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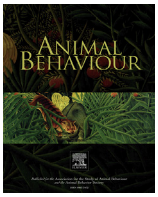

Sexual size dimorphism of brood-parasitic nestlings does not affect host chick survival, size or fledging phenology

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Intraspecific variability in parasite virulence holds significant ecological and evolutionary implications as it can result in uneven costs incurred by individuals of a host species. Obligate brood parasites, birds that lay their eggs in the nest of another species and do not raise their own young, differ in interspecific virulence, as some species kill all host nestmates directly while other species do not. However, variation in the intraspecific virulence of a brood-parasitic species has rarely been investigated. One source of this variability could arise through sexual size dimorphism, because many brood-parasitic species are sexually dimorphic in size starting at the nestling stage, and nestling size often impacts competitive ability in the brood. Here, we investigated the sex-specific effects of nestling brood-parasitic brown-headed cowbirds, *Molothrus ater*, on one of their hosts, the prothonotary warbler, *Protonotaria citrea*. We predicted that larger male cowbird nestlings would cause host chicks to have lower survival and size and altered life history in the form of delayed fledging. Using experimentally parasitized broods, we found that although male brood-parasitic cowbird nestlings are heavier than females, there was no effect of cowbird sex on the survival, size or fledging phenology of the host nestlings. Furthermore, there were no differences in fledging phenology between the male and female cowbird chicks. Instead, we found that wing length, which was similar between the parasitic nestlings' sexes, was an important predictor for age and order of fledging. These findings show that the extent of development is critical for the timing of fledging of both parasitic and host species. Notably, the lack of effect of cowbird nestling sex on host chick survival and fledging suggests that the presence of a parasite is more influential for host nestlings than the size of parasites.

Parasites differ widely in the degree of fitness losses that they inflict upon their hosts, which is known and quantified as virulence (Frank & Schmid-Hempel, 2008). Parasitic virulence varies between species and systems but can also vary within a species even when using the same host (Colinet et al., 2010). For example, the bacterium *Pasteuria ramosa*, the parasitic wasp *Leptopilina boulardi* and the blood fluke *Schistosoma mansoni* all show intraspecific variation in their virulence towards their hosts the water flea *Daphnia magna*, the fruit fly *Drosophila melanogaster* and the freshwater snail *Biomphalaria glabrata*, respectively (Colinet et al., 2010; Gower & Webster, 2005; Little et al., 2008). The existing variation in fitness costs that hosts incur owing to the characteristics of the parasite is essential to understand coevolutionary cycles between the two species. One possible source of variation in parasitic virulence is sexual size dimorphism. For example, female *Nanobalcis worsfoldi* parasitic snails are larger than males, which could result in different prevalence or parasitic burden caused by the two sexes, although this has yet to be investigated (Sales & Queiroz, 2021). The virulence of parasites of different sexes has not been given the same attention as the effects of parasitism experienced differently by hosts of different sexes (Duneau & Ebert, 2012), but it could be just as important for host–parasite coevolution. Sexual dimorphism of parasites could result in disparate selective pressures on hosts that are more likely to encounter or be infected by one parasite sex and for the evolution of host selection respective to parasite sex.

In one parasitic strategy, known as obligate brood parasitism, parasites rely on other individuals to raise their offspring (Pollock et al., 2021). Brood parasitism is found in several insect and fish species; however, it is most widely studied in birds, where over 100

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species are obligate brood parasites and 17% of all bird species serve as their hosts (Antonson et al., 2022; Soler, 2017). Virulence differs extensively between these brood-parasitic species, as some are nestmate-eliminators, meaning that the brood-parasitic chick evicts or kills all other host eggs and young, while some cohabitate with host nestlings, as the brood-parasitic chick does not directly kill host young (De Mársico et al., 2017; Kilner, 2005; Moskát et al., 2017). While the latter type of parasitism may be less virulent, the parasitic chick still often causes the host to suffer lower reproductive success by outcompeting the host chicks for parental provisions (Payne & Payne, 1998; Rivers & Peer, 2016); thus, host nestlings in parasitized nests often suffer decreased growth and increased mortality compared to their nonparasitized counterparts (Hauber, 2003; Hoover, 2003b; Scharf et al., 2021). Brood parasites can additionally impact host population dynamics by causing female-biased host nestling mortality, resulting in a male-biased sex ratio of host fledglings (Zanette et al., 2005).

For nestmate-cohabitating brood parasites, chick size may be an important characteristic in their success. Brood-parasitic nestlings usually outcompete smaller host nestlings (Lorenzana & Sealy, 1999) but struggle to overcome the size advantage when host nestlings are larger (Lichtenstein & Sealy, 1998; Rivers, 2007). Host parents may prefer to feed the largest chick in the nest, regardless of its species (Rivers, 2007). Therefore, if one sex is larger than the other sex in parasitic chicks, it is possible that parasites may cause sex-specific virulence on host nestling survival. If one sex is more virulent, this could have important effects on host population dynamics if the sex ratio of parasitic eggs is temporally or spatially skewed. Because female birds have been shown to have some control over the sex of the eggs they lay (Pike & Petrie, 2003), it is possible that female brood parasites could be selective about which hosts they parasitize relative to the sex of the egg they are carrying and that host parents of certain quality would be more likely to receive a female or male chick.

