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RESEARCH ARTICLE

Opportunistic conspecific brood parasitism in a box-nesting population of Prothonotary Warblers (*Protonotaria citrea*)

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ABSTRACT

Conspecific brood parasitism (CBP), although prevalent in some avian taxa, is easily overlooked when it occurs in low frequencies, and therefore the ecology of this behavior has only occasionally been described in passerines. We describe the occurrence of CBP in a population of Prothonotary Warblers (*Protonotaria citrea*) breeding in nest boxes, demonstrate associated fitness costs, and investigate parasite strategy. We genotyped individuals at 6 microsatellite loci and used Cervus software to determine log-likelihood of maternity (LOD scores) for offspring and social mothers. We set critical cutoff LOD scores at 95% confidence for exclusion of the social mother and assignment of a parasite mother from the breeding population. Of 805 nestlings (233 family groups during 2009–2013), we found that 12.7% had genotypes that were incompatible with their social mother. Females with unrelated nestlings (hosts) fledged fewer biological offspring within the host year than nonhost females despite fledging more total offspring, but being a host was not significantly associated with total reproductive success over 5 yr of breeding. We were able to identify only ~30% of parasite females, which suggests that the majority of parasites may be floaters (i.e. non-nesters) in the population or nesting in nearby natural cavities. We found no evidence of host selection based on host age, arrival at the breeding site, or nest-box productivity in the previous year. This opportunistic behavior is likely facilitated by the nesting ecology of this population, in that nest sites are limited, conspicuous, and relatively dense. Future studies investigating CBP in populations using natural cavities can help elucidate the drivers of this behavior.

Keywords: breeding ecology, cavity nesters, conspecific brood parasitism, *Protonotaria citrea*

Parasitismo de cría conespecífico y oportunista en una población anidante de cajas de *Protonotaria citrea*

RESUMEN

Aunque es prevalente en algunos taxones de aves, el parasitismo de cría coespecífico se pasa por alto fácilmente cuando ocurre en bajas frecuencias. Por lo tanto, la ecología de este comportamiento apenas ha sido descrita ocasionalmente en aves paserinas. En este estudio describimos la incidencia del parasitismo de cría coespecífico en una población de *Protonotaria citrea* que se reproduce en nidos de cajas, demostramos los costos asociados con la aptitud e investigamos la estrategia de parasitismo. Determinamos el genotipo de individuos en seis locus de microsatélites y usamos el programa Cervus para determinar la verosimilitud de la maternidad (puntajes LOD) de las crías y las madres sociales. Establecimos los valores de corte críticos de LOD en 95% de confianza para la exclusión de la madre social y la asignación de una madre parásita de la población reproductiva. Encontramos que de 805 polluelos (233 grupos familiares de 2009 a 2013), el 12.7% tenía genotipos incompatibles con el de su madre social. Las hembras con polluelos no emparentados (hospederas) criaron menos descendencia biológica en el año en que fueron hospederas que las hembras no hospederas a pesar de criar más polluelos en total, pero el ser hospedera no estuvo significativamente relacionado con el éxito reproductivo total en cinco años de reproducción. Pudimos identificar solo ~30% de las hembras parásitas, lo que sugiere que la mayoría de los parásitos podrían ser flotantes (i.e. no anidantes) en la población o anidantes en cavidades naturales cercanas. No encontramos evidencia de selección de hospedero basada en edad del hospedero, llegada al sitio de reproducción o productividad de la caja de anidación en el año anterior. Este comportamiento oportunista probablemente es facilitado por la ecología de anidación en esta población en la que los sitios de anidación son limitados, conspicuos y relativamente densos. Estudios futuros que investiguen el parasitismo coespecífico en poblaciones usando cavidades naturales pueden ayudar a elucidar las causas de este comportamiento.

Palabras clave: anidantes de cavidades, ecología reproductiva, parasitismo de cría coespecífico, *Protonotaria citrea*

INTRODUCTION

Conspecific brood parasitism (CBP), in which a female lays one or more eggs in the nest of another female of the same species, is an important facultative female reproductive strategy in many avian taxa. While breeding ecology likely facilitates this behavior (Semel et al. 1988, Semel and Sherman 2001), life history characteristics—the balance of costs to the hosts and benefits to parasites—determine whether it can persist in a population (Eadie and Fryxell 1992, de Valpine and Eadie 2008). Because of potential costs associated with raising unrelated young incurred by the hosts, the prevalence of CBP has been linked to the amount of parental care required by chicks (Lyon and Eadie 2008), the behavior being more common in species with precocial young. It is also more commonly described in colonial and cavity-nesting species (Yom-Tov 2001), likely because of the proximity of breeding pairs and the ease of locating neighboring nests. Conspecific brood parasitism has been documented, though less frequently, in species with substantial parental care (Lyon 1993, Weaver and Brown 2004) and in noncolonial open-cup nesters (Yom-Tov 2001). This suggests that the behavior may be widespread at low frequencies and may only reach higher frequencies when costs to the hosts are balanced by benefits to parasites.

