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Adult sex dictates juvenile–adult interactions in brood-parasitic brown-headed cowbirds, *Molothrus ater*

Mac L. Chamberlain [a, *], Wendy Schelsky [a, b, c], Autumn Bush [a], Shanelle Johnson-Cadle [a], Shelby Lawson [a, d], Mark E. Hauber [e]

[a] *Department of Ecology, Evolution, and Behavior, School of Integrative Biology, University of Illinois Urbana-Champaign, Urbana, IL, U.S.A.*
[b] *Illinois Natural History Survey, Prairie Research Institute, University of Illinois Urbana-Champaign, Urbana, IL, U.S.A.*
[c] *The Grainger College of Engineering, University of Illinois Urbana-Champaign, Urbana, IL, U.S.A.*
[d] *Department of Integrative Biology, Oregan State University, Corvallis, OR, U.S.A.*
[e] *Advanced Science Research Center and Programs in Biology and in Psychology, Graduate Center of the City University of New York, New York, NY, U.S.A.*

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Obligate brood-parasitic birds provide critical ontogenetic and life history complications for members of their own species, especially in the context of social development. For example, parasitic brown-headed cowbirds exhibit complex social dynamics, with just-independent juveniles relying on early conspecific adult interactions for their successful social development. Our study examines these brood parasites' juvenile–adult interactions and the potential role of genetic relatedness in cowbird ontogeny by investigating co-capture patterns and DNA data collected across 3 years (2022–2024) in east-central Illinois, U.S.A. Using microsatellite-based parentage analyses and Queller–Goodnight relatedness estimates (QGM), we examined the genetic relationships of 122 adults and seven juveniles and compared these with co-capture occurrences. Of the 478 potential adult–juvenile pairings, only three pairings among two juveniles showed high QGM relatedness values suggestive of parent–offspring relationships; follow-up parentage analyses confirmed that only two of our juveniles had putative parents (95% confidence) within our sampled adult cowbird population. Juveniles were seldom co-captured with adults with whom they had higher than expected relatedness and were never co-caught with putative parents. Using the admittedly small sample sizes of captured juveniles, we found that generalized linear mixed models produced no statistical evidence supporting an effect of relatedness on juvenile–adult co-capture rates in contrast to earlier findings. In contrast, we found strong statistical evidence suggesting that juveniles are more likely to associate with adult females than with adult males ($P = 0.001$), supporting prior behavioural studies suggesting that juvenile preferences are driven by both self-referent phenotype matching and attraction to adult female vocalizations. Our findings do not support the assumption that relatedness consistently drives juvenile–adult associations in brown-headed cowbirds, and suggests, instead, that other factors may play a more prominent role. These results underscore the need for further research on socio-ontogenetic mechanisms in this and other avian obligate brood parasites.

Parental care for vulnerable young takes a variety of forms across many diverse lineages (Gonzalez-Voyer & Kolm, 2010). In contrast, the single most common parental care strategy in birds is biparental care by a socially monogamous adult pair (Burley & Johnson, 2002; Cockburn, 2006). This inherently conspecific social environment allows recently hatched birds to successfully undergo filial and sexual imprinting on both their parents and their siblings during the dependent period (Chamberlain et al., 2024). Imprinting processes facilitate the acquisition of essential conspecific recognition cues as well as reproductive, antipredator and foraging behaviours and sexual preferences (Klinghammer & Hess, 1964; Licklitter & Gottlieb, 1985; Lorenz, 1937). Parents providing their offspring with the cues to successfully identify and interact with conspecifics benefits the young's fitness not only through clear reproductive benefits later in life but also through various other social, antipredator, roosting and foraging benefits (Clark & Mangel, 1984; Göth & Hauber, 2004).

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* Corresponding author.
E-mail address: maclc2@illinois.edu (M. L. Chamberlain).

