

# Genetic elucidation of host use by individual sympatric bronzed cowbirds (*Molothrus aeneus*) and brown-headed cowbirds (*M. ater*)

K. Ellison, S.G. Sealy, and H.L. Gibbs

**Abstract:** Species of avian brood parasites that use one or several species of hosts are called host specialists and generalists, respectively. To determine host use of individual bronzed cowbirds, *Molothrus aeneus* (Wagler, 1829), and brown-headed cowbirds, *Molothrus ater* (Boddaert, 1783), we assigned maternity to eggs using microsatellite DNA markers. We measured patterns of host use by individual sympatric cowbirds. This allowed us to determine whether these species competed for host nests and the number of females laying at nests that were already parasitized by conspecifics. We monitored 1447 nests of 42 potential host species and found that each species of cowbird used primarily four host species, with minimal overlap in the species used, yet at least some individuals acted as generalists. Individual cowbirds tended to avoid laying again at nests each had already parasitized, and multiple parasitism was frequently due to same-day laying by more than one female (19% and 44% of 27 and 39 cases for brown-headed and bronzed cowbirds, respectively). Our results suggest that both cowbirds can differentially parasitize host species; however, host use does not appear to be refined, as many eggs were laid in already-parasitized nests when unparasitized nests of other suitable host species were available.

**Résumé :** Les oiseaux parasites des nids qui utilisent individuellement une ou plusieurs espèces d'hôtes sont dits respectivement des parasites spécialisés et généralistes. Afin de préciser l'utilisation des hôtes chez des individus du vacher bronzé, *Molothrus aeneus* (Wagler, 1829) et du vacher à tête brune, *Molothrus ater* (Boddaert, 1783), nous avons établi la maternité des oeufs à l'aide de marqueurs microsatellites de l'ADN. Nous avons déterminé les patrons d'utilisation des hôtes chez des vachers individuels sympatriques. Cela nous permet de savoir s'il y a chez ces espèces une compétition pour les nids des hôtes et de recenser le nombre de femelles qui pondent dans des nids déjà parasités par des oiseaux de leur espèce. Dans 1447 nids de 42 espèces potentielles d'hôtes, chaque espèce de vacher utilise principalement quatre espèces d'hôtes, avec un minimum de chevauchement dans le choix des espèces; cependant, au moins quelques individus se comportent comme des généralistes. Les vachers individuels tendent à éviter de pondre une seconde fois dans un nid qu'ils ont déjà parasité; le parasitisme multiple (19 % de 27 cas chez le vacher à tête brune et 44 % de 39 cas chez le vacher bronzé) est souvent dû à la ponte par plus d'une femelle au cours d'une même journée. Nos résultats indiquent que les deux vachers peuvent parasiter de façon différentielle les espèces d'hôtes; toutefois, l'utilisation des hôtes ne semble pas très précise, car plusieurs oeufs sont pondus dans des nids déjà parasités, alors qu'il y a des nids non parasités d'espèces d'hôtes appropriés qui sont encore disponibles.

[Traduit par la Rédaction]

## Introduction

Some avian brood parasites use a single host species, whereas others parasitize several species (Friedmann 1929; Rothstein and Robinson 1998). The cowbird genus *Molothrus* Swainson, 1832 comprises five extant species, of which the two most basal and the three most recently derived each use fewer than 10 species and more than 93 species as hosts, respectively (Fig. 1). Patterns of host use among these cow-

birds have been attributed to a winnowing of the number of hosts over time through the evolution of host defenses — the coevolutionary hypothesis (Rothstein 1990; Rothstein et al. 2002). As an alternative, Lanyon (1992) hypothesized that host generalism is a derived state among the cowbirds. To understand more fully the evolution of host use, however, knowledge of the behavior of individuals is crucial.

For instance, common cuckoos, *Cuculus canorus* L., 1758, use more than 220 species as hosts (Lowther 2005; Payne 2005), but individual common cuckoos parasitize only one or a few host species and are considered, individually, to be specialists (Moksnes and Røskoft 1995; Gibbs et al. 2000). This specialism is a result of matrilineal lines for host use (Gibbs et al. 2000) and time (60 My) for the refined coevolution between cuckoos and some of their hosts observed today (Rothstein et al. 2002). By contrast, Friedmann (1929) assumed that individual brown-headed cowbirds, *Molothrus ater* (Boddaert, 1783), used multiple hosts and Fleischer (1985) confirmed this by comparing allozymes among eggs laid by different females. Based on microsatellite DNA and mtDNA variation, Gibbs et al. (1997) found

Received 20 January 2006. Accepted 2 June 2006. Published on the NRC Research Press Web site at <http://cjz.nrc.ca> on 17 October 2006.

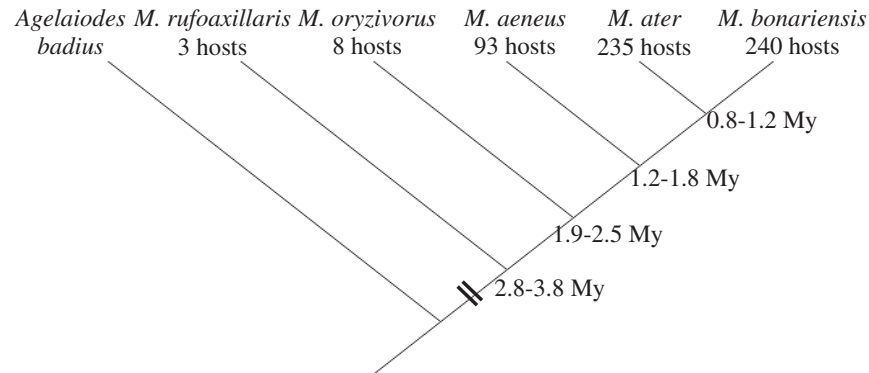
**K. Ellison**<sup>1,2</sup> and **S.G. Sealy**. Department of Zoology, University of Manitoba, Winnipeg, MB R3T 2N2, Canada.

**H.L. Gibbs**. Department of Evolution, Ecology and Organismal Biology, Ohio State University, 318 West 12th Avenue, Columbus, OH 43210, USA.

<sup>1</sup>Corresponding author (e-mail: [ksellison@wisc.edu](mailto:ksellison@wisc.edu)).

<sup>2</sup>Present address: Department of Wildlife Ecology, University of Wisconsin, 1630 Linden Drive, Madison, WI 53706, USA.

**Fig. 1.** Phylogeny for *Molothrus* spp. based on cytochrome *b* gene sequences (Lanyon 1992, Johnson and Lanyon 1999). Host data are from Lowther (2005). Divergence estimates at nodes were calculated by Rothstein et al. (2002). The double line indicates the break for an outgroup, the nonparasitic baywing (*Agelaiodes badius* (see Lowther 2001), previously known as the bay-winged cowbird, *Molothrus badius*).



that genetically distinct host races did not occur among brown-headed cowbirds reared by two hosts. Several recent studies have further demonstrated that brown-headed cowbirds act individually as host generalists (Hahn et al. 1999; Woolfenden et al. 2003; Strausberger and Ashley 2005).