When CBP occurs at a low frequency, it can be difficult to detect. Because parasitic eggs and nestlings are not conspicuous (as they are in interspecific brood parasitism), careful field observations, comprehensive genetic analyses, or a combination of these is needed to identify parasitic nestlings. The infrequent detection of CBP in passerines has led some investigators to reject mother–offspring mismatches as genotyping errors rather than true cases of CBP (e.g., Stutchbury et al. 1997), thereby contributing to the idea that this behavior is rare in these systems. To our knowledge, CBP has only been documented in one warbler species, the Western Yellow-breasted Chat (Miño et al. 2011).

Given the difficulty of detecting this behavior when it occurs at low frequencies, few studies have described the ecology of CBP in altricial species. In systems with low levels of parental care, studies have found little or no cost of raising unrelated young (Sorenson 1993, Dugger and Blums 2001, Roy Nielsen et al. 2006); in these cases, CBP becomes costly only when it occurs at high frequencies, increasing clutch size to a degree that prevents even distribution of incubation temperature and thus lowers hatching success. In species with substantial parental care (i.e. provisioning of altricial nestlings), potential costs to the host are greater. Lyon (2003) estimated fitness costs of CBP in American Coots, a species with intensive parental care, and found that broods with parasitic chicks had lower biological offspring survival than nonparasitized broods. Brown and Brown (1997) investigated fitness costs of CBP in colonial Cave and Cliff swallows but focused primarily on adult survival and found no effect of CBP.

To understand how hosts may be affected by CBP, it is necessary to also investigate the drivers of this behavior (i.e. how and why females lay parasitically). The benefits of laying parasitically include boosting fecundity (Lyon and Eadie 2008), spreading risk of egg or nestling predation (Andersson and Åhlund 2012), and obtaining some reproductive output when constrained by suboptimal conditions (making "the best of a bad job"; Lyon and Eadie 2008). In colonial and cavity-nesting species, nest sites are often a limiting resource (Semel and Sherman 2001); increased competition for nest sites can drive CBP through both nest takeovers (after 2 females initiate a clutch in the same nest) and deliberate parasitism by some females that are outcompeted (floater females) (Sandell and Diemer 1999, Saitou 2001). Older females tend to arrive at the breeding grounds earlier (Stewart et al. 2002), and therefore we expect floaters to be younger in these systems (Sandell and Diemer 1999).

Here, we analyze the occurrence of CBP in a population of an altricial, cavity-nesting species, the Prothonotary Warbler (*Protonotaria citrea*). This population breeds in nest boxes, making nest sites both limited and conspicuous, thus potentially facilitating CBP. Repeated field observations of >1 egg appearing in a nest within 24 hr as well as preliminary evidence of mother–offspring mismatch from a study investigating extrapair paternity (Heidrich 2013) suggested that CBP was occurring and prompted the genetic analyses presented here. We expected that the intensive parental care of this species would make CBP costly to the host, so we compared annual reproductive success between host and nonhost females. We also investigated characteristics of the host females, host nest boxes, and parasites to better understand how and why CBP occurs in this population.

METHODS

Study System and Field Methods

The study population has been breeding in nest boxes in several locations near Richmond, Virginia, USA, since 1988 (Blem and Blem 1991). From 2009 to 2013, 65 nest boxes were monitored at Deep Bottom Park in Henrico County, Virginia. All boxes were checked 3 times wk^{-1} during the breeding season to determine nest initiation, hatching, and completion dates for each clutch, as well as to monitor reproductive output and success of individual females. Females captured at the box during incubation and nestlings between ages 6 and 8 days were banded with a U.S. Fish and Wildlife Service aluminum leg band. Adults were given a unique 3-color combination of plastic leg bands, and nestlings were given 1 cohort-specific color band to indicate hatch year. Blood samples were collected for each individual via brachial venipuncture and blotted onto a Whatman FTA Card for storage.

Numerical ages (in years) of females were determined from long-term recapture data. At first capture, adults were aged on the basis of plumage characteristics (following Pyle 1997). Actual ages were known for individuals that either hatched at our study site or were aged as second-year at first capture. Birds of unknown age at first banding were assigned a numerical age as follows: after-hatch-year birds were given a numerical age of 1.5 (i.e. $\geq$1 yr old), and after-second-year were given a numerical age of 2.5 (i.e. $\geq$2 yr old).

