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Turner, A. M., M. E. Hauber, and D. G. Reichard. 2026. Repeated parasitism of individual female Dark-eyed Juncos (*Junco hyemalis*) by Brown-headed Cowbirds (*Molothrus ater*) over two decades. Journal of Field Ornithology 97(4):3. https://doi.org/10.5751/JFO-00853-970403

Avian Behavior, Ecology, and Evolution

Repeated parasitism of individual female Dark-eyed Juncos (*Junco hyemalis*) by Brown-headed Cowbirds (*Molothrus ater*) over two decades

Parasitismo repetido en hembras individuales de *Junco hyemalis* por parte de *Molothrus ater*, durante más de dos décadas

Abbigail M. Turner[1,2], *Mark E. Hauber*[1,3] *and Dustin G. Reichard*[4]

ABSTRACT. Repeated interactions between individual brood parasites and their hosts within and between breeding seasons are an understudied dynamic of most host-parasite systems due to a lack of longitudinal data. Dark-eyed Juncos (*Junco hyemalis*) are a common host that accepts and raises offspring of obligate brood parasitic Brown-headed Cowbirds (*Molothrus ater*). The Ketterson-Nolan lab at Indiana University has collected long-term, publicly available data on this host-parasite system in Virginia, USA, which allowed us to examine the occurrence and effect of repeated cowbird parasitism on color-banded individuals of a host species across 22 years. Based on previous data collected elsewhere on this and other cowbird hosts, we hypothesized that some individual juncos in this system would experience repeated parasitism within and between breeding years and that the likelihood of repeated parasitism would be negatively related to the reproductive success of the host. We did not detect a statistical pattern of repeated parasitism of female juncos within the same breeding year. In contrast, we found that juncos that were parasitized in their first year were statistically more likely to be parasitized in subsequent breeding years relative to females that had not been parasitized at the onset of their breeding. Current reproductive success was not related to the likelihood of future parasitism. However, the number of breeding years was a significantly positive predictor of whether a female junco was ever parasitized in her lifetime. Our findings align with other studies on Brown-headed Cowbird hosts; however, the factors that affect the patterns of repeated brood parasitism of the same individual hosts remain unknown in this system.

RESUMEN. Las interacciones repetidas entre parásitos de cría y sus hospedadores, tanto dentro como entre temporadas reproductivas, constituyen una dinámica poco estudiada en la mayoría de los sistemas hospedador-parásito, debido a la escasez de datos longitudinales. *Junco hyemalis* es un hospedador común que acepta y cría a las crías de *Molothrus ater*, un parásito de cría obligado. El laboratorio Ketterson-Nolan de la Universidad de Indiana ha recopilado datos de largo plazo, disponibles públicamente, sobre este sistema hospedador-parásito en Virginia, Estados Unidos, lo que nos ha permitido examinar la ocurrencia y los efectos del parasitismo repetido por *M. ater* en individuos de una especie hospedadora marcados con anillos de color durante 22 años. Con base en datos previos obtenidos en otros sitios para esta y otras especies hospedadoras de *M. ater*, planteamos la hipótesis de que, en este sistema, algunos individuos de *J. hyemalis* experimentarían parasitismo repetido dentro y entre años reproductivos, y que la probabilidad de dicho parasitismo estaría negativamente relacionada con el éxito reproductivo del hospedador. No detectamos un patrón estadístico de parasitismo recurrente en las hembras durante una misma temporada reproductiva. En contraste, encontramos que las hembras parasitadas durante su primer año tenían una probabilidad estadísticamente mayor de ser parasitadas en temporadas reproductivas posteriores que aquellas que no lo habían sido al inicio de su vida reproductiva. El éxito reproductivo actual no se relacionó con la probabilidad de sufrir parasitismo en el futuro. Sin embargo, el número de años reproductivos fue un predictor positivo y significativo de que una hembra fuera parasitada al menos una vez durante su vida. Nuestros hallazgos concuerdan con los resultados de otros estudios realizados en hospedadores de *M. ater*; sin embargo, los factores que determinan los patrones de parasitismo de cría repetida en los mismos individuos hospedadores siguen siendo desconocidos en este sistema.