Indeed, many brood parasites show sexual dimorphism in size; for example, in 83.6% of parasitic cuckoo species, males are larger than females (Krüger et al., 2007). This size dimorphism can already be present at the chick stage; for example, common cuckoo, *Cuculus canorus*, chicks are the same body mass at hatching, but males gain higher asymptotic mass before fledging than females (Požgayová et al., 2018). Within the nestmate-cohabitator brood parasites, larger body mass in male chicks was found in the brown-headed cowbird, *Molothrus ater* (Tonra et al., 2008; but see Weatherhead, 1989), shiny cowbird, *Molothrus bonariensis* (Tuero et al., 2013) and screaming cowbird, *Molothrus rufoaxillaris* (De Mársico et al., 2010). Thus, male and female brood-parasitic nestlings may exhibit different competitive strategies as males might require more food or be more competitive based on their larger size and could have different life history characteristics at this stage (Tonra et al., 2008).

The dynamics between competing nestlings in a brood can be studied through mortality and growth, but critical life history phase changes can also be an interesting lens through which to view competition. For birds, fledging is a key event that represents the shift from a sessile, dependent existence into one in which the bird must navigate the environment, avoiding predators and environmental hazards while also finding their parents for food (Jones et al., 2020). In most species, very little is known about the ontogeny of fledging outside of the general length of the nestling period (Mainwaring, 2016; Martin et al., 2018), even though this life stage transition results in a survival bottleneck, with 40–60% of fledglings dying within 4 days of leaving the nest (Jones et al., 2017; Naef-Daenzer et al., 2001). Several studies have shown that the extent of nestling development, specifically wing length, is an important predictor of fledging order and age. Nestlings with longer wings fledge at younger ages than nestlings with shorter wings (Johnson et al., 2004; Radersma et al., 2011; Scharf et al., 2022). Nestling mass can also predict fledging order within a brood, with larger nestlings fledging before their smaller nestmates (Lemel, 1989). Size differences also have effects after fledging. At fledging, heavier great tits, *Parus major*, and coal tits, *Parus ater*, had higher survival during their first winter (Naef-Daenzer & Grüebler, 2016; Radersma et al., 2015). Similarly, fledglings with longer wings were better at flying longer distances and had higher survival (Jones & Ward, 2020; Martin et al., 2018). Because avian brood parasitism often causes adverse effects on host nestling growth (Hoover, 2003b), investigating these characteristics of fledging can elucidate further downstream consequences of parasitism on host life histories.

In this study, we aimed to evaluate whether (1) male and female parasite nestlings differ in their size and fledging characteristics; (2) male and female parasite nestlings have different effects on host size, sex ratio, survival and fledging characteristics; and (3) wing development affects parasite fledging phenology similarly as it does in hosts. We experimentally parasitized nests of the prothonotary warbler, *Protonotaria citrea*, an acceptor host of the brown-headed cowbird, and measured nestling size, survival and fledging. In a previous study in this system, we found that cowbird parasitism increased mortality, decreased size and altered fledging phenology in warbler host nestlings compared to (experimental) conspecific parasitism by another, unrelated warbler chick (Scharf et al., 2022). In particular, host nestlings fledged at older ages and the brood had a greater fledging latency when parasitized with a cowbird. For this study, we predicted that cohabitative parasitism by the typically larger male cowbirds would cause (1) decreased growth, (2) increased mortality, (3) altered sex ratio and (4) altered fledging phenology in warbler host nestlings compared to parasitism by the smaller female cowbirds. Specifically, we predicted that male cowbird parasitism would increase the fledging latency of the whole nest and raise the age of host nestlings at fledging.

METHODS

Study Site and Species

This study was performed in bottomland swamps located in the Cache River watershed of southern Illinois, U.S.A. Manipulations for this experiment were performed between May and August of 2020–2022 at three separate study sites. Prothonotary warblers, which are socially monogamous neotropical migrants (Petit, 2020), are secondary cavity nesters that readily nest in artificial nestboxes made from 1.9-litre beverage cartons that are placed in these swamps (Hoover & Schelsky, 2020). Prothonotary warblers are prone to brood parasitism by brown-headed cowbirds, and at our study sites, up to 60% of nestboxes are naturally parasitized with at least one cowbird egg (Hoover 2003a). When parasitizing a warbler nestbox, cowbirds typically lay an egg without removing a host egg (Hoover 2003a). Prothonotary warblers at these study sites typically lay two clutches in a breeding season, with the first clutch usually containing five eggs and the second clutches being smaller (Petit, 2020). First broods are more likely to be parasitized, as the number of cowbird eggs laid peaks early in the season and declines sharply (Hoover & Reetz 2006). Warbler eggs hatch together on the same day, with the exception of second clutches where incubation starts when the penultimate egg is laid. Cowbird eggs have rapid embryonic development (McMaster & Sealy, 1998) and normally hatch 1 day earlier than the warbler clutch but can also hatch 2 days earlier or on the same day as the host brood.