Compared with the relatively well-studied brown-headed cowbird (Rothstein and Robinson 1998), information about parasitism by the bronzed cowbird, *Molothrus aeneus* (Wagler, 1829), is limited (Friedmann 1929; Carter 1986; Peer and Sealy 1999a; Chace 2005) and it is not clear whether individual bronzed cowbirds are generalists. Therefore, more detailed information on host use by individual bronzed cowbirds is needed to evaluate whether this species comprises specialists or a mixture of individuals with different host-use strategies (see Lowther 1995; Strausberger and Ashley 2005). Also, because many nests parasitized by both species receive more than one cowbird egg (Carter 1986; McLaren et al. 2003; Ellison 2004), identifying the number of females that lay in each nest is important. For instance, do individuals have limited access to nests and thus need to lay more than one egg per nest? Or do several females lay in the same nest? Each scenario has different implications for the evolution of cowbird host use, particularly among nestlings that compete for food (see Kilner et al. 2004), and both types of parasitism occurred at nests of the song sparrow, *Melospiza melodia* (Wilson, 1810), used by brown-headed cowbirds in Manitoba (McLaren et al. 2003). Therefore, we tracked host use by individual bronzed cowbirds and brown-headed cowbirds by assigning parentage using microsatellite DNA.

## Methods

### Study site

We measured host use by cowbirds during four breeding seasons (1999–2002) at Fort Clark Springs, Kinney Co., Texas (29°18'N, 100°43'W). Bronzed and brown-headed cowbirds have co-occurred in the area for more than 50 years (Oberholser 1974). We used DNA to identify eggs laid by different individuals and, to increase our sampling for species parasitized by the cowbirds, we monitored nests and host fledgling groups (see Carter 1986) in areas surrounding a 27 ha area where clutches were manipulated. Because we

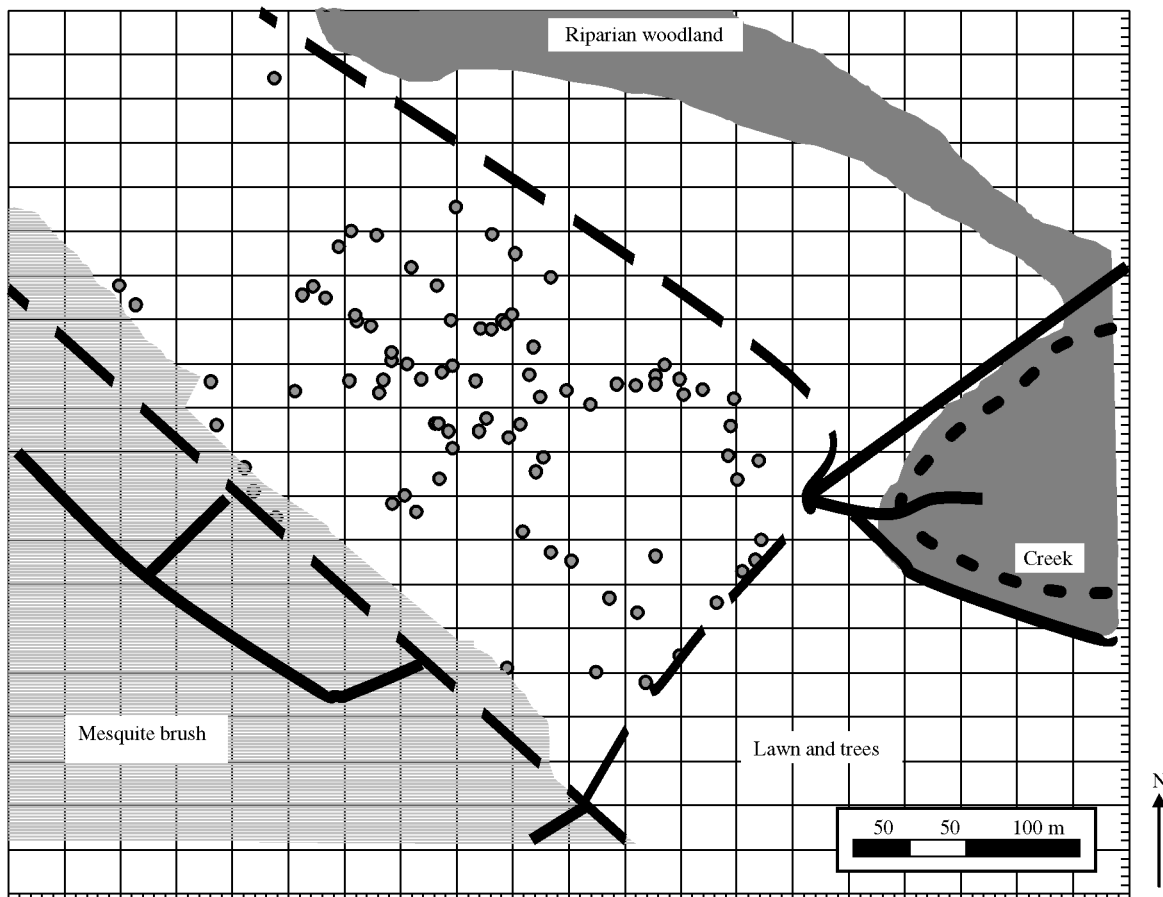
destroyed eggs to obtain DNA, the two methodologies were applied in different plots (Fig. 2). In the “core” area, nests were located by identifying areas with territories for which we lacked nests. Territories were identified by spot mapping (International Bird Census Committee 1970) songbird locations twice per week. We did not survey barn swallows, *Hirundo rustica* L., 1758, purple martins, *Progne subis* (L., 1758), or doves because they are infrequently parasitized by cowbirds and are poor fosterers for cowbirds. All cowbird eggs that we collected were from nests located within the core area (Fig. 2).

The core area consisted of a manicured golf course dominated by trees <7 m tall (honey mesquite, *Prosopis glandulosa* Torr., Texas huisache, *Acacia farnesiana* (L.) Willd., and other *Acacia* spp.). Cowbird fledging success was measured in surrounding areas within 2 km of the core area. Also, as a control for the effects of switching cowbird eggs with model eggs (details below), we did not manipulate cowbird eggs in any nests in 1999. Thus, data on cowbird fledging success in 1999 were collected in both the core and the surrounding areas. To further assess the diversity of species used as hosts, we surveyed host fledgling groups (see Carter 1986). We considered host species those that fed young cowbirds incapable of sustained flight. Fledgling cowbirds may be fed by individuals other than their fosterers (Sealy and Lorenzana 1997), but this is exceedingly rare (2%,  $n = 519$ ) compared with the usual case, in which cowbirds are fed by their fosterers (100%,  $n = 14$ ; Eastzer et al. 1980; Woodward 1983; Smith and Arcese 1994). Thus, we considered our host survey methods to be relatively accurate. The surrounding areas were a mixture of residential lots, riparian woodland of Texas live oak, *Quercus virginiana* P. Mill., and pecan, *Carya illinoensis* (Wangenh.) K. Koch, and Trans-Pecos brushland with mostly honey mesquite (<4 m tall) and grass (1 m).