Key Words: *Brown-headed Cowbird; Dark-eyed Junco; repeated parasitism*

INTRODUCTION

Avian obligate brood parasites are completely reliant on heterospecific hosts to raise their offspring successfully and vary widely in their virulence (Kilner 2005). In turn, host species vary in their susceptibility to brood parasitism (Spottiswoode et al. 2012). Hosts that accept some or all parasitic eggs can be at risk of being re-parasitized, both within the same clutch by the same or different parasitic females (i.e., "multiple parasitism") and across breeding attempts throughout their lives (Carter 1986, McLaren et al. 2003, Ellison et al. 2006, Hauber et al. 2012, Gloag et al. 2014, Ursino et al. 2020). Both specialist and generalist brood parasites are known to engage in repeated parasitism in which the same individual host female's subsequent nests are parasitized within and/or between breeding years (Hauber 2001, McLaren et al. 2003, Molina-Morales et al. 2013, de la Colina et al. 2016). Thus, individual hosts experience multiple and/or repeated parasitism to varying degrees across both time and space (Carter 1986, Molina-Morales et al. 2013, de la Colina et al. 2016).

[1]Department of Evolution, Ecology, and Behavior, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA, [2]Division of Forestry, Indiana Department of Natural Resources, Indianapolis, Indiana, USA, [3]Advanced Science Research Center and Programs in Biology and Psychology, Graduate Center of the City University of New York, New York, New York, USA, [4]Department of Biological Sciences, Ohio Wesleyan University, Delaware, Ohio, USA

For example, only a small number of Eurasian Magpies (*Pica pica*) are repeatedly parasitized by their brood parasite, the Great Spotted Cuckoo (*Clamator glandarius*), between breeding years (Molina-Morales et al. 2013), whereas repeated parasitism of the same Eastern Phoebe (*Sayornis phoebe*) hosts by Brown-headed Cowbirds (*Molothrus ater*) is a statistical pattern across years (Hauber et al. 2004).

Many factors can affect the probability of future parasitism either within or between breeding seasons. Past parasitism exposure has been shown to increase the likelihood of subsequent parasitism by individual hosts (Smith 1981, Hauber et al. 2004, Hoover et al. 2006). The availability of greater host numbers and densities at the population level can also increase future parasitism (McLaren et al. 2003, Ellison et al. 2006, Rivers et al. 2012, de la Colina et al. 2016), along with individual parasites' breeding site fidelity (e.g., Woolfenden et al. 2003; see Hauber et al. 2020 for a review). Further, aspects of the nesting environment (i.e., habitat, landscape composition, and nest concealment) can affect the likelihood of multiple and repeated multiple parasitism for some host species (Burhans 1997, Hauber 2001, Chace et al. 2005, Patten et al. 2006, 2011, Howell et al. 2007, Wagner et al. 2013). Host reproductive success could also be a factor; for example, in a Brown-headed Cowbird-host system, where parasitism rates declined over the season, failed first host breeding attempts provided realized opportunities to re-parasitize their re-nesting attempts sooner and more often (Hoover et al. 2006). In addition, older host females are more likely to have gained the experience to avoid or defend against parasitism (Hauber et al. 2004, Molina-Morales et al. 2013, de la Colina et al. 2016, Martinez et al. 2020; but see Smith et al. 1984). Host female age may also affect the likelihood of future parasitism by affecting other aspects of female reproductive behavior during nest site selection and parenting (Smith 1981, Grim 2002, Parejo and Avilés 2007).

Although multiple and repeated parasitism may appear costly to the brood parasites, there is mixed support for the idea of whether cowbirds (*Molothrus* spp.) should avoid engaging in this pattern (Ursino et al. 2020). From a mechanistic perspective, cowbirds must temporally synchronize their egg laying with that of the host they are parasitizing (Boldrick et al. 2026), which may increase the likelihood of multiple parasitism when multiple females target the same individual. Consequently, multiple parasitism creates competition between parasitic offspring themselves and adds to that individual host's parasite load, resulting in both the parasites' and the host's loss of fitness (McLaren et al. 2003, Rivers et al. 2012, Gloag et al. 2014, Ursino et al. 2020). Additionally, if adult cowbird abundance is high (Rivers et al. 2012) and host availability is scarce, multiple and repeated parasitism may also be more likely to occur (McLaren et al. 2003, de la Colina et al. 2016). From a different ultimate perspective, however, mathematical modeling shows clear benefits of repeated parasitism for the parasites because this behavior decreases the fitness benefits of antiparasitic behaviors (e.g., egg rejection) evolving in the host, by increasing the evolutionary time it takes for resistance to spread in repeatedly parasitized host lineages (Hauber et al. 2004, Hoover and Hauber 2007).