Experimental Manipulations

Warbler nests were randomly selected for experimental parasitism in 2020 and 2021. In 2022, all active warbler nests were experimentally treated whenever feasible. We experimentally parasitized nests because it is possible that females have some control over the sex of the eggs they lay (Pike & Petrie, 2003); hence, host selection by cowbirds could result in host pairs of certain traits disproportionately receiving male or female cowbird chicks. Experimental parasitism involved removing unincubated naturally laid cowbird eggs from warbler nests and moving each to a different unparasitized nest within 1 week of the egg being laid. Eggs were carefully transported in compartments made of a memory foam block nested in a plastic container. Some nests that experienced natural parasitism were also experimentally parasitized, but the natural cowbird egg was swapped out with a cowbird egg laid in another nest. At the time of egg transfer, we did not know the sex of the cowbird embryo, but we generally expected a 50–50% sex ratio based on previous research (Louder et al., 2020). All experimentally parasitized nests were given one cowbird egg each.

Prothonotary warbler nests were experimentally parasitized during the laying stage and before or within 1 day of the onset of incubation. Prothonotary warblers never reject cowbird eggs or young and typically only abandon a nest parasitized by a cowbird if the cowbird egg is laid before any warbler eggs (Hoover, 2003a). All cowbird eggs were introduced to nests after the first warbler egg was laid, so no nests were abandoned owing to our experimental parasitism. Nestboxes were monitored every other day to check for newly initiated clutches, incubation date and hatching date, as well as the number of eggs and the mortality of nestlings.

When most of the warbler and cowbird nestlings within a nest were 6 days of age posthatching (range 5–8 days), we banded each with an aluminium band and a passive integrative transponder (PIT) tag. This age was chosen to prevent the chance of force-fledging, as cowbird parasites can be 2 days older than the warbler brood and fledge at as early as 8 days of age. For each warbler and cowbird nestling, a measurement of the weight (g), tarsus length (mm) and unflattened first primary feather length (mm) were also taken at this time.

We parasitized 86 nests with cowbird eggs, and our experimental manipulations, which were blind to the sex of the cowbird embryo, yielded $N = 15$ nests with a male and $N = 18$ nests with a female cowbird chick in the brood that had fledging data for the cowbird. We had two additional male cowbird nests in which the male cowbird successfully fledged but were not included owing to radio-frequency identification system (RFID) reader battery failure and failure of the reader to record. These males were included in measurement analyses, yielding 17 male and 18 female cowbird nests for size comparisons. The remaining samples were lost due to predation of the incubating female warbler ($N = 5$), nest eviction of the incubating female warbler by a heterospecific competitor ($N = 1$), hatching failure of the cowbird egg ($N = 9$), death of the cowbird chick from starvation or cold ($N = 29$), nest predation ($N = 1$), battery failure of the RFID readers ($N = 1$), failure of the RFID reader to log the cowbird ($N = 1$) or by general abandonment of the nest ($N = 6$). The likelihood of cowbird chick death was similar between years (54%, 50% and 38% deaths due to starvation or cold compared to successful fledging events in 2020, 2021 and 2022, respectively; Fisher's exact test: $P = 0.437$).

RFID Dataloggers

To record the fledging date and time of nestlings, we used RFID systems attached to nestboxes with PIT tags that were integrated into a leg colour band for each bird (Eccel Technology Ltd, Groby, U.K.). The RFID system was made from an Arduino circuit board (Bridge et al., 2019), a copper antenna loop that circled the nestbox entrance and a 12 V battery and battery converter kept inside a waterproof battery storage box. The RFID reader was set to scan for the presence of a PIT tag within the vicinity of the antenna (i.e. in the nestbox entrance) every 2 s. If a bird with a PIT tag was detected in the nestbox entrance, the RFID reader would record the identity of the PIT tag every 8 s starting from when the bird arrived in the entrance until it left. The RFID reader recorded only one PIT tag at a time, but the copper antenna surrounding the entrance of the nestbox was 42 mm, meaning that it would be unusual for two birds to be fully sitting in the entrance at once. To extend battery life, readers were set to sleep between 2200 and 0500 hours. Previous research found that in this system the earliest known fledging time was 0640 hours and the latest was 1955 hours, which are both well within the operating hours of the RFID reader (Scharf et al., 2022). The last RFID log of each nestling aged 8 days or older was defined as their time of fledging. Nestlings were not included in the RFID analysis if the timer on the reader was not correct and did not record the appropriate time, the battery died prematurely, or if the nestling was accidentally force fledged ($N = 1$ nest). In total, there were 27 nests with fledging data for the whole brood and an additional six nests with partial fledging data as one or more warblers were not recorded, and one nest had fledging data for the warblers but not the cowbird.