Maternal Exclusion

DNA was extracted from blood samples following Smith and Burgoyne (2004, method 4), using two 1.2 mm punches of dried blood collected on Whatman FTA cards and sequential washes of DNAzol (Life Technologies, Carlsbad, California, USA), ethanol, and DDH_20; genomic DNA was eluted into water. DNA was amplified in 10 µL PCR reactions with reagents at the following final concentrations: 1X Invitrogen reaction buffer, 3 mM $MgCl_2$, 0.15 mM dNTPs, 2.5 units Taq polymerase, and 0.5 µM each of the forward and reverse primers; 6 µL DDH_2O sample^{-1} was also included. Amplification of DNA was performed with an initial heating of 94°C for 3 min, followed by a cycle of 94°C for 1 min, the locus-specific annealing temperature for 45 s, and 72°C for 1 min. This cycle was repeated 30 times, followed by an end temperature of 72°C for 5 min.

All individuals were genotyped at 6 microsatellite loci that were developed for related taxa (Dawson et al. 1997, Winker et al. 1999, Stenzler et al. 2004) but have previously been used successfully in this species (Beck 2010, Schelsky 2010). Fragment analysis was conducted on a 3730 DNA Analyzer (Applied Biosystems, Foster City, California, USA), and raw fragment sizes were read from trace files using STRand 2.4.59 (Toonen and Hughes 2001). Raw fragment sizes were binned to alleles in R using the package MsatAllele (Alberto 2009). Loci with peaks that could not be reliably assigned to a bin were excluded for that individual. Latent genetic diversity (expected and observed heterozygosity [H_E and H_O] and inbreeding F_{IS}) was summarized for both adult and offspring populations. Inbreeding was measured directly in the adult population, whereas a permutation approach, in which a single nestling was randomly selected from each clutch, was adopted for the offspring generation to remove bias due to assumed co-ancestry.

Maternal exclusion analysis (to determine whether the social mother could be excluded as a possible biological mother of each nestling) was performed using Cervus 3.0.7 (Kalinowski et al. 2007), based on LOD scores (the sum of log-likelihood ratios at each locus) for each mother–offspring pair. Cervus implements a simulation-based approach that incorporates uncertainty due to missing data and genotyping error to determine confidence tables of LOD scores for both assigning and excluding sampled parents; we based our analyses on LOD cutoff values for 95% confidence of both exclusion (to exclude social mothers) and assignment (to identify parasite females). For a detailed description of the likelihood estimations and implementation of this program for CBP and extrapair paternity analyses, see Kalinowski et al. (2007) and Lemons et al. (2015), respectively. Simulations of 10,000 mother–offspring pairs were run with the following parameters: 90% of females sampled, 78% of loci typed, genotyping error rate of 0.10. The proportion of females sampled was based on field data (we obtained a blood sample from 92.5% of females observed incubating in nest boxes from 2009 to 2013), proportion of loci typed was estimated directly from our genetic data (calculated in Cervus and averaged across all loci), and we set genotyping error rate high to incorporate uncertainty arising from unknown genotyping error rates in our dataset and observations of loci out of Hardy-Weinberg equilibrium (Table 1).

We excluded from our analyses any mother–offspring pairs whose genotypes were compared at $<$3 loci. Multi-locus exclusion probability was calculated for all possible combinations of 3 loci and averaged. Any nestling whose social mother had an LOD score less than the 95% confidence cutoff value for exclusion was considered parasitic. For all parasitic nestlings, we also used Cervus to determine pairwise LOD scores for that nestling and all sampled females ($n = 117$). We used the critical LOD cutoff at 95% confidence for assignment generated by the initial simulation to determine the most likely mother (i.e. parasite female) for that nestling; any mother–offspring pair with an LOD score greater than the critical LOD cutoff value was considered a match.

For any broods with $>$1 nestling found to be incompatible with the social mother, we were interested in whether those eggs were laid by a single female or by multiple parasitic females. We measured relatedness (r) among parasitic nestlings in the same brood using Kingroup 2 (Konovalov et al. 2004, Konovalov and Heg 2008) to calculate maximum-likelihood estimates of relatedness and to test 2 hypotheses of how pairs of nestlings were related, either full sibling ($r = 0.5$) or half sibling ($r = 0.25$), following Goodnight and Queller (1999). This program facilitates the testing of a primary hypothesis against a null hypothesis using likelihood ratios and a simulation-based approach to estimate P values at a significance level of 0.05. We used 1,000 simulated pairs as recommended by Konovalov et al. (2004) and tested each hypothesis against the null hypothesis that the pairs were unrelated.