### Host use

The number of cowbird eggs per nest was recorded in the core area. To ensure that we collected all cowbird eggs within the core, all songbird nests were checked daily during 6- to 10-day periods centered on the potential host's first egg day. We compared the number of eggs laid per nest with random Poisson series to determine whether cowbirds

**Fig. 2.** Core area (within broken lines) for cowbird egg collection, 2000–2002, Fort Clark Springs, Texas. Brushy habitat (shaded areas) abutted manicured lawn and ornamental plantings (unshaded areas). Trees in the core area are represented by circles, and roads are represented by solid lines.



distributed their eggs in a numerically random pattern among host nests. We followed the Kolmogorov–Smirnov procedure for such a comparison outlined by Lea and Kattan (1998).

We monitored nests to detect the use of host species that may reject cowbird eggs by watching or videotaping nests of potential hosts prior to sunrise, when cowbirds lay (Scott 1991; Peer and Sealy 1999b). Hi8 and digital video cameras were mounted on tripods 3–5 m from nests, and observers watched nests from >10 m away. We also checked the contents of nests 10–30 min after sunrise to further minimize chances of missing cowbird eggs that were quickly ejected by potential hosts (see Scott 1977). Of the species commonly parasitized, only the Bullock’s oriole, *Icterus bullockii* (Swainson, 1827), is known to eject foreign eggs (Rohwer et al. 1989; Sealy and Underwood 2004).

**Identification of egg-laying individuals**

Cowbirds were captured in seed-baited walk-in traps and uniquely color-banded, and blood was sampled. DNA was obtained from the blood of adults and young and from the eggs collected from host nests (Alderson et al. 1999a). Cowbird eggs found on the rim of nests or directly below nests were included in the genetic analysis (details below) because they represent host use. Cowbird eggs were collected when found to prevent them from being destroyed by predators or

damaged by cowbirds and were replaced by painted wooden eggs of similar mass, dimensions, and appearance (see Peer et al. 2002) to minimize nest abandonment by hosts. No eggs were collected in 1999; hence, no egg models were placed in nests. This was necessary because model eggs may affect cowbird behavior at nests because they cannot be punctured (Massoni and Reboresda 2002), and their appearance may affect the probability of subsequent parasitism (see Ortega et al. 1994). To test whether model cowbird eggs affected multiple parasitism, we compared rates of multiple parasitism during the control (1999) and treatment years.

Cowbirds that laid within the core area were identified by comparing adult and offspring alleles at microsatellite DNA loci (Alderson et al. 1999a; Woolfenden et al. 2003). Eggs were artificially incubated for 3–5 days and then stored at –20 °C. DNA was extracted from embryos and shells (sensu Sambrook et al. 1989). DNA from embryos yielded maternal and paternal haplotypes (Alderson et al. 1999a, 1999b), whereas DNA from shells and embryos was used to obtain maternal (shell) genotypes and paternal haplotypes (i.e., non-maternal half of embryo genotype) (Strausberger and Ashley 2001). Samples were PCR-amplified and genotyped at four or five microsatellite DNA loci using polyacrylamide gel electrophoresis and autoradiography. For one locus, Maμ 16, DNA was quantified by

**Table 1.** Abundance (mean no. of males/count) of potential host species of bronzed and brown-headed cowbirds and nest data for the core area during cowbird egg laying, 2000–2002.

Species and mean female mass (g)*	Abundance (SD)	% parasitism (n)	Bronzed cowbird ( <i>Molothrus aeneus</i> , 57.4 g)		Brown-headed cowbird ( <i>M. ater</i> , 32.0 g)*	
			Eggs	Fledglings <sup>†</sup>	Eggs	Fledglings <sup>†</sup>
<b>Species counted during censuses</b>						
Vermilion flycatcher ( <i>Pyrocephalus rubinus</i> , 16.0)	5.3 (2.4)	6 (104)	0	0	9	7
Ash-throated flycatcher ( <i>Myiarchus cinerascens</i> , 27.2)	0.8 (1.0)	0 (7)	0	0	0	0
Western kingbird ( <i>Tyrannus verticalis</i> , 39.6)	2.2 (1.7)	0 (21)	0	0	0	0
Scissor-tailed flycatcher ( <i>T. forficatus</i> , 43.2)	1.9 (1.8)	0 (10)	0	0	0	0
Bell's vireo ( <i>Vireo bellii</i> , 9.0)	8.1 (2.5)	74 (118)	0	0	108	20
Cactus wren ( <i>Campylorhynchus brunneicapillus</i> , 38.9)	1.1 (1.0)	0 (6)	0	0	0	0
Bewick's wren ( <i>Thryomanes bewickii</i> , 9.9)	1.3 (1.0)	0 (6)	0	0	0	1
Blue-gray gnatcatcher ( <i>Poliophtila caerulea</i> , 5.7)	1.5 (1.5)	82 (11)	0	0	12	53
Eastern bluebird ( <i>Sialia sialis</i> , 31.6)	0.5 (0.7)	0 (6)	0	0	0	0
Northern mockingbird ( <i>Mimus polyglottos</i> , 48.5)	9.6 (4.5)	0 (122)	0	0	0	0
Chipping sparrow ( <i>Spizella passerina</i> , 12.3)	1.6 (1.3)	0 (21)	0 <sup>‡</sup>	0	0	2
Lark sparrow ( <i>Chondestes grammacus</i> , 29.0)	8.5 (3.3)	2 (200)	2 <sup>‡</sup>	0	2	0
Northern cardinal ( <i>Cardinalis cardinalis</i> , 44.3)	0.2 (0.5)	50 (3)	2	4	1	0
Blue grosbeak ( <i>Guiraca caerulea</i> , 27.5)	0.4 (0.7)	67 (3)	2	0	2	2
Painted bunting ( <i>Passerina ciris</i> , 11.8)	1.7 (1.1)	20 (21)	0	0	4	1
Great-tailed grackle ( <i>Quiscalus mexicanus</i> , 107.0)	1.1 (1.7)	0 (6)	0	0	0	0
Orchard oriole ( <i>Icterus spurius</i> , 19.6)	2.7 (2.1)	79 (34)	73	34	1	0
Hooded oriole ( <i>I. cucullatus</i> , 24.3)	1.0 (1.0)	82 (30)	62	24	1	0
Bullock's oriole ( <i>I. bullockii</i> , 33.6)	1.6 (1.4)	33 (40)	15	6	0	0
House finch ( <i>Carpodacus mexicanus</i> , 21.4)	1.5 (1.6)	0 (51)	1 <sup>‡</sup>	0	0	0
Lesser goldfinch ( <i>Carduelis psaltria</i> , 9.5)	0.4 (0.6)	0 (8)	0	0	0	0
Totals		n/a (828)	157	68	140	86
<b>Species not counted during censuses</b>						
Mourning dove ( <i>Zenaidura macroura</i> , 115.0)		0 (15)	0	0	0	0
White-winged dove ( <i>Z. asiatica</i> , 153.0)		0 (19)	0	0	0	0
Inca dove ( <i>Columbina inca</i> , 47.5)		0 (3)	0	0	0	0
Common ground-dove ( <i>C. passerina</i> , 30.1)		0 (4)	0	0	0	0
Couch's kingbird ( <i>Tyrannus couchii</i> , 45.0)		0 (2)	0	0	0	0
Barn swallow ( <i>Hirundo rustica</i> , 15.8)		0 (6)	0	0	0	0
Yellow-throated vireo ( <i>Vireo flavifrons</i> , 18.0)		0 (2)	0	0	0	0
Black-tailed gnatcatcher ( <i>Poliophtila melanura</i> , 5.1)		50 (2)	0	0	0	1
Yellow-breasted chat ( <i>Icteria virens</i> , 25.1)		25 (12)	0 <sup>‡</sup>	0	0	1
Olive sparrow ( <i>Arremonops rufivirgatus</i> , 23.6)		0 (1)	0	0	0	0
Black-throated sparrow ( <i>Amphispiza bilineata</i> , 13.5)		0 (1)	0	0	0	0
Pyrrhuloxia ( <i>Cardinalis sinuatus</i> , 34.3)		66 (3)	0	2	0	0
Indigo bunting ( <i>Passerina cyanea</i> , 14.1)		100 (1)	0	0	0	0
Totals		n/a (71)	0	2	0	2

\*Data from Dunning (1993); mass for appropriate subspecies of brown-headed cowbird from Fleischer and Rothstein (1988).

