairs in reproductive investment, but this was not the case. In addition, specific events during previous reproduction such as losing a nest during different stages of reproduction (e.g., egg predation, nestling mortality) may be associated with variation across individuals in breeding experience. Unfortunately we do not have the data to test for these effects. We feel that our classification of reproductive experience is reasonable for this system, but it prevents us from disentangling any effects of age from breeding experience.

Overall, our results do not support the hypothesis that pair longevity is associated with increased reproductive investment. Rather, reproductive investment decisions appear to be based on individual age, experience, or both. Incorporating more finite measures of breeding experience, age, paternity, offspring survival, and causes of divorce may allow a more in-depth understanding of the importance of each factor in reproductive success.

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Author contribution AMP and VVP designed the experiment and wrote the first draft of the manuscript. AMP analyzed all data. All authors participated in data collection and contributed to writing the finalized version of the manuscript.

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Data availability All data used in this study are available at https://github.com/apitera/MOCHPairLongevity.

Code availability Only published software packages were used in this study; no custom code was used. However, R code used in these analyses is available at https://github.com/apitera/MOCHPairLongevity.

Declarations

Ethics approval This study was in accordance with the University of Nevada, Reno Institutional Animal Care and Use committee (protocol no. 00046 and 00603) and local and federal guidelines (California Fish and Wildlife Permit SC-5210 (DocID: D-0019571790–9)). We released all birds immediately after banding, handled as little as possible and only when necessary, and showed no evidence of detrimental effects of banding or nest monitoring.

Consent to participate Not applicable.

Consent for publication All coauthors consented to submitting this manuscript for publication.

Competing interests The authors declare no competing interests.

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