

## ORIGINAL ARTICLE

# Relatedness constrains virulence in an obligate avian brood parasite

James W. RIVERS<sup>1,#,\*</sup> and Brian D. PEER<sup>2</sup>

<sup>1</sup> Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, California 93106, USA

<sup>2</sup> Department of Biological Sciences, Western Illinois University, Macomb, Illinois 61455, USA

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**Abstract** Virulence, the amount of harm a parasite inflicts on its host, is integral to elucidating the evolution of obligate avian brood parasitism. However, we lack information regarding how relatedness is linked to changes in behavior and the degree of harm that brood parasites cause to their hosts (i.e., virulence). The kin competition hypothesis combines theory from offspring signaling and parasite virulence models and states that the begging intensity of co-infecting parasites is driven by their relatedness, with concomitant changes in the degree of virulence expressed by parasitic young. We tested this hypothesis using the Brown-headed Cowbird *Molothrus ater*, an obligate brood parasitic bird whose virulence at the nestling stage is mediated by vigorous begging displays that are used to outcompete host young during feeding bouts. We found support for both predictions of the kin competition hypothesis: first, the begging intensity of cowbirds was greater in a population where cowbirds typically competed against unrelated host nestmates, relative to a population where they often competed against kin. Second, the greater intensity of begging in cowbirds was positively associated with decreased growth in host offspring during the developmental period. Given the dearth of studies on virulence in avian brood parasites, our results notably extend our understanding of how relatedness is linked to parasite behavior and virulence, and they highlight how spatially-isolated host populations can harbor different levels of virulence that are driven by competitive interactions between co-infecting parasites.

**Key words** Begging, Brown-headed cowbird, Kin competition hypothesis, *Molothrus ater*, Virulence

Obligate brood parasites are rare among birds, comprising approximately 1% of all named avian species (Davies 2000). Members of this group lay their eggs into the nests of heterospecific hosts and, after hatching, their parasitic offspring actively usurp parental care from their host parents (Rothstein 1990; Davies 2000). The harm to hosts caused by avian brood parasites, also known as virulence, occurs either through specialized behaviors of parasitic nestlings or through removal of host offspring (typically eggs) by parasitic adults. The degree of virulence is usually greater from the actions of parasitic nestlings than adults (Kilner 2005; Peer et al. 2013), and parasitic young can be placed into one of two distinct groups

(Kilner 2005). In the first group, termed nestmate killers, young parasites direct aggression toward nestmates and kill them by physically evicting them from the nest (Davies 2000) or by stabbing them with specialized bill structures (Friedmann 1955; Morton & Farabaugh 1979; Spottiswoode & Koorevaar 2012). In both cases, the result is the elimination of potential competitors in the nest, allowing nestmate killers to obtain all food provisioned by host parents. Such specialized killing behaviors do not exhibit variation in their expression except under exceptional circumstances (e.g. Rutila et al. 2002), and they are limited to older parasitic lineages that have experienced long coevolutionary histories with their hosts (Sorenson & Payne 2002). In the second group, termed non-evictors, parasitic offspring do not display aggression towards nestmates. Instead they exploit host provisioning rules by using vigorous begging displays that

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# Corresponding author, E-mail: jim.rivers@oregonstate.edu

\* Present address: Department of Forest Ecosystems and Society, Oregon State University, Corvallis, Oregon 97331, USA

divert food resources away from host young, resulting in a disproportionately large food share going to the parasitic nestling (Dearborn 1998; Lichtenstein & Sealy 1998; Kilner et al. 2004; Rivers 2007). Thus, virulence in non-evictors is mediated entirely through their begging displays (Kilner 2005; Peer et al. 2013) via factors that are linked to offspring begging intensity, including the relatedness between a parasite and its nestmate competitor(s) (Bremermann & Pickering 1983; Kilner 2005; Buckling & Brockhurst 2008; Alizon et al. 2009).