TABLE 1. Microsatellite loci used for maternal exclusion in Prothonotary Warblers breeding at Deep Bottom Park in Henrico County, Virginia, USA, 2009–2013. Included are number of alleles per locus, expected and observed heterozygosity (H_E and H_O), inbreeding coefficient (F_{IS}), and P value from chi-square test for Hardy-Weinberg equilibrium (HWE). Summary statistics for nestling genotypes represent averages from 1,000 permutations of randomly sampling 1 offspring clutch^{-1} ($n = 312$ individuals included in each permutation) to account for presence of siblings.

	Locus	Alleles	H_E	H_O	F_{IS}	HWE P
Adults	Dpu01	26	0.837	0.599	0.285	<0.001
	Dpu03	11	0.634	0.542	0.145	<0.001
	Dpu16	23	0.918	0.826	0.101	<0.001
	VeCr04	15	0.491	0.417	0.151	0.0054
	VeCr06	10	0.613	0.571	0.067	0.879
	VeCr07	16	0.908	0.786	0.134	<0.001
	Mean	16.8	0.733	0.624	0.147	–
Nestlings	Dpu01	28.5	0.801	0.550	0.312	<0.001
	Dpu03	19.8	0.658	0.553	0.160	<0.001
	Dpu16	22.5	0.907	0.841	0.073	0.058
	VeCr04	15.8	0.465	0.409	0.120	<0.001
	VeCr06	13.3	0.599	0.600	-0.001	0.265
	VeCr07	18.0	0.917	0.819	0.107	0.001
	Mean	19.65	0.724	0.628	0.132	–

Reproductive Success of Host Females

We compared annual reproductive success between host and nonhost females using Wilcoxon rank-sum tests. To determine the number of biological offspring fledged for each female, we subtracted the number of mismatched offspring in her broods from the total number of nestlings she fledged each year. We tested for differences in both total brood size fledged and total biological offspring fledged between hosts and all nonhost females breeding in the same year. We assessed whether host females were less likely to double-brood in the host year using a chi-square test of independence. For each clutch, we calculated the proportion of eggs in the clutch that successfully hatched and fledged, and tested for a difference in hatching and fledging success between nests with and without CBP using a Wilcoxon rank-sum test.

We summed the number of young fledged (considered fledged if they survived to day 9) for all females to determine total reproductive output across all years of breeding from 2009 to 2013. We then used Poisson generalized linear models and an Akaike's Information Criterion (AIC) model selection framework to test the relationship between total reproductive success of a female and the following predictor variables: total number of years breeding in our nest boxes, total number of broods fledged, number of broods with CBP, and her mean age (in years) across all years of breeding data. A model set of all combinations of these predictors was constructed, and second-order AIC values (AIC_c) were calculated for each model. Model weights (w_i) were calculated as the likelihood of the model divided by the sum of likelihoods in the model set. Following the suggestion of Burnham and Anderson (2002), we considered any model with a difference in AIC_c (ΔAIC_c) < 2 to be supported by the data. When there was more than one model with $\Delta\text{AIC}_c <$ 2, model-averaged parameter estimates were calculated.

Parasite Strategy

To assess whether parasites were older, potentially higher-quality females (laying parasitically to boost fecundity) or younger, lower-quality females (laying parasitically to "make the best of a bad job"), we analyzed age in 2 ways: Ages of parasites were compared to those of all other nonparasite females breeding in the same year (Wilcoxon rank-sum test) as well as to the ages of the host female whose nest they parasitized (Wilcoxon signed-rank test).

To investigate potential host-selection strategies of parasite females, we tested the likelihood of a nest containing a parasitic nestling based on characteristics of the host female or nest box. We used generalized linear mixed models in an AIC model selection framework as described above to identify characteristics that may predict whether a nest is more likely to contain a parasitic nestling. Using host status (a binary indicator of whether or not each brood had CBP) as the response variable, we created a model set using nest initiation date (ordinal date), host age (in years), number of young fledged from the box in the previous year, and a binary indicator of whether the box experienced CBP in the previous year as predictors. Nest initiation date was scaled within each year to the earliest nest initiation date for that year (i.e. for each year, the first day with egg laying = day 0). We also included random effects of year and female band number in all models, because some females were included in this analysis more than once.

All means are reported ± SD, and all analyses were performed in R 3.2.1 (R Development Core Team 2015) unless otherwise noted.

