

# Indirect estimates of breeding and natal philopatry in an obligate avian brood parasite

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**Abstract** In theory, obligate brood parasitic birds are freed from several of the temporal and spatial constraints of parental care for dependent young. Yet, similar to parental bird species, adults in several avian brood parasites show a territorial spacing system while breeding, including site fidelity within and across years. Banding-based capture and sighting studies are also suggestive of non-parasite-like lower levels of natal philopatry in avian brood parasites. We analyzed the potential correlation of physical distance with genetic structure of a Brown-headed Cowbird *Molothrus ater* population, sampling parasitic chicks from nests across different temporal and spatial scales of its common host, the Eastern Phoebe *Sayornis phoebe*, near Ithaca, New York, USA. In support of extensive breeding but not historical patterns of natal philopatry, we detected no significant covariation of genetic similarity by distance of nestling parasites at the scale beyond that of individual host nest sites. These results contribute towards a

baseline for future behavioral and genetic comparisons of whether and how parasitic versus parental reproductive strategies impact patterns of avian population genetic structure across space and time.

**Keywords** Breeding range · Brood parasite · Dispersal · Site fidelity · Territoriality

## Zusammenfassung

### Indirekte Bewertung von Brüten und angeborener Ortstreue bei einem obligatorischen Brutparasiten

Theoretisch unterliegen obligate Brutparasiten nicht all‘ den zeitlichen und räumlichen Zwängen von Vögeln, die für ihre Jungen Brutpflege leisten. Dennoch zeigen adulte Tiere einiger Brutparasiten-Arten während der Brutzeit ein territoriales Verhalten, zu dem, ähnlich den brutpflegenden Vogelarten, Ortstreue innerhalb eines Jahres und über mehrere Jahre hinweg gehört. Auch Beobachtungen und Fänge beringter Tiere weisen auf niedrigere Stufen angeborener Ortstreue bei Brutparasiten hin. In unserer Studie analysierten wir für Populationen des Braunkopf-Kuhstärkings (*Molothrus ater*) mögliche Korrelationen von räumlichen Abständen mit genetischer Ähnlichkeit, indem wir in der Nähe von Ithaca, New York, in unterschiedlichen räumlichen und zeitlichen Maßstäben Parasiten-Küken aus Nestern ihres üblichen Wirtsvogels, des Weißbauch-Phoebetryanns (*Sayornis phoebe*), untersuchten. Wir fanden keine signifikante Kovarianz von genetischer Ähnlichkeit mit räumlicher Verteilung. Dieses Ergebnis bildet einen Ausgangspunkt für zukünftige vergleichende verhaltensbiologische und genetische Untersuchungen der Frage, ob und inwieweit parasitische versus brutpflegerische

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Fortpflanzungsstrategien einen Einfluss auf die genetische Struktur von Vogelpopulationen in Zeit und Raum haben.

## Introduction

By laying their eggs in the nests of other birds, avian social parasitic birds are freed from many of the spatial and temporal constraints faced by parental bird species, including nest building and caring for dependent young (Ortega 1998; Hauber and Dearborn 2003). Yet, adult brood parasites in several lineages exhibit breeding site fidelity, or breeding philopatry (Pearce 2007), within and between breeding seasons (Soler et al. 1995; Lyon 1997; Langmore et al. 2007; Fossoy et al. 2011). Breeding philopatry may benefit females or pairs of brood parasites through partially or fully exclusive access to nests of territorial hosts (Hahn et al. 1999; Woolfenden et al. 2001; Hauber 2002; Strausberger and Ashley 2003) and, thus, minimize the cost of begging competition for foster parental care between unrelated parasitic nestmates (Smith and Arcese 1994; Soler and Soler 1999; Hauber et al. 2000; Trine 2000; Hoover 2003). Breeding philopatry by female parasites may also be beneficial by causing naive hosts to misimprint on parasitic eggs and, thus, become acceptors in subsequent breeding attempts (Lotem 1993; Lawes and Marthews 2003; Hauber et al. 2004; Hoover and Hauber 2007; Strausberger and Rothstein 2009).