DNA Analysis

Blood samples were taken from the brachial vein of each nestling when the majority in the nest were 6 days old (range 5–8 days old). Each blood sample was less than 100 μl and stored in 100% ethanol at room temperature until DNA extraction. The samples were extracted using Qiagen DNeasy Blood & Tissue kits (Qiagen Inc., Valencia, CA, U.S.A.).

To sex each nestling, we genotyped all warbler and cowbird nestlings at the *CHD* gene using the 1237L forward and P2 reverse primers (Griffiths et al., 1998; Kahn et al., 1998). The *CHD* gene is present on the Z and W chromosomes (female birds are heterogametic ZW whereas males are homogametic ZZ) and yields differently sized fragments depending on which chromosome the amplicon(s) reside(s). We used DNA from known-sex adult warblers and cowbirds as positive controls, as adults are sexually dichromatic.

Ethical Note

This research was permitted by the University of Illinois at Urbana-Champaign IACUC (#19032, #19039, #22020) and conducted under a federal bird banding permit #23931, Cypress Creek National Wildlife Refuge research and monitoring special use permits (#2020-002R, 2021-001R, 2022-004-R), state permits from the Illinois Department of Natural Resources (NH20–22.5777), Illinois Department of Natural Resources site permits (SS20-27, SS21-32, SS22-37) and an Illinois Nature Preserves Commission permit. Nests were visited every other day and for only a few minutes each to limit human exposure while also obtaining precise hatching and nest failure dates. Our RFID systems allowed us to get precise fledging data without human interaction aside from changing the batteries every 2 weeks. All suggested guidelines provided by the U.S. Geological Survey Bird Banding Lab were followed for handling, banding and blood sampling of chicks.

Statistical Analysis

We used R version 4.3.0 (R Core Team 2017) for all statistical analyses and figure creation. Packages ‘nlme’ (Pinheiro et al., 2022), ‘lme4’ (Bates et al., 2015), ‘FactoMineR’ (Lê et al., 2008) and ‘lmtest’

(Zeileis & Hothorn, 2002) were used for statistical analyses, and ‘ggplot2’ (Wickham, 2009), ‘ggsignif’ (Ahlmann-Eltze & Patil, 2021) and ‘ggpubr’ (Kassambara, 2023) were used for figures.

First, we investigated sex differences in body size and fledging characteristics between the cowbird nestlings. We used linear mixed models with sex as a predictor variable (i.e. fixed effect) and mass, fledging age or fledging order as response variables. Hatching asynchrony of the cowbird was used as a covariate, and a variable that combined site and year (i.e. ‘siteyear’) was used as a random effect. Response variables were checked for homogeneity of variance, and the residuals of all the response variables were inspected for normality. Because nestlings were not all the same age when they were measured, nestling age was always included as a covariate in models of size. For the fledging age of cowbirds, there was an outlier fledging at least 2 days earlier than all other birds that was potentially driving certain relationships, so we compared models with and without the outlier. Fledging order was a rank adjusted number from 0 to 1 for the order that each bird within a nest fledged, with 0 being birds that fledged first and 1 being birds that fledged last (Radersma et al., 2011). Although fledging order was uniformly distributed, it was not heteroscedastic (Breusch – Pagan test: $\chi^2_2 = 5.27$, $P = 0.07$ for sex model, $\chi^2_3 = 3.61$, $P = 0.31$ for wing length model), and general linear models have previously been used to investigate fledging order (Radersma et al., 2011).

Second, we investigated the effect of cowbird sex on warbler sex ratios, survival, body size and fledging characteristics. We used binomial mixed models to determine the effect of cowbird sex on the proportion of warbler nestlings that died per nest and to determine the effect of parasitism treatment and cowbird sex on the sex ratio of prothonotary warbler fledglings per nest. We also used the number of fledglings and number of hatchlings as weights to account for the number of trials making each proportion and siteyear a random effect. To determine the effect of cowbird sex on warbler size and fledging age, we used linear mixed models with cowbird sex as a predictor variable and nest ID nested within siteyear as a random effect. For testing the effect of cowbird sex on warbler size, we performed a principal component analysis with warbler mass, first primary feather length and tarsus length and used the first principal component containing the three variables that all loaded positively and explained 87.34% of the variance. Mass, wing length and tarsus length contributed similarly at 32.6%, 34.8% and 32.5% and had qualities of 0.85, 0.91 and 0.85, respectively. Nest fledging latency (h) was calculated per nest as the amount of time elapsed between the last fledged bird and the first bird to fledge in the nest. We tested two sets of these models, one in which the cowbirds were included in the overall nest fledging latency calculation and one in which only warblers were used to calculate the overall fledging latency. The age in days at measurement was included as a covariate in our warbler size model; cowbird hatching asynchrony was used as a covariate in the size, latency and fledging age models; and number of fledglings was included as a covariate in the latency models. To determine whether there was an effect of cowbird mass alone, we ran the above set of models, substituting an interaction of cowbird mass and age of mass measurement for cowbird sex.

