

Female mountain bluebirds (*Sialia currucoides*) paired to more colourful males produce male-biased broods

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Abstract

Sex allocation theory predicts that females should bias the sex ratio of their offspring in response to differences in the reproductive value of sons versus daughters. Consistent with this prediction, females of many species appear to bias offspring sex ratios in response to mate attractiveness and condition. Male mountain bluebirds (*Sialia currucoides*) display full body UV-blue structural plumage colouration, which is associated with attractiveness, condition, and reproductive success. Over four breeding seasons, we found females paired with more colourful males produced increasingly male-biased broods and provisioned offspring at a higher rate. Surprisingly, however, we also found females with duller plumage and those mated to first-year males produced more malebiased broods. These results provide support for sex allocation in mountain bluebirds and suggest female reproductive decisions may be influenced by the attractiveness of her mate. However, this system is clearly complex and more work is needed to understand the roles of male age and female colouration in the signalling systems of mountain bluebirds.

Keywords

mountain bluebird, *Sialia currucoides*, sex ratio, sex allocation, plumage, structural colouration.

1. Introduction

When the reproductive value of sons and daughters differs, sex allocation theory predicts that females should invest more heavily in the higher-value gender and bias offspring sex ratios to incur fitness benefits (Trivers & Willard, 1973). Which gender is of higher reproductive value to a female depends on both intrinsic factors (e.g., her own condition) and extrinsic factors (e.g., mate quality and resource availability) (Trivers & Willard, 1973;

Burley, 1981). When males have high variance in reproductive success, females capable of producing high-quality offspring may benefit from producing more sons than daughters, while a female only capable of producing low-quality offspring may benefit from producing more daughters than sons (Trivers & Willard, 1973). In nature, biased sex allocation in response to parental condition and habitat quality has been documented in many vertebrates, including mammals (Robert & Schwanz, 2011) and numerous bird species (Alonso-Alvarez, 2006).

In taxa with genetic sex determination, such as birds, brood sex ratios may be adjusted pre-laying, in the form of biased ovulation or fertilization, or post-laying, in the form of biased offspring mortality (Pike & Petrie, 2003; Alonso-Alvarez, 2006). During meiosis, sex chromosomes segregate in a Mendelian fashion, which should result in an equal probability of producing a son or a daughter (Pike & Petrie, 2003; Alonso-Alvarez, 2006). Bachtrog et al., 2014). Consequently, it would seem unlikely that sex ratios could be biased pre-laying, yet biased offspring sex ratios have been noted in many bird species at laying (Alonso-Alvarez, 2006).

Birds exhibit ZW sex determination, in which males are the homogametic sex and the sex chromosome inherited from the mother determines offspring sex. Previous studies of avian sex allocation have found that female birds appear to modify brood sex ratios in response to factors such as individual condition or quality, mate condition or quality, and habitat quality (Alonso-Alvarez, 2006). Though the physiological mechanisms allowing females to control sex-specific egg development are poorly understood (reviewed in Pike & Petrie, 2003; Alonso-Alvarez, 2006; Navara, 2013), the environmental factors associated with biased sex ratios are being unravelled on a species-specific basis.

Much research into variation in brood sex ratio among birds has focused on the role of male attractiveness. In species with female mate choice, male attractiveness is often associated with sexually-selected plumage characteristics, with attractive males being those that display more exaggerated forms of plumage (Burley, 1986). Male plumage can signal the direct (e.g., parental care, resource holding potential) and/or indirect (e.g., good genes) benefits a female will gain by mating with a particular male (Griffith & Pryke, 2006). Thus, a female paired to an attractive male may benefit from producing a male-biased brood if sons inherit the features of the male parent. Because females experience relatively low variation in reproductive success, a daughter would be less affected by the inheritance of lower-quality genes than a son, suggesting a female-biased brood would be adaptive when a female is paired to a less attractive male. Male plumage ornamentation has been positively correlated with male-biased broods in several species, including collared fly-catchers (*Ficedula albicollis*; Ellegren et al., 1996), red-breasted flycatchers (*Ficedula parva*; Mitrus et al., 2015), common yellowthroats (*Geothlypis trichas*; Taff et al., 2011), blue tits (*Cyanistes caeruleus*; Sheldon et al., 1999; Griffith et al., 2003; Delhey et al., 2007), and barn swallows (*Hirundo rustica*; Romano et al., 2015).