The spatial aspects of host–parasite interactions also critically impact population dynamics of both brood parasites and their hosts (May and Robinson 1985; Robinson et al. 1995; Strausberger and Ashley 1997; Grim 2002; Roskaft et al. 2002; Hoover et al. 2006). To date, we know from data based on banding, resighting and mark/recapture methods that, in the obligate brood parasitic Brown-headed Cowbird (*Molothrus ater*), adult females and males show strong breeding site fidelity within and between years (range of 22–86% adults recaptured/resighted between years in northeastern North America; Darley 1982; Dufty 1982, 1988; Hauber and Russo 2000). In contrast, regarding direct or indirect information on natal philopatry in brood parasitic birds, there are only a handful of published datasets, with records from Ohio, USA, on Brown-headed Cowbirds reporting no returns of banded young as adults to the study site (0% of  $n = 35$  fledged banded nestlings; Nice 1937) and from British Columbia, Canada, also documenting low levels of local returns (5% of females and none of males of  $n = 127$  banded nestlings of presumed equal sex ratio; Smith and Arcese 1994).

Comparing natal and breeding philopatry with banding data on juvenile (hatch-year) and adult Brown-headed Cowbirds in California, USA, also revealed that, across years, a consistently smaller proportion (6.1%) of locally

banded juveniles returned to the same vocal-dialect region than did adult birds (11.1%) (Anderson et al. 2005). Similar relative patterns regarding greater adult versus less extensive fledgling philopatry and sex-specific survival were also present in long-term data on banding and re-trapping fledgling (females: 7.3%, males: 15%) and adult Brown-headed Cowbirds (40–50% for residents) from Colorado, USA (Ortega and Ortega 2009). These data are suggestive of lower effective levels of natal philopatry than breeding philopatry in this parasitic species, and provide a mechanism to explain the extensive morphological, vocal, and genetic measures of significant gene flow detected and reported between local breeding populations of this cowbird species (Fleischer and Rothstein 1988; Fleischer et al. 1991; O’Loughlen and Rothstein 2002).

These published magnitudes of overall breeding versus natal philopatry estimated for Brown-headed Cowbirds (hereafter: Cowbirds) are similar to those reported for several non-parasitic, migratory parental songbird species that are sympatric with Cowbirds throughout North America (~50% adults vs. 5% nestlings; Weatherhead and Forbes 1995; Winkler et al. 2005; Hoover and Reetz 2006). Hence, alternative reproductive strategies (i.e. parasitism vs. parental breeding) may not be critical in shaping the geographic and population structure of continental songbirds. However, unlike in insular-breeding songbirds where philopatry can be measured directly and definitively (e.g., Richardson et al. 2010), in many landbirds with contiguous breeding habitats, direct measures of natal philopatry pose significant methodological shortcomings by overestimating the mortality of birds dispersing beyond the boundaries of a study site (Weatherhead and Forbes 1995; Winkler et al. 2005). These constraints are compounded for brood parasites, for which studies on fecundity and host use have extensively relied on locating most or all host nests within relative small sites occupied by a handful of adult parasites (Fleischer 1985; Arcese et al. 1996; McLaren et al. 2003; Strausberger and Ashley 2003, 2005; Woolfenden et al. 2003; Ellison et al. 2006), even though, for practical purposes, focusing on a small study site with many host species and nesting territories allows researchers to exhaustively search and sample the reproductive effort of individual territorial parasitic adults (e.g., Smith and Arcese 1994; Strausberger and Ashley 1997, 2003, 2005; Alderson et al. 1999). Nonetheless, local host-nest searching efforts are typically done at a cost of reducing sampling effort at broader geographic scales (Hauber 2000, 2001; Hauber and Russo 2000).

