Effects of a novel ectoparasite on condition and mouth coloration of nestling barn swallows

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Parasites have profound and widespread implications for the ecology and evolution of hosts, and human activity has increased the frequency of interactions between hosts and parasites that have not co-evolved. For example, by building habitat attractive for nesting, humans might have facilitated range expansion by cliff swallows (*Petrochelidon pyrrhonata*) and barn swallows (*Hirundo rustica*) in North America, concurrently allowing a haematophagous ectoparasite of cliff swallows, the swallow bug (*Oeciacus vicarious*), to infest the nests of barn swallows. We found that in barn swallow nests infested with swallow bugs, nestlings weighed less and had lower haematocrit, and the within-brood variation in body mass and tarsus length was higher. Information about these negative effects might be available to parents via mouth coloration, a condition-dependent component of the begging signal. We found that nestlings from infested broods had lower-intensity carotenoid-based and ultraviolet mouth colours, although most elements of colour were unrelated to parasites. Host switching by the swallow bug offers excellent opportunities to understand the direct and indirect effects of a novel parasite and might also afford insights into how parasites cope with selective pressures exerted by closely related hosts with key ecological differences.

ADDITIONAL KEYWORDS: barn swallow – begging – host switching – mouth colour – parasite.

INTRODUCTION

The fitness challenges imposed by parasites have ubiquitous and far-reaching effects on the evolution and ecology of hosts (Clayton & Moore, 1997; Rivero & Gandon, 2018). Parasites might drive the evolution of adaptations that allow potential hosts to avoid and remove parasites (Bush & Clayton, 2018), could explain the evolution of male ornaments (Hamilton & Zuk, 1982; Folstad & Karter, 1992) and might even explain the maintenance of sex itself (Salathé *et al.*, 2008). As newly interacting parasites and hosts co-evolve, the harm a parasite causes the host generally declines for some period of time, although trade-offs acting on both players can lead virulence to stabilize at any absolute level (Toft & Karter, 1990; Brown *et al.*, 2021). When a host encounters a new parasite, documenting the effects of this interaction can offer insights into the causes and consequences of host switching, how virulence evolves and how new parasites shape the

ecology of hosts (Toft & Karter, 1990; Woolhouse *et al.*, 2005; Brown *et al.*, 2021).

Human activities can facilitate contact between hosts and parasites that would be isolated in nature (Lymbery *et al.*, 2014; Galipaud *et al.*, 2017; Rogalski *et al.*, 2017). One hypothesized example of recent host switching driven by human activity is the expansion of the cimicid swallow bug (*Oeciacus vicarius*) from its typical host, the cliff swallow (*Petrochelidon pyrrhonata*), to other members of the family Hirundinidae. The life cycle of this haematophagous ectoparasite is dependent upon feeding from nestlings, and it colonizes by crawling within colonies and by clinging to the feet of adults to move between colonies (Loye, 1985). Historically, the North American breeding ranges of three colonial or semi-colonial swallows, cliff, cave (*Petrochelidon fulva*) and barn (*Hirundo rustica*) swallows, were characterized by little overlap (Brown & Brown, 2020; Brown *et al.*, 2020; Strickler & West, 2020). The ranges of all three species, however, have expanded in the last century, an expansion hypothesized to have been driven, at least in part, by the construction of bridges, culverts and buildings that

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increased available habitat for nest building (Brown & Brown, 2020; Brown *et al.*, 2020; Strickler & West, 2020). Crucially for the expansion of the swallow bug to non-cliff swallow hosts, this boom of anthropogenic habitat has also brought swallow species that would historically have used different habitats for breeding into direct contact with each other and with each other's nest-specialist parasites (Weaver & Brown, 2005; Kopachena *et al.*, 2007; Brown & Brown, 2020).

Although historically rare (Usinger, 1966; Smith & Eads, 1978), swallow bug infestations in barn swallow nests have increased in recent years, coincidental with the increased range overlap of barn and cliff swallows (Orr & McCallister, 1987; Kopachena *et al.*, 2007; Brown & Brown, 2020). In addition to encountering swallow bugs dispersing at nesting sites shared with cliff swallows, barn swallows can be exposed to parasites by re-use of nests (Safran, 2004), including the remnants of old cliff swallow nests (Samuel, 1971; M. B. Dugas, personal observation). In cliff swallows, parasitized nestlings have lower body mass and haematocrit and reduced growth, are less likely to survive to fledging and less likely to survive their first year of independence (Brown & Brown, 1986, 1996), although virulence is declining (Brown *et al.*, 2021). Relatively little is known about the effect of swallow bugs on barn swallow nestlings, but feather growth (but not body condition) is reduced in parasitized nests, especially in large broods (Kopachena *et al.*, 2000).