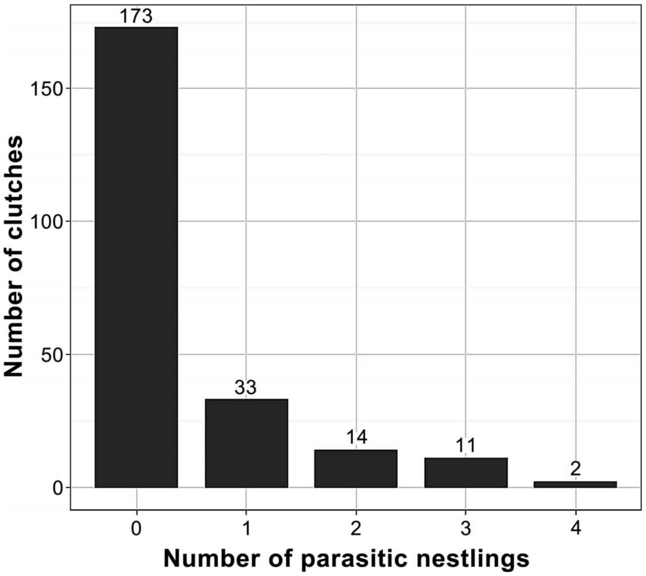

FIGURE 1. Histogram of the number of parasitic nestlings (those with genotypes incompatible with the social mother) found in a single brood of Prothonotary Warblers at Deep Bottom Park in Henrico County, Virginia, USA, 2009–2013. Of the 233 broods sampled, 60 contained ≥1 parasitic nestling.

TABLE 2. Number of individuals sampled and results of maternal exclusion analysis by year for Prothonotary Warblers breeding at Deep Bottom Park in Henrico County, Virginia, USA, 2009–2013. Total offspring sampled include only those that were part of a complete family group (i.e. blood samples were taken for all offspring and the social mother) and whose genotypes were compared to that of the social mother at ≥3 (of 6) microsatellite loci.

	Year	Total	Number parasitic	Proportion parasitic
Nestlings	2009	27	6	0.222
	2010	165	17	0.103
	2011	188	19	0.103
	2012	198	31	0.159
	2013	227	29	0.129
	Total	805	102	0.127
Broods	2009	8	2	0.250
	2010	47	9	0.191
	2011	59	13	0.228
	2012	59	19	0.328
	2013	60	17	0.288
	Total	233	60	0.262

RESULTS

Maternal Exclusion

We genotyped the offspring and social mothers from 235 broods during 2009–2013. Estimates of allelic diversity and inbreeding coefficients were comparable to results of other studies of this species using these loci (Beck 2010, Schelsky 2010), with average adult diversity of $H_e = 0.73$, though with moderate levels of inbreeding ($F_{IS} = 0.14$; Table 1). Similar levels of diversity and inbreeding were observed in offspring from permutated sampling (to minimize nestmate relatedness from introducing bias in estimating F_{IS}). This level of observed inbreeding corresponds to an average relatedness among pairs of adults equal to double first cousins, aunt/uncle–nephew/niece, grandparent–grandchild, or half siblings (Hedrick 2011).

The mean number of alleles per locus was 27.8 (Table 1; minimum = 18, maximum = 40). The average multilocus exclusion probability for all combinations of 3 loci was 0.940 (± 0.055). We initially identified 7 broods for which all nestlings tested were found to have incompatible genotypes with the social mother; however, 5 of those 7 were incomplete family groups; eggs or nestlings were lost before sampling because of hatching failure, partial predation, or some other cause. Biological offspring may have died before sampling, leaving only parasitic nestlings. There were 2 broods for which all nestlings were tested and found to have incompatible genotypes with the social mother, and for these we assumed we had identified the wrong social mother and excluded them from all analyses. Misidentification of the social mother in these 2 cases could have happened if the female was captured at the box while prospecting for nest sites instead of incubating or if she was incorrectly recorded as being observed at the nest box. Of the 233 remaining broods, 60 contained ≥1 parasitic nestling. Most CBP broods had either 1 or 2 parasitic nestlings (Figure 1), though the number of parasitic nestlings in a clutch ranged from 1 to 4. Of the 805 mother–offspring pairs compared at ≥3 loci, 102 were excluded at 95% confidence (Table 2), indicating that 12.7% of nestlings were parasitic and 25.7% of broods were parasitized. The proportion of parasitic nestlings was not different across years ($\chi^2 = 5.8$, df = 4, $P = 0.22$), nor was the proportion of broods with ≥1 parasitic nestling ($\chi^2 = 3.0$, df = 4, $P = 0.56$).

Twenty-seven of the broods with CBP ($n = 60$ total) contained >1 parasitic nestling (Figure 1). The mean pairwise relatedness between these parasitic nestlings in the same brood was 0.40 ± 0.339. Based on our hypothesis test procedure in Kingroup, 24 of these broods contained nestling pairs that were determined to be full siblings and 1 contained a nestling pair that were likely half siblings (all $P < 0.05$).