Here, we argue that sampling individuals at sufficient distances to encompass the breeding ranges of several adult parasites is spatially more relevant and statistically more feasible for the analysis of parasite-specific patterns of

natal philopatry, dispersal, and population structure (Winkler et al. 2005; Hoover and Reetz 2006; Schlossberg 2009). Specifically, in our work, we analyzed genetic estimates of relatedness of avian brood parasite chicks across different scales of host-nest distances to study and test whether natal and breeding philopatry by Cowbirds resulted in spatially-scaled genetic population structure. We sampled DNA of parasite chicks from nests of predominantly one host species within a contiguous habitat, but across different spatial scales of inter-nest distances, so as to include parasitism events either by the same or by different female parasites (Gibbs et al. 1997). From these data, we evaluated two hypotheses regarding spatial and temporal patterns of cowbird distribution dynamics. First, we considered whether both natal philopatry and breeding site fidelity are prevalent in Cowbirds. Specifically, we tested the explicit prediction, following the spatial-genetic analyses of Double et al. (2005) and Dutech et al. (2005), that, among nestling Cowbirds, genetic relatedness negatively covaries with increasing geographic distance between host nests. Second, we predicted that extensive breeding, but not natal, philopatry would result in statistical patterns of genetic structure among cowbird nestlings that are limited to the spatial scale of individual host nests within each female parasite's territory (Hahn and Fleischer 1995; McLaren et al. 2003; Strausberger and Ashley 2003, 2005; Hauber et al. 2004).

## Methods

We studied cowbird parasitism in the vicinity of Ithaca (42°26'N, 76°30'W), New York, USA, from 2000 to 2003 (for more details, see Hauber 2001; Hauber et al. 2004). Cowbird chicks were located predominantly in nests of the Eastern Phoebe (*Sayornis phoebe*, hereafter: Phoebe) (89% of  $n = 57$  samples from 45 nests), with additional single Cowbird chick samples obtained from parasitized nests of Song Sparrows (*Melospiza melodia*) ( $n = 3$ ), Dark-eyed Juncos (*Junco hyemalis*) ( $n = 1$ ), and House Finches (*Carpodacus mexicanus*) ( $n = 2$ , cross-fostered as eggs into non-parasitized Phoebe nests so that the chicks survived to fledging; Kilner et al. 2004). Multiple parasitism events in this data set were only detected in Phoebe nests; thus, to avoid the confound of covariation between host species use and parasite genetic structure (Strausberger and Ashley 2005) within our samples, we also repeated our genetic analyses with cowbird chicks sampled from Phoebe nests only (see below). Previous research at this site on Eastern Phoebes indicated significant spatial structure of cowbird parasitism within and across years (Hauber 2001; Hauber et al. 2004). Therefore, our additional analyses included comparisons of chicks sampled from multiply

parasitized broods, using a single randomly chosen sample per nest, and using nests within the same year only.

Suitable breeding habitats of cowbird hosts were visited from the onset of the host and parasite breeding seasons (starting at around 1 May of each study year; Hauber 2001, 2003). When nests were located, we monitored the content and thus the subsequent fate of breeding attempts every 3–4 days. For each nest we recorded its location on a spatial grid to allow calculation of pairwise geographic distances between nest sites. Other than nests located within the same Phoebe territory (distance: 0 km), all other nests were 1–50 km apart, which was deemed far enough to be beyond the dimensions of the breeding range of individual female Cowbirds (~200 m diameter, following Gibbs et al. 1997). This scale of minimal distances between different individual female Cowbirds' territories had also been suggested by other published work from Ithaca (Friedmann 1929), from other upstate New York study sites (Dufty 1982; Hahn et al. 1999), and from other regions within the Cowbird's range (Darley 1983; Rothstein et al. 1986; Raim 2000). Our sampling paradigm, therefore, implies that parasitism events in separate host nest sites were likely owing to eggs laid by different Cowbird females. Some nest contents at our site were manipulated by moving Cowbird eggs or chicks between clutches for a separate set of experiments (Hauber 2003; Kilner et al. 2004), but the genetic identity and the spatial location of chick DNA samples were always assigned to the host nest of original parasitism.