Here, we studied the occurrence of repeated parasitism of Dark-eyed Juncos (*Junco hyemalis,* hereafter juncos) both within the same breeding year and between years by Brown-headed Cowbirds (hereafter cowbirds), a generalist, obligate brood parasite that breeds across much of temperate North America. Over 100 species successfully raise cowbird offspring (Lowther 2020), and these hosts vary in their ability to respond to parasitism and manage the costs they endure (Rothstein 1975). Many cowbird host species accept the parasitic egg and, as a result, have decreased reproductive success compared to non-parasitized nests (Lorenzana and Sealy 1999, Hauber 2003, Turner et al. 2022), with smaller host species being especially negatively affected when raising a cowbird (Lorenzana and Sealy 1999, Hauber 2003, Kilner 2003). Female cowbirds benefit from the foster-parental care given to their offspring by their hosts and are adapted to taking advantage of this opportunity within and across host nesting attempts. For example, female cowbirds demonstrate a variety of strategies to find host nests (Norman and Robertson 1975, Strausberger 1998), often stealthily laying before dawn to avoid detection by hosts (Scott 1991) and preferentially parasitizing certain species and host individuals (Smith 1981, Hauber et al. 2004).

Repeated parasitism of an individual host by the same or different female cowbirds can occur between subsequent breeding attempts (assuming the host has more than one clutch), including across breeding years (McLaren et al. 2003, Ellison et al. 2006, Hauber et al. 2012, Gloag et al. 2014). Data on repeated cowbird parasitism of the same host individual are rare due to the limited availability of long-term data sets and color-banded host populations (Hauber et al. 2004, Wagner et al. 2013). However, individuals of specific host species in which these criteria have been met show that individuals can be repeatedly parasitized by cowbirds between nesting attempts, both within (Hauber 2001, Hoover et al. 2006) and between breeding years (Hauber et al. 2004, Hoover et al. 2006, Wagner et al. 2013). Eastern Phoebes are such an example: they are susceptible to repeated parasitism both within and between breeding years (Hauber 2001, Hauber et al. 2004, Wagner et al. 2013). Additionally, parasitism status in one year has been associated with parasitism status in the subsequent years in Prothonotary Warblers (*Protonotaria citrea*), Red-winged Blackbirds (*Agelaius phoeniceus;* Hoover et al. 2006), and in a California, USA urban population of juncos (Hauber et al. 2004).

Juncos have become common cowbird hosts in the past 60 years, with their ranges recently overlapping in the wilderness of the Allegheny Mountains in Virginia, USA (Wolf et al. 1987). Female juncos are multi-brooded and construct open cup nests on or near the ground in a concealed location (Nolan et al. 2020). Female juncos are the only sex to build nests and provide care to the eggs until hatching; they also accept cowbird eggs and the resulting parasitic nestlings and raise them alongside their own young. However, junco nest success decreases when parasitized by a cowbird (Wolf et al. 1987, Lorenzana and Sealy 1999, Turner et al. 2022) and increases when cowbird eggs are removed from the nest by researchers (Turner et al. 2022).

Cowbird parasitism of individually color-banded junco nests has been extensively documented since the 1980s by researchers at Mountain Lake Biological Station (MLBS) in Virginia, USA, led by Ellen Ketterson and the late Val Nolan Jr. at Indiana University (Wolf et al. 1987, Turner et al. 2022). Adult juncos in this population exhibit high rates of breeding site fidelity and offer a

Table 1. Summary of statistical tests, models, and outputs.