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In contrast to the biparental care seen across most bird species, 1% of the ~10 000 avian species have lost parental care behaviours through the evolution of obligate brood parasitism (Davies, 2010). These parasites lay their eggs in the nests of other bird species, leaving the host parent(s) to incubate and raise the foreign offspring (Davies, 2010; Payne, 1977; Rothstein, 1990). If these brood-parasitic offspring were to filially or sexually (mis)imprint on their foster parents, it would lead to maladaptive species recognition and heterospecific social associations (Slagsvold et al., 2002). Because of this atypical avian social milieu in developing obligate brood parasites, it had long been assumed that species-specific behaviours in obligate brood parasites were innate (Davies, 2010; Ortega, 1998). This assumption was challenged when studies on self-referent phenotype matching in parasitic young revealed that future associative preferences could be influenced by the subject's own (experimentally manipulated) phenotype early in development (Hauber et al., 2000). Thus, conspecific social preferences in brood-parasitic species can be, at least partly, modified over the course of the juvenile's development based on the individuals' own experiences. Over the last few decades, more and more evidence has accumulated showing that, despite previous considerations (e.g. Davies, 2010; Ortega, 1998), early interactions between brood-parasitic juveniles and conspecific adults are crucial for a juveniles' adaptive social development (Freeberg, 2004; Louder et al., 2019; Soler & Soler, 2000). Although the ontogenetic timing of these interactions varies, adults of the brown-headed cowbird, the great spotted cuckoo, *Clamator gladarius*, and at least 10 other brood-parasitic species have been opportunistically observed interacting with and even feeding fledglings and postfledgling juveniles (Lorenzana & Sealy, 1998).

Considering these recurrent observations, it has been suggested that brown-headed cowbirds ('cowbirds' hereafter) may be able to seek out, recognize and preferentially associate with closely related juveniles (West et al., 1981). If this were the rule rather than the exception, the parent cowbird's facilitation of juvenile progeny's social development could be an example of kin-directed preferential behaviour. Alternatively, it is possible that cowbird juveniles follow any adult conspecifics that they encounter due to the high conspecific preference seen in laboratory trials and the lack of attacks that juvenile cowbirds may otherwise experience directed at them by adult heterospecifics (Hauber, 2002). Juvenile cowbirds typically remain with their host parents and/or foster family for 20–30 days postfledging before dispersing independently and seeking out conspecific groups (Louder et al., 2015). Evidence suggests that interactions between parasitic nestlings and adult cowbirds do not occur either at the host nest (Hauber, 2002), or during the early postfledging period when juveniles are still under host care (Hauber, 2002; Louder et al., 2015), indicating that interactions with conspecific adults most likely occur after juveniles become independent (Hahn & Fleischer, 1995). Little is known about natal dispersal ecology in cowbirds; however, because adult female cowbirds can be territorial during the breeding season (Thompson, 1994), it is likely, but not necessary, that these adult–juvenile pairs may share relatedness due to spatial proximity (Hahn & Fleischer, 1995; Hauber et al., 2012).

To assess genetic relatedness between co-captured juveniles and female adult cowbirds, Hahn and Fleischer (1995) performed a DNA fingerprinting study between juveniles and adult females trapped together. Despite their small sample size, the results showed that the most highly related female captured with a juvenile shared significantly more bands with that juvenile than with a randomly assigned adult from the population. Hahn and Fleischer's study only comprised pairings of juveniles with adult females; no adult males were included in their analysis. We, therefore, do not know whether a potential genetic association pattern between adult males and co-caught juveniles also holds or is exclusive to adult females. It stands to reason that, because interactions with both adult males and adult females are required for the social development of captive juvenile cowbirds (Freeburg, 2004) and because most female cowbirds are genetically monogamous with a single male sire (Louder et al., 2019), both adult males and females should show similar patterns to preferentially associate with closely related juveniles. Additionally, Hahn and Fleischer's approach to exclusively compare the most-related juvenile–adult co-captured pairing against the relatedness that juvenile shares with a single, randomly chosen adult introduces significant bias and risks leaving out important genetic distributional patterns with regard to how the focal juvenile is genetically related to all sampled adults within the population.

Here, we both replicate and expand upon Hahn and Fleischer's (1995) approach and report on a co-capture study coupled with a microsatellite-based genetic relatedness analysis on three consecutive breeding seasons of brown-headed cowbirds, including relatedness of juveniles with both adult males and females captured. We determine whether adults and juveniles co-caught in our population exhibited more related genetic patterns as was found by Hahn and Fleischer (1995) and whether any genetic association patterns are influenced by the sex of the adult or the juvenile cowbird.

METHODS

Study Site and Capture Methods

This study was performed at Kennekuk County Park (Danville) in east-central Illinois, U.S.A. (40°11′54″N, 87°43′30″W), a locality that contains ~1250 ha of grassland, shrubland and forest. We used two methods to capture birds: (1) stationary walk-in ladder traps, measuring 2.4 × 1.8 × 1.5 m with 12.7 cm gaps between ladder slots and baited with a mix of sunflower and white millet seed, and (2) stationary mist nets (35 mm mesh, 12 m length) with a female cowbird chatter playback as a lure. Cowbirds were trapped at three different locations (in 2022) and six different locations (in 2023 and 2024) on this property, with trapping locations averaging 1.22 km apart (0.52–2.56 km). All ladder traps and mist net sites were spatially distinct both within and across each year (Fig. 1).