Although non-evictors typically compete against unrelated host young, in some areas it is common for parasitic nestlings to compete against conspecifics for food in host nests (Kattan 1997; Martinez et al. 1998; Rivers et al. 2010). The kin competition hypothesis combines theory from offspring signaling (Godfray 1991, 1995; Johnstone & Godfray 2002) and parasite virulence models (Anderson & May 1982; Bremermann & Pickering 1983; Frank 1996; Kilner 2005; Buckling & Brockhurst 2008; Alizon et al. 2009) and states that the begging intensity of parasitic nestlings that co-infect a host nest is influenced by the degree to which they are related, with downstream effects on the expression of parasite virulence. This hypothesis makes two predictions. First, as relatedness increases between parasitic nestmates, their begging displays become reduced in intensity because of inclusive fitness costs (Bremermann & Pickering 1983; Godfray 1991, 1995; Frank 1996; Johnstone & Godfray 2002; Buckling & Brockhurst 2008). Second, a decrease in parasite begging intensity leads to a concomitant decrease in per-capita virulence, which can be detected as diminished growth and survival in host young (Dearborn 1998; Lichtenstein & Sealy 1998; Hauber 2003; Kilner et al. 2004). Careful evaluation of the links between kin selection, parasite behavior, and virulence is fundamental to elucidating the evolutionary relationships between hosts and parasites (Anderson & May 1982; Combes 2001). However, no study has been undertaken in spatially isolated parasite populations to test the kin competition hypothesis, limiting our understanding of how begging behavior and virulence of avian brood parasites change across spatial scales and due to variation in local environmental conditions.

Parasitism by the Brown-headed Cowbird *Molothrus ater* (hereafter cowbird) in the mid-continental region of the United States is temporally consistent over long temporal scales (i.e., >5 years; Jensen & Cully 2005; Rivers et al. 2010), yet it is highly vari-

able over small spatial scales (<70 km; Chace et al. 2005; Jensen & Cully 2005). This results in a mosaic of cowbird populations that consistently differ in the degree to which their hosts experience multiple parasitism. In cowbird populations where multiple parasitism is common, individual females often re-lay in host nests (Alderson et al. 1999; Hahn et al. 1999; McLaren et al. 2003; Ellison et al. 2006; Rivers et al. 2012), which can result in high levels of within-brood relatedness and a large proportion of parasitic young that compete against kin for food (Rivers et al. 2012).

In this study, we tested the kin competition hypothesis by comparing the intensity of cowbird begging displays and virulence expression between a population in which cowbirds typically compete against non-kin for food and a second population in which cowbirds frequently compete against kin. The cowbird is an ideal species for testing the kin competition hypothesis because it is a non-evictor (Peer et al. 2013) and its virulence is mediated entirely through begging displays that divert food away from hosts and to cowbirds, indirectly causing harm to host offspring (Kilner et al. 2004; Rivers 2007). Our previous work found that multiple parasitism was common in a high-density cowbird population (Rivers et al. 2010), and in this population many cowbirds were raised alongside close kin (i.e., full siblings; Rivers et al. 2012). Thus, we predicted that four distinct components of the cowbird begging display (i.e., latency to beg, begging score, call rate, and call amplitude) would be more intense in the population where cowbirds were raised without kin, relative to the population where cowbirds were often reared with close relatives. We also predicted that an increase in cowbird begging intensity resulted in increased virulence to host young as measured by a reduction in body size, which has been linked to survival during the post-fledging period (Magrath 1991; Verboven & Visser 1998; Naef-Daenzer et al. 2001). We tested the kin competition hypothesis through controlled experiments that tested cowbirds across a gradient of short-term need to ensure that the degree of hunger was standardized when evaluating begging displays (Rivers et al. 2013). Finally, we evaluated whether the two populations differed in two costs that are thought to constrain the expression of offspring begging displays (Chappell & Bachman 2002; Haskell 2002; Johnstone & Godfray 2002): nestling body size, a proxy for growth costs (Chappell & Bachman 2002; Clark 2002), and nest predation rate

of the preferred cowbird host, a proxy for predation costs (Haskell 2002). Our study represents the first empirical test of how relatedness is linked to virulence and mediated by a behavioral mechanism in an obligate avian brood parasite, in turn providing information that is currently missing from our understanding of how virulence evolves in nature.