Although many studies have found evidence supporting the idea that birds are able to adaptively manipulate brood sex ratios, others have failed to find such evidence (Koenig & Dickinson, 1996; Saino et al., 1999; Ewen et al., 2004; Rosivall et al., 2004; Korsten et al., 2006; Czyź et al., 2012). The current literature is contradictory: one study will find a relationship in a certain species, while another study on the same species, but on a different population or during a different year will not (e.g., blue tit: Griffith et al., 2003; Korsten et al., 2006; collared flycatcher: Ellegren et al., 1996; Rosivall et al., 2004; barn swallow: Saino et al., 1999; Romano et al., 2015). These conflicting results highlight the need for replication across populations and over time.

Brood sex ratios have been studied in both western (*Sialia mexicana*; Dickinson, 2004) and eastern bluebirds (*Sialia sialis*; Lombardo, 1982). No evidence for adaptive brood sex ratio adjustment was observed in eastern bluebirds (Lombardo, 1982); in western bluebirds, biased brood sex ratios were related to the presence of helpers-at-the-nest (Dickinson, 2004). Although closely related, mountain bluebirds (*Sialia currucoides*) do not breed cooperatively, suggesting brood sex ratios may be affected by different factors. Mountain bluebirds are sexually dimorphic and male UV-blue plumage colouration is associated with body size and reproductive success (Balenger et al., 2009a), suggesting that it may be adaptive for females to bias brood sex ratios in response to male plumage colouration.

We studied a population of mountain bluebirds breeding in interior British Columbia, Canada over four breeding seasons to ask whether female mountain bluebirds produce sex-biased broods in response to mate attractiveness. In accordance with sex allocation theory (Trivers & Willard, 1973; Burley, 1981), we predicted that females paired with more colourful males will produce male-biased broods. In addition, we asked whether males or females adjust parental investment (provisioning rate) in response to their offspring sex ratio, and whether parental investment is associated with male plumage colouration.

2. Materials and methods

2.1. Study species

Mountain bluebirds are medium-sized, secondary cavity nesting songbirds that readily make use of nest boxes in open grasslands, often along fence posts. Mountain bluebirds display UV-blue plumage and are sexually dimorphic (Power & Lombardo, 1996). Breeding males possess full-body UV-blue plumage, while breeding females have a generally more subdued brown-grey plumage, with UV-blue on their rump, tail, and flight feathers that is duller than that of males (Power & Lombardo, 1996). Unlike carotenoid and melanin based plumage, which depend primarily upon pigment deposition, variation in UV-blue plumage is dependent upon melanin arrangement and feather microstructure (Prum, 2006). An individual's nutritional state during moult can affect the resulting feather structure and colour (Keyser & Hill, 1999; Siefferman & Hill, 2005, 2007; Doyle & Siefferman, 2014). Consequently, structurally based plumage appears to be an honest signal of an individual's condition and quality, as is true for colours based on dietary pigments (Keyser & Hill, 2000; Siefferman & Hill, 2003).

In a population of mountain bluebirds from Wyoming, USA, male plumage colouration was positively correlated with male wing size and total reproductive success (i.e., the sum of within-pair and extra-pair offspring), suggesting that brightly coloured males are in better condition and of higher quality than dull males (Balenger et al., 2009a). Mountain bluebirds are so-cially monogamous and show weak negative assortative mating in regard to plumage colour (Morrison et al., 2014). Extra-pair paternity rates are high in this species (72% of broods; Balenger et al., 2009b), and males that sire extra-pair young have brighter UV-blue plumage than males that do not (Balenger et al., 2009a; O'Brien & Dawson, 2011), suggesting the opportunity for sexual selection to act on male UV-blue plumage (Balenger et al., 2009b).