Trapping in all 3 years began the third week of April and continued through the last week of July to roughly represent the local passerine (host and cowbird) breeding season (Jones et al., 2022). These capture onset and cutoff dates were selected to reduce the likelihood of capturing transient or migratory individuals, allowing us to concentrate our efforts on resident adults and juveniles that were locally bred and fledged. Banding efforts during all summers were split between morning days, when traps and nets were opened at approximately 0800 hours and closed after a maximum of 5 h, and evening days, when traps and nets were opened at approximately 1500 hours and closed at sunset. Within these trapping windows, ladder traps were checked every 60–90 min and mist nets were checked every 30 min. All captured individuals were processed at the trap upon discovery and immediately released.

Banding efforts were not distributed evenly across years. We utilized two ladder traps and one mist net location during the summer of 2022 with a combined total of 223 h (morning) and 55 h (evening). The summer of 2023 had a substantially larger effort utilizing three ladder traps and three mist net locations with combined total of 300 h (morning) and 265 h (evening). Finally, the summer of 2024 used two ladder traps and four mist net locations with a combined total of 284 h (morning) and 168 h (evening). Due to the greater proportion of morning hours compared to evening hours and the high productivity of ladder traps compared to mist nets, 79% of captures across all 3 years came from morning ladder traps.

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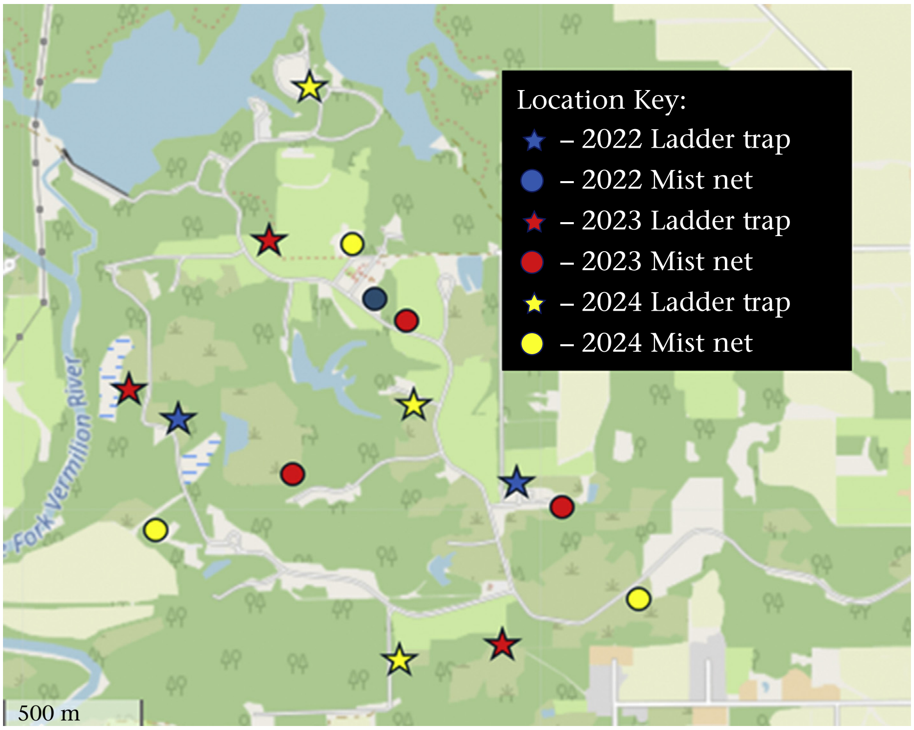

Figure 1. A map of trapping locations within Kennekuk County Park (Danville, IL, U.S.A.; 40°11′54″N, 87°43′30″W). Stars indicate locations of ladder traps while circles represent locations of mist nets. Similarly, blue markers indicate locations used in the summer of 2022, red markers indicate locations used in 2023 and yellow markers indicate locations used in 2024.

All captured birds were aged based on plumage characteristics and adult birds were also sexed based on plumage characteristics (Pyle, 1997). All subjects were banded using metal U.S. Geological Survey (USGS) bands and given a unique combination of three plastic colour leg bands. We measured wing chord (mm) using a wing rule, bill and tarsus length (mm) using dial callipers and weight (g) using either an electric balance or a spring scale. Blood samples for molecular sexing (juveniles) and microsatellite analyses (both juveniles and adults) were taken from the brachial vein during each individuals' initial capture and immediately stored in 95% ethanol at ambient temperature for further genetic analyses (see below).