## Microsatellite analyses

Each Cowbird chick at ~5 days was subjected to banding and measurement (not reported here), and blood sample collection (~100  $\mu$ L) through the puncture of the brachial vein. Blood was stored in Longmire's lysis buffer and housed at ambient temperatures or 4°C until DNA extraction. We used the DNEasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) to isolate DNA from all samples following the manufacturer's prescribed protocol. The sex of Cowbird chicks in this sample was not determined. All methods for PCR amplification followed those given in Strausberger and Ashley (2001, 2003, 2005). Specifically, DNA was amplified at seven loci (Table 1). Loci CB.1, CB.12, CB.15 and their primer sequences are described in Longmire et al. (2001),  $Ma\mu$  20 is described in Gibbs et al. (1997) and Alderson et al. (1999), and  $Ma\mu$  25,  $Ma\mu$  29, and  $Dp\mu$  15b are described in Alderson et al. (1999). We then performed PCR for all loci in a total volume of 7.5  $\mu$ L with 20–40 ng DNA, 1 $\times$  PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 0.08 mM of each dNTP, 1.5 mM  $MgCl_2$ , 0.66  $\mu$ g Bovine Serum Albumin, 0.27  $\mu$ M each primer with the following cycling parameters: 94°C for

**Table 1** Characteristics of microsatellite loci used in this study

Locus	<i>n</i>	<i>K</i>	$H_e$	$H_o$	<i>P</i> value for HWE test
CB.1	57	21	0.941	0.877	0.002
CB.12	57	26	0.941	0.895	0.280
CB.15	57	23	0.907	0.930	0.905
Dpμ 15b	49	14	0.896	0.857	0.2663
Maμ 20	52	14	0.889	0.596	<0.0001
Maμ 25	56	29	0.943	1.000	0.808
Maμ 29	57	20	0.907	0.789	0.121

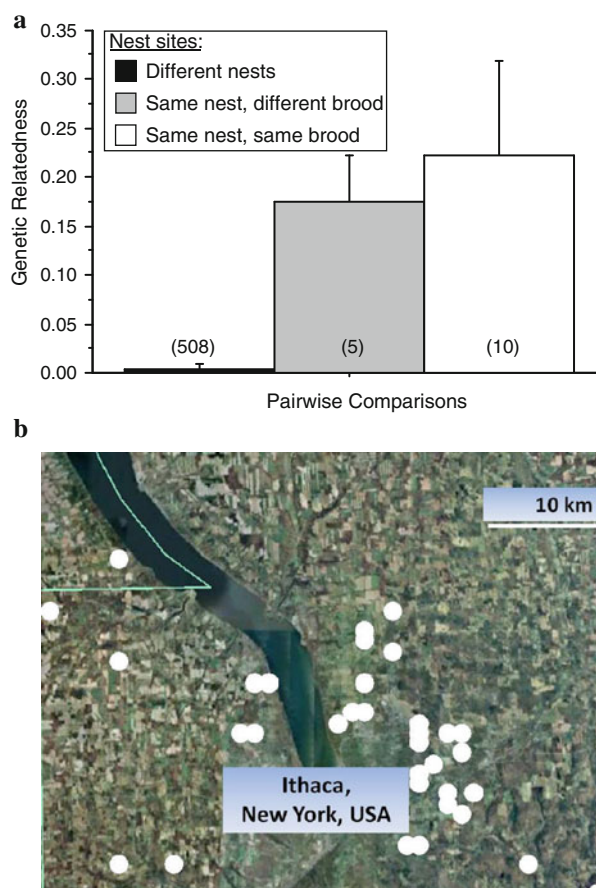
*n* number of individuals scored, *K* number of alleles,  $H_e$  and  $H_o$  expected and observed heterozygosity, respectively, for the Ithaca population of Brown-headed Cowbirds (*Molothrus ater*)

5 min followed by 35 cycles of 94°C for 20 s, 49°C (Dpμ 15, Maμ 25, and Maμ 29) 55°C (CB.1, CB.12, and CB.15) or 60°C (Maμ 20) for 20 s, and 72°C for 1 min. A 5-min extension at 72°C concluded each PCR.