2.2. Field methods

Field work for this project was conducted during the 2011–2014 breeding seasons (May–July) in Kamloops, British Columbia, Canada (885–1116 m

asl; 50°37'N, 120°19'W), using nest box routes established and maintained by the Kamloops Naturalist Club. Nest boxes were monitored every one to three days to determine first egg date, clutch size, hatch date, number of nestlings and fledging success. Five to ten days after eggs hatched, adult males and females were captured at the nest using nest box traps. Adults were banded with a single Canadian Wildlife Service (CWS) aluminium band and a unique combination of three plastic colour bands. We classified adults as either second-year (SY) or after-second-year (ASY) by examining the moult limits of the primary and greater coverts, as described by Pyle (1997). To evaluate individual body size and condition, we measured mass, unflattened wing chord, tail length and tarsus length. For colour analysis, we collected ten rump feathers and a single tail feather from each adult. Nestlings were banded with a single CWS aluminium band 9 to 13 days after hatching. At the time of banding, we collected blood samples from adults and nestlings by piercing the ulnar vein and drawing 15–25 μ l of blood into a micro-capillary tube.

All work was approved by the Thompson Rivers University Animal Care and Use Committee and was conducted under a Canadian Federal Master Banding Permit and Scientific Collection Permit.

2.3. Parental care

To examine patterns of parental care, we conducted video recordings of morning provisioning trips for two-hour periods during the early (3–5 days after hatching) and late (14–16 days after hatching) nestling phases, for a total of four hours per nest. Nest watch videos were recorded using a Handy-cam DCR-SX45 (Sony, Tokyo, Japan) or a HD Hero2 or 3 (GoPro, San Mateo, CA, USA). Nest watches started between 06:20 and 12:45 (mean \pm SD = 09:17 \pm 85 min). Video cameras were placed on the ground approximately two meters in front of the nest box and aimed at the box entrance. A trained observer analysed nest watch videos to determine rates of provisioning by the male and female (separately). Provisioning rates were measured as the number of trips to the nest per hour per nestling, following Morrison et al. (2014). Female visits to the nest during the early nestling phase that lasted longer than 30 s were recorded as brooding. Adults were not captured at nests within 48 h prior to nest watches to avoid modification of parental behaviour.

2.4. Feather colour analysis

We mounted rump and tail feathers on low-reflectance black paper. Rump feathers were mounted in an overlapping pattern that mimicked the way feathers would normally lie on the bird. We quantified male and female plumage colouration by measuring reflectance across the avian visual spectrum (300–700 nm) using an Ocean Optics JAZ spectrometer (Dunedin, FL) with a PX-2 xenon light source. The fibre optic probe was held in a non-reflective probe holder to measure feathers from a standard 90° angle and 5.9 mm distance. We took ten readings for each plumage area (tail and rump) from haphazard locations within the blue regions.

Using the R-based colour analysis program RCLR v.28 (Montgomerie, 2008), we calculated three colour variables for each feather reading (brightness, hue and chroma) and then, for each of these variables, averaged the values obtained over the ten readings from each feather sample. Brightness was measured as the average percent reflectance across the avian visual spectrum (300-700 nm). Chroma was measured as the proportion of reflectance within the blue range (400-510 nm) and ultraviolet range (300-400 nm) relative to the total light reflected across the avian visual range. Finally, hue was measured as the wavelength at maximum reflectance. Due to high colinearity among these three colour variables, we used principal component analysis to collapse the three variables into a single factor. Because the first principal component (PC1) explained most of the variance for each plumage area, we used PC1 to represent overall plumage colouration variation (Table 1). A greater PC1 value corresponded to increases in brightness and chroma, but a decrease in hue (i.e., maximum reflectance shifted more towards the UV end of the spectrum).

2.5. Molecular methods

We stored blood taken from adults and nestlings at 4°C in ethanol (2011–2014) or dried on filter paper (2014 only). Total genomic DNA was extracted using the standard protocol for the E.Z.N.A Blood DNA Mini Kit (Omega Bio-Tek, Norcross, GA, USA) and stored at -20°C.

We determined nestling sex by using polymerase chain reaction (PCR) to amplify two homologous avian sex chromosome genes. P8 (5'-CTCCCAAG GATGAGRAAYTG-3') reverse and P2 (5'-TCTGCATCGCTAAATCCTTT-3') forward primers were used to amplify the chromo-helicase-DNA-binding

Table 1.

Results from a principal components analysis of measures of rump and tail plumage colouration (brightness, hue and chroma).