Reproductive Success of Host Females

Although females in this population often have multiple broods in a single breeding season, no female was found to be a host for >1 brood in the same year. Clutch size of parasitized nests was slightly larger, as expected, but the difference in clutch size between parasitized and nonparasitized nests was not statistically significant (parasitized = 4.6 ± 0.79, nonparasitized = 4.4 ± 0.95, $P = 0.23$).

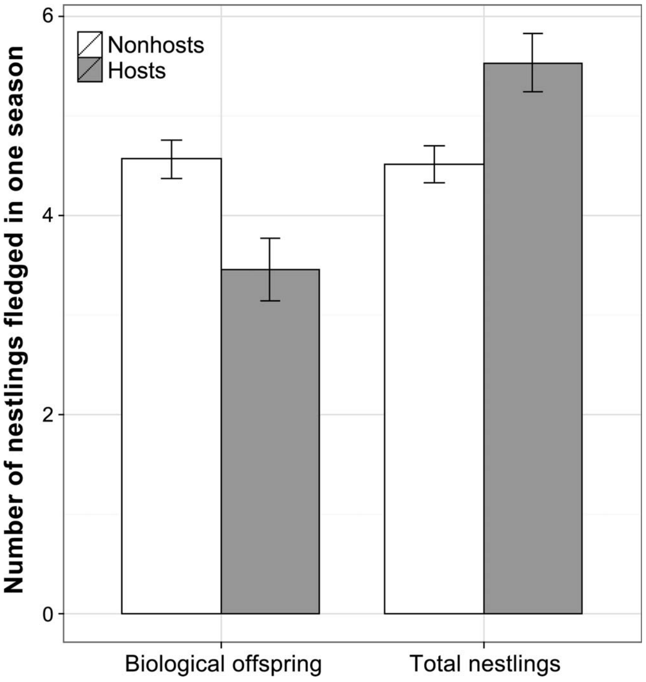

FIGURE 2. Number of biological offspring and total number of nestlings fledged in 1 yr by host and nonhost female Prothonotary Warblers breeding at Deep Bottom Park in Henrico County, Virginia, USA, 2009–2013. Height of the bars indicates mean number fledged and error bars represent SE.

Host females ($n = 55$) fledged, on average, more total offspring than nonhost females ($n = 127$) breeding in the same year (mean host = 5.5 ± 2.09, mean nonhost = 4.5 ± 2.05, $P = 0.002$), but they fledged significantly fewer biological offspring in those years than nonhost females (Figure 2; mean host = 3.7 ± 2.01, mean nonhost = 4.5 ± 2.05, $P = 0.005$). Host females were also less likely to double-brood in the host year than nonhost females ($\chi^2 = 5.9$, df = 1, $P = 0.01$). There was no difference in hatching success (CBP = 0.90 ± 0.15, non-CBP = 0.89 ± 0.19, $P = 0.70$) or fledging success (CBP = 0.84 ± 0.22, non-CBP = 0.86 ± 0.23, $P = 0.27$) of eggs between clutches with CBP ($n = 60$) and those without CBP ($n = 173$).

We did not identify a single top model from the model set predicting total reproductive output as a function of total number of broods, number of broods with CBP, mean age, and years breeding; 3 models were identified with $\Delta\text{AIC}_\text{c} < 2$ (Table 3). The variance inflation factor from the global model ($\hat{c} = 1.07$) indicated minimal overdispersion and the goodness-of-fit chi-squared test for the global model was not statistically significant ($P = 0.09$), indicating adequate model fit. Three models received support with $\Delta\text{AIC}_\text{c} < 2$, all with more support than the null model (Table 3). Total number of broods was the only strong predictor of reproductive output ($\beta = 0.25$, 95% confidence interval [CI]: 0.19 to 0.32); neither mean age ($\beta = 0.02$, 95% CI: –0.06 to 0.09) nor total number of years breeding ($\beta = 0.07$, 95% CI: –0.07 to 0.21) was significantly associated with total number of young fledged. CBP was not included as a parameter in the top models.

TABLE 3. Model set of Poisson generalized linear models predicting total reproductive output of female Prothonotary Warblers breeding at Deep Bottom Park in Henrico County, Virginia, USA, 2009–2013. Predictor variables include the total number of broods, the number of years breeding, the number of broods with conspecific brood parasitism (CBP), and the female's mean age (in years) across those 5 yr. The 3 models with $\Delta\text{AIC}_\text{c}$ were averaged to calculate parameter estimates.