Finally, to determine the effect of cowbird wing length on cowbird fledging characteristics, we used the same models as in the first set with wing length instead of cowbird sex and with the added covariate of age at measurement. We set $\alpha < 0.05$ for all analyses.

RESULTS

Sex Differences between Cowbirds

We parasitized 86 nests with cowbird eggs and our experimental manipulations, which were blind to the sex of the cowbird embryo, yielded $N = 15$ nests with a male and $N = 18$ nests with a female cowbird chick in the brood that had fledging data for the cowbird.

Female cowbirds were significantly lighter than male cowbirds ($\beta = 3.526$, $t_{25} = 3.550$, $P = 0.002$; Fig. 1a). However, there were no statistical sex differences in the cowbird nestlings' fledging age ($\beta = 0.257$, $t_{24} = 0.699$, $P = 0.492$; without outlier: $\beta = 0.632$, $t_{23} = 2.043$, $P = 0.053$) or fledging order ($\beta = 0.083$, $t_{19} = 0.498$, $P = 0.624$).

Effect of Parasite Sex on Warbler Nestlings

There was no difference in the proportion of warbler deaths in each nest from hatching to fledging between nests parasitized with a male versus female cowbird ($\beta = 0.554$, $t_{32} = 1.055$, $P = 0.292$; Fig. 2a). Between nests parasitized with a cowbird and nests that were not parasitized, there was no difference in the sex ratio of prothonotary warbler fledglings ($\beta = -0.159$, $t_{100} = -0.694$, $P = 0.488$). The sex ratio of prothonotary warbler fledglings was 0.479 in parasitized nests and 0.465 in nonparasitized nests, with higher values being more female-biased. In addition, there was no difference in the sex ratio of warbler fledglings between nests parasitized with a male or female cowbird ($\beta = -0.007$, $t_{32} = -0.018$, $P = 0.986$; Fig. 2b). The sex ratio of prothonotary

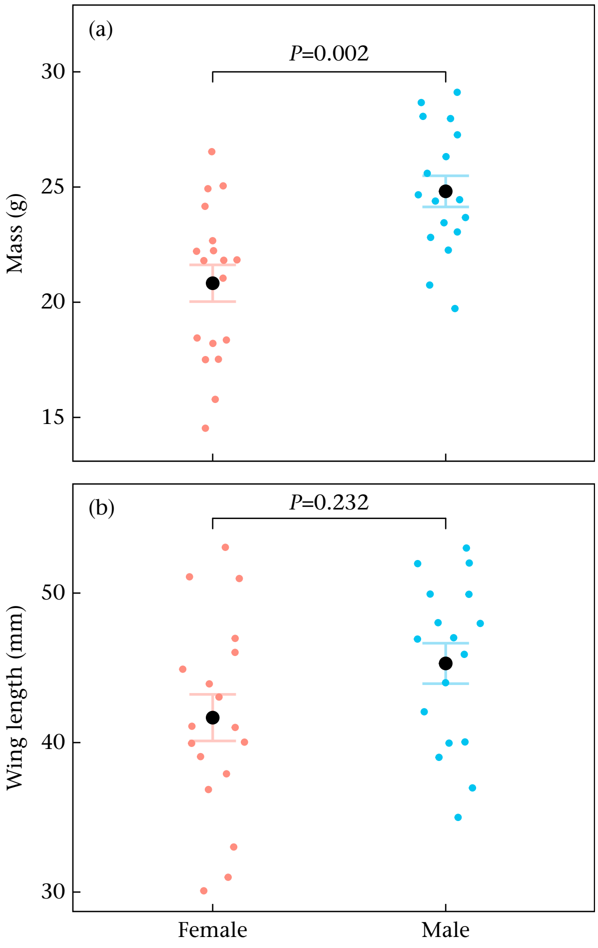

Figure 1. Comparison of (a) nestling mass and (b) wing length between male and female cowbirds. Black points represent the mean, with bars representing standard error. Comparisons are shown with their corresponding *P* value. Each point denotes an individual nestling.