### Statistical analyses

Relatedness values were calculated using Kinship v.1.3 (Goodnight and Queller 1999). The Kinship software calculates Queller and Goodnight's (1989) relatedness statistic ( $r_{XY}$ ), an unbiased estimate of the true relatedness between two individuals X and Y relative to the population (sample) mean. By definition, the average sample (population-level) relatedness in these analyses is set at 0 (Queller and Goodnight's 1989), which means that either positive or negative values of relatedness can be obtained for chick-chick pairwise comparisons. We used all chicks sampled whose DNA was amplified in our analyses because we had no a priori directional predictions about the genetic structure of Cowbirds at the spatial scale utilized in our study. Whether the distribution of relatedness values was statistically different from 0 was first examined between Cowbird chicks within broods, and then it was evaluated for chicks from the same nest site within the same breeding season and across years (Fig. 1a). Only chicks from Eastern Phoebe nests were included for these latter two sets of comparisons, with  $\alpha = 0.05$  set for all analyses.

If Cowbirds showed both natal and breeding philopatry, we would expect to find an inverse relationship between Cowbird chick relatedness and geographic distance between sampled nests (Double et al. 2005; Temple et al. 2006; Ortego et al. 2008; see also Dutech et al. 2005). The relatedness matrix output from Kinship was used for genetic distance data, while a geographic distance matrix was calculated between nest sites from the X and Y coordinates on the spatial grid described above. We began our analyses by conducting 2nd order polynomial regression analyses of pairwise genetic distances against pairwise geographic distances. This is because we sampled Cowbird host nests in a relatively uniform habitat matrix, where Cowbirds can disperse regardless of direction towards suitable breeding and foraging habitats (Hauber 2001;



**Fig. 1** Genetic relatedness of Brown-headed Cowbird (*Molothrus ater*) chicks in Eastern Phoebe (*Sayornis phoebe*) nests across all comparisons of broods from different nest sites, from the same nest sites, and from within the same broods (a) (mean + SE, numbers of comparisons are in parentheses), located around Ithaca, New York (white circles; b)

Fig. 1b). Therefore, the 2nd order distance term represents the area rather than the linear dimension of dispersal opportunities.

However, given the deviation from normal distribution and the non-independence of pairwise-based datasets, to test the potential covariation of genetic and spatial distance more robustly, we continued to explore our data by performing a Mantel test (Mantel 1967) using the ISOLDE program as implemented in Genepop v.1.2 (Raymond and

Rousset 1995), with  $10^4$  permutations to calculate probability values from Spearman rank correlations. We also conducted these analyses separately for each of the 2 years in which we had the largest sample sizes of parasitized Phoebe nests (2000 and 2001, see above).

We followed up these tests with two sets of additional permutational linear models (Mantel 1967; Lapointe and Legendre 1992) to evaluate the significance of the observed non-parametric rank test statistics, using the calculated pairwise relatedness values and geographic distances. We first grouped genetic distance data into bins of pairwise geographic distances of 0, 1–10, 11–20, and >20 km, because these groupings resulted in similar sample sizes. Following an initial one-way analysis of variance, we randomly permuted the data  $10^3$  times in the rows and columns of the dependent (genetic relatedness) matrix variable and compared the values of the 0 km distance bin with those of all other distances. We then repeated this test using only the >0 km distance pair data bins to determine if relatedness values varied across these scales of pairwise geographic distances. This set of tests was conducted in SAS v.8.2.