Ethical Note

All procedures involving live birds were approved by the University of Illinois at Urbana-Champaign Institutional Animal Care and Use Committee (Protocol number 22043) and conducted under federal and state permits (Sub/Permit number 23681-C). All personnel were fully trained and followed institutional, state and federal animal care guidelines. Subjects were free-living brown-headed cowbirds captured in Danville, Illinois, U.S.A. Capture efforts were designed to minimize stress and handling time; traps and nets were monitored frequently, and all birds were processed immediately upon discovery. Standard morphometric and blood sampling protocols were used, and each bird was banded with a USGS metal band and a unique combination of plastic colour bands. No injuries or adverse effects were observed, and all individuals were released at the site of capture.

Genetic Analysis and Juvenile Sexing

Blood samples were stored in 95% ethanol at ambient temperature until the following September. DNA was then extracted from blood samples using Qiagen DNeasy Blood and Tissue kits (Qiagen, Valencia, CA, U.S.A.) following a modified protocol for blood stored in ethanol for nucleated red blood cells and then further stored in a −20 °C freezer until the PCR reactions were run. Juvenile birds were sexed using the *CHD* gene, which resides on the avian sex chromosomes; in birds, males are the homogametic sex (i.e. ZZ) whereas females are heterogametic (i.e. ZW), and the *CHD* amplicon varies in size depending on which chromosome it resides (Ellegren, 1996).

To determine genetic relatedness between individuals, we used nine microsatellite loci that were generated via PCR amplification procedures described previously (Louder et al., 2015). Three loci (*CB1*, *CB12*, *CB15*) were sourced from Longmire et al. (2001); Alderson et al. (1999) provided descriptions for three additional loci (*Mau 10*, *Mau 25*, *Mau 29*); two loci (*Mau 101*, *Mau 104*) were documented in Strausberger and Ashley (2001); Strausberger and Ashley (2003) described our last locus, *Mau 102*. Each locus's forward primer was fluorescently labelled (6-FAM, HEX or NED) and analysed on an Applied Biosystems (Thermo Fisher, Waltham, MA, U.S.A.) 3730xl DNA analyzer at the Carver Biotechnology Center at the University of Illinois at Urbana-Champaign to determine fragment sizes.

Genotypes were assigned through automatically or manually calling alleles using GeneMapper version 3.7 (Thermo Fisher). To include only resident and potentially breeding adults, we limited our data set by removing data from all individuals in the analysis that were exclusively captured before 8 May for each year. This date was chosen as the earliest potential cowbird egg laying date at Kennekuk County Park based on data from Dr Todd Jones, who had previously worked within this same study site and system (T. J. Jones, 2019; Jones et al., 2022). Remaining adults in the data set are hereafter referred to as 'focal adults'.

Genotypes of focal adults were compared using GenAlEx version 6.503 (Peakall & Smouse, 2012) to produce mean Queller—Goodnight (QGM) estimated pairwise relatedness scores

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(Queller & Goodnight, 1989). Pairwise QGM relatedness scores were calculated for all adult–juvenile pairs regardless of whether they were captured together. An estimated QGM score of 1 corresponds to a genetically identical twin (or clone), a score of 0.5 corresponds to full sibling or parent–offspring relationship and a score of 0.25 corresponds to a half sibling relationship.

To further assign any parent–offspring relationships, we conducted a known-adult sex parentage analysis using the computer software CERVUS version 3.0.7 (Kalinowski et al., 2007). CERVUS is a popular software for parental assignment that uses genotyped loci and a likelihood-based approach to calculate log likelihood ratios (LOD) for inferring parentage (Marshal et al., 1998; Slate et al., 2000). We performed a separate analysis for each year and defined ‘candidate parents’ as focal adults who were caught at least once during that trapping season. Our simulations used the recommended 10 000 tests, the proportion of loci typed specific to each year (ranging between 0.965 and 0.980), and the recommended assumed proportion of mistyped loci of 0.01 (Marshal et al., 1998). Using anecdotal observations of resighted colour-banded individuals, we estimated our proportion of population sampled to be 0.75. Due to our lack of confidence in this estimate and the unstandardized methods used to derive it, we varied this proportional value between 0.25, 0.4 and 0.8, and it did not alter our parental assignments.

Our parentage analyses resulted in combined nonexclusion probabilities (likelihoods of failing to exclude a nonrelative and categorizing them as a potential parental) for the first parent assignment, second parent assignment and parent pair assignments. Across both years, these values were no higher than 0.00018, 3.13E-6 and 2.86E-10 respectively.