Statistical test	Model	Output
Repeated parasitism within the same breeding year		
GLMM binomial, logit link	Parasitism status of subsequent attempts in same breeding ~ Status of 1st breeding attempt + year (random)	$\chi^2 = 1.69$, df = 1, $P = 0.10$, $N = 432$
Quasibinomial GLM, logit link	Repeated parasitism score ~ parasitism status of 1st breeding attempt	$F = 3.76$, df = 1, $P = 0.10$, $N = 449$
Quasibinomial GLM, logit link	Repeated parasitism score ~ parasitism status of 1st breeding year	$F = 30.14$, df = 1, $P < 0.001$,† $N = 447$
Repeated parasitism between breeding years		
GLMM binomial, logit link	Parasitism status of attempts in subsequent breeding years ~ status of 1st breeding year + year (random)	$\chi^2 = 7.55$, df = 1, $P = 0.006$,† $N = 224$
Reproductive success and repeated parasitism		
GLMM binomial, logit link	Parasitism status of subsequent nesting attempts within the same year ~ reproductive success of 1st breeding attempt + year (random)	$\chi^2 = 1.47$, df = 1, $P = 0.23$, $N = 362$
GLMM binomial, logit link	Parasitism status of subsequent breeding years ~ reproductive success of 1st breeding year + year (random)	$\chi^2 = 2.10$, df = 1, $P = 0.15$, $N = 198$
GLMM binomial, logit link	Reproductive success of subsequent breeding attempts within the same year ~ reproductive success of 1st breeding attempt + year (random)	$\chi^2 = 1.45$, df = 1, $P = 0.23$, $N = 302$
GLMM binomial, logit link	Reproductive success of subsequent breeding years ~ reproductive success of 1st breeding year + year (random)	$\chi^2 = 2.57$, df = 1, $P = 0.11$, $N = 167$
Age and probability of parasitism		
GLM binomial, logit link	Lifetime parasitism status ~ number of breeding years 1–7	$\chi^2 = 13.15$, df = 6, $P = 0.04$,† $N = 454$
Post-hoc comparisons:	1st year breeder vs. 4th year breeder	estimate = -1.22 ± 0.41, $Z = -2.98$, $P = 0.046$†
	2nd year breeder vs. 4th year breeder	estimate = -1.20 ± 0.43, $Z = -2.79$, $P = 0.078$
GLM binomial, logit link	Lifetime parasitism status ~ number of breeding years 1–4	$\chi^2 = 9.57$, df = 3, $P = 0.02$,† $N = 443$
Post-hoc comparisons:	1st year breeder vs. 4th year breeder	estimate = -1.27 ± 0.41, $Z = -2.98$, $P = 0.02$†
	2nd year breeder vs. 4th year breeder	estimate = -1.20 ± 0.43, $Z = -2.79$, $P = 0.03$†

† Statistically significant at $\alpha = 0.05$.

suitable system for studying repeated parasitism events within and across years involving the same individual female, given that females choose the nest location and construct and incubate the nest (Nolan et al. 2020). In addition, most females maintain the same mate for the entire breeding season, and in cases in which both members of a pair return to breed again in a subsequent year, approximately 80% reestablish the same pair bond (Ketterson et al. 1996). Using these long-term data on the cowbird-junco system at MLBS, we predicted that: (1) first-time breeding juncos with initial cowbird parasitism experience would be more likely to be parasitized in subsequent breeding attempts and breeding years, (2) lower reproductive success of female juncos would significantly increase the likelihood of repeated parasitism occurring within the same breeding year, and (3) the likelihood of experiencing repeated parasitism would decrease with host age.

METHODS

Data collection

We used the publicly available data set associated with the MLBS long-term junco study (https://hdl.handle.net/2022/15253) and extracted data on junco female identity (through banding records), nest success, reproductive outcome, and cowbird parasitism status (using the same methods applied to the data set compiled for Turner et al. 2022). We obtained data from 454 females with a range of breeding years (1–7 years, only 11 of which are with 5–7 years of breeding). Data were recorded on parasitism status (parasitized or not) of the first nesting attempt in their first breeding year, parasitism of subsequent nesting attempts within the same year (yes or no), and parasitism of subsequent nesting attempts between years (yes or no, if the female bred for two or more years). However, the data were not consecutive for all females across all years. For example, a female could have been observed in years t and $t + 2$, but not in year $t + 1$. Juncos are intra-annually multibrooded (Nolan et al. 2020), with average nesting attempts of 1.4 nests/yr in the study population from 1990 to 2012. To assess whether juncos that were parasitized in their first nesting attempt/yr were more likely to have a subsequent nest parasitized, we also calculated a repeated parasitism score (values from 0 to 1) for each female by excluding the female's first nest and determining the ratio of the number of parasitized nests divided by the total number of subsequent nesting attempts. We also calculated a lifetime parasitism score, which was the ratio of the total number of parasitized nests divided by the total number of nesting attempts monitored across all years per known female.

Statistics

All statistical tests were performed using RStudio version 2025.09.2 (Posit Team 2025). For a summary of all statistical tests and outputs, see Table 1. To determine whether females with parasitized first nests were more likely to be parasitized in subsequent nests within the same year, we used a generalized linear mixed model (GLMM), with subsequent parasitism attempts within the same year as the response variable (parasitized or not parasitized), parasitism of the first breeding attempt as a predictor, and year as a random effect. Model significance for all GLMMs was evaluated using likelihood ratio tests by comparing the full model to a reduced model (without the predictor of interest). We also ran a quasibinomial generalized linear model (GLM) to analyze whether a female's repeated parasitism score (0 to 1) is predicted by the parasitism status of her first breeding attempt or first breeding year (parasitized or not parasitized). To test model significance for the quasibinomial, we used an F-test.