## MATERIALS AND METHODS

### 1) Study locations and species

Our experiments were conducted over several years from 15 May to 31 July on cowbird populations breeding at the Konza Prairie Biological Station in northeastern Kansas, USA (2004–2007; hereafter, high-relatedness population) and at the Freeman Energy reclaimed mine site in western Illinois, USA (2007–2008; hereafter, low-relatedness population). Cowbird parasitism has been stable for >40 years and >10 years in the high- and low-relatedness populations, respectively (Zimmerman 1983; Rivers et al. 2010; Brian D. Peer unpublished data). These populations are distinct because cowbirds do not move >25 km during the breeding season (Curson et al. 2000) and our study areas were separated by approximately 520 km. We were restricted to these two populations for this study because they were the only sites available to us that fulfilled the following criteria: (1) pre-existing data were available on the extent of cowbird parasitism in the host community, (2) sites consisted of prairie grasslands and contained a cowbird host community that was typical of this habitat, (3) the Dickcissel *Spiza americana* was the numerically dominant and preferred cowbird host in the local community, and (4) the populations differed in the extent to which cowbird nestlings competed against kin for food in host nests. Regarding the last criterion, our previous work in the high-relatedness population found that when a cowbird competed against a conspecific in a host nest, which occurred for >75% of cowbirds (i.e. 2030 of 2661 cowbird offspring from N=2612 total nests; Rivers et al. 2012), there was a 40.4% (95% CI: 28.4–52.4%) likelihood that the two competing cowbirds were full siblings (Rivers et al. 2012). Genetic data were unavailable from the low-relatedness population; however, even if all cowbirds observed in each multiply-parasitized nest from this population were assumed to be full siblings, the likelihood that two competing cowbirds were full siblings would still be significantly lower than in the high-relatedness population (21.9%, 95%

CI: 17.4–26.4%; James W. Rivers & Brian D. Peer unpublished data). We note that this assumption is unlikely given the cowbird's promiscuous mating system in our study area, so our estimate of the likelihood that two co-infecting cowbirds are full siblings in the low-relatedness population is highly conservative (Elliott 1980; Strausberger & Ashley 2003) and is likely to be even lower in reality. Mean parasitism rates in the high-relatedness population (55.7%, N=2612 nests; Rivers et al. 2010) was markedly higher than the mean parasitism rate in the low-relatedness population (19.4%; N=124 nests; James W. Rivers & Brian D. Peer unpublished data). Additionally, the proportion of cowbirds sharing the nest with at least one other cowbird was significantly greater in the high-relatedness population (76.3%, N=2612 total nests; Rivers et al. 2012) relative to the low-relatedness population (33.0%, N=124 total nests;  $X^2=136.2$ ,  $P<0.001$ ). Therefore, the two populations differed significantly in the propensity that an individual cowbird shared its nest with another cowbird, and the likelihood that multiple cowbirds that shared a nest were closely related.

### 2) Creation of experimental broods

We searched for nests of breeding passerines using a combination of systematic searches and behavioral observations. Nest predation rates were high in our study, so we moved most cowbird eggs to egg incubators to maximize the number of nestlings available for experiments. Our initial analysis found no difference between the begging of nestlings that hatched in an incubator and those that hatched from nests in the field (James W. Rivers unpublished data), so we combined young from both groups in all analyses. On the morning of hatching, we manipulated nests so that they contained one cowbird and two Dickcissel nestlings, with all nestlings hatching on the same day to standardize age. We completed all manipulations of nest contents by noon local time to minimize age differences among nestlings within a nest. At the same time when broods were created, we measured each nestling (right tarsus length and body mass) to assess growth; however, logistical constraints restricted our measurement of hatchlings to N=5 broods in the high-relatedness population and N=9 broods in the low-relatedness population. When creating experimental broods, we only used a single cowbird from multiply-parasitized nests to minimize possible relatedness confounds (Rivers et al. 2012); we fostered additional nest contents to other nests or collected

under permit. We selected a brood of three nestlings because it is a common brood size for parasitized Dickcissel nests in the high-relatedness population (Zimmerman 1983; Rivers et al. 2003). Because there is no procedure that allows for accurate, rapid assessment of the gender of hatchling passerine nestlings under field conditions, we were unable to control for nestling sex and instead assumed a 50:50 sex ratio for nestlings used in this study.

### 3) Experimental procedures

On the afternoon of nestling day five (where day zero was the day of hatching), we brought all nestlings from each experimental brood into a laboratory to assay begging behaviors under standardized, climate-controlled conditions; an additional nestling from a non-experimental nest was swapped into each focal nest to guard against parental abandonment. Thirty minutes after being removed from the nest, we re-measured each nestling (right tarsus length and body mass) and then fed it up to eight mealworms to standardize hunger level. We marked Dickcissels on the bill with a non-toxic marker for individual identification during trials, and then placed all nestlings into an artificial nest within an open pine box whose sides prevented nestlings from seeing the experimenter. After 10 min, and every 10 min thereafter for a 90 min period, we stimulated nestlings to beg by tapping gently on the side of the nest to mimic the arrival of a parent with food; all nestlings begged readily to this stimulus during pilot trials. We did not feed nestlings during trials to assess how begging intensity changed relative to short-term need (see Clark 2002). We began recording behaviors immediately after nestlings were placed in the artificial nest via a miniature videocamera attached to a camcorder; we recorded begging calls using the program Syrinx via a lapel microphone placed ~10 cm above the nest that was attached to a laptop computer. After each trial was completed, we fed all nestlings to satiation with mealworms and then returned them to their nests. Our additional work with cowbirds indicates that laboratory- and field-based behavioral assays provide similar results (Rivers et al. 2014), so we assumed that our findings from laboratory trials accurately reflect behaviors expressed under natural conditions and can therefore be linked directly to parasite virulence.