Statistical Analyses

Sex ratios were calculated for each year by transforming counts of captures into a proportional ratio, with the number of females standardized to 1 (e.g. a ratio of 25 females to 32 males is expressed as 1:1.28).

To determine whether pairwise relatedness covaried with adult–juvenile co-captures, we used a generalized linear mixed model with a binomial distribution (‘lme4’ package; Bates et al., 2015) in R version 4.1.2 (R Core Team, 2021). A binary value was given to adult–juvenile pairs based on co-capture status (0 = not co-captured; 1 = co-captured) and was used as the response variable, with the pairwise relatedness estimates, sex of the adult and sex of the juvenile used as predictor variables and juvenile identity (ID) and sampling year used as random effects.

Finally, to evaluate the sensitivity of our model to detect effects of kinship, we conducted a post hoc power analysis using the ‘simr’ package in R (Green & MacLeod, 2016). We extended our fitted GLMM to simulate a larger sample size of 50 juvenile individuals, while maintaining the original model structure and variance parameters.

RESULTS

Capture and Genetic Results

In 2022, we captured 25 adult females, 33 adult males and 3 fledgling males during the trapping season, 8 May – 30 July (1:1.32 female to male adult ratio). In 2023, we captured 25 adult females, 51 adult males, 2 fledgling females and 2 fledgling males during the same summer months (1:2.04 female:male adult ratio). In 2024, we captured 28 adult females and 71 adult males and no fledglings (1:2.53 female:male adult ratio). Because no fledglings were caught in 2024, we excluded 2024’s capture and genetic data from the analyses below. In total, our analyses include 122 unique adult individuals and 7 juvenile individuals captured between 2022 and 2023. Of the 478 potential adult and juvenile pairings across both years, only 21 were co-captured together at least once. All juvenile capture events ranged between 12 June and 12 July, with a mean date of 6 July for both years.

We found high levels of heterozygosity within our 2022 and 2023 focal adult populations, with 74.5% of identified loci being heterozygous and 126 unique alleles identified across all nine microsatellites. Overall mean pairwise relatedness between focal adults and juveniles was low in both capture years, with QGM scores averaging −0.05 (SD = 0.13) in 2022 and −0.01 (SD = 0.16) in 2023 (Fig. 2). Out of 478 total possible pairings between juveniles and adults, only 25 pairings were 0.25 or higher (potential half sibling, uncle/aunt or niece/nephew). Of those 25 pairings, only three were 0.5 or higher (potential parent–offspring) across both years. One juvenile in 2022 (Juvenile 60) showed high relatedness with one adult male (QGM > 0.50, indicating a potential parent–offspring or full sibling relationship), and one juvenile in 2023 (Juvenile 57) had similarly high relatedness with one adult male and one adult female.

Our parentage analysis resulted in similar relationship conclusions. In 2022, parentage assignment at the 95% confidence level identified both a mother and a father for Juvenile 60 with no other potential parents. Similarly, in 2023, parentage assignment at the 95% confidence level identified both a mother and a father for Juvenile 57 with no other potential parents. Unlike our QGM analysis, the parentage analysis identified one additional potential father at the 80% confidence level for a third juvenile (Juvenile 52). No parents were assigned for the remaining juveniles at either of these confidence levels. None of the potential offspring–parent pairs were ever captured together, nor were the potential breeding adult pairs ever captured together. The five putative parents were captured between one and four times each, with capture dates ranging between 18 May and 10 July, which supports the appropriateness of our overall sampling period (8 May – 30 July) and lends further credence to the assumption that potential parents are present and can be captured even early in the season, prior to the emergence of fledglings.

Results of Statistical Modelling

Juvenile sex was not a significant predictor ($z = -0.832$, $P = 0.401$) of adult–juvenile co-capture rates, and there were no significant interactions between any of the predictor variables (juvenile sex, adult sex and QGM relatedness; all $z < 0.79$, all $P > 0.34$). Including juvenile sex in the model caused our model’s maximum absolute gradient, or ‘max|grad|’, to be 0.006, which surpassed the default tolerance level in R of 0.002. This high max|grad| value suggests that our model had difficulty converging and may suffer from overfitting, a likely case given our single-digit juvenile sample size and a relatively small sample of adult–juvenile co-captures. We subsequently removed juvenile sex from the model, which resolved this fault. All subsequent results utilized a model that was identical to the one described in our Statistical Analysis section above, excluding juvenile’s sex as a predictor.