<sup>†</sup>From fledgling surveys outside core area (1999–2002); tallies do not include fledglings detected as eggs.

<sup>‡</sup>First record of bronzed cowbird parasitism on this species (parasitized chipping sparrow and yellow-breasted chat nests were outside of core area).

automated sequencing on an ABI PRISM<sup>®</sup> 310 Genetic Analyzer (Applied Biosystems, Foster City, California) with fluorescently labeled primers (Longmire et al. 2001; Strausberger and Ashley 2001). The number of females laying at the same nest was often determined by observation, because each cowbird can lay only one egg per day (Sturkie 1976); thus, more than one female was involved when more than one egg appeared in a nest on the same day. We also used the PARENTAGE (see Marchetti et al. 1998) and KINSHIP (Goodnight and Queller 1999) programs (see Alderson et al. 1999a, 1999b) to statistically assign parentage based on genetic data. PARENTAGE excludes nonmatching candidate genotypes to yield high-resolution parentage assignments based on calculations

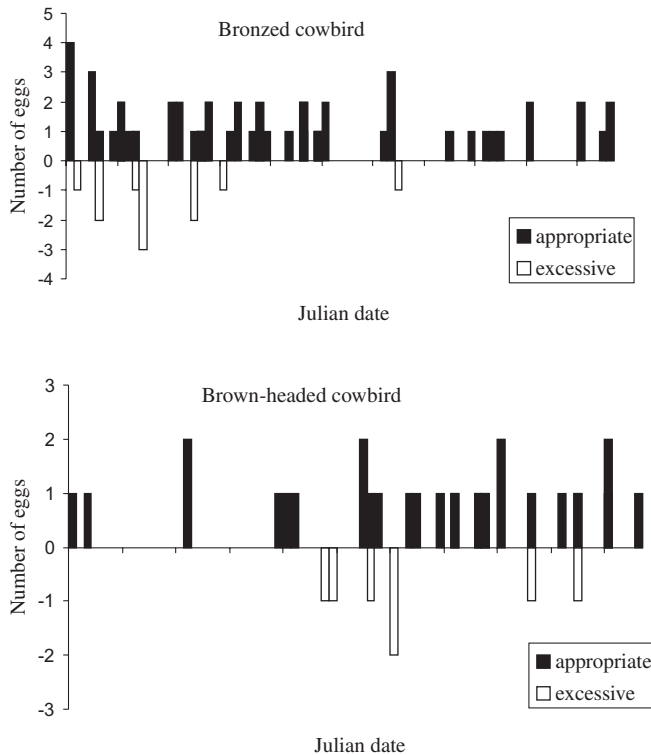
sensu Chakraborty et al. (1988); see Alderson et al. (1999a). We used KINSHIP to calculate relatedness likelihood values (Goodnight and Queller 1999), which were used to identify half- and full-siblings (sensu McLaren et al. 2003).

#### Females involved in multiple parasitism

We used observational laying data, maternal haplotypes, and output from the PARENTAGE and KINSHIP programs to determine the likelihood that eggs were laid by the same or different females (sensu McLaren et al. 2003). For this analysis we were interested only in the number of laying females; thus, this method differed from that used for parental assignment, described previously. Specifically, alleles



**Fig. 3.** Timing and frequency of same-day multiple parasitism (more than one egg laid at a nest on the same day) by bronzed and brown-headed cowbirds. For each day, cowbird eggs laid after parasitized clutches had reached the maximum size that could normally be cared for (two and three for brown-headed and bronzed cowbirds, respectively) were classified as excessively laid (open bars); all other eggs were classified as appropriately laid (solid bars). Same-day laying was classified as appropriate because cowbirds were presumably physiologically committed to laying at these nests. The initial Julian date is 27 April.



were compared at four loci to calculate the probability that offspring were related maternally.

**Synchrony of cowbird laying**

We considered the timing of cowbird laying to be appropriate when cowbird eggs were laid between the first day of host laying (LD 1) and the third day of incubation (ID 3). This period exceeds that used by McLaren et al. (2003) by 1 day because for most host clutches at our site, incubation spanned up to 14 days, whereas McLaren et al. (2003) considered only the song sparrow, which has a 12-day incubation period.

Among the nests that were multiply parasitized, we compared the appropriateness of cowbird laying in each category of multiple parasitism, i.e., single-female nests versus multiple-female nests, using contingency tests. We analyzed data by nest, each classified as “all parasitism appropriate” or “one or more cases inappropriate”, and by egg, each scored as laid “appropriately” or “inappropriately”. For classification of eggs, we included only eggs laid after the first cowbird egg appeared in each nest. In cases where two cowbird eggs appeared on the same day, neither was included.

**Results**

**Host use**

During 1999–2002, we found 1447 nests including 330 and 238 eggs laid by bronzed and brown-headed cowbirds, respectively (2000–2002 data in Table 1). Bronzed cowbirds parasitized primarily orchard orioles, *Icterus spurius* (L., 1766), and hooded orioles, *Icterus cucullatus* Swainson, 1827, whereas brown-headed cowbirds parasitized blue-gray gnatcatchers, *Poliptila caerulea* (L., 1766), Bell’s vireos, *Vireo bellii* Audubon, 1844, and vermilion flycatchers, *Pyrocephalus rubinus* (Boddaert, 1783). Only nests of northern cardinals, *Cardinalis cardinalis* (L., 1758), were used regularly by both cowbird species; however, only three northern cardinal nests were available in the core area. Bronzed cowbirds also parasitized nests of yellow-breasted chat (*Icteria virens* (L., 1758)), Bullock’s oriole, lark sparrow (*Chondestes grammacus* (Say, 1823)), chipping sparrow (*Spizella passerina* (Bechstein, 1798)), and house finch (*Carpodacus mexicanus* (Müller, 1776)). The distribution of eggs laid by brown-headed cowbirds differed significantly from a random series ( $D = 0.63, P < 0.001$ ), whereas that of eggs laid by bronzed cowbirds did not ( $D = 0.15, P = 0.08$ ).

Fourteen and 20 bronzed and brown-headed cowbird eggs, respectively, were found beneath nests. Observations during cowbird laying attempts revealed that cowbirds sometimes did not lay eggs in nests (bronzed cowbird, 5%,  $n = 41$ ; brown-headed cowbird, 22%,  $n = 23$ ). In four cases, eggs missed nests (two for each cowbird species). Also, brown-headed cowbirds twice laid eggs atop Bell’s vireos, and one brown-headed cowbird egg was laid on the ground. In all seven of these cases, laying failure was associated with host nest defense. Although cowbirds removed host eggs ( $n = 3$  observations), none removed cowbird eggs or models.