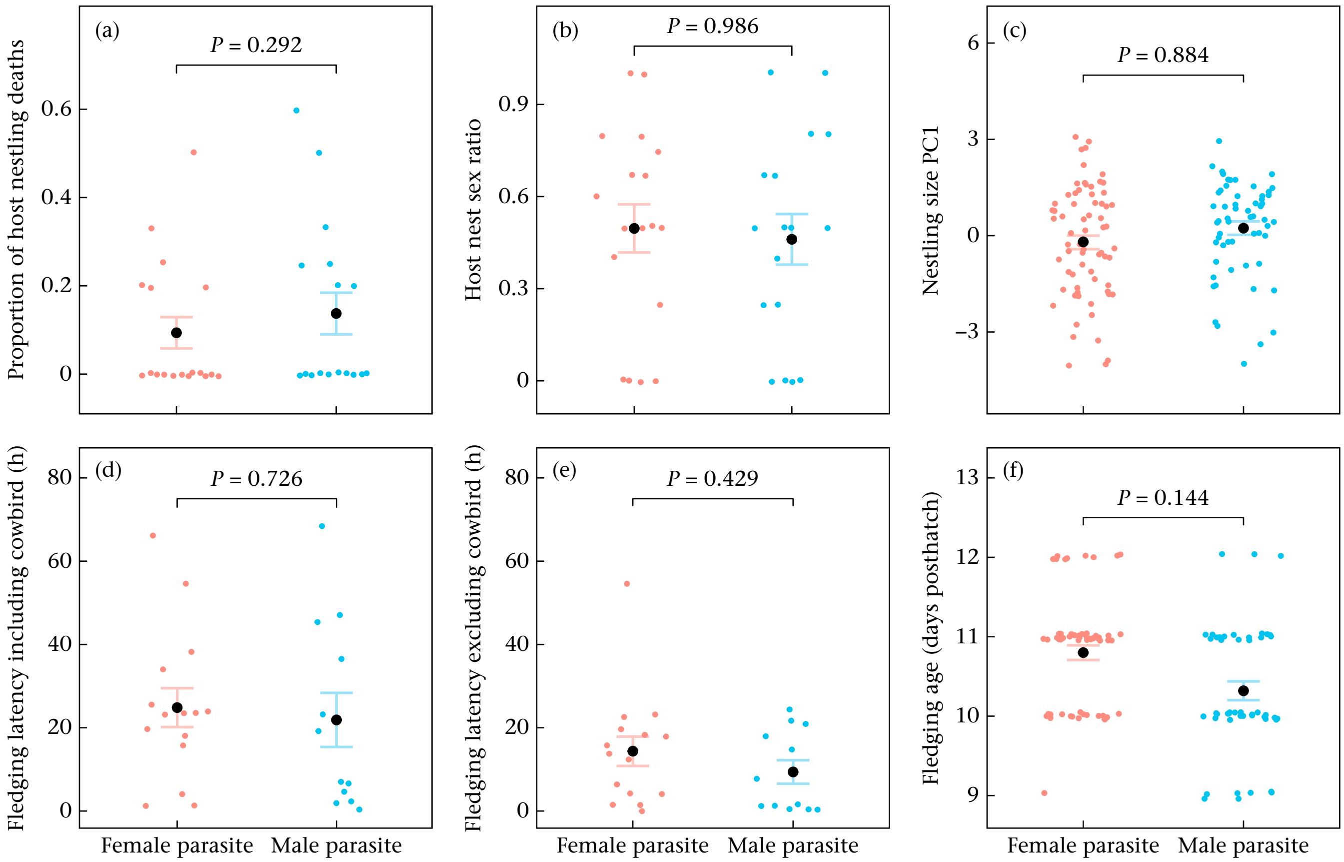

Figure 2. Comparison of (a) proportion of warbler nestling deaths for each nest from hatching to fledging; (b) sex ratio of warblers at the fledging stage for each nest; (c) warbler nestling size, first principal component from a principal component analysis of nestling mass, tarsus length and wing length at 6 days old; (d) fledging latency including cowbirds, calculated as the time elapsed between the first cowbird or warbler in a brood fledging and the last; (e) fledging latency excluding cowbirds; and (f) age at fledging of prothonotary warblers that were parasitized with a male or female cowbird. Black points represent the mean, with bars representing standard error. For (a), (b), (d) and (e), each point denotes a nest, and for (c) and (f), each point denotes an individual bird.

warbler fledglings was 0.461 in male cowbird parasitized nests and 0.496 in female cowbird parasitized nests.

Sex of the cowbird parasite did not influence warbler host nestling size ($\beta = 0.057$, $t_{26} = 0.147$, $P = 0.884$; Fig. 2c), overall fledging latency ($\beta = 2.359$, $t_{17} = 0.356$, $P = 0.726$; Fig. 2d), fledging latency excluding the cowbird ($\beta = -3.584$, $t_{17} = -0.810$, $P = 0.429$; Fig. 2e) or fledging age ($\beta = -0.345$, $t_{23} = -1.512$, $P = 0.144$; Fig. 2f). Substituting the interaction of cowbird mass and age of mass measurement for cowbird sex also did not show any effects on any of the above characteristics ($P > 0.05$; Table A1).

Wing Length

There was no sex difference in wing length of the cowbirds ($\beta = 1.880$, $t_{25} = 1.226$, $P = 0.232$; Fig. 1b). Cowbirds with longer wings fledged at significantly younger ages ($\beta = -0.139$, $t_{23} = -3.980$, $P < 0.001$; Fig. 3a; without outlier: $\beta = -0.114$, $t_{22} = -3.511$, $P = 0.002$) and earlier in the order ($\beta = -0.059$, $t_{18} = -2.891$, $P = 0.010$; Fig. 3b).