Adult sex had a highly significant effect, with juveniles being more likely to be caught with adult females than with adult males ($z = -3.30$, $P = 0.001$; Fig. 3). Relatedness (QGM) was not a significant predictor in our model ($z = 0.837$, $P = 0.403$; Fig. 4). We again found no evidence for a statistical interaction between the two predictor variables QGM relatedness and adult sex after removing juvenile sex from our model ($z = -0.233$, $P = 0.816$). Because many of our juveniles did not have the opportunity to be observed interacting with closely related adults (i.e. their parents

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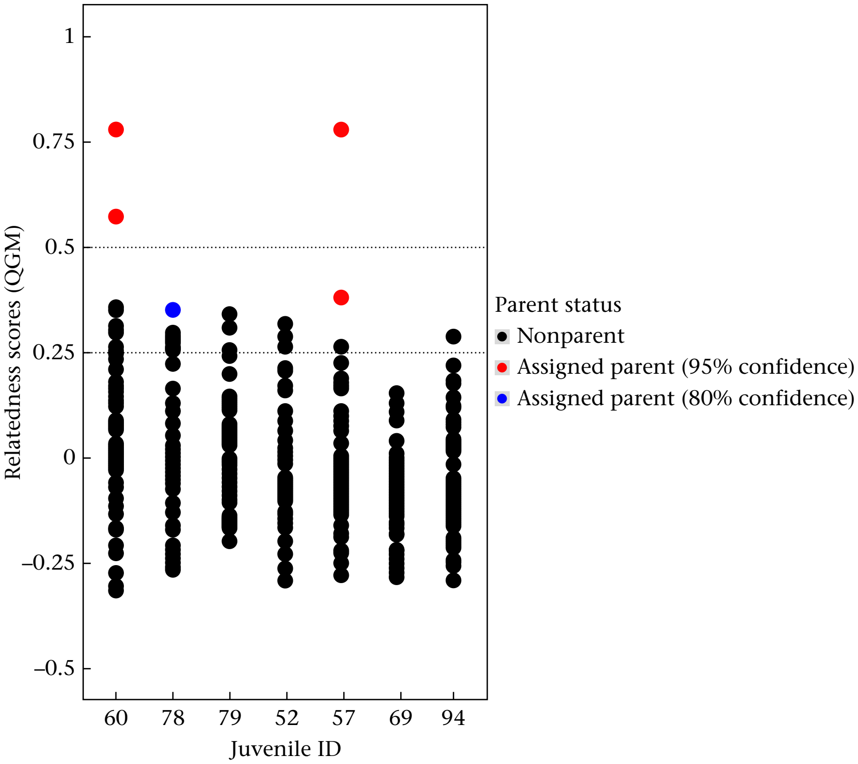

Figure 2. A distribution of Queller–Goodnight (QGM) scores showing the relatedness for seven different brown-headed cowbird juveniles captured in east-central Illinois across 2022 and 2023 compared to the focal adult cowbirds captured that year. A QGM score of 0.5 corresponds to a full sibling or parent–offspring relationship and a score of 0.25 corresponds to a half sibling relationship. Using a likelihood-based parentage analysis, putative parents were assigned at 95% confidence (red) and 80% confidence (blue).

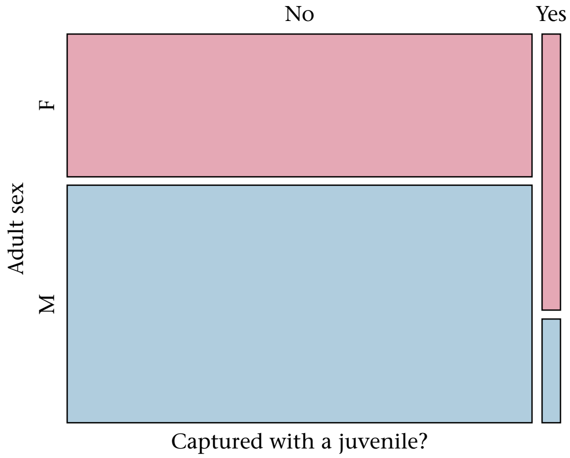

Figure 3. A mosaic plot showing the adult sex distribution of juvenile–adult pairs captured together and those who were not. The height and width of each section is proportional to the 478 total potential juvenile–adult pairings. Despite a skew towards males in the overall adult population, we see a heavy bias towards adult females in potential juvenile–adult pairings captured together.

were not found within our sampled population), we performed a post hoc analysis using the same model structure but including only the three juveniles with putative parents in our sample. Our original model design was overcomplex for this reduced sample size, so it was simplified such that co-capture status was our response variable, QGM relatedness was our only fixed effect and juvenile ID and sample year were random effects. We again found no evidence for an influence of QGM relatedness on co-capture status ($z = 0.055$, $P = 0.956$).