Model parameters	AIC_c	$\Delta\text{AIC}_\text{c}$	w_i
Broods	523.35	0	0.35
Years Breeding + Broods	524.56	1.21	0.19
Broods + Age	525.26	1.92	0.13
Broods + CBP	525.41	2.07	0.12
Years Breeding + Broods + Age	526.67	3.32	0.07
Years Breeding + Broods + CBP	526.72	3.37	0.06
Broods + CBP + Age	527.41	4.06	0.05
Global model	528.88	5.53	0.02
Years Breeding + CBP	549.79	26.45	0
Years Breeding	549.99	26.64	0
Years Breeding + Age	550.02	26.67	0
CBP + Age	609.93	86.59	0
Age	631.98	108.63	0
CBP	700.4	177.06	0
Intercept only	752.83	229.48	0

Parasite Strategy

We identified 32 parasite females out of our breeding female population (assigned at 95% confidence). Parasites were not older or younger than nonparasites breeding in the same year ($P = 0.47$). Parasites were also not significantly older than the host female in whose nest they laid eggs (mean age difference = 0.2 yr, $P = 0.25$). Of these parasites, 6 nested in a nest box in the same year they laid parasitically, 15 used a nest box in a previous year, and 11 used a nest box in a subsequent year.

The model with the most support for predicting whether or not a brood contained a parasitic nestling included host female age and whether the box experienced CBP in the previous year (Table 4). The goodness-of-fit chi-square test for the global model was not significant ($P = 0.13$), indicating that the model adequately fit our data. However, the 95% confidence intervals surrounding the parameter estimates for both female age and CBP in the previous year contained zero, which indicates that these factors are not significantly associated with the presence of a parasitic nestling (host female age: $\beta = 0.28$, 95% CI: –0.14 to 0.7; CBP in the previous year: $\beta = 0.79$, 95% CI: –0.46 to 2.04).

TABLE 4. Model set of binomial generalized linear mixed models predicting whether a brood contained $\geq$1 parasitic nestling, for 233 Prothonotary Warbler broods at Deep Bottom Park in Henrico County, Virginia, USA, 2009–2013 (CBP = conspecific brood parasitism). Predictor variables include age of the host female (in years), total number of nestlings fledged from the nest box in the previous year, and ordinal date of clutch initiation (day of first egg laying). Nest initiation date was standardized within year such that the earliest date for each year was day 0. All models also included random effects of year and host female band number, because females were included in this analysis more than once.

Model parameters	AIC_c	ΔAIC_c	w_i
Female Age + CBP in Previous Yr	71.91	0	0.62
Female Age + Nest Initiation Date + CBP in Previous Yr	74.26	2.34	0.19
Global model	75.64	3.72	0.10
Success in Previous Yr + CBP in Previous Yr	77.4	5.49	0.04
Nest Initiation Date + CBP in Previous Yr	77.66	5.74	0.04
Nest Initiation Date + Success in Previous Yr + CBP in Previous Yr	79.67	7.75	0.01
Female Age + Success in Previous Yr	128.28	56.37	0
Female Age + Nest Initiation Date + Success in Previous Yr	130.07	58.16	0
Success in Previous Yr	135.64	63.72	0
Nest Initiation Date + Success in Previous Yr	137.61	65.69	0
Female Age + Nest Initiation Date	244.17	172.26	0
Female Age	247.2	175.28	0
Nest Initiation Date	249.56	177.64	0
Intercept and random effects only	257.23	185.32	0

DISCUSSION

Our results represent only the second documentation (to our knowledge) of CBP in a member of the warbler family (CBP was previously documented in Western Yellow-breasted Chat; Miño et al. 2011). By our estimates, 12.7% of nestlings in our study population of Prothonotary Warblers were not related to their social mother. Host females raising these unrelated young fledged fewer biological offspring in that year, even though they raised more total nestlings than nonhosts; this suggests that hosts experienced increased parental demand with decreased genetic reward. However, we did not directly quantify the energetic investment associated with incubating and feeding additional young, and the results of the total reproductive output model suggest that long-term costs are minimal. Our results also suggest that this behavior occurs opportunistically (i.e. without deliberate targeting of hosts), in that we found no patterns of host selection based on host or nest-box characteristics.

Nests with CBP did not contain significantly more eggs, which suggests either that parasites remove eggs or that host females lay fewer eggs when parasitized. Although egg removal (Lombardo et al. 1989) and egg transfer (Weaver and Brown 2004) by conspecific brood parasites have been documented, this is unlikely to occur in the study population. Nest cups typically sit well below the entrance to the box, so egg removal would require carrying the egg out of the nest box, a behavior that is unlikely in a species as small as the Prothonotary Warbler. It is more likely that hosts reduce clutch size by halting laying, as documented in American Coot (Lyon 2003) and Barrow's Goldeneye (Jaatinen et al. 2009). Indeterminate egg laying, and specifically "addition indeterminate" (halting laying in response to egg addition), has been documented in several passerines (Kendra et al. 1988, Kennedy 1991). Reduction in clutch size and lower likelihood of double-brooding within the host season leads to the observed lower annual reproductive output by hosts when compared to nonhosts in the same year.