More than one female laying at a nest on the same morning was more common among bronzed cowbirds (44%,  $n = 39$ ) than among brown-headed cowbirds (19%,  $n = 27$ ) (Fig. 3). On two occasions, two bronzed cowbirds attempted to lay at one nest at the same time (5%,  $n = 39$ ): once at the nest of a hooded oriole and once at the nest of an orchard oriole. In both instances, the cowbirds apparently laid without disruption, as the number of fresh cowbird eggs per nest equaled the number of cowbirds observed to have visited the nests. At the hooded oriole nest, the second of two bronzed cowbirds clung to the underside of the nest while the other cowbird was on the nest. The second bird lightly pecked the first, which then left, and the second then settled in the nest and laid. Our view of the bronzed cowbirds at the orchard oriole nest was obscured, but they were in contact with each other while atop the nest.

**Host use by individuals**

We genotyped 233 and 163 samples from bronzed and brown-headed cowbirds, respectively, at a minimum of four microsatellite loci (Table 2). This facilitated a relatively high power of exclusion when assigning parentage (Table 2). Eleven bronzed cowbirds and five brown-headed cowbirds were assigned multiple eggs. Of those assigned more than one egg, 73% of bronzed cowbirds and 60% of brown-

**Table 2.** Measures of variability at microsatellite DNA loci.

Locus	Bronzed cowbird			Brown-headed cowbird			% heterozygosity*		
	No. of alleles	bp	<i>n</i>	No. of alleles	bp	<i>n</i>	Observed	Expected	PIC
Maμ 1	25	194–270	222	23	194–270	160	78	94	94
Maμ 12	22	169–233	216	28	179–269	146	76	90	89
Maμ 15	8	276–304	187	23	240–324	96	78	79	76
Maμ 16	18	368–824	80	9	327–788	75	59	98	98
Maμ 25	15	129–169	136	24	125–219	93	71	80	80
Total	88	129–824	233	107	125–788	163	72	88	87

\*Expected heterozygosity (Nei 1987) and PIC (point information criteria, a measure of the information related to heterozygosity across all loci; Hearne et al.

headed cowbirds parasitized more than one host species (Fig. 4). Of the 139 cowbirds banded as young, two (2%) bronzed cowbirds were observed in subsequent years, but no breeding by these birds was detected.

### Females involved in multiple parasitism

Sixty-three percent ( $n = 76$ ) and 36% ( $n = 99$ ) of nests were multiply parasitized by bronzed and brown-headed cowbirds, respectively (Table 3). Most parasitized nests received 1–2 eggs (bronzed cowbird, 72%,  $n = 55$  nests; brown-headed cowbird, 97%,  $n = 96$  nests), with an average of 2.1 and 1.2 eggs per parasitized nest for bronzed and brown-headed cowbirds, respectively (Table 3). Host use was not affected by the substitution of models for real cowbird eggs. Multiple parasitism differed significantly between the control year and some treatment years, yet all significant differences were due to increased multiple parasitism (Table 4). This reveals that the presence of model eggs did not reduce cowbird parasitism. Also, both cowbirds were nearly equally abundant each year, ranking 14th and 15th among species detected during 10 morning surveys each season (see Ellison 2004). Therefore, frequencies of multiple parasitism did not reflect seasonal differences in cowbird numbers.

The exclusion analysis determined that most eggs laid in multiply parasitized nests had been laid by different females. However, because not all eggs at multiply parasitized nests yielded DNA (bronzed cowbird, 48%,  $n = 50$  nests; brown-headed cowbird, 51%,  $n = 39$  nests), sampling may have been biased toward multiple parasitism by different individuals owing to the number of female assignments made by laying day (41% and 25% of assignments for bronzed and brown-headed cowbirds, respectively; Table 5). Nonetheless, when considering only nests where maternity was assigned to all eggs, only 12% ( $n = 25$ ) and 15% ( $n = 20$ ) of multiply parasitized nests contained eggs laid by one female (bronzed and brown-headed cowbirds, respectively).

### Synchrony of cowbird laying

Most nests eventually parasitized (72%,  $n = 177$ ) were found prior to parasitism and most cowbird eggs laid were synchronized with the hosts' nest cycle; nine and one eggs laid by bronzed and brown-headed cowbirds, respectively, were laid in nests already abandoned. Bronzed and brown-headed cowbirds laid their eggs coincidentally with an appropriate period of host nesting (89% ( $n = 98$ ) and 79%

( $n = 121$ ) of bronzed and brown-headed cowbird eggs, respectively).

Not all eggs in each nest could be assigned maternity (Table 5). Considering only nests for which all cowbird eggs were assigned, bronzed cowbirds were equally likely to parasitize the same nest again ( $\chi^2 = 0.037$ ,  $P = 0.847$ ,  $n = 27$  nests). By contrast, brown-headed cowbirds were significantly less likely to parasitize the same nest again ( $\chi^2 = 4.167$ ,  $P = 0.041$ ,  $n = 24$  nests). Among eggs laid in multiply parasitized nests, there was no difference between the numbers laid appropriately and inappropriately by bronzed cowbirds ( $\chi^2 = 2.793$ ,  $P = 0.095$ ), whereas brown-headed cowbird eggs were significantly more likely to be laid appropriately ( $\chi^2 = 4.568$ ,  $P = 0.033$ ). Julian dates of nest initiation for nests that received one or more inappropriately laid cowbird eggs (bronzed cowbird,  $129.3 \pm 30.1$ ,  $n = 8$ ; brown-headed cowbird,  $138.2 \pm 21.4$ ,  $n = 26$ ) did not differ significantly from those for nests that received only appropriately timed cowbird eggs (bronzed cowbird,  $140.8 \pm 26.8$ ,  $n = 37$ ,  $U = 184.0$ ,  $P = 0.29$ ; brown-headed cowbird,  $142.5 \pm 22.3$ ,  $n = 95$ ,  $U = 1365.0$ ,  $P = 0.42$ ).