### 4) Quantification of begging behavior and nestling body condition

We only considered begging that occurred in the 15 s following each stimulation during the 90 min trials (i.e., nine stimulations/trial) because most food items are provisioned within this period by cowbird hosts (James W. Rivers unpublished data). We defined latency to beg as the time elapsed from the onset of tapping on the nest to the start of begging. To calculate a begging score, we first assigned a begging posture rank on a scale of 0–3 for each 1 s of the 15 s period when begging was quantified, where 0=not begging, 1=gaping without neck stretched, 2=gaping with neck stretched to three-fourths of its length and belly in contact with nest cup, and 3=gaping with neck stretched more than three-fourths of its length and belly not in contact with nest cup (see Rivers 2007). We then summed the 15 begging posture ranks to create a begging score that could range from 0–45 (Rivers et al. 2013). We restricted our comparison of call rate to the last stimulation of the trial because begging calls were not typically given until the latter part of the trials. One of us (JWR) quantified begging behaviors from videotapes and audio recordings taken at both sites to eliminate possible inter-observer biases. At the end of each trial, we measured the maximum begging call amplitude (dB) of the cowbird by removing both Dickcissels from the nest, placing a hand-held sound meter 30 cm away from the cowbird nestling still in the nest, and stimulating it to beg. We used different sound meters in each population, but they provided the same results when tested together over a range of frequencies and amplitudes (James W. Rivers & Brian D. Peer unpublished data).

Nestling begging can be influenced by body condition (Clark 2002), so we used morphological data to assess whether cowbirds in our experimental broods differed from those raised in reference broods. We did not have data for reference broods from our study populations so instead we calculated the mean body mass on nestling day five (tarsus data were unavailable) from the 18 cowbird hosts listed in Kilpatrick (2002) for comparison with cowbirds raised in experimental broods. We adopted this approach because cowbirds are generalist brood parasites that are raised by a diversity of host species under a wide range of environmental conditions; therefore, they lack a single reference host brood against which our experimental nestlings could be compared (see Rivers et al. 2013).

## 5) Statistical analysis

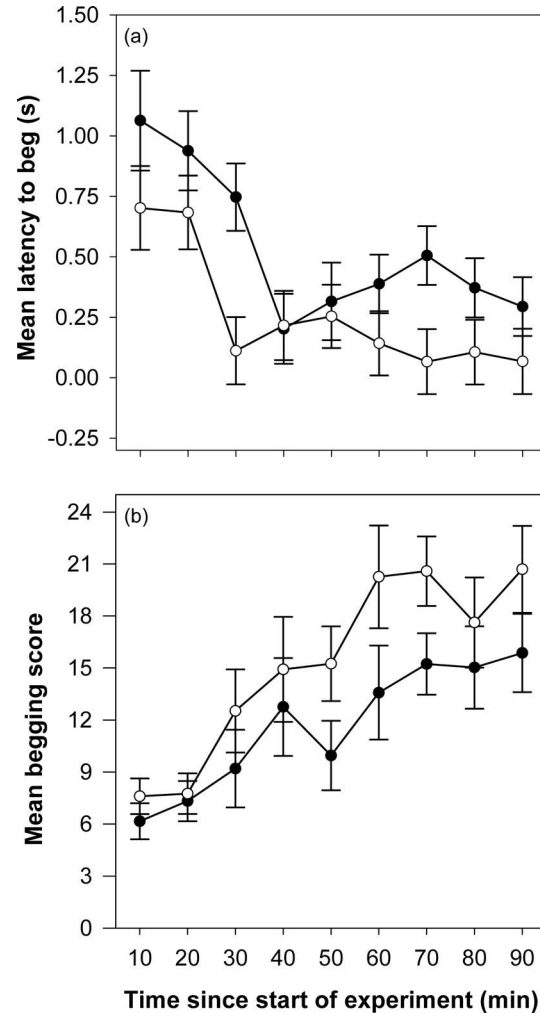
We used the PROC MIXED modeling function in SAS v.9.2 to assess latency to beg and begging score because they represented repeated measures during trials, and we used the correlation structure that generated the lowest AICc value among candidate correlation structures. We constructed models that included population as a fixed effect (two levels: high-relatedness, low-relatedness), stimulation as the repeated measures factor (nine levels: 1...9), and nest as a random effect. Begging can be influenced by nest-mate behaviors, so we initially summed the begging scores of both Dickcissel nestmates for each stimulation within each trial for use as a covariate. However, we found no significant population\*covariate interaction in our initial analysis so this term was subsequently dropped. We used *t*-tests to compare single measures of begging responses and morphological measurements between the two populations, and to compare mass in experimental broods to reference values from the literature. We report least squares marginal means (from repeated measures analysis) or arithmetic means (from *t*-tests) and their associated standard errors (SEs). Finally, we report parameter estimates ( $\beta$ ) from the full model (i.e., adjusted for covariates) and their associated 95% confidence intervals (CIs); we set the alpha level for all tests at  $P < 0.05$ .