## Results

### Overall variation in genetic relatedness between Cowbird nestlings

As defined by the formulae of calculating population wide relatedness (Queller and Goodnight 1989), our combined sample of Cowbird chicks from the different host species, nest sites, and years showed an average relatedness value

that was not statistically different from zero ( $t = 1.05$ ,  $P = 0.294$ ,  $n = 57$  samples from 45 nests) (Fig. 1a; Table 2). Specifically, for distances greater than the scale of individual female Cowbirds' territories ( $\geq 1$  km), relatedness values were also statistically indistinguishable from 0 for parasite chicks sampled from different Phoebe nests in the year 2000 (mean  $\pm$  SE:  $0.018 \pm 0.015$ ;  $t = 1.21$ ,  $P = 0.230$ ,  $n = 20$ ) and in 2001 ( $-0.011 \pm 0.007$ ;  $t = 1.57$ ,  $P = 0.116$ ,  $n = 29$ ) (Fig. 2b).

In contrast, despite our small sample sizes, the genetic estimates of pairwise relatedness were significantly positive for Cowbird chicks sampled from either the same Phoebe broods (one-sample  $t = 2.30$ ,  $P = 0.047$ ,  $n = 10$ ) or from the same Phoebe nest site across different breeding attempts ( $t = 2.30$ ,  $P = 0.022$ ,  $n = 5$ ) (Fig. 1). Critically, these results also validate our implicit assumption that our genetic analyses of detectable levels of variability and structure. Accordingly, even when relying on small sample sizes and the limited number of microsatellite loci genotyped in this study, our methods and data generated sufficient power to detect statistically non-random patterns of genetic relatedness at different spatial scales of Cowbird parasitism across hosts' nest sites (Fig. 1a).

### Variation of genetic relatedness by geographic distance

We detected no statistical pattern of linear covariation of pairwise genetic distances of Cowbird nestlings against geographic distances sampled in our study [2nd order polynomial regression with geographic distance:  $R^2 = 0.002$ ,  $P = 0.182$ ; simple regression with geographic distance:

**Table 2** Statistical outputs of the observed metrics for which random permutations of genetic relatedness comparisons were tested

ANOVA table					
	<i>df</i>	Sum of squares	Mean sum of squares	<i>F</i> stat	<i>P</i> value
Model	3	1.87	0.62	21.0	<0.001
Error	1,592	47.3	0.03		
Total	1,595	49.2			
Comparison of least square mean estimates using <i>t</i> statistics (with <i>P</i> values) for each distance bin					
	0 km	1–10 km	11–20 km		
1–10 km	-7.80 (<0.001)				
11–20 km	-7.70 (0.001)	0.286 (0.775)			
>20 km	-7.81 (<0.001)	-0.337 (0.736)	-0.583 (0.560)		
Least square mean estimates of relatedness and 95% confidence intervals (CI)					
Distance bin	LSME relatedness	95% CI lower	95% CI upper		
0 km	<b>0.248</b>	<b>0.187</b>	<b>0.308</b>		
1–10 km	0.0001	-0.014	0.014		
11–20 km	0.003	-0.011	0.017		
>20 km	-0.004	-0.021	0.014		

Relatedness values for only the distance bin of 0 km were positive and higher than for all other bins (shown in bold)

$R^2 = 0.002$ ,  $P = 0.112$ ; simple regression with (geographic distance)<sup>2</sup>:  $R^2 = 0.001$ ,  $P = 0.226$ ].

None of our permutation tests, using two-tailed Spearman rank correlations between genetic relatedness and geographic distance ( $\geq 1$  km), showed a statistically consistent pattern of covariation, either when compared for the totality of our sample ( $n = 57$ ,  $P = 0.415$ ) (Fig. 2a), or analyzed separately for the 2 years with the most Cowbird samples from Phoebe nests (year 2000:  $n = 20$ ,  $P = 0.055$ ; year 2001:  $n = 29$ ,  $P = 0.356$ ) (Fig. 2b).