We studied the effect of the swallow bug infestation on nestling barn swallows, considering both proxies for individual nestling condition (body mass, tarsus length and haematocrit) and how infestation was associated with properties of the brood (size of brood and variation in individual body mass). We also asked whether infestation was associated with the coloration of nestling mouths, a component of the begging signal that contains information and influences parents in passerines generally (reviewed by Caro *et al.*, 2016) and in barn swallows specifically (Saino *et al.*, 2000, 2003, 2008; de Ayala *et al.*, 2007). Swallow bugs can elicit an immune response in their hosts (Fassbinder-Orth *et al.*, 2013), and disturbances to the immune system influence mouth colour in birds, possibly owing to competition for carotenoids between colour and immune function (Saino *et al.*, 2000, 2003; Piault *et al.*, 2008; Romano *et al.*, 2011). By comparing barn swallow broods with and without swallow bug infestation, we tested the predictions that these novel parasites would be associated with higher within-brood variation in nestling size and perhaps smaller broods (Knowles *et al.*, 2010), and that chicks from infested nests would be of worse quality and display less intense mouth colours.

MATERIAL AND METHODS

STUDY SPECIES

The barn swallow is a small (17–20 g), migratory passerine. Pairs typically breed in a solitary manner or in small groups with some distance between neighbours (Safran, 2007). The open cup nest is built of mud balls attached to or resting on natural or (now more commonly) man-made structures, and both parents deliver insect boluses to broods of three to seven nestlings during the 15- to 27-day nestling period (Brown & Brown, 2020). We studied 119 barn swallow nestlings from 28 nests distributed across 13 bridges and culverts in Cleveland, Pottawattomie, Caddo and Grady counties, OK, USA in June–July 2009. We used one to six nests per site, which were the vast majority of active nests at each site during the study period. Cliff swallows were breeding at four of these sites, and all were within ~2 km of active cliff swallow colonies and/or contained evidence of previous cliff swallow use (e.g. nest 'scars' on concrete). Nesting eastern phoebes (*Sayornis phoebe*) were present at one site and nesting house sparrows (*Passer domesticus*) at another.

We visited nests regularly until egg laying was complete. To establish the day of hatching, we checked daily beginning the day before the anticipated hatch date. At day 10 post-hatching, we estimated swallow bug density and measured nestling phenotype. This is the age at which the effects of swallow bugs on cliff swallows have been assessed most commonly (Brown & Brown, 1996) and is within the age range during which barn swallow nestling mouth colour has been considered in previous work (Saino *et al.*, 2003, 2008; de Ayala *et al.*, 2007). North American barn swallow nests host a suite of ectoparasites other than swallow bugs (e.g. *Ornithonyssus sylvarium*; Brown & Brown, 2020); although we noted no substantial infestations of these parasites, we did not quantify them.

To avoid disturbing the parents, we briefly removed a portion of each brood to a car parked nearby but out of view of the parents. We weighed each nestling to the nearest 0.01 g on an electronic balance, measured the left and right tarsus to the nearest millimetre with digital callipers, then sampled mouth coloration (details in next subsection). After measurement of mouth colour, we obtained a small (~75 µL) blood sample from the brachial vein, centrifuged the sample immediately and recorded the haematocrit. We then returned the nestlings and sampled the remainder of each brood. Nestlings were away from the nest for < 30 min. Body mass, mean tarsus length and haematocrit are all likely to be influenced by parasites generally and by this parasite in particular (Møller, 1990; Brown & Brown, 1996; Saino, 1998). Body mass is a good predictor of

fitness prospects in nestling birds (Schwagmeyer & Mock, 2008), including barn swallows (Teglhøj, 2020). Haematocrit is unlikely to be strongly associated with fitness prospects, but any relationship is expected to be positive (Cuervo *et al.*, 2007).