To evaluate if females parasitized in their first breeding year were more likely to be parasitized in subsequent years, we used a GLM with parasitism status in subsequent years as the response variable (parasitized or not parasitized) and parasitism status of the first

breeding year (had a successful nest or not) as a predictor. We did not include year as a random effect because it accounted for zero of the variance and produced model warnings. A likelihood ratio test was used to assess model significance.

A GLMM was used to analyze the effect of junco reproductive success on the likelihood of subsequent parasitism. The model included parasitism status of the subsequent breeding attempts within the same year (parasitized or not parasitized) as a response variable, reproductive success of the first breeding attempt (fledge or fail) as the predictor, and year as a random effect. A GLM was used to assess between-year differences, with parasitism status in subsequent years as the response, and reproductive success in the first breeding year (fledge or fail) as the predictor and year as a random effect.

To test whether future reproductive success was affected by the first breeding attempt/yr, we used another set of GLMMs. Within the same year, we used reproductive success of the first nesting attempt (fledge or fail) as the response, and reproductive success of subsequent nesting attempts (fledge or fail) as a predictor, with year as a random effect. Between breeding years, we used reproductive success of any nest in their first breeding year (fledge or fail) as the response, and reproductive success of any nest in subsequent years (fledge or fail) as a predictor, with year as a random effect.

Lastly, we used a GLM to analyze the lifetime parasitism status (parasitized or not parasitized in their lifetime) predicted by breeding years. We ran this GLM twice, with breeding years 1–7 (N = 454) and only breeders with 1–4 years (N = 443).

RESULTS

Repeated parasitism within the same breeding year

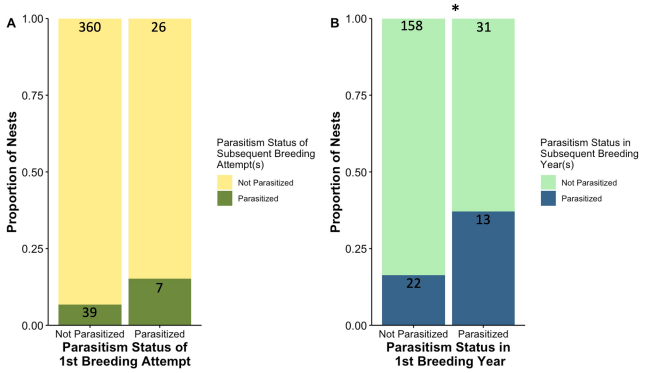
Female juncos that were parasitized in their first nest were not significantly more likely to have subsequent nesting attempts parasitized in the same year (χ^2 = 1.69, df = 1, P = 0.10; Fig. 1A). In contrast, females that were parasitized at least once in their first breeding year were more likely to be parasitized in subsequent years (χ^2 = 7.55, df = 1, P = 0.006; Fig. 1B).

Females who had their first-ever nesting attempt parasitized did not have significantly different repeated parasitism scores than females whose first nest was not parasitized (F = 3.76, df = 1, P = 0.10; Fig. 2A). However, the parasitism status of a female's first breeding year (parasitized or not) was a significant predictor of a greater repeated parasitism score in subsequent years combined (F = 30.14, df = 1, P < 0.001; Fig. 2B).

Reproductive success and repeated parasitism

We found that females with successful first nests were not significantly more likely to have subsequent nests that were parasitized in the same year (χ^2 = 1.47, df = 1, P = 0.23; Fig. 3A). Females that fledged juncos from any nesting attempt in their first breeding year were not more likely to be parasitized in subsequent breeding years (χ^2 = 2.10, df = 1, P = 0.15; Fig. 3B). Additionally, females that fledged juncos from their first-ever breeding attempt were not statistically more likely to fledge additional juncos in their first year (χ^2 = 1.45, df = 1, P = 0.23), nor did we find this effect for breeding attempts in subsequent years (χ^2 = 2.75, df = 1, P = 0.11).

Fig. 1. (A) Proportion of individually marked female juncos' nests that were repeatedly parasitized within the same year. Females that were parasitized in their first nests were not significantly more likely to have subsequent nesting attempts parasitized in the same year (χ^2 = 2.76, df = 1, P = 0.10). (B) Proportion of female juncos' nests that were repeatedly parasitized between breeding years. Females that were parasitized in their first breeding year were more likely to be parasitized in subsequent years (χ^2 = 7.07, df = 1, P = 0.008).