We found some evidence of inbreeding within the population, and given this level of relatedness, it's not surprising that some loci are out of Hardy-Weinberg equilibrium. Despite this, we are confident in our maternal exclusions because the likelihood calculations implemented by Cervus allowed us to incorporate uncertainty due to genotyping error as well as observed allele frequencies in the adult population (Kalinowski et al. 2007). The focus of the present study was simply to quantify the prevalence of CBP at one site and not to make comparisons between sites or subpopulations, analyses that have a greater potential to be biased by Hardy-Weinberg disequilibrium (Hedrick 2011).

For the parasites we identified, there was no evidence that they were older or younger than the average female or the host they targeted; however, our ability to generalize this result is limited because so few were identified. Although other studies of CBP have found higher proportions of nesting parasites (66.7%, Lyon 1993; 50%, Reichart et al. 2010), we identified relatively few parasites from our breeding females. The low number of parasites identified and the fact that so few of them nested in the same year suggests that parasites are either floater females that are unable to obtain nest sites or females nesting in nearby natural cavities that never use a nest box. We have observed unbanded females in the study area and also around active nest boxes (A. M. Tucker and L. P. Bulluck personal observation), potentially prospecting for future nest sites. Nest boxes are surrounded by suitable nesting habitat, but they are likely preferred over natural cavities (Hoover 2006, Beck 2013) because they are maintained with small openings and placed on metal poles over water to decrease predation (2009–2013: 16.1% of clutches predated, 2.2% with cowbird parasitism). Although we sampled the majority of females (92.5%) breeding in nest boxes, we did not perform extensive passive mist netting to capture individuals that did not utilize a nest box. If females were unable to secure a nesting location, it is unlikely they would return in a subsequent year to breed (Hoover 2003), resulting in parasitic nestlings from unidentified mothers. Of course, a combination of multiple parasite strategies is possible, with some floater females dumping eggs, some nesting parasites using nearby natural cavities, and some nesting parasites using nest boxes. Mixed parasite strategies could also help explain why we found no evidence of host selection based on nest or female characteristics.

Our analyses did not suggest that more productive boxes were targeted by parasites, but the high density of good-quality nest boxes in our system has likely led to competition for a limited number of nest boxes. Prothonotary Warblers are secondary cavity-nesters and therefore rely on the presence of cavities or nest boxes to breed. At our study site, 92–94% of boxes were occupied by Prothonotary Warblers each year from 2010 to 2013, which suggests that nest sites might be a limiting factor for females in this population. Cooper et al. (2009) found that Prothonotary Warblers breeding in natural cavity nests had territory sizes ranging from 1.98 (± 0.12) to 4.57 (± 0.36) ha. The average distance between our nest boxes is 53.4 m (approximate average circular territory size = 0.22 ha), resulting in a considerably higher nest density than was found in a natural system. Additionally, CBP has been shown to be more common in Wood Ducks that use nest boxes than in those that breed in natural cavities (Semel et al. 1988). Additional studies of this species breeding in natural cavities, and in nest boxes at lower densities, may help elucidate whether the level of parasitism observed here was associated with higher breeding density and competition for nest boxes.

It is also possible that mismatched offspring observed in broods result not from deliberate egg dumping but from nest-box takeover during laying. Semel and Sherman (2001) determined that what appeared to be cases of CBP in a population of box-nesting Wood Ducks resulted from nest takeover by a more dominant female, through which the previous female's eggs became incorporated into the new clutch. We occasionally observed complete burial of eggs under new nests ($\sim$3% of clutches buried), which suggests that nest takeover occurs with some regularity in this population. In some cases the previous clutch's eggs may not be buried but are incorporated into the next clutch, with only the second female observed at the nest box. Broods with >1 mismatched nestling, and especially those in which the parasitic nestlings were related, may be cases of nest-box takeover. Future studies examining both behavioral responses of females to egg additions and the role of female aggression and dominance in nest-box acquisition are needed to understand the behaviors driving our observations of mismatched offspring.

Our results suggest that CBP may be more common than previously thought and highlight many areas for future research. Understanding the drivers and consequences of CBP in different systems will help us not only place this behavior in the context of varied and dynamic avian mating strategies, but also understand its role in the evolution of obligate interspecific brood parasitism (Hamilton and Orians 1965, Robert and Sorci 2001, Shaw and Hauber 2009).

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Author contributions: A.M.T., S.K.H., and L.P.B. conceived the study, collected the data, and wrote the manuscript. A.M.T. and R.J.D. designed the genetic methods. A.M.T., R.J.D., and L.P.B. analyzed the data. S.K.H., R.J.D., and L.P.B. contributed substantial materials, resources, and/or funding.

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