Quantification of variation in the density of swallow bugs on barn swallow nests proved challenging. Swallow bugs (and their faeces) were often visible on nests. However, in pilot efforts in 2007–2008, counts at the same nest could vary across orders of magnitude in only a few days, with a handful of bugs visible one day and several hundred later in the week. Likewise, we removed several nests after chicks fledged and found substantial infestations in nests on which we had observed only a few bugs (similar difficulties have been observed by others: C. R. Brown, personal communication). As we had little confidence that we could obtain quantitative estimates of infestation in active nests, we categorized nests into 'swallow bugs present' or 'swallow bugs absent' for subsequent analysis. We determined 'presence' by spraying the nest lightly with water during incubation and again on day 10 to encourage swallow bug emergence, and we confirmed 'absence' by removing and searching the nest after fledging.

MEASUREMENT AND QUANTIFICATION OF NESTLING MOUTH COLOUR

We sampled the reflectance of nestling mouth tissue (320–700 nm) using a USB4000 spectrometer (Ocean Optics, Dunedin, FL, USA). A deuterium–tungsten halogen lamp (DT-MINI-2-GS; Ocean Optics) produced light that illuminated a ~5 mm^2 circle of tissue via a bifurcated fibre optic probe held at a 90° angle to and fixed distance from the tissue (Andersson & Prager, 2006). Reflectance was quantified relative to a white standard (WS-1) and recorded in SPECTRASUITE software (Ocean Optics). We sampled nestling flange and gape tissue four times each, twice from the maxilla and twice from the mandible (Fig. 1), and used median reflectance curves from each tissue for further analysis.

We used reflectance to calculate three variables that are good receiver-independent measures of structural and pigment-based coloration and/or influence parents (see Supporting Information, Expanded methods; Dugas & McGraw, 2011). Brightness (Endler, 1990) measures the total light reflected by tissue, may contain information about individual condition (e.g. Pirrello *et al.*, 2017) and is likely to be the primary mediator of visual conspicuousness (Dugas, 2015a). In barn swallows, nestlings with brighter flanges are heavier and have longer tarsi and greater feather growth (de Ayala *et al.*, 2007). Relative ultraviolet (UV) intensity is
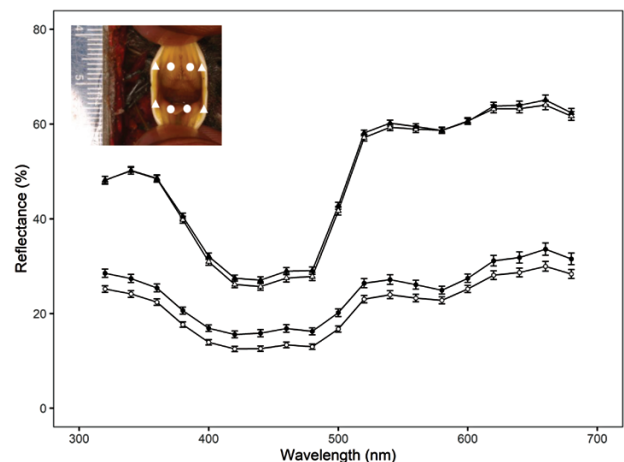
Figure 1. Mouth colour of barn swallow nestlings at day 10 post-hatching. Reflectance (mean ± SE) at 10 nm intervals of the gape (open circles, swallow bugs absent; filled circles, swallow bugs present) and flanges that border it (open triangles, bugs absent; filled triangles, bugs present). Inset is a photograph of a representative nestling barn swallow mouth, with approximate locations of colour sampling of the flange (triangles) and gape (circles).

a comparison of reflectance in UV-A wavelengths (320–400 nm) with long-wavelength reflectance (600–699 nm), providing an index of UV intensity relative to the baseline reflectance of unpigmented tissue (Bleiweiss, 2008). Although there is little indication that UV coloration reveals nestling quality, barn swallow parents favour nestlings with intact mouth colours over those with experimentally reduced flange UV reflectance (de Ayala *et al.*, 2007). Chroma (Endler, 1990) is a proxy for the carotenoid content of nestling mouth tissue (Dugas & McGraw, 2011) and is positively associated with condition proxies and favoured by barn swallow parents (Saino *et al.*, 2000, 2003).