Consistent with our overall capture patterns, most ($N = 6$) juveniles in our study were caught using seed-baited ladder traps, with only a single individual captured via mist netting. This discrepancy in capture methods may introduce some bias to our results. However, given the small sample size of juveniles, our models lack the statistical power to account for capture method as a covariate. Notably, excluding the sole mist-netted juvenile from the data set did not alter our conclusions (relatedness: $z = 1.05$, $P = 0.290$; adult sex: $z = -2.73$, $P = 0.006$).

Adult sampling began on 8 May, which may have included early-season adults who left the study site before juveniles became independent of their host parents. If so, these adults would not have had the opportunity to be captured alongside juveniles, potentially biasing our sample. To address this, we conducted a post hoc analysis identical to our original models, but restricted to individuals captured on or after our earliest fledgling capture date (12 June). This adjustment did not change our conclusions (relatedness: $z = 0.068$, $P = 0.946$; adult sex: $z = -2.460$, $P = 0.014$).

Regarding our power analysis, based on 300 simulations, the estimated power to detect a significant effect of relatedness (QGM) was 63.3% (95% CI: 57.6–68.8%), which falls below the conventional 80% threshold. This result suggests that even with substantially more ($N = 50$) juveniles than our observed sample ($N = 7$), our model would still be underpowered to reliably detect moderate effect sizes of kinship. Therefore, while we found no evidence for kin associations, we acknowledge that these null results should be interpreted with caution due to limited statistical power.

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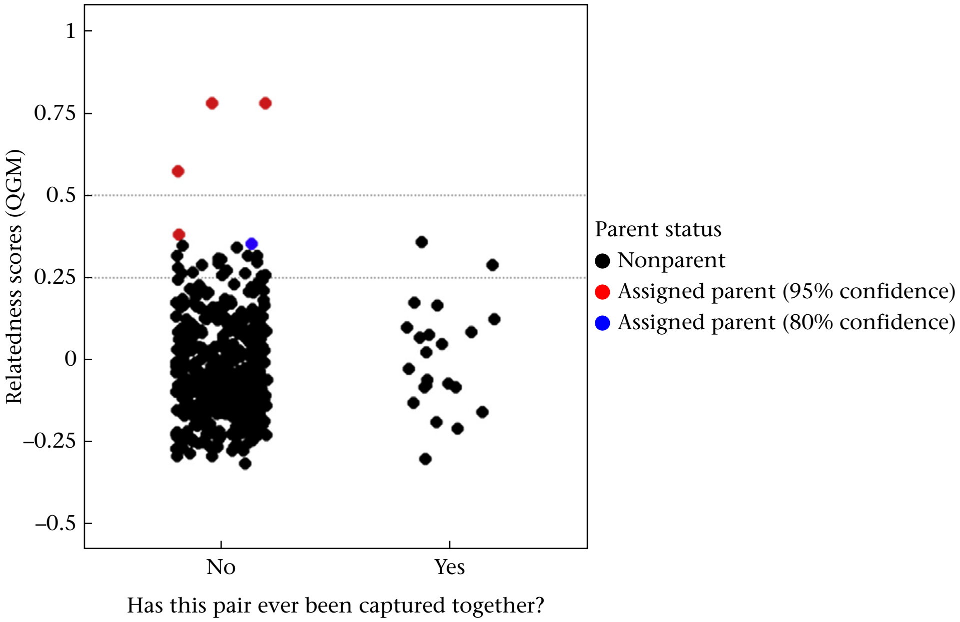

Figure 4. A distribution of Queller—Goodnight (QGM) scores showing the relatedness of 478 potential juvenile—adult pairings sampled in 2022 and 2023 with regard to whether that pair was captured together at least once. A QGM score of 0.5 corresponds to a full sibling or parent—offspring relationship and a score of 0.25 corresponds to a half sibling relationship. Using a likelihood-based parentage analysis, putative parents were assigned at 95% confidence (red) and 80% confidence (blue).