## RESULTS

We created a total of 28 experimental cowbird broods in the high-relatedness population, and 20 experimental broods in the low-relatedness population. However, nearly half (22/48=46%) of those nests were lost due to predation before experiments could be conducted. Thus, our sample size was limited to 14 broods in the high-relatedness population and 12 in the low-relatedness population.

### 1) Begging display intensity

We found that latency to beg was significantly shorter for cowbirds in the low-relatedness population ( $\beta = -0.28$  [95% CI:  $-0.45, -0.10$ ],  $F_{1,23.5} = 10.15$ ,  $P = 0.004$ ; stimulation effect:  $\beta = -0.07$  [95% CI:  $-0.40, 0.26$ ],  $F_{8,160.0} = 4.69$ ,  $P < 0.001$ ; site\*stimulation interaction:  $\beta = -0.35$  [95% CI:  $-0.72, 0.03$ ],  $F_{8,156.0} = 1.03$ ,  $P = 0.416$ ; Fig. 1A). In addition, cowbirds in the low-relatedness population had a greater and marginally significant begging score than those in the high-relatedness population ( $\beta = 3.57$  [95% CI:  $-0.31, 7.44$ ],



**Fig. 1.** (a) Mean ( $\pm$ SE) latency to beg and (b) begging score of brown-headed cowbirds from a high-relatedness population (filled circles) and a low-relatedness population (open circles) when tested under laboratory conditions.

$F_{1,25.4} = 3.59$ ,  $P = 0.070$ ; stimulation effect:  $\beta = 0.66$  [95% CI:  $-1.00, 2.31$ ],  $F_{8,85.6} = 7.98$ ,  $P < 0.001$ ; site\*stimulation interaction:  $\beta = 4.22$  [95% CI:  $0.02, 8.43$ ],  $F_{8,79.2} = 0.82$ ,  $P = 0.591$ ; Fig 1B). We found that call rate ( $16.0 \pm 2.86$  calls/15 s) and maximum call amplitude ( $64.6 \pm 0.68$  dB) were significantly greater in the low-relatedness population as well (rate:  $6.9 \pm 2.40$  calls/15 s,  $t_{1,24} = -2.4$ ,  $P = 0.022$ , Fig. 2a; amplitude:  $57.5 \pm 1.95$  dB,  $t_{1,20} = -3.4$ ,  $P = 0.003$ , Fig. 2b). We detected no difference in Dickcissel begging score between the two populations ( $\beta = -3.96$  [95% CI:  $-12.01, 4.10$ ],  $F_{1,28.7} = 1.01$ ,  $P = 0.323$ ); this measure was consistent during the course of experimental trials (population\*stimulation interaction:  $\beta = -5.25$  [95% CI:  $-13.53, 3.04$ ],  $F_{8,46.8} = 0.43$ ,  $P = 0.897$ ), and