Age and the probability of parasitism

The number of breeding years was a significant predictor of whether a female was ever parasitized in her lifetime, with younger females being more likely to experience parasitism (χ^2 = 13.15, df = 6, P = 0.04; Fig. 4). Post hoc comparisons showed a significant difference between females that bred for one year and for four years (estimate = −1.22 ± 0.41, Z = −2.98, P = 0.046). While not significant, we detected a nearly significant comparison between parasitism of second-time breeders and fourth-year breeders (estimate = −1.20 ± 0.43, Z = −2.79, P = 0.078). We found a similar qualitative result when we excluded 5–7 year breeders (χ^2 = 9.57, df = 3, P = 0.02, N = 11), with significant comparisons between females that bred for one year (estimate = −1.27 ± 0.41, Z = −2.98, P = 0.02) or two years (estimate = −1.20 ± 0.43, Z = −2.79, P = 0.03) compared to fourth-time breeders.

DISCUSSION

Likelihood of repeated parasitism

Female juncos with prior parasitism experience in their first breeding year were more likely to be parasitized again in subsequent breeding years. Studies on other populations of juncos (e.g., Hauber et al. 2004) and on other species of cowbird hosts (Smith 1981, Hauber et al. 2004) have found similar results, whereby females parasitized in their first year are more likely to be parasitized in subsequent years. We did not find this relationship to be statistically significant within the same breeding year, although our results trended in that direction. In Prothonotary Warblers, but not in Red-winged Blackbirds, the likelihood of subsequent breeding attempts being parasitized within the same year is negatively related to the reproductive success of the first nesting attempt (Hoover et al. 2006). In Prothonotary Warblers, Hoover et al. (2006) also found that failed

Fig. 2. (A) Repeated parasitism score of females parasitized repeatedly in the same year. Female juncos who had their first-ever nesting attempt parasitized did not have significantly higher subsequent parasitism scores than females whose first nests were never parasitized ($F = 3.76$, df = 1, $P = 0.10$). (B) Repeated parasitism score of female juncos repeatedly parasitized between years. Females parasitized in their first year were significantly more likely to have higher subsequent parasitism scores ($F = 30.14$, df = 1, $P < 0.001$).

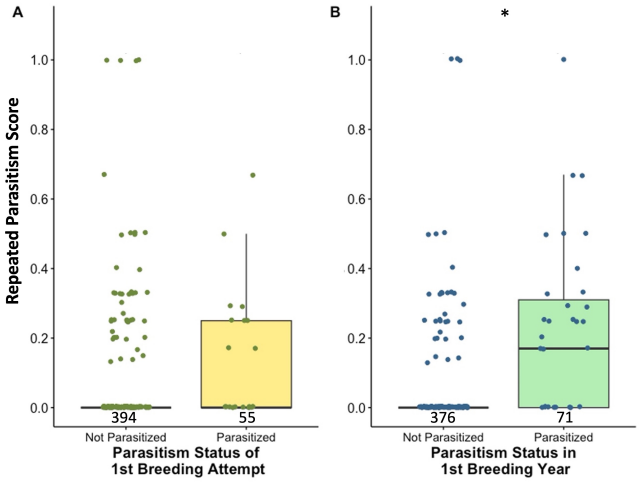
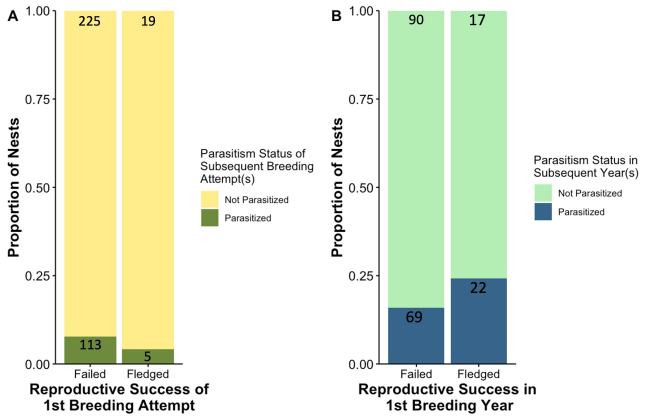


Fig. 3. (A) Reproductive success of the first breeding attempt and the proportion of nests with subsequent parasitism within the same breeding year. Females with failed first breeding attempts were not significantly more likely to experience repeated parasitism in the same year ($\chi^2 = 2.51$, df = 1, $P = 0.11$). (B) Reproductive success during the first breeding year and the proportion of females that experienced parasitism in subsequent breeding years. We did not detect a significant difference in future parasitism between females that failed or fledged juncos ($\chi^2 = 2.75$, df = 1, $P = 0.10$).