	Eigenvalue	Proportion of variance	Colour variable	Factor loading
Male tail PC1	1.84	0.61	Brightness UV + blue chroma Hue	0.64 0.51 -0.57
Female tail PC1	1.68	0.56	Brightness UV + blue chroma Hue	$0.54 \\ 0.54 \\ -0.65$
Male rump PC1	2.07	0.69	Brightness UV + blue chroma Hue	$0.49 \\ 0.64 \\ -0.60$
Female rump PC1	2.17	0.72	Brightness UV + blue chroma Hue	$0.45 \\ 0.64 \\ -0.63$

Because the first principal component (PC1) was found to explain most of the variation, PC1 was used to represent overall plumage colour variation.

(CHD) genes of the Z (CHD-Z) and W (CHD-W) sex chromosomes (Griffiths et al., 1998). Due to the inclusion of an intron, the P8 and P2 primers amplify regions approximately 300 and 375 bp in size from the CHD-Z and CHD-W genes, respectively, allowing for amplicon separation and sex determination (Griffiths et al., 1998). Following separation, we identified males by the presence of a single 300-bp band and identified females by the presence of both a 300-bp band and a 375-bp band.

PCR amplification was carried out in a total volume of 25 μ l, the final reaction conditions being: 10.35 μ l H₂O; 3.0 μ l (3.0 mM) MgCl₂; 5.0 μ l (1×) buffer; 0.5 μ l (0.2 mM) nucleotide mix; 0.15 μ l (0.75 U) *Taq* polymerase; 2.5 μ l (1 μ M) each P2 and P8 primers; 1 μ l genomic DNA (mean ± SD = 233 ± 193 ng/ μ l). All reagents were supplied in the GoTaq PCR Core System II (Promega, Madison, WI, USA). An initial denaturing step at 94°C for 5 min was followed by 30 cycles of 94°C for 30 s, 51°C for 45 s, and 72°C for 45 s. A final run of 48°C for 1 min and 72°C for 5 min completed the program. PCR products were separated by gel electrophoresis for 90–120 min at 90 V in a 2.5% agarose gel (10 × 7 cm) stained with GelRed Nucleic Acid Stain (Biotium, Hayward, CA, USA). When tested using DNA from adult birds of known sex (N = 32; 21 females and 11 males), we found the protocol to have 100% accuracy (32/32 birds correctly sexed).

2.6. Statistical analysis

Because we were interested in female control of brood sex ratio, rather than differential offspring mortality, we restricted our analyses to broods containing four or more offspring. A minimum brood size of four offspring was chosen due to increased bias in broods containing fewer nestlings. Of our total sample of 89 broods, 75 broods (84.3%) contained four or more nestlings. We further restricted our analyses to broods from which both the male and female attending the nest had been caught and identified (68 broods, 79%).

Here, we refer to population sex ratio as the overall proportion of male and female nestlings in the population; brood sex ratio is based on the proportion of male and female nestlings within a brood. Mean brood sex ratio for each breeding season was calculated as the sex ratio averaged over all broods for that year.

Statistical analyses were performed using JMP 12 statistical analysis software (SAS Institute, 2015). Because the data did not meet the assumptions of normality, we used Wilcoxon signed-rank tests to determine if population sex ratio and brood sex ratios deviated from unity. A Kruskal-Wallis test was used to determine if brood sex ratios differed among years. To examine whether plumage colouration was associated with parental provisioning rates, we constructed linear mixed models with provisioning rate (early male, early female, late male, late female) as the response variable, rump PC1, tail PC1, and age as main effects, with corresponding interaction terms, and individual identity (band number) as a random effect. To examine whether plumage colouration predicted brood sex ratios, we constructed generalized linear mixed models with binomial error distribution and a logit link function, using nestling sex (M/F) as the response variable, tail colour, rump colour, and age as main effects and a random effect of nest ID nested within female band number. Models were run for males and females separately and we also included interaction terms, which were subsequently removed if nonsignificant. To ask whether parents adjusted provisioning rates in response to offspring sex ratio, we constructed linear mixed models with provisioning rate as the response variable, brood sex ratio as the main effect, and individual identity as a random effect. We also included an effect of nest watch time and interaction terms, which were subsequently removed if non-significant.

3. Results

Table 2.