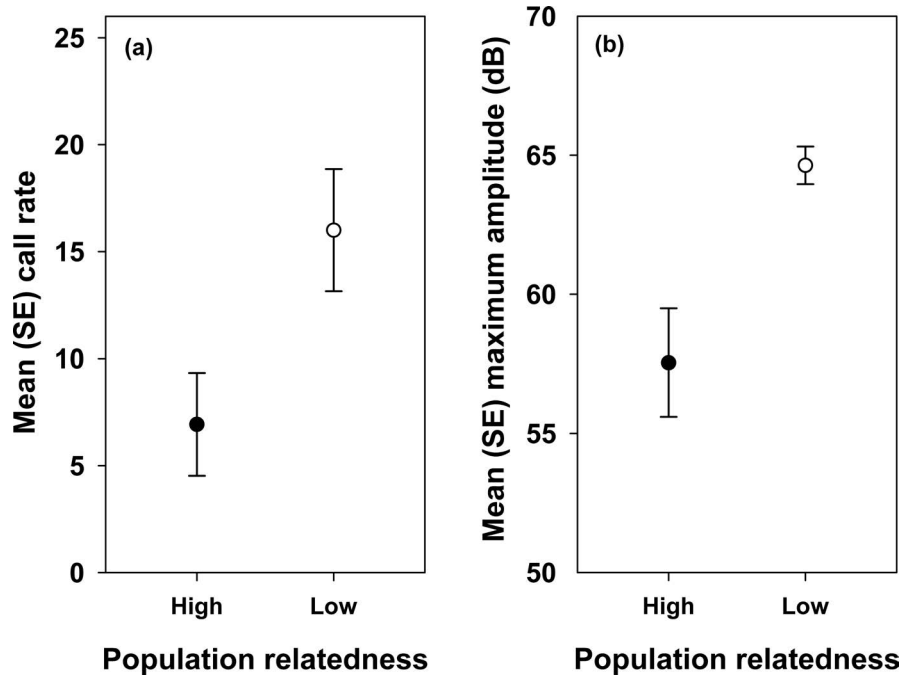


Fig. 2. (a) Mean ( $\pm$ SE) begging call rate (calls per 15 s) (b) maximum begging call amplitude (dB) of brown-headed cowbirds from a high-relatedness population (filled circles) and a low-relatedness population (open circles) when tested after 90 min of food deprivation.

was lower than cowbird begging scores in both populations (data not shown).

## 2) Parasite virulence

We detected no population-level differences in Dickcissel nestlings for either body mass (high-relatedness:  $2.7 \pm 0.14$  g, low-relatedness:  $2.6 \pm 0.15$  g;  $t_{1,11} = 0.56$ ,  $P = 0.587$ ) or right tarsus length (high-relatedness:  $7.3 \pm 0.21$  mm, low-relatedness:  $7.5 \pm 0.17$  mm;  $t_{1,11} = 0.72$ ,  $P = 0.486$ ) when measured on the morning of hatching. When we re-measured nestlings on nestling day five, we detected no population-level difference in right tarsus length (high-relatedness:  $20.4 \pm 0.16$  mm, low-relatedness:  $20.0 \pm 0.23$  mm;  $t_{1,24} = 1.42$ ,  $P = 0.168$ ). However, Dickcissel body mass in the low-relatedness population ( $13.7 \pm 0.37$  g) was significantly lower than Dickcissel nestlings in the high-relatedness population ( $15.2 \pm 0.30$  g;  $t_{1,24} = 3.23$ ,  $P = 0.004$ ; Fig. 3), a difference of  $> 10\%$ .

## 3) Population-specific mediators of begging intensity

We detected no difference between the high-relatedness ( $18.7 \pm 0.39$  g) and low-relatedness populations for cowbird body mass ( $18.7 \pm 0.54$  g,  $t_{1,24} = -0.07$ ,  $P = 0.948$ ), nor did we detect any differ-