DISCUSSION

We found that although male cowbirds are significantly heavier than female cowbirds at the mid-nestling stage, the sex of the parasite did not affect the size, survival, sex ratio or fledging of their host nestmates. Male and female cowbirds showed no differences in their fledging characteristics. Cowbird wing length at the mid-nestling stage was an important predictor for fledging characteristics, with longer-winged birds fledging at earlier ages and earlier in the order within the host brood; however, wing differences were not attributable to cowbird sex. These results together suggest that the overall presence of a heterospecific brood parasite cowbird, rather than the size dimorphism between its sexes, appears to be the most relevant to host warbler survival, growth and fledging.

Cowbird parasitism has previously been shown to have many negative effects on prothonotary warbler host nestlings, including increased hatching failure (Hoover, 2003b), decreased growth (Hoover, 2003b; Scharf et al., 2022), higher proportion of nestling deaths (Antonson et al., 2022; Hoover, 2003b; Scharf et al., 2021, 2022), lower immune responses (Scharf et al., 2021) and lower rates of recruitment into the adult population (Hoover & Reetz, 2006). Many of these effects are attributed to the size difference between the parasite and host, as cowbird chicks are usually 2.0–2.5 times larger than warblers (Hoover, 2003b) and might intercept many provisions from their host nestmates. If male cowbirds were more competitive because they are larger than females, this could have important demographic effects on warbler populations because the sex ratio of parasitic eggs becomes more male-biased later in the breeding season (Louder et al., 2020). Additionally, this could affect population dynamics between brown-headed cowbirds, as male cowbirds could potentially have an advantage if the other cowbirds within the same nest are female

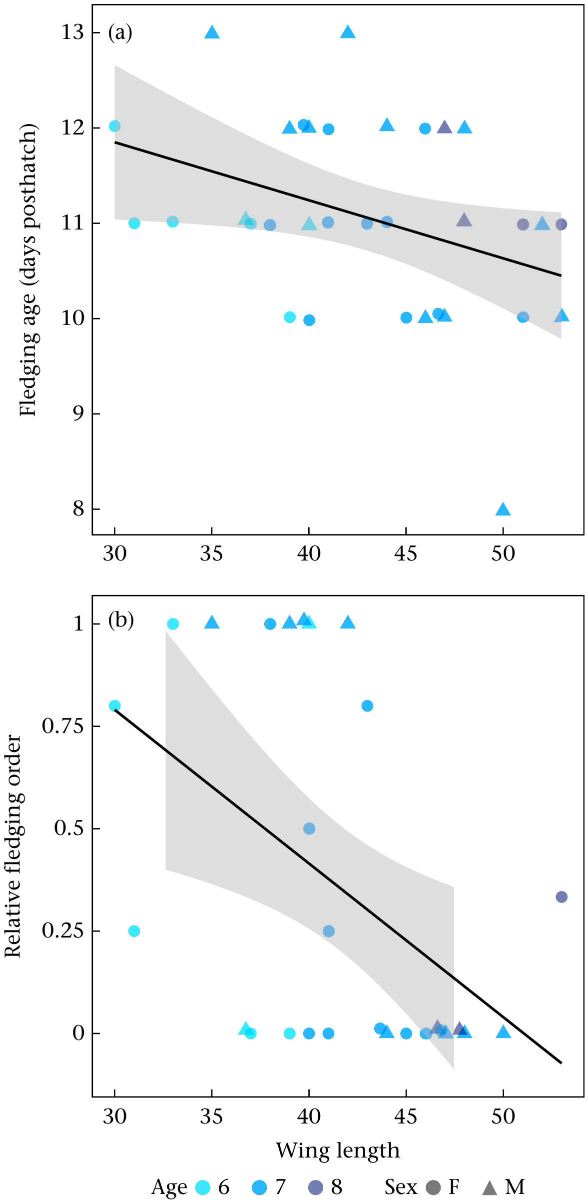

Figure 3. The effect of a cowbird nestling's wing length on their (a) age of fledging and (b) relative fledging order within a brood (with 0 being first fledged). The colour shading indicates the age of the cowbird when the measurement was taken. Each point represents an individual nestling and the regression lines show a significant relationship. F: female; M: male.

in areas where multiple parasitism of a single nest is common. In our previous study, we found that while the experimental addition of a cowbird nestling caused warbler nestlings to be smaller, have lower survival and fledge at older ages, the experimental addition of an unrelated warbler nestling caused no differences in any of these metrics compared to the control (Scharf et al., 2022). Thus, it appeared that the adverse effects on warbler nestlings were not simply due to brood enlargement, but rather were specific to the larger, parasitic cowbirds. However, here we found no effect of size-dimorphic cowbird sex on the size, survival or fledging of prothonotary warbler nestlings. This could be because within our study, the smallest mass difference between a cowbird and a host nestmate was still large; the cowbird was 29% heavier. Rivers et al. (2010) found that in parasitized broods of small, intermediate and large hosts, the largest nestling received the majority of food items regardless of whether it was the parasite. Cowbirds were always the largest nestlings in our nests; hence, the effects of parasite size on host nestling characteristics might be more apparent in hosts of a similar size, where cowbirds may or may not be the largest nestling in the brood.