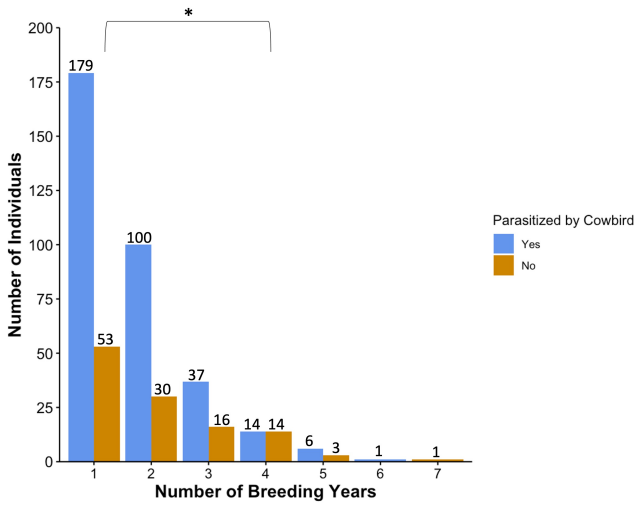
first nests are more likely to experience repeated parasitism within the same year. We did not detect a similar statistical pattern here.

Typically, cowbird parasitism declines throughout the breeding season, and failed attempts present an earlier opportunity to re-parasitize than do successful nests (Brooker and Brooker 1996, Hoover et al. 2006). However, we did not find a relationship between junco reproductive success and subsequent parasitism within or between years. The main driver for nest failure in this population of juncos is depredation, mostly by small mammals and raptors, and not directly as a result of cowbird parasitism (Clotfelter et al. 2007, Nolan et al. 2020). We found a few cases of cowbird depredation of junco nests, but our past study did not find evidence for retaliatory mafia behavior by cowbirds in this system (Turner et al. 2022). Therefore, our results prompt us to wonder what it is about individual female junco traits, including their behavior, that may make them more or less likely to experience repeated parasitism.

The possibility of repeated cowbird parasitism may be affected by the attributes of the host individual's territory and the microsite at which each nest is constructed. Habitat fragmentation, landscape composition (Robinson et al. 2000, Patten et al. 2006, 2011, Howell et al. 2007), or degree of nest concealment (Burhans 1997, Hauber 2001, Wagner et al. 2013) can all influence parasitism rates. For example, Eastern Phoebe nests built under eaves are parasitized more than nests built over water under bridges (Hauber 2001), and nests built in edge habitats are parasitized more often than in open prairie (Patten et al. 2011). In contrast, studies of other cowbird hosts have not found support for nest concealment influencing the likelihood of parasitism (Smith 1981, Burhans and Thompson 1998, Clotfelter 1998, Banks and Martin 2001, Shaffer et al. 2003). This latter result could be due to the multiple strategies that female cowbirds deploy to find suitable host nests (Norman and Robertson 1975, Strausberger 1998). Additionally, repeated parasitism within and between years can be affected by the availability of hosts and nests during the breeding season (McLaren et al. 2003, Ellison et al. 2006, Rivers et al. 2012, de la Colina et al. 2016), although it is rare for the same female cowbird to repeatedly parasitize the same nesting attempt (Ellison et al. 2006, Hauber et al. 2012, Gloag et al. 2014; but see Rivers et al. 2012, Rivers and Peer 2016).

Adult juncos in the MLBS study population have high breeding site fidelity, both within and between breeding years (Nolan et al. 2020), which may make them a reliable and consistent target for repeated parasitism by the also territorial and philopatric breeding adult female cowbirds (Woolfenden et al. 2003, Hauber et al. 2012). For example, Prothonotary Warblers are more likely to be parasitized when they use the same nest boxes in subsequent breeding years (Hoover et al. 2006). Due to the difficulty of monitoring banded, radio-tracked, and molecularly marked individual female cowbirds (e.g., de la Colina et al. 2016, Louder et al. 2019, Chamberlain et al. 2025), we know relatively little about the breeding philopatry of adult cowbirds. However, results from a limited sample of color-banded individuals show high breeding site fidelity rates of > 50% in adults but low rates (< 5%) of natal philopatry (Hauber et al. 2020). In addition, results from Woolfenden et al. (2003) and Strausberger and Ashley (2005) suggest that at least some female cowbirds exhibit non-random host use. Thus, in areas where there is high breeding philopatry of both the host and parasite species, the likelihood of repeated parasitism within and between breeding years could be higher.