3.1. Population and brood sex ratios

We determined the sex of 334 nestlings from 68 broods from the 2011–2014 breeding seasons (Table 2). We could not assign gender to nine nestlings from eight broods because no DNA was available or because we failed to obtain PCR products from those individuals. Broods included in the analysis ranged in size from three to six nestlings (range, mean \pm SD; overall: 3–6, 5.46 \pm 0.68, N = 68; 2011: 4–6, 5.19 \pm 0.75, N = 26; 2012: 4–6, 5.29 \pm 0.73, N = 14; 2013: 3–6, 4.8 \pm 0.91, N = 16; 2014: 4–6, 4.92 \pm 0.51, N = 12). Brood sex ratios ranged from 0 (exclusively female) to 1 (exclusively male) (Figure 1). Neither population nor mean brood sex ratios were found to deviate from 0.50 within or across the four study years (Table 2). Because mean brood sex ratio did not differ among years ($H_3 = 0.47$, p = 0.93), data were pooled in subsequent analyses (Table 2).

3.2. Parental plumage colour and brood sex ratio

When asking if male plumage colouration predicted brood sex ratios, we found nests with males displaying more colourful tails contained a higher proportion of male offspring ($\chi^2 = 7.29$, p = 0.007; Table 3; Figure 2A). This relationship was either significant or approached significance in three out of four years (2011: $\chi^2 = 2.92$, p = 0.09; 2012: $\chi^2 = 3.85$, p = 0.05; 2013: $\chi^2 = 3.69$, p = 0.05; 2014: $\chi^2 = 0.12$, p = 0.73). We also found a significant effect of male age; SY males had more male-biased broods than

Year	Broods	Nestlings sexed	Population sex ratio	W	р	Mean brood sex ratio ± SD	W	р
2011	26	133	0.51	67.5	0.86	0.51 ± 0.20	29.5	0.46
2012	14	71	0.52	57.0	0.73	0.54 ± 0.24	13.5	0.53
2013	16	73	0.47	-95.0	0.57	0.49 ± 0.23	5.5	0.85
2014	12	57	0.59	165.0	0.15	0.58 ± 0.23	14.5	0.23
Pooled	68	334	0.52	946.0	0.55	0.53 ± 0.22	221.5	0.17

Summary of mountain bluebird sex ratio data for the 2011–2014 breeding seasons.

Wilcoxon signed-rank tests were performed to determine if population sex ratios and brood sex ratios deviated from unity.



Figure 1. Distribution of brood sex ratios (proportion of male offspring) for the 2011–2014 breeding seasons. The mean brood sex ratio (0.53) did not deviate from unity.

ASY males (Table 3). In addition, there was a marginal male age × male rump PC1 interaction (p = 0.06). To examine this potential interaction, we separated males by age and found a significant effect of male rump PC1 in SY males, where more colourful SY males produced more male-biased broods ($\chi^2 = 5.68$, p = 0.02); there was no relationship between ASY rump PC1 and sex ratio ($\chi^2 = 0.02$, p = 0.88; Figure 2B). In contrast to males, females with lower rump PC1 values produced more male-baised broods ($\chi^2 = 4.75$, p = 0.03; Figure 3); there was no effect of tail PC1 or female age (Table 3).

3.3. Parental provisioning rates and brood sex ratio

Finally, we asked whether parents adjusted provisioning rates in response to offspring sex ratios. We found no effect of sex ratio on provisioning rates by males or females during either the early or late nestling stage (all p > 0.34; Table 4).

Table	3.
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Results of generalized linear mixed models examining the influence of plumage colouration and age on sex ratio.

Variable	Estimate	SE	χ^2	р	Ν
Male plumage colouration					
Intercept	-0.28	0.14	3.86	0.05	324
Male tail PC1	-0.30	0.11	7.29	0.007^{*}	324
Male rump PC1	-0.06	0.12	0.23	0.63	324
Male age	0.38	0.15	6.70	0.01*	324
Male age \times male rump PC1	0.19	0.10	3.55	0.06	324
Female plumage colouration					
Intercept	-0.07	0.12	0.38	0.53	330
Female tail PC1	-0.04	0.10	0.20	0.66	330
Female rump PC1	0.20	0.09	4.75	0.03*	330
Female age	-0.07	0.12	0.28	0.59	330

We used nest ID nested within female identity as a random effect. Non-significant interactions were removed to derive final models.

* Significant interaction, p < 0.05.