DISCUSSION

In contrast to Hahn and Fleischer's (1995) findings, our analyses did not reveal an influence of relatedness on juvenile—adult associations. However, we urge caution in interpreting this result, as our sample size of juvenile birds was limited. Although few pairwise genetic comparisons in our data set approached the expected thresholds for parent—offspring or full sibling relationships, and our parentage analysis identified putative parents for only two of seven juveniles at the 95% confidence level, these patterns may reflect limitations in sampling rather than definitive evidence against the role of genetic relatedness. It is also possible that the microsatellite markers we used to measure relatedness do not have a fine enough resolution to distinguish between key relationships accurately. We consider this unlikely, however, due to the successful use of the same suite of microsatellite loci in other published studies (Louder et al., 2015, 2019). The high degrees of polymorphism and heterozygosity in our sampled population along with our parentage analyses' extremely low nonexclusion probabilities provide further support for the discriminatory power of our markers and the robustness of our parental assignments. Our juveniles' parents may have also been present in the population but never sampled. New-to-our-trap adult cowbirds were captured and individuals without colour bands were visually observed up until the very last week of trapping for all summers, confirming the notion that we were unable to trap the entire adult population and that our juveniles' parents were potentially unsampled. Lastly, because few of our juvenile—adult co-captures resulted in relatedness values ≥0.5 and only two of our juveniles were assigned at least one putative parent, it is possible that most if not all of our juveniles were produced elsewhere and dispersed into our study location independently of their parents. With our current data, we are unable to definitively differentiate among these possibilities, and we therefore emphasize the need for additional studies to clarify the potential role of relatedness in shaping early social interactions in brown-headed cowbirds.

However, unlike the unsupported influence of relatedness in our adult—juvenile interactions, we did find strong evidence that juvenile cowbirds are more likely to be captured with adult females than with adult males. This higher likelihood is despite a skewed male sex ratio in our captured adult population. Our findings are in line with what we would expect based on the plumage similarity between juvenile and female adult cowbirds and given that Hauber et al. (2000) showed that naïve, laboratory-raised juvenile cowbirds use self-referent phenotype matching to preferentially associate with adult birds that visually look more like themselves.

Additionally, Hauber et al. (2001) and Hauber (2002) showed through laboratory trials and field observations that juvenile cowbirds have an innate attraction towards the cowbird's own chatter call (a primarily adult female vocalization; Burnell & Rothstein, 1994), despite a lack of previous acoustic experience. Early exposure to conspecific chatter calls has also been shown to initiate and facilitate the acquisition of species-specific behaviours in brown-headed cowbirds as well as the shiny cowbird, *Molothrus bonariensis* (Hauber et al., 2001; Louder et al., 2019; Crudele et al., 2023), further reinforcing the specific importance of early interactions with adult females, related or not, relative to interactions with males during this stage of the juvenile's development.

Freeberg's (2004) work has shown that juveniles require interactions with both adult males and adult females and that this interaction is crucial for their successful development of species-specific courtship behaviours and mating preferences. As such, it is possible that our sampling was performed too early in the juveniles' developmental process, considering that further sex-based associative learning preferences may only be present later in the social ontogeny when the juvenile's relevant sexual, vocal and reproductive behaviours are being developed. Moreover, given that our observed juvenile preferences towards adult female interactions were driven in part by self-referent phenotype matching, it may be that juvenile males lose this associative preference, or rather redirect it towards adult males, later on in their development after undergoing their first postjuvenile moult. Taken

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together, these findings underscore the complexity of juvenile–adult associations in cowbirds, suggesting that early social preferences may be shaped more visibly by behavioural and phenotypic cues than by genetic relatedness, although further research is needed to clarify the potential influence of kinship.

In conclusion, our study characterizes patterns of the intricate dynamics of adult–juvenile interactions in brood-parasitic birds. Despite previous conclusions that species-specific behaviours in obligate brood parasites are primarily innate, our findings are consistent with a more nuanced socio-ontogenetic process that emphasizes early juvenile–adult interactions, whereby adult females and juveniles seem to have a vested interest in socializing together more so than do males and that such intergenerational interactions do not need to be limited to closely related individuals. The findings from our study challenge previous assumptions and underscore the importance of further research into the ontogenetic mechanisms subserving social interactions and their benefits in obligate brood parasites. We also acknowledge potential limitations of our study, particularly the small sample size of juvenile birds caught across the 3 years of study. Further research, with larger sample sizes, across different cowbird populations and throughout different ontogenetic stages, is needed to replicate and expand upon our findings.

Author Contributions

Mac L. Chamberlain: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Wendy Schelsky**: Writing – review & editing, Supervision, Conceptualization. **Autumn Bush**: Writing – review & editing, Data curation. **Shanelle Johnson-Cadle**: Writing – review & editing, Data curation. **Shelby Lawson**: Writing – review & editing, Data curation. **Mark E. Hauber**: Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Data Availability

The data set for this study is available on Figshare at https://doi.org/10.6084/m9.figshare.28087466.v1.

Declaration of Interest

None.

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