STATISTICAL ANALYSIS

Initially, we tested for differences between nests with and without swallow bugs in brood size and relative difference in nestling mass (RDNM), calculated for a brood as the range in nestling mass divided by mean nestling mass (Skagen, 1987). We also calculated a similar metric (hereafter, 'RDNT') using tarsus length instead of mass. Both RDNM and RDNT were highly correlated with the coefficient of variation (both $r = 0.9$, $N = 28$, $P < 0.001$), another measure of within-brood variation (Knowles *et al.*, 2010). For each response variable, we used linear mixed models with swallow bug presence (present or absent) entered as a fixed factor, Julian date included as a covariate (with

brood size as an additional covariate in the models for RDNM and RDNT) and colony as a random effect.

We also used linear mixed models to test for differences in nestling body mass, mean tarsus length and haematocrit. Tarsus and haematocrit were not correlated ($r = 0.02$, $N = 119$, $P = 0.803$), whereas mass was moderately correlated with haematocrit ($r = 0.23$, $N = 119$, $P = 0.013$) and mean tarsus length ($r = 0.37$, $N = 119$, $P < 0.001$). A common condition proxy is residuals from mass regressed on tarsus, but it was highly correlated with mass in this sample ($r = 0.88$, $N = 119$, $P < 0.001$), hence we chose to exclude it from our analyses. We ran separate models for mass, tarsus and haematocrit, including the fixed factor of swallow bug presence, brood size and Julian date as covariates, and the random effects of colony and nest nested within colony.

To test for the predicted differences between mouth coloration of barn swallow chicks from nests with and without swallow bugs, we used linear mixed models with each colour parameter (brightness, relative UV intensity and chroma) as the dependent variable, the fixed factor of swallow bug presence, covariates of brood size, nestling mass and Julian date, and the random effects of colony and nest within colony. Given that carotenoids colour tissue by selective absorption of light, both brightness and relative UV intensity are, *ceteris paribus*, negatively associated with tissue carotenoid content (Andersson & Prager, 2006; Dugas & McGraw, 2011). To estimate relationships independently of this constraint, we included chroma as a covariate for analyses in which brightness or relative UV intensity was the dependent variable. The inclusion of this covariate prioritizes estimation of the physical property of tissue above estimation of what the parents might see (e.g. estimated brightness of pigment-free tissue vs. total brightness of tissue); we present the results of analyses without this covariate in the Supporting Information (Table S1).

We conducted all analyses in R v.4.0.1 (R Core Team, 2020) using the packages lme4 (Bates *et al.*, 2015) and MuMIn (Barton, 2019). Degrees of freedom for fixed effects were estimated using Satterthwaite's approximation.

RESULTS

Swallow bugs and brood-level properties

Brood size was similar in barn swallow nests with and without swallow bugs ($F_{1,24.2} = 1.86$, $P = 0.185$; Fig. 2), and broods were marginally larger earlier in the season ($F_{1,23.5} = 3.67$, $P = 0.068$, $\beta \pm$ SE $= -0.03 \pm 0.01$). Within-brood mass variation as estimated by RDNM was greater in nests with swallow bugs present ($F_{1,24} = 5.01$, $P = 0.035$; Fig. 2), whereas Julian date ($F_{1,24} = 0.35$,

$P = 0.560$) and brood size ($F_{1,24} = 2.71$, $P = 0.113$) were not related to this measure. Within-brood variation in tarsus length (RDNT) was greater in nests with swallow bugs present ($F_{1,24} = 6.38$, $P = 0.019$; Fig. 2) and lower earlier in the season ($F_{1,24} = 8.31$, $P = 0.008$), but unrelated to brood size ($F_{1,24} = 0.55$, $P = 0.465$).

Swallow bugs and individual phenotype

Nestlings were heavier and had higher haematocrit in nests without swallow bugs, but tarsus length did not differ (Table 1; Supporting Information, Fig. S1). Swallow bug presence was associated with few aspects of barn swallow nestling mouth coloration. In nests without swallow bugs, nestling flanges were lower in relative UV intensity and the gape was marginally more chromatic (i.e. carotenoid rich; Table 2; Supporting Information, Fig. S2). Regardless of swallow bug presence, heavier nestlings had less intense UV reflectance in both gape and flange tissue and their flanges were brighter (Table 2). Sequential removal of non-significant covariates did not change these patterns qualitatively, except that the marginal effect of swallow bug infestation on gape chroma was significant when the covariate of mass was removed ($F_{1,25.5} = 5.55$, $P = 0.026$, $\beta \pm$ SE $= -0.025 \pm 0.011$). When we did not include the covariate of chroma (which corrects for expected effects of carotenoids on brightness and relative UV intensity), the relationship between flange relative UV intensity and swallow bug presence was no longer significant (Supporting Information, Table S1).