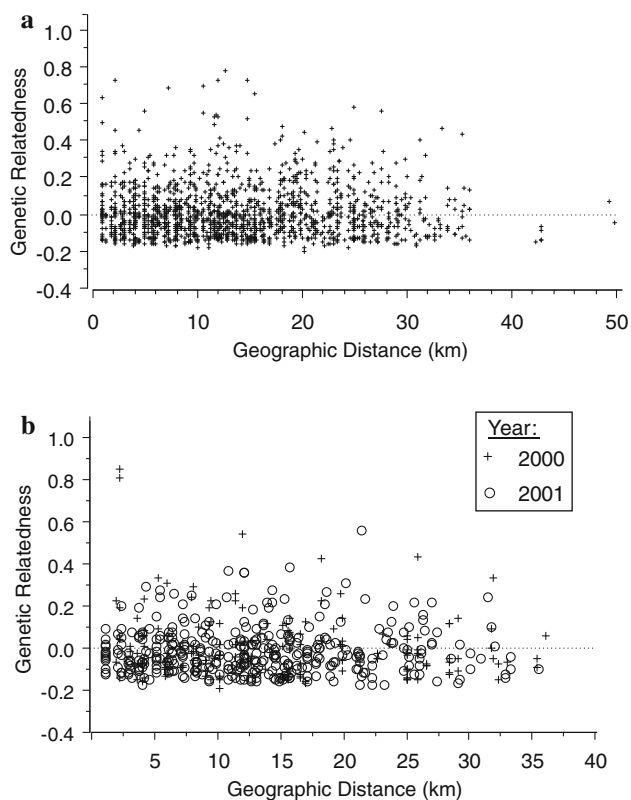
Similar to these findings from the non-parametric tests, we found that pairwise relatedness values were significantly different between geographic distance bins of 0, 1–10, 11–20, and  $>20$  km, as the random permutation tests revealed that the observed  $F$  statistic value fell above the distribution of the expected values ( $P = 0.001$ ) (Table 2). Post hoc analyses, using least square mean estimates and Bonferroni corrections for multiple-comparisons, indicated that relatedness values for only the distance bin of 0 km were positive and higher than for all other bins (Table 2). When restricting these latter analyses to Cowbird chicks from different host nests and nesting sites, pairwise relatedness values were not statistically different between

geographic distance bins of 1–10, 11–20, and  $>20$  km ( $F_{2,1562} = 0.23$ ,  $P = 0.798$ ). Permutation tests of the resampled  $F$  statistics revealed that the observed value fell within the distribution of the expected values ( $P = 0.796$ ). Post hoc analyses indicated that relatedness values were not different between any of this subset of ( $\geq 1$  km) distance bins (all  $P > 0.5$ ).

## Discussion

Social parasitism is a reproductive strategy employed by less than 2% of avian species (Davies 2000). As such, we had hypothesized that brood parasitic birds might be characterized by dispersal behaviors, as indicated by patterns of natal and breeding philopatry, that are dramatically different from other, nesting and parental avian taxa. In contrast, our genetic data are in line with our overview of the handful of previous behavioral studies indicating consistently extensive breeding site fidelity and lower levels of natal philopatry in this generalist avian brood parasite, the Brown-headed Cowbird. Overall, these results also suggest that spatial and temporal dispersal patterns of adult and juvenile parasite may be similar to those of other migratory, non-parasitic, continental species of landbirds (reviewed in Schlossberg 2009). Although we did not analyze Cowbirds' parentage in this study, parasitic Cowbirds also appear to be similar to parental songbird species in apparent social and moderate-to-high levels of genetic monogamy (e.g., Alderson et al. 1999). Divergent patterns of extra-pair paternity, however, would not have affected our predictions based on hypotheses of breeding and natal philopatry in Cowbirds and the resulting spatial structure in genetic relatedness.

Prior trapping and observational data from our Ithaca study site and from research conducted elsewhere in nearby New York State and Ontario, Canada, were also consistent with a territorial adult space-use and breeding philopatry between years by female Brown-headed Cowbirds, where territories are defended in response to intruding female conspecifics (Friedmann 1929; Dufty 1982; Darley 1983; Hahn et al. 1999; Hauber and Russo 2000; Hauber et al. 2001; Hauber 2002). In support of this prior behavioral evidence, despite our small sample sizes and relatively few microsatellite loci analyzed, the molecular analyses here confirmed and revealed that consecutive Cowbird breeding attempts within the same host territories (nests) were genetically more similar than the average population level of relatedness (Fig. 1a). These within host nest/site genetic results are also consistent with previous research that documented higher genetic relatedness between Cowbird chicks sampled from the same nests as well as from the same nest sites between breeding attempts (McLaren et al.