Fig. 4. Number of breeding years was a significant predictor of whether a female was ever parasitized in her lifetime (χ^2 = 13.15, df = 6, P = 0.04). Post hoc analysis showed a significant difference between females that bred in one vs. four years (232 females bred in one year, 130 in two, 53 in three, and 28 in four years). Only 11 females bred in five or more years (N = 9 in five years, N = 1 in six and seven years).



The availability of other hosts in the community could affect the likelihood that females experience repeated parasitism (Robinson et al. 2000, Woolfenden et al. 2004, Chace et al. 2005, Igl and Johnson 2007). If other potential host populations change over time with parasite abundance, we would expect that juncos would experience varying degrees of parasitism or repeated parasitism. Unfortunately, our long-term data set does not include the abundance of other host species, and so we were unable to account for how community changes over time affect the likelihood of repeated parasitism of juncos. However, at least some aspects of the cowbirds' host selection process are non-random (Woolfenden et al. 2003, 2004, Chace et al. 2005, Igl and Johnson 2007). For example, cowbirds in Manitoba, Canada parasitize Song Sparrows (*Melospiza melodia*) more frequently and intensely than other species, even though they are not the most abundant host in the area (Woolfenden et al. 2004). In the Great Plains, Red-winged Blackbirds are the preferred host, even though they exhibit strong front-loaded nest defense behaviors against parasitism (Igl and Johnson 2007, Lawson et al. 2020). In these studies, both host species were also significantly more likely to experience multiple parasitism, suggesting that there are differences in host value that inform host preference.

Female host longevity

We found that lifetime parasitism score decreased with increasing number of breeding years (i.e., female junco longevity), implying that older, returning female juncos are less likely to experience cowbird parasitism than younger, single-year breeders. This result is consistent with observations from Eurasian Magpies, a common host of the brood-parasitic Great Spotted Cuckoo, in which older females are also less likely to be parasitized than younger females (Martínez et al. 2020). This relationship could be due to traits associated with a particular female, given that naïve breeders tend to be foreign egg accepters and build nests in lower quality areas (Grim 2002, Parejo and Avilés 2007, Moskát et al. 2014). Contrary to our findings, the likelihood of parasitism increases with age in at least one other cowbird host, the Song Sparrow (Smith 1981, Smith et al. 1984). This outcome could potentially be explained by the propensity of older female sparrows to increase their activity when presented with a cowbird mount relative to younger females in the same simulated interaction, a behavior hypothesized to be maladaptive (Smith et al. 1984) because Brown-headed Cowbirds pay attention to hosts' near-nest activities when choosing nests to parasitize (Clotfelter 1998, Banks and Martin 2001), which could be affected by increasing female age.

There are multiple other factors that could explain why the age of the host female is not consistently related to parasitism rate across species. One possibility could be that longer-lived females have more nesting attempts to parasitize than younger females (Smith and Arcese 1994) or that older females have more nesting attempts in years with lower levels of parasitism. Accordingly, in the MLBS system, the annual cowbird parasitism rate of juncos varied significantly over the 22 years included in this study (Turner et al. 2022). Another potential explanation is that certain aspects of nest site selection and placement could be affected by female age (Parejo and Avilés 2007). Female Song Sparrow yearlings, for example, selected nest sites only in certain microgeographic zones on Mandarte Island, Canada, whereas older females were parasitized more often in all six zones analyzed (Smith et al. 1984). Additionally, longer-lived females could switch nesting sites within or between years to avoid parasitism (Molina-Morales et al. 2013). However, this explanation does not seem to be likely in our study population because > 95% of females were highly repeatable in their breeding site location within and between years.

CONCLUSION

This data set provides a unique opportunity to analyze long-term data on reproductive success and parasitism rates of Dark-eyed Juncos by Brown-headed Cowbirds. We found that juncos who were parasitized in their first breeding year were more likely to be parasitized in subsequent years, but not in nesting attempts within the same year. Also, older female juncos, those with a higher number of breeding years, were less likely to be parasitized. More studies are needed to fully assess the seasonal and interannual dynamics behind repeated brood parasitism in both generalist and specialist parasitic species.

Acknowledgments:

We thank Prof. Ellen Ketterson and her research team at Indiana University for access to data and nest logs. We also thank all the United Junco Workers who endeavored tirelessly to collect these data in the field.

Data Availability:

Our data can be accessed on Figshare at https://doi.org/10.6084/m9.figshare.29695685.

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