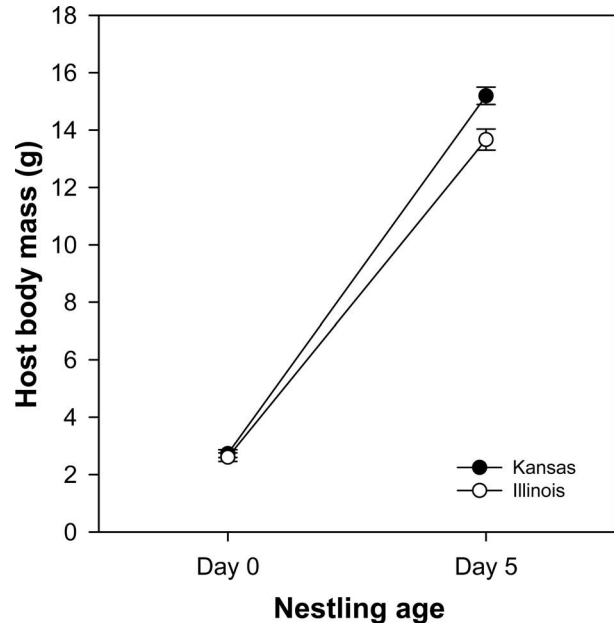


Fig. 3. Mean ( $\pm$ SE) body mass of Dickcissel nestmates that competed against brown-headed cowbirds in high-relatedness (filled circles) and low-relatedness populations (open circles) when measured at the time broods were created (nestling day 0;  $N = 5$  high-relatedness and  $N = 9$  low-relatedness broods) and immediately prior to begging trials (nestling day 5;  $N = 14$  high-relatedness and  $N = 12$  low-relatedness broods).

ence between the populations with respect to cowbird right tarsus length (high-relatedness:  $19.9 \pm 0.36$  mm, low-relatedness:  $20.5 \pm 0.35$  mm;  $t_{1,24} = -1.27$ ,  $P = 0.216$ ). The body mass of cowbirds in each of our study populations was significantly greater than cowbird body mass averaged from published values ( $15.0 \pm 0.57$  g;  $P < 0.001$  for both comparisons). We also detected no difference in nest predation rates for the Dickcissel between the two populations: 68% of 125 nests were depredated in the high-relatedness population and 61% of 132 nests were depredated in the low-relatedness population ( $X^2 = 1.53$ ,  $P = 0.217$ ).

## DISCUSSION

We found that both predictions of the kin competition hypothesis were supported in our study. First, all four measures of begging we considered (i.e., latency to beg, begging score, call rate, and call amplitude) increased in intensity over the course of experimental trials in both populations; however, cowbird begging was significantly more intense in the low-relatedness population than in the high-relatedness population. Second, divergent begging displays in the two populations led to variation in the virulence experienced by host offspring during the developmental period: Dickcissels that competed against more intensively begging cowbirds in the low-relatedness population experienced significantly greater mass loss than those in the high-relatedness population. Importantly, host young from the two populations did not differ in size when broods were created on the morning of hatching, yet Dickcissels in the low-relatedness population were  $> 10\%$  smaller when re-measured five days later, indicating an effect that arose during the nestling stage. Nestling body size can have a strong, positive effect on post-fledging survival (Magrath 1991; Verboven & Visser 1998; Naef-Daenzer et al. 2001), so the decrease we observed may have had important fitness consequences for Dickcissel young after leaving the nest. Taken together, this provides strong evidence that greater cowbird begging intensity observed in the low-relatedness population resulted in greater virulence being inflicted on host young as predicted by the kin competition hypothesis.

The divergence in cowbird begging displays we observed could also result from differences in population-specific conditions such as growth or predation costs (Godfray 1991, 1995; Haskell 2002; Johnstone & Godfray 2002). However, cowbirds in both populations were similar in size and  $\sim 25\%$  heavier than

age-specific values that were averaged across a range of hosts (Kilpatrick 2002), suggesting that cowbird growth was not constrained in either population. Nest predation rates of the Dickcissel, a preferred cowbird host (Rivers et al. 2010), was similar in the two populations, suggesting that selection on begging displays from nest predators was similar in both populations. That we did not detect any differences in proxies for growth or predation costs, combined with the marked divergence in mean relatedness levels of cowbird nestmates in the two populations (Rivers et al. 2012; see above), strongly implicates inclusive fitness costs as the factor that drove observed differences in begging display intensity. In turn, this provides strong support for the difference in virulence levels experienced by hosts being linked to changes in a behavioral mechanism (i.e., begging display) that was brought about by kin selection. Nevertheless, logistical constraints prevented us from testing whether some unknown factor (e.g., food resources) may have diverged between the sites and contributed to the variation in begging displays that we observed. Thus, further study of this topic in additional populations will be helpful in working through the finer details of how local environmental conditions might also mediate parasite begging and resultant virulence.