## Discussion

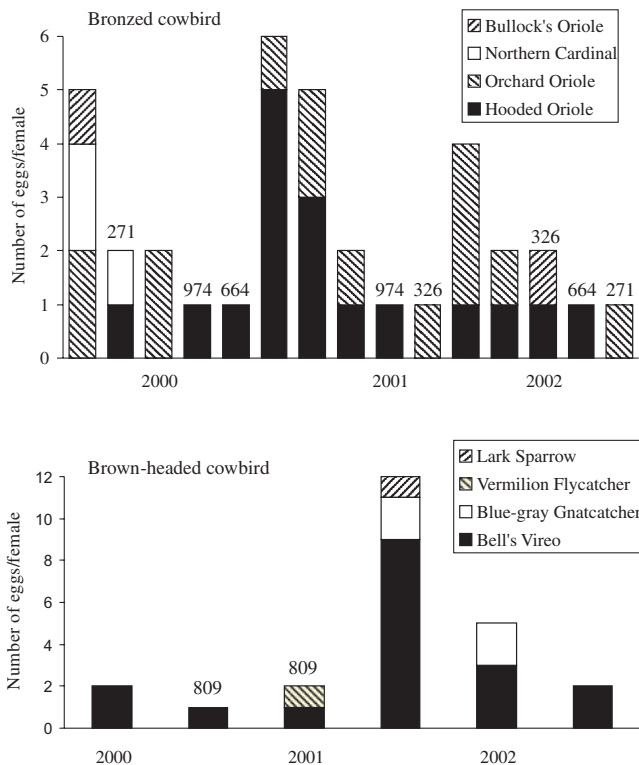
### Host use by individuals

Individual bronzed cowbirds parasitized nests of host species that differ in size (20–44 g), plumage color, and nesting structure (Table 6). Evolutionary analyses of host use by species of *Molothrus*, therefore, have correctly assumed that the three most recently derived molothrine species act individually as generalists. Although it is possible that populations of specialists and generalists coexist (Ney-Nifle et al. 2005) and that some individual brown-headed cowbirds are specialists, most bronzed and brown-headed cowbirds are generalists (Strausberger and Ashley 2005; this study). Implicitly, these cowbirds have not encountered strong selection, either positive or negative, from any group of potential host species (Joseph et al. 2002). Despite this, however, bronzed cowbirds apparently use only certain host species, where available, generally *Icterus* spp. (see also Friedmann 1929) or finches (Sealy et al. 1997). Elsewhere, where suitable orioles are uncommon, mimids and northern cardinals were most often parasitized (Carter 1986; Peer and Sealy 1999a). Moreover, the use of certain hosts over others frequently involved multiple parasitism, despite the availability of unparasitized nests of other apparently suitable host spe-

			Exclusion probability			
Brown-headed cowbird			Bronzed cowbird		Brown-headed cowbird	
Observed	Expected	PIC	1st parent	2nd parent	1st parent	2nd parent
89	97	97	0.784	0.879	0.879	0.935
87	97	97	0.673	0.803	0.887	0.940
76	92	91	0.419	0.597	0.708	0.828
95	98	98	0.908	0.952	0.908	0.952
86	92	90	0.439	0.617	0.698	0.822
87	95	95	0.998	1.000	1.000	1.000

1992) were calculated using Cervus 1.0 (Marshall et al. 1998).

**Fig. 4.** Host use by bronzed cowbirds and brown-headed cowbirds, for eggs genetically assigned to females at Fort Clark Springs, Texas. Numbers above bars are the identification numbers for females that laid in multiple years.



cies. Friedmann et al. (1977) and Strausberger (1998) suggested that multiple parasitism was associated with a cowbird species' differential parasitism or "preference" for a particular host.

Geographic variation in host use largely reflects the availability of hosts; however, our study included >900 nests of 18 potential host species. Host size may affect parasitism, as bronzed cowbirds rarely use species under 10 g (Lowther 2005) and competition from hosts too large may reduce cowbird success. For instance, Peer and Sealy (2004a) experimentally cross-fostered hatchling brown-headed cowbirds (2.2 g) and bronzed cowbirds (3.5 g) into the nests of great-tailed grackles, *Quiscalus mexicanus* (J.F. Gmelin, 1788) (6.2 g); brown-headed cowbird nestlings did not survive, whereas bronzed cowbird nestlings did. Host size may also affect the frequency of multiple parasitism, as larger

hosts may be able to rear more than one cowbird (Weatherhead 1989; Lorenzana and Sealy 1999; Mermoz and Reborada 2003; Astié and Reborada 2006).

Competition with brown-headed cowbirds for nests likely affected the number of host species used by bronzed cowbirds (Ellison 2004). However, both species of cowbird continued to use only certain host species, which resulted in multiple parasitism, despite the availability of unparasitized nests of other potential host species nearby. The primary difference between the hosts for each cowbird was body size: bronzed cowbirds parasitized species >20 g, whereas brown-headed cowbirds rarely did so (Ellison 2004).

**Females involved in multiple parasitism**

As species, the three generalist cowbird species (bronzed cowbirds, brown-headed cowbirds, and shiny cowbirds, *M. bonariensis* (J.F. Gmelin, 1789)) frequently lay more than one egg per nest (Lea and Kattan 1998; Ortega 1998; McLaren et al. 2003). In Texas, bronzed cowbirds laid multiple eggs in nests far more often than brown-headed cowbirds. This seems best explained by the use of larger hosts by bronzed cowbirds. However, most multiple parasitism involved more than one female laying at the same nest, sometimes on the same morning (Fig. 3). Thus, are cowbirds constrained by the number of available host nests, or are they not able to determine which nests other cowbirds have parasitized? Often many nests are available simultaneously, but a distinction must be made between those that are at the right stage for successful parasitism (LD 3 to ID 2 or 3) (McLaren et al. 2003; this study) and those that are not. Frequently many nests of suitable host species were available (Table 1); however, the availability of primary hosts became limited across each season.

The high frequency of multiple parasitism suggests that any selection for cowbird counter-defenses from host egg discrimination would initially be weak relative to background losses to nestling competition. Yet, from a host's perspective, selection to detect foreign eggs would be greater than if single parasitism predominated and presumably more host young fledged. Host tolerance of frequent multiple parasitism appears best explained by the hypotheses that hosts lack defenses against parasitism owing to either an evolutionary lag or an equilibrium (reviewed by Rothstein and Robinson 1998).

Most females did not lay again in nests they had already parasitized. This suggests that individuals are not limited by the availability of host nests. It is intriguing that cowbirds

**Table 3.** Cowbird egg distribution among the nests of the primary hosts of each cowbird.

	No. of cowbird eggs per nest						Mean no. of eggs per nest
	1	2	3	4	5	6	
<b>Bronzed cowbird</b>							
Orchard oriole	7	10	4	2	3	0	2.38
Hooded oriole	9	9	6	1	1	1	3.56
Bullock's oriole	8	3	0	0	0	0	1.27
Total	24	22	10	3	4	1	2.13
<b>Brown-headed cowbird</b>							
Vermilion flycatcher	9	0	0	0	0	0	1.00
Bell's vireo	56	16	2	0	0	0	1.27
Blue-gray gnatcatcher	7	3	0	0	0	0	1.30
Total	72	19	2	0	0	0	1.25

**Note:** Four and two nests of three other species were singly and doubly parasitized by bronzed cowbirds, respectively. Likewise, 11 nests of seven other species were singly parasitized by brown-headed cowbirds (see Table 1).

**Table 4.** Frequencies of multiple parasitism during seasons with (2000–2002) or without (1999) substitution of models for cowbird eggs.

Season	Frequency of multiple parasitism (%)	
	Bronzed cowbird ( <i>n</i> )	Brown-headed cowbird ( <i>n</i> )
1999	46.5 (43)	15.6 (32)
2000	71.4 (28)*	48.0 (25)**
2001	66.7 (24)	40.6 (32)**
2002	40.0 (15)	18.5 (27)

**Note:** The only significant differences from the control season occurred during one and two seasons for bronzed and brown-headed cowbirds, respectively. \*,  $P < 0.05$ ; \*\*,  $P < 0.001$ .

continued to parasitize species whose nests were already parasitized rather than seek nests of other species, especially when we realize that over 40 potential host species nested at the site (Table 1).