DISCUSSION

The range of barn swallows in North America has expanded. Together with use of anthropogenic habitat, this expansion has seen these birds increasingly encounter a novel parasite that co-evolved with cliff swallows (Kopachena *et al.*, 2000, 2007). In barn swallow nests with swallow bug infestations, nestlings roughly halfway through development weighed less and had lower haematocrit, a result consistent with the haematophagous habit of this parasite. Direct comparison with a more southern population is difficult because of different analytical approaches, but generally we seem to have found stronger effects of swallow bugs on barn swallows than previous work (Kopachena *et al.*, 2000). These small previously reported effects were, however, most apparent in large broods (Kopachena *et al.*, 2000), a result consistent with our finding that within-brood variation in nestling mass and tarsus length was higher in infested broods. A key puzzle remains how the effects of swallow bugs on barn and cliff swallows compare. Infestations in

Figure 2. Boxplots of brood size (A), relative difference in nestling mass (RDNM; B) and relative difference in nestling tarsus length (RDNT; C) in barn swallow nests with swallow bugs absent (*N* = 16) and present (*N* = 12). *Significant (*P* < 0.05) difference.

Table 1. Results of linear mixed models assessing the relationship between condition proxies and swallow bug presence, brood size and Julian date for barn swallow nestlings

Parameter	*F*	d.f.	*P*-value	β ± SE
Mass				
Swallow bugs present	6.64	1,19.1	**0.019**	−1.78 ± 0.69
Brood size	2.64	1,24.6	0.117	0.63 ± 0.39
Julian date	0.04	1,22.8	0.852	–
Tarsus length				
Swallow bugs present	0.75	1,17.7	0.399	–
Brood size	1.18	1,26.3	0.288	–
Julian date	1.91	1,25.3	0.179	0.08 ± 0.07
Haematocrit				
Swallow bugs present	6.79	1,20.8	**0.017**	−0.05 ± 0.02
Brood size	0.86	1,22.6	0.363	–
Julian date	0.19	1,20.9	0.664	0.003 ± 0.007

Colony and nest within colony were included as random effects. Significant (*P* < 0.05) effects are shown in bold.

barn swallow nests in Texas appear to be of comparable size to those in cliff swallows in similar colonies from Nebraska, although the effects on nestlings might be less severe (Kopachena *et al.*, 2007). In the same region (and some of the same colonies) that we studied here, we found that the mass and haematocrit of the second largest nestling in cliff swallow nests (the only brood member measured) was unrelated to swallow bug abundance (Dugas & Border, in press). Therefore, although a precise and direct comparison of effects on cliff and barn swallows is yet to be carried out, patterns at this point suggest higher virulence in the novel host, mirroring the results of experimental exposure of a novel swallow host to the European swallow bug (*Oeciacus hirundinis*) (Merino *et al.*, 2001). Also of interest will be assessing the effects of the swallow bug on barn swallow adults. Given that in adult cliff swallows ectoparasites reduce annual survival (Brown *et al.*, 1995), increase dispersal (Brown & Brown, 1992) and reduce the number of broods reared per season (Brown & Brown, 2015). Contemporary comparisons of cliff and barn swallows will also need to account for a recent decline in the virulence of swallow bugs on cliff swallow hosts (Brown *et al.*, 2021).