3.4. Parental provisioning rates and plumage colouration

In most models, neither male nor female provisioning rates (both early and late) were associated with male tail or rump plumage colouration (only the relationship between male tail PC1 and late female provisioning approached significance at p = 0.08; all other relationships p > 0.26). However, for female provisioning during the early nestling period, there were significant effects of male tail PC1 and an interaction between male age and tail PC1 (tail PC1: N = 61, $F_{1,56} = 4.86$, p = 0.03; tail PC1 × male age: N = 61, $F_{1,56} = 11.75$, p = 0.001), but not rump PC1 (N = 61, $F_{1,56} = 0.44$, p = 0.51). After separating by age classes, the relationship between tail PC1 and early female provisioning was significant for ASY males (N = 46, $r^2 = 0.24$, p = 0.0006; Figure 4), but not SY males (N = 15, $r^2 = 0.08$, p = 0.31).

4. Discussion

Over four breeding seasons, we observed support for sex allocation in mountain bluebirds: females paired with more colourful males produced more male-biased broods. Our results were somewhat surprising, however, in that



Figure 2. (A) Broods contained a greater proportion of male offspring when females paired to males with bright UV-blue tail plumage (SY and ASY males pooled). (B) SY males (open circles, grey line) with brighter rump plumage produced more male-biased broods, while there was no relationship between rump colouration and brood sex ratio in ASY males (closed circles).



Figure 3. Females with duller UV-blue tail plumage produced broods with a greater proportion of male offspring.

SY males produced more male-biased broods and a relationship between rump colouration and male-biased broods was only present in SY males. Another unexpected finding was that females with less colourful rumps produced male-biased broods. However, Morrison et al. (2014) also found perplexing relationships with female colour in the same population of mountain bluebirds, with an observation of negative assortative mating (SY female tail PC1 was negatively associated with male rump PC1). Despite the relationship between male plumage colouration and offspring sex ratio, which is consistent with adaptive sex ratio adjustment, we did not observe any evidence that either females or males adjust their parental investment in response to brood sex ratios.

Relationships between plumage ornamentation and brood sex ratio have been reported previously in several species. Of the studies investigating male ornamentation as a driver of sex allocation, those finding significant relationships were generally conducted over three or more breeding seasons (range 1-8, mean \pm SD = 3.9 ± 2.6 , N = 6) (Ellegren et al., 1996; Sheldon et al.,

Table 4.

Results of a linear mixed model examining the influence of offspring sex ratio on provisioning rates of male and female mountain bluebirds. We included nest ID nested within female identity as a random effect.

Variable	Estimate	SE	t	р	Ν
Early male provisioning					
Intercept	1.36	0.22	6.05	< 0.0001*	60
Sex ratio	-0.18	0.40	-0.47	0.64	60
Early female provisioning					
Intercept	0.33	0.33	0.99	0.33	60
Sex ratio	0.29	0.30	0.97	0.34	60
Nest watch time	0.08	0.03	2.55	0.01*	60
Late male provisioning					
Intercept	1.81	0.47	3.83	0.0003*	54
Sex ratio	0.72	0.84	0.86	0.39	54
Late female provisioning					
Intercept	2.22	0.48	4.63	< 0.0001*	54
Sex ratio	0.21	0.85	0.25	0.80	54

* Significant value, p < 0.05.

1999; Griffith et al., 2003; Delhey et al., 2007; Mitrus et al., 2015; Romano et al., 2015), while those finding no evidence for such a relationship were conducted over three or fewer breeding seasons (range 1–3, mean \pm SD = 2.0 \pm 0.8, N = 4) (Saino et al., 1999; Rosivall et al., 2004; Korsten et al., 2006; Czyź et al., 2012). A comparison between studies on the same species illustrates the importance of the inclusion of multiple breeding seasons: in barn swallows, Romano et al. (2015) found evidence to support sex allocation in relation to male ornamentation over eight breeding seasons. Here, we observed support for sex allocation in response to male mountain bluebird plumage colouration over four breeding seasons. This relationship was either significant or approached significance in all but one of our study years. Thus, data spanning multiple breeding seasons may be necessary to reveal overall trends of sex allocation both within species and populations.