We did not find any effect of overall cowbird parasitism or cowbird sex on the sex ratio of prothonotary warbler fledglings. By contrast, Zanette et al. (2005) found that the nests of the song sparrow, *Melospiza melodia*, parasitized with a brown-headed cowbird had fewer surviving female than male nestlings. This was attributed to the sexual dimorphism of song sparrows, as male song sparrow adults and nestlings are larger and may have a competitive advantage over females. By contrast, we found only a small difference in wing length between the male and female prothonotary warbler nestlings (Scharf et al., 2022); therefore, female prothonotary warblers are likely not subject to the competitive disadvantage that female song sparrows are.

As a generalist brood parasite, brown-headed cowbirds have plastic developmental rates depending on which host species they are raised by (Jones & Ward, 2021; Kilpatrick, 2002; Winnicki et al., 2021). Cowbirds experience carryover effects from the nestling to the postfledging stage, as they have shorter nestling periods, fledge with shorter wings and exhibit higher postfledging mortality when raised in the nests of host species with higher mortality risk (Jones & Ward, 2021). These relationships are not specific to cowbirds, and it is known in other species that wing length is especially important for postfledging survival (Jones & Ward, 2020; Martin et al., 2018) and that longer-winged nestlings typically fledge earlier (Johnson et al., 2004; Radersma et al., 2011). Here, we found that, in agreement with the published literature, wing length predicted cowbird fledging age and fledging order, with longer-winged cowbirds fledging earlier, regardless of sex. Wing length might be especially important for cowbirds in the nests of prothonotary warblers, as prothonotary warblers often nest above water in bottomland swamps (Petit & Petit, 1996). These swamps are characterized by relatively open understories dominated by bald cypress, *Taxodium distichum*, and tupelo, *Nyssa aquatica*, trees (Hoover, 2003b). While prothonotary warbler chicks show some swimming ability after fledging (Petit, 2020), cowbirds do not exhibit this behaviour and will likely drown if they land in water. Thus, it is crucial that in this host–parasite system, cowbirds have sufficient flying ability when they fledge. These results also parallel what we previously found about their host: prothonotary warblers with longer wings also fledged earlier in age and order (Scharf et al., 2022). Thus, cowbirds follow similar developmental 'rules' for fledging as their host.

Sexual size dimorphism in nestling cowbirds did not impact fledging phenology. Here, male and female cowbirds had similarly sized wings at the mid-nestling stage, but males were heavier. In a study on great tits, the males were also heavier than females; however, there was no effect of sex on fledging time, asynchrony or order (Radersma et al., 2011). The lack of differences in the fledging phenology between the sexes might be attributable to the lack of sex difference in wing length at the nestling stage, as wing length is generally the most important predictor of fledging (Johnson et al., 2004; Radersma et al., 2011; Scharf et al., 2022).

Many comparisons of virulence among brood parasites in the current literature occur between species, most obviously between nestmate killer and nest-cohabitator species (Kilner, 2005; Moskát et al., 2017), or when comparing the effect of nest-cohabitator virulence across a gradient of host sizes (Hauber, 2003; Lorenzana & Sealy, 1999). Variation in nestling virulence within one parasitic species and one host has not been studied to the best of our knowledge, and here we report that size-dimorphic parasite sex has no effect on host nestling characteristics such as size, survival and fledging. Understanding the factors that affect the virulence of nest-cohabitator brood parasites is essential to understand parasite–host interactions and how all hosts may not incur the same costs. This is especially true for parasites that are sexually dimorphic as this could have effects on both parasite and host demography and behaviour. Future research is needed on sex dimorphic brood parasites in the nests of other host species, particularly hosts that are closer in size to the parasite itself.

Author Contributions

H.M. Scharf: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **M.E. Hauber:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **E. Blumentritt:** Writing – review & editing, Investigation. **W.M. Schelsky:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Data Availability

The data sets generated and/or analysed during the current study are available in the FigShare depository: https://doi.org/10.6084/m9.figshare.24511384.v1.

Declaration of Interest

The authors declare that there are no conflicts of interest.

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Appendix

Table A1
Cowbird mass effects on prothonotary warbler nestmates

Response variable	β	*df*	*Z*/*t*	*P*
Proportion of host deaths before fledging	3.455	30	0.612	0.540
Sex ratio	0.016	30	0.030	0.976
Host size	−0.409	24	−0.654	0.519
Fledging latency (including cowbird)	9.934	15	0.710	0.489
Fledging latency (excluding cowbird)	3.791	15	0.476	0.641
Fledging age	0.478	21	1.217	0.237

Effect sizes, degrees of freedom, *Z* or *t* values and *P* values associated with the effect of cowbird parasite mass on the size, survival and fledging characteristics of host prothonotary warbler nestlings.