Table 2. Results of linear mixed models assessing the relationship between colour parameters of nestling barn swallow mouth coloration and swallow bug presence

Parameter	F	d.f.	P-value	β ± SE
Flange				
Brightness				
Swallow bugs present	0.53	1,14.7	0.477	–
Mass	11.08	1,94.8	**0.001**	0.006 ± 0.002
Brood size	0.05	1,19.8	0.825	–
Julian date	2.43	1,21.5	0.134	0.0007 ± 0.0004
Chroma	112.27	1,108.7	**< 0.001**	−0.961 ± 0.091
Relative ultraviolet intensity				
Swallow bugs present	5.10	1,21.8	**0.034**	−0.038 ± 0.020
Mass	6.04	1,93.8	**0.016**	−0.008 ± 0.003
Brood size	3.86	1,27.0	0.060	0.019 ± 0.010
Julian date	1.94	1,23.7	0.177	−0.0010 ± 0.0007
Chroma	66.27	1,110.1	**< 0.001**	−1.312 ± 0.161
Chroma				
Swallow bugs present	0.14	1,26.6	0.713	–
Mass	2.67	1,101.5	0.105	0.003 ± 0.002
Brood size	0.04	1,28.6	0.835	–
Julian date	0.001	1,25.0	0.973	–
Gape				
Brightness				
Swallow bugs present	1.13	1,21.5	0.301	–
Mass	0.25	1,101.1	0.620	–
Brood size	0.23	1,26.1	0.633	–
Julian date	0.01	1,24.8	0.914	–
Chroma	22.97	1,110.1	**< 0.001**	−0.541 ± 0.113
Relative ultraviolet intensity				
Swallow bugs present	3.21	1,16.7	0.092	−0.052 ± 0.029
Mass	4.32	1,84.4	**0.041**	−0.012 ± 0.006
Brood size	3.74	1,21.5	0.067	0.033 ± 0.017
Julian date	0.02	1,22.7	0.883	–
Chroma	5.21	1,107.6	**0.024**	−0.580 ± 0.254
Chroma				
Swallow bugs present	4.13	1,27.3	0.051	−0.023 ± 0.011
Mass	0.35	1,89.9	0.557	–
Brood size	1.02	1,30.2	0.321	–
Julian date	2.58	1,26.4	0.120	−0.0007 ± 0.0004

Colony and nest within colony were included as random effects. Significant ($P < 0.05$) effects are shown in bold.

Swallow bug infestation did not affect all barn swallow family members equally; instead, it was associated with an increase in the within-brood variation in nestling mass and tarsus length. This increased variation could have arisen by direct effects of the parasites on nestlings, changes in how nestlings allocate resources to development (Saino *et al.*, 1998), differences in the allocation of parental care within broods or, more likely, interactions among these mechanisms (Dugas, 2015b). For example, female blue tits (*Cyanistes caeruleus*) medicated to reduce *Plasmodium* infections deliver more food to their brood and, presumably via more equitable distribution, reduce within-brood mass variation (Knowles *et al.*, 2010). Parent birds respond in diverse ways to resource abundance and shortfalls, with an increase in equity being only one possible result (Caro *et al.*, 2016). Parents that adjust provisioning and allocation strategies in response to changing circumstances stand to accrue fitness benefits (Mock & Forbes, 1995) and

might make such adjustments based on information revealed by offspring signals (Mock *et al.*, 2011).

The coloration of avian soft parts is generally a good candidate to convey dynamic information about individual condition (Iverson & Karubian, 2017). The 'yellowness' of the gape tissue of barn swallow nestlings was less intense in nests parasitized by the novel swallow bug. This result gives further support to the hypothesis that the carotenoid richness of nestling mouth tissue signals high quality, joining positive relationships with nestling size and immune function in barn swallows (Saino *et al.*, 2000, 2003; de Ayala *et al.*, 2007), size/age and circulating carotenoids in cave swallows (Dugas *et al.*, 2018) and mass in cliff swallows, a species in which the extent of swallow bug infestation is also negatively associated with these colours (Dugas & Border, in press). It is a bit less clear whether there are conserved (or simple) relationships between condition, broadly defined, and the structural elements that are likely to underlie variation in brightness and UV reflectance (Prum & Torres, 2003) in these close relatives. The brightness of flange tissue is positively associated with nestling mass in barn swallows (de Ayala *et al.*, 2007; present study) and cliff swallows, and in the latter both flange and gape brightness are negatively associated with swallow bug density (Dugas & Border, in press). Although these patterns suggest that brighter tissue reveals high quality, the brightness of cave swallow mouths is negatively associated with mass/age and circulating vitamin E (Dugas *et al.*, 2018). Here, we found that UV flange coloration was negatively associated with swallow bug infestation, but also less intense in heavier nestlings (as was UV colour in the gape, *contra* de Ayala *et al.*, 2007). In cave swallows, this element of flange colour is also negatively associated with mass (Dugas *et al.*, 2018), but the opposite was true in cliff swallows (and the relationship with swallow bugs is complex: Dugas & Border, in press). Among-species comparisons (e.g. Avilés *et al.*, 2008) offer important insights into broad patterns shaping the evolution of morphological elements of the begging display, and comparisons among close relatives that seem to differ in these relationships might prove helpful in understanding how and why condition is linked to each element, in addition to the extent to which these relationships are constrained.