**Fig. 2** Genetic relatedness of Brown-headed Cowbird chicks sampled from different host nests near Ithaca, New York, across all our samples (a) and from Eastern Phoebe nests sampled either in 2000 or in 2001 (b)

2003; Strausberger and Ashley 2003, 2005; Ellison et al. 2006), implying further support for breeding philopatry (Dufty 1982), including predominantly exclusive access of adult Cowbirds to host nests (Hahn et al. 1999) within and between years.

In contrast to detected patterns of breeding philopatry, published and recent behavioral, morphological, and now genetic data on natal philopatry are increasingly in support of moderate-to-low levels of isolation-by-distance relatedness of brood parasitic Brown-headed Cowbirds at broader geographic scales (Fleischer and Rothstein 1988; Fleischer et al. 1991; Anderson et al. 2005; Ortega and Ortega 2009; this study). Importantly, our genetic analyses were based on the type of data that had been previously unavailable in the published literature for Cowbirds regarding the genetic similarity between chicks beyond the scales of either individual host nests and a handful of local adult female parasites' breeding range (Hauber 2001). Nonetheless, we found no consistent pattern of the predicted negative covariation of genetic relatedness and geographic distance predicted by theoretical models and reported empirically for other, natively philopatric landbirds (Double et al. 2005; Ortega et al. 2008), neither with our relatively large sample sizes of hosts broods (Fig. 2a) nor with the more limited within-host within-year comparisons (Fig. 2b).

However, there are several explanations for the lack of such statistical relationships, including: (1) biologically low levels of natal philopatry by Cowbirds across its North American range (Nice 1939; Smith and Arcese 1994; Anderson et al. 2005; this study); alternatively, varying levels of natal philopatry in Cowbirds may not result in detectable genetic structuring at the scale examined by our study because of (2) the non-linearity of spatial and genetic relatedness, (3) genetic structuring at different spatial scales from that examined by our study, or (4) the immigration of breeders, perhaps biased by greater sex specific dispersal of female birds (Schlossberg 2009) and/or greater female mortality (Ortega and Ortega 2009), into the local parasite population (Anderson et al. 2005).

We used both linear and non-parametric analyses to begin to evaluate spatial-genetic variation between Cowbird chicks in our study, but the results from these tests were also confirmed by the results of the extensive combination of variance and randomization tests (Table 2). Therefore, explanation (2) is unlikely to apply to our results. In addition, counter to explanation (3), the spatial scale of our study encompassed parasitized host nests no more than 50 km apart, with most nests within 3–12 km of each other (Fig. 2a). These distances are similar in magnitude to the range of natal dispersal by migratory continental passerines near Ithaca and at other temperate study sites (Winkler et al. 2005; Hoover and Reetz 2006) and are also similar to the geographic distance sampled in a previous analysis of avian spatial genetic structure, which

detected significant decreases in relative relatedness at the scale of 1–10 km ( $\sim 0.1$  per 1 km; Woxvold et al. 2006). We, therefore, suggest that, to address the biological relevance of the remaining alternative explanations regarding (1) versus (4) for our patterns reported here in Cowbirds, future work should combine (a) genetic analyses of both nuclear and mitochondrial markers to examine sex-specific patterns of dispersal (Marchetti et al. 1998; Gibbs et al. 2000; Daniel et al. 2007) with (b) direct observations of the movements of identifiable brood parasite chicks of known sex (Tonra et al. 2008). These latter methods will likely be a combination of mixing approaches of classical banding and resighting methods (Nice 1937; Smith and Arcese 1994; Anderson et al. 2005) and recent technological developments in the individual tracking of small animals through long-life radio transmitters, satellite trackers, pit tags, geolocators, and long-distance transponders (Wikelski et al. 2007; Stutchbury et al. 2009).

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