Contrary to our expectations, we found that females paired to SY males produced more male offspring. This is counterintuitive, as females may receive indirect genetic benefits by mating with older males (Brooks & Kemp, 2001) and, thus, often prefer older males as both social and extra-pair part-

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Figure 4. When paired to ASY males with bright tail plumage, females provisioned more frequently during the early nestling phase.

ners (Kempenaers et al., 1997; Brooks & Kemp, 2001; Freeman-Gallant et al., 2011). As a result, females paired to older males would be predicted to produce male-biased broods. In addition, the relationship between rump colouration and offspring sex ratio was only present in SY males, which is somewhat surprising. However, Taff et al. (2011) found a similar relationship between male age, plumage ornamentation and brood sex ratio in common yellowthroats. One possible explanation is because first-year males moult rump feathers shortly after fledging, there was greater variability in condition amongst first-year males, making rump colouration a more reliable signal of quality in SY males than ASY males. However, this fails to account for the lack of an age effect in tails, which are grown under different conditions in SY and ASY males (in the nest vs. free-living, respectively).

The negative relationship observed between female rump colour and brood sex ratios is perplexing. Trivers & Willard's (1973) seminal hypotheses regarding sex allocation stated that females in good condition should produce male-biased broods, assuming offspring condition and survival is correlated with maternal condition (Trivers & Willard, 1973). This relationship between maternal condition and brood sex ratio has been well studied and validated in several species (Nager et al., 1999; Whittingham & Dunn, 2000; Alonso-Alvarez & Velando, 2003; Pike & Petrie, 2005). Thus, based on sex allocation theory, more colourful females (presumably in better nutritional condition during the time of moult) should produce male-biased broods. The fact that we observed the opposite clearly indicates the need for more research into the signalling system of mountain bluebirds.

Female provisioning behaviour was positively associated with mate attractiveness. During the early stages of nestling development, females paired to older, ASY males with brighter tail plumage colouration provisioned their offspring at a higher rate. This result is somewhat surprising, as previous work by Morrison et al. (2014) in our study population, and by Balenger et al. (2007) in a population of mountain bluebirds from Wyoming, USA, failed to find a relationship between male or female plumage colouration and provisioning rates.

One potential explanation for the relationship between female provisioning and male colouration is differential resource allocation. Under the Differential Allocation Hypothesis, individuals maximize fitness through strategic partitioning of parental investment (Burley, 1986). Females paired to highly attractive males may increase parental investment and, in return, attractive males may be able to decrease parental investment (Linville et al., 1998; Jawor et al., 2004; Siefferman & Hill, 2005). Though plausible, we did not observe any evidence of differential allocation of parental investment driving increased maternal investment (as measured by provisioning rate). These results are consistent with the findings of Balenger et al. (2007) and Morrison et al. (2014); they similarly found no direct association between male and female plumage colouration and provisioning rates.

An alternative explanation for the relationship between female provisioning and male colouration is that provisioning rates may be related to territory quality. In blue grosbeaks (*Passerina caerulea*), the quality of the territory a male is able to establish is associated with individual condition and UV-blue plumage colouration (Keyser & Hill, 2000). In addition, males on high quality territories with abundant prey provision at increased rates (Keyser & Hill, 2000). As mountain bluebirds also display UV-blue plumage colouration, it is plausible that male colouration may be associated with quality of the territory a male is able to obtain. Thus, one possibility is that females paired with colourful males are rearing young on high quality territories with easily accessible prey and as a result, are able to provision at a higher rate. At present, no measures of territory quality have been taken in our study population. An important next step in deciphering the underlying cause(s) of this relationship would be to assess food availability and other indicators of habitat quality in each male bluebird's territory.

Sex allocation theory predicts that females should increase investment and adjust the sex ratio of their broods in response to the differential reproductive value of sons and daughters. As predicted, we found that female mountain bluebirds produced more male-biased broods when paired with attractive males (i.e., higher tail UV-blue plumage colouration). In addition, we found females paired with more colourful males provisioned their offspring more frequently. However, there was no evidence that either males or females adjusted provisioning rates in response to brood sex ratios. Together, these results provide support for sex allocation in mountain bluebirds, and suggest male attractiveness may influence female reproductive decisions. However, more work is clearly needed to understand the role of male age and female plumage colouration in the signalling system of mountain bluebirds.

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