The condition dependence of nestling mouth coloration, along with parental responses to variation in this trait, offers key support for the hypothesis that some components of begging evolve to signal high quality (Mock *et al.*, 2011; Caro *et al.*, 2016). Both within-tissue patterns of variation (Dugas, 2010) and comparisons between adults and nestlings (Dugas *et al.*, 2018) in swallows suggest that the brightness, UV richness and

carotenoid richness of flange colour have all evolved in the context of visual communication between offspring and parents. Although we have no test of the parental response to colour in this population, it is notable that the two colour elements negatively associated with swallow bug infestation, the 'yellowness' of the gape and the UV richness of flange colour, are both favoured by parents in other populations (Saino *et al.*, 2000; de Ayala *et al.*, 2007). The effect associated with swallow bugs is certainly more modest than that generated by experimental manipulation, suggesting that caution is warranted in predicting how parents might respond to within- or among-brood variation of this magnitude. However, there is good reason to suspect this range of variation could be biologically important, as the effect of swallow bug infestation on colour was equivalent to the difference between being the heaviest and lightest member of a brood (the effect of mass here was also similar to that reported in another bird by Dugas & McGraw, 2011).

The negative effects of swallow bugs on barn swallow host nestlings indicate that infestations could exert selective pressures on populations of naïve hosts (Merino *et al.*, 2001; Kopachena *et al.*, 2007), perhaps including additional/new selection on parental favouritism within and among broods. The recent nature of this selective pressure, along with potential replication in cave swallows (Weaver & Brown, 2005), will present the chance to test predictions about the evolution of convergent and novel anti-parasite mechanisms (Bush & Clayton, 2018), including the immune response (Fassbinder-Orth *et al.*, 2013), perhaps reflecting the different breeding ecologies of these closely related birds. Adaptations of the parasite to the novel host will also be of interest. Nest shape, for example, varies considerably within the swallows (Winkler & Sheldon, 1993), and differences between the gourd-shaped nest of cliff swallows and the open cup of barn swallows presumably present different challenges to this nest-bound parasite (e.g. bugs are typically clustered at the end of the nest entrance tunnel on cliff swallow nests, presumably an aid in dispersal). Intriguingly, the same anthropogenic habitat use that might have facilitated host switching might also have increased parasite loads and driven the evolution of reduced virulence in cliff swallows (Brown *et al.*, 2021), further complicating selective pressures on hosts and parasites alike. As human activity continues to drive contact between hosts and parasites that did not co-evolve (Rogalski *et al.*, 2017), this will present the opportunity, or perhaps the obligation, to ask these exciting questions about how, why and when parasites affect host fitness (Toft & Karter, 1990; Woolhouse *et al.*, 2005).

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DATA AVAILABILITY

Data underlying all analyses presented here are available in the Supporting Information (Tables S1 and S2).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Results of linear mixed models assessing the relationship between select colour parameters of nestling barn swallow mouth coloration and swallow bug presence, without the inclusion of chroma as a covariate. Colony and nest within colony were included as random effects. Sequential removal of non-significant covariates did not change these patterns qualitatively. Significant effects are indicated in bold.
Table S2. Excel file of analysis data.
Figure S1. Boxplots showing differences in mass (A), tarsus length (B) and haematocrit (C) of barn swallow nestlings from nests with or without the presence of swallow bugs. *Significant ($P < 0.05$) difference in a linear mixed model that included the covariates of brood size and Julian date and the random effects of colony and nest within colony.
Figure S2. Boxplots showing coloration of the flange (A–C) and gape (D–F) tissue of barn swallow nestlings from nests with and without swallow bugs present. *Significant ($P < 0.05$) difference in a linear mixed model that included the covariates of brood size, Julian date and nestling mass and the random effects of colony and nest within colony.