An important question that emerges from our study is how a reduction in begging display intensity of cowbirds in the high-relatedness population could be maintained by natural selection given that the costs of reduced cowbird begging in the absence of kin could be potentially high. However, a closer examination of our study system illustrates several factors that contribute to the maintenance of reduced begging in the high-relatedness population. The following are of note: (1) cowbird eggs typically hatch prior to host eggs (Briskie & Sealy 1990), (2) cowbirds usually compete against physically smaller hosts (Ortega 1998; Rivers et al. 2010), and (3) host parents bias their feeding to the largest nestling in their brood, which is typically a cowbird in parasitized nests (Dearborn 1998; Lichtenstein & Sealy 1998; Rivers 2007). Moreover, cowbirds in the high-relatedness population exhibited more intense begging displays than nearly all of their hosts (Rivers 2007; see Rivers et al. 2013 for the lone exception). Taken together, this means that cowbirds in this population maintain a competitive advantage over host offspring during feeding events, allowing them to obtain a disproportionate share of the food parents deliver (Dearborn 1998; Lichtenstein & Sealy 1998; Kilner et al. 2004;

Rivers 2007).

Cowbirds may compete against unrelated cowbirds in host nests under conditions of multiple parasitism, so a reduction in begging intensity could be costly and difficult to maintain under high rates of multiple parasitism. However, most cowbird hosts in the high-relatedness population can rear multiple cowbirds (Ortega 1998; Rivers et al. 2010); indeed, the Dickcissel can raise as many as four cowbirds from a single nest (Temple 2002). As noted above, the greater competitive ability of cowbirds relative to host young results in cowbirds receiving a disproportionate share of food brought to the nest (Dearborn 1998; Lichtenstein & Sealy 1998; Kilner et al. 2004; Rivers 2007). Thus, during conditions of multiple parasitism, host offspring are outcompeted by cowbird nestmates and ultimately incur the cost of the increased competition for food. A single cowbird competing against several unrelated cowbirds could incur costs of reduced begging, but our data indicate that such a situation is rare, as >80% of 1,425 parasitized nests harbored only 1-2 cowbirds (Rivers et al. 2012). Thus, the great majority of cowbirds compete against, at most, only one other cowbird. Taken together, the cowbird's ability to readily outcompete its hosts, the ability of most hosts to raise multiple cowbirds, and parasitism dynamics that typically result in the great majority of cowbirds competing against only one conspecific, help to explain how reduced begging can be maintained in the high-relatedness population. We suspect that the reduced begging displays observed in the high-relatedness population we studied are not unique and are instead likely to be found in other populations where multiple parasitism is common and cowbirds regularly compete against kin.

Our findings complement the few empirical studies that have found that relatedness constrains begging behavior of offspring (Briskie et al. 1994; Boncoraglio & Saino 2008; Boncoraglio et al. 2009). However, our results are especially interesting because they demonstrate that the begging behavior of brood parasites, a group long considered to represent the height of selfishness in altricial birds (Davies 2000), is constrained in the same manner as non-parasitic species during the developmental period. This finding suggests that relatedness among nestmates is a key factor that limits offspring begging displays, even among virulent obligate brood parasites. Moreover, it also demonstrates that a high level of relatedness between competing nestmates alone may be adequate to impose constraints on begging intensity, as cow-

birds are never raised by their genetic parents. It is also worth noting that our results run counter to theoretical models of signaling by parasitic young because such models are based on the assumption that parasites are raised without conspecifics (Harper 1986; Motro 1989; Godfray 1995; Holen et al. 2001). Although this may be the case for many species of brood parasites, there are some species and populations in which multiple parasitism is common (Kattan 1997; Martinez et al. 1998; Schuetz 2005; Rivers et al. 2010; Stevens et al. 2013). Constrained begging displays in avian brood parasites may therefore be more common than previously thought.

Our study provides the first evidence that virulence in an obligate avian brood parasite is linked to the intensity of begging displays, which, in turn, appears to be mediated by kin selection. This finding is important because it supports both theoretical models and empirical studies that have found a reduction in virulence when parasites compete against relatives for critical resources (Bremermann & Pickering 1983; Nowak & May 1994; Frank 1996). In particular, our study is noteworthy because it was conducted in wild populations of a free-ranging vertebrate macroparasite whereas previous studies were focused on microparasites in controlled laboratory settings (e.g., Gardner et al. 2004; Lopez-Villavicencio et al. 2010; Rumbaugh et al. 2012). Despite the differences between our study and previous investigations, a pattern is emerging in which relatedness serves to mediate the degree of virulence expressed across a wide spectrum of parasites. Although obligate avian brood parasites are markedly more complex in their behavior and orders of magnitude larger in size and life expectancy than microparasites (Rothstein 1990; Rothstein & Robinson 1998; Davies 2000), it appears that the virulence of both groups is shaped by environmental factors in similar ways. Thus, our study confirms the strong influence that relatedness can have in shaping begging behavior of a developing parasite and, in turn, the degree of harm it causes to its host.

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