Our observations suggest that the costs of multiple parasitism to cowbirds are not reduced through avoidance or immediate removal of the eggs of a competitor. We found that it was common for more than one female to lay at the same nest on the same morning, but the coincident arrival of cowbirds at nests was rare. However, when two bronzed cowbirds visited a nest at the same time, neither disrupted laying by the other or left without laying. Because we collected cowbird eggs, we do not know whether cowbirds subsequently visited nests to puncture or remove eggs (see Ortega 1998; Tewksbury et al. 2002). However, several cowbird eggs found under nests had been laid atop hosts ( $n = 2$ ) or outside the nests ( $n = 32$ ). Furthermore, although cowbirds removed host eggs, none removed cowbird eggs or models.

Because multiple parasitism was common and usually involved laying by different females, host use by bronzed cowbirds appears to reflect elements of randomness and selectivity: nests were frequently parasitized with little difference from a random series and the species used as hosts were parasitized differentially despite their relatively low abundance. However, it is important to note that most of the species not used as hosts did *not* possess traits to coun-

ter the costs of parasitism; thus, bronzed cowbirds' focused parasitism on a few species was not because of host defenses.

### The evolution of host diversity

Rothstein et al. (2002) hypothesized that coevolution, rather than a phylogenetic trend (Lanyon 1992), more clearly explains the pattern of molothrine host numbers. Under the hypothesis of coevolution, ancestral cowbirds would have used many species as hosts until, over time, some host species developed defenses that reduced costs associated with parasitism. Explicit coevolution must be reciprocal and, thus, to be considered a result of coevolution, a brood-parasitic trait must be derived solely as a response to a defensive trait among hosts (Rothstein 1990). Thus, via coevolution, a cowbird could stop using host species with adequate defenses and favor those with which it has greater success. Thereby, the number of effective hosts available to a species of cowbird would have decreased over time.

In Texas, many hosts that accept cowbird eggs and rear cowbirds elsewhere were not parasitized, despite the 2.1 cowbird eggs/nest among the nests of orioles. However, more time may be required for coevolution to be manifested in the reduction of host number for bronzed cowbirds (Rothstein et al. 2002). It is informative to consider host use by the most basal among the molothrines (Fig. 1), the screaming cowbird (*Molothrus rufoaxillaris* Cassin, 1866).

The screaming cowbird uses primarily three host species (Fraga 1996; Mermoz and Reboreda 1996), and its young apparently mimic the nestlings of one of its hosts, the baywing, *Agelaioides badius* (Vieillot, 1819) (Lowther 2001) (Hudson 1920; Friedmann 1929). However, because screaming cowbirds fledge from nests of another host, the chopi blackbird, *Gnorimopsar chopi* (Vieillot, 1819) (Sick 1993; Fraga 1996), and are equally successful parasitizing nests of baywings (Fraga 1998) and brown-and-yellow marshbirds, *Pseudoleistes virescens* (Vieillot, 1819) (Mermoz and Reboreda 1996; Mermoz and Fernández 2003), the remarkable similarity between nestlings of baywings and those of screaming cowbirds may have been



**Table 5.** Number of female bronzed (A) and brown-headed cowbirds (B) that laid at multiply parasitized nests, by number of cowbird eggs per nest.

(A) Bronzed cowbirds.						
No. of females	2 eggs	3 eggs	4 eggs	5 eggs	6 eggs	7 eggs
1	3	0	0	0	0	0
≥1	1	3	0	0	0	0
2	11 (4)	3	0	0	0	0
≥2	0	5 (4)	1	0	0	0
3	0	1 (1)	3 (1)	0	0	0
≥3	0	0	1 (1)	0	0	2 (1)
4	0	0	0	2 (1)	0	0
≥4	0	0	0	1 (1)	0	0
5	0	0	0	1 (1)	1 (1)	0

(B) Brown-headed cowbirds.		
No. of females	2 eggs	3 eggs
1	3	0
2	17 (4)	0
≥2	—	4 (2)

**Note:** Number of cases in which different individuals were identified by same-day laying is shown in parentheses. Inequalities (≥) represent minimal estimates for nests where the exact number of laying females could not be determined.

**Table 6.** Characteristics of species parasitized by bronzed cowbirds and brown-headed cowbirds at Fort Clark Springs, Texas.

Species	Mass (g)*	Plumage color (male/female)†	Type of nest	Egg appearance
Vermilion flycatcher	16.0	Red/grey	Cup	Maculated
Bell's vireo	9.0	Grey-green	Pendant	Maculated
Bewick's wren	9.9	Brown	Cavity	Maculated
Blue-gray gnatcatcher	5.7	Grey	Cup	Maculated
Chipping sparrow	12.3	Brown	Cup	Maculated
Lark sparrow	29.0	Brown	Cup	Maculated
Northern cardinal	44.3	Red/brown	Cup	Maculated
Blue grosbeak	27.5	Blue/brown	Cup	Unspotted
Painted bunting	11.8	Red/green	Cup	Maculated
Orchard oriole	19.6	Chestnut/yellow‡	Pendant	Maculated
Hooded oriole	24.3	Orange/yellow‡	Pendant	Maculated
Bullock's oriole	33.6	Orange/yellow‡	Pendant	Maculated
House finch	21.4	Red and grey/brown and grey	Cup	Maculated

\*Mass data from Dunning (1993).

†One color listed for sexually monomorphic species.

‡The plumage of yearling males is yellow until after their first breeding season.

derived secondarily. Indeed, nestling discrimination by hosts is extremely rare (Langmore et al. 2003), whereas egg discrimination is more common (Rothstein 1990; Peer and Sealy 2004b).

Specialization by the screaming cowbird does not appear to be a result of widespread host defenses. Most songbirds with which it co-occurs accept eggs of both screaming and shiny cowbirds (Mason 1986). Egg rejection would be expected, however, because it is the most widespread defense in reducing costs of parasitism and rejection traits persist in the absence of parasitism (Briskie et al. 1992; Rothstein 2001; Underwood et al. 2004). For some species, such as the great-tailed grackle and the boat-tailed grackle, *Quiscalus major* Vieillot, 1819, rejection appears to have been

maintained for 0.8 My (Peer and Sealy 2004a). Alternatively, a mechanism such as host species imprinting, as suggested for common cuckoos (Teuschl et al. 1998; Vogl et al. 2002; Skjelseth et al. 2004), may have restricted the basal species to a few hosts. Indeed, collectively, studies of host use by shiny cowbirds within communities of the Caribbean have documented geographic variation in which certain species were differentially parasitized for decades, regardless of their abundance (Manolis 1982; Wiley 1988; Cruz et al. 1995; Lowther and Post 1999). Island populations of the Caribbean that were likely founded by only a few individuals appear to have persisted in their differential use of primarily two or three new hosts, despite the availability of many other potential hosts (Post and Wiley 1977a, 1977b;

Cruz et al. 1995). As in our study, such differential use of hosts occurred despite multiple parasitism of differentially parasitized hosts, even when unparasitized nests of suitable hosts were available.

### Future directions

To better understand the persistence of multiple parasitism, a community-based approach is needed. This would allow comparison among nests available at the time of laying. Likewise, a study of cowbird nest-searching activities would reveal the time devoted to locating nests as well as the potential for cowbird detection and avoidance of costs associated with multiple parasitism. For instance, can cowbirds recognize nests that will be or already are parasitized? Likewise, studies focusing on the effect of natal conditions on host selection by individuals may yield interesting results. Particularly among the basal cowbirds, elucidation of host selection by individuals may improve our understanding of the evolution of generalism among the more derived cowbirds. Genetic analysis of screaming cowbird populations using different hosts (*sensu* Gibbs et al. 1997, 2000; Joseph et al. 2002) may reveal historic patterns of specialism on different host species.

### Acknowledgements

We thank the landowners at Fort Clark Springs for their hospitality and the many field assistants who helped collect our field data. The manuscript benefited from comments by M.V. Abrahams, D.C. Dearborn, J.F. Hare, G.R. Klassen, B.M. Strausberger, and an anonymous reviewer. This study was funded by a Natural Sciences and Engineering Research Council of Canada grant to S.G.S. and F.M. Chapman Awards of the American Museum of Natural History to K.E. All portions of this study complied with US federal and Texas state law and were carried out with the approval of our respective university animal-care committees.

### References

- Alderson, G.W., Gibbs, H.L., and Sealy, S.G. 1999a. Parentage and kinship studies in an obligate brood parasitic bird, the brown-headed cowbird (*Molothrus ater*), using microsatellite DNA markers. *J. Hered.* **90**: 182–190. doi:10.1093/jhered/90.1.182. PMID:9987928.
- Alderson, G.W., Gibbs, H.L., and Sealy, S.G. 1999b. Determining the reproductive behavior of individual brown-headed cowbirds using microsatellite DNA markers. *Anim. Behav.* **58**: 895–905. doi:10.1006/anbe.1999.1220. PMID:10512663.
- Astíe, A.A., and Reboreda, J.C. 2006. Costs of egg punctures and parasitism by shiny cowbirds (*Molothrus bonariensis*) at creamy-bellied thrush (*Turdus amaurochalinus*) nests. *Auk*, **123**: 23–32.
- Briskie, J.V., Sealy, S.G., and Hobson, K.A. 1992. Behavioral defenses against avian brood parasitism in sympatric and allopatric host populations. *Evolution*, **46**: 334–340. doi:10.2307/2409854.
- Carter, M.D. 1986. The parasitic behavior of the bronzed cowbird in south Texas. *Condor*, **88**: 11–25.
- Chace, J.F. 2005. Host use by sympatric cowbirds in southeastern Arizona. *Wilson Bull.* **117**: 375–381.
- Chakraborty, R., Meagher, T.R., and Smouse, P.E. 1988. Parentage analysis with genetic markers in natural populations. 1. The expected proportion of offspring with unambiguous paternity. *Genetics*, **118**: 527–536. PMID:3163316.
- Cruz, A., Manolis, T.D., and Andrews, R.W. 1995. History of shiny cowbird *Molothrus bonariensis* brood parasitism in Trinidad and Tobago. *Ibis*, **137**: 317–321.
- Dunning, J.B. 1993. Handbook of avian body masses. CRC Press, Boca Raton, Fla.
- Eastzer, D., Chu, P.R., and King, A.P. 1980. The young cowbird: average or optimal nestling? *Condor*, **82**: 417–425.
- Ellison, K. 2004. Host use by sympatric cowbirds (*Molothrus aeneus* and *M. ater*). Ph.D. thesis, Department of Zoology, University of Manitoba, Winnipeg.
- Fleischer, R.C. 1985. A new technique to identify and assess the dispersion of eggs of individual brood parasites. *Behav. Ecol. Sociobiol.* **17**: 91–99.
- Fleischer, R.C., and Rothstein, S.I. 1988. Known secondary contact and rapid gene flow among subspecies and dialects in the Brown-headed Cowbird. *Evolution*, **42**: 1146–1158. doi:10.2307/2408999.
- Fraga, R.M. 1996. Further evidence of parasitism on chopi blackbirds (*Gnorimopsar chopi*) by the specialized screaming cowbird (*Molothrus rufoaxillaris*). *Condor*, **98**: 866–867.
- Fraga, R.M. 1998. Interactions of the parasitic screaming and shiny cowbirds (*Molothrus rufoaxillaris* and *M. bonariensis*) with a shared host, the bay-winged cowbird (*M. badius*). In *Parasitic birds and their hosts: studies in coevolution*. Edited by S.I. Rothstein and S.K. Robinson. Oxford University Press, New York. pp. 173–193.
- Friedmann, H. 1929. The cowbirds: a study in the biology of social parasitism. C.C. Thomas, Springfield, Ill.
- Friedmann, H., Kiff, L.F., and Rothstein, S.I. 1977. A further contribution to knowledge of the host relations of the parasitic cowbirds. *Smithson. Contrib. Zool.* **235**: 1–75.
- Gibbs, H.L., Miller, P., Alderson, G., and Sealy, S.G. 1997. Genetic analysis of brown-headed cowbirds *Molothrus ater* raised by different hosts: data from mtDNA and microsatellite DNA markers. *Mol. Ecol.* **6**: 189–193. doi:10.1046/j.1365-294X.1997.00171.x.
- Gibbs, H.L., Sorenson, M.D., Marchetti, K., de L. Brooke, M., Davies, N.B., and Nakamura, H. 2000. Genetic evidence for female host-specific races of the common cuckoo. *Nature (London)*, **407**: 183–186.
- Goodnight, K.F., and Queller, D.C. 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Mol. Ecol.* **8**: 1231–1234. doi:10.1046/j.1365-294X.1999.00664.x. PMID:10447863.
- Hahn, D.C., Sedgwick, J.A., Painter, I.S., and Casna, N.J. 1999. A spatial and genetic analysis of cowbird host selection. *Stud. Avian Biol.* **18**: 204–217.
- Hearne, C.M., Gosh, S., and Todd, J.A. 1992. Microsatellites for linkage analysis of genetic traits. *Trends Genet.* **8**: 288–294.
- Hudson, W.H. 1920. Birds of La Plata. J.M. Dent, London, U.K.
- International Bird Census Committee. 1970. Recommendations for an international standard for a mapping method in bird census work. In *Bird census work and environmental monitoring*. Bull. Ecol. Res. Comm. 9, Swedish Nat. Sci. Res. Council., Stockholm. Edited by S. Svensson. pp. 49–52.
- Johnson, K.P., and Lanyon, S.M. 1999. Molecular systematics of the grackles and allies, and the effect of additional sequence (cyt *b* and ND2). *Auk*, **116**: 759–768.
- Joseph, L., Wilke, T., and Alpers, D. 2002. Reconciling genetic expectations from host specificity with historical population dynamics in an avian brood parasite, Horsfield's bronze-cuckoo *Chalcites basalis* of Australia. *Mol. Ecol.* **11**: 829–837. doi:10.1046/j.1365-294X.2002.01481.x. PMID:11972768.

- Kilner, R.M., Madden, J.R., and Hauber, M.E. 2004. Brood parasitic cowbird nestlings use host young to procure resources. *Science* (Washington, D.C.), **305**: 877–879. doi:10.1126/science.1098487. PMID:15297677.
- Langmore, N.E., Hunt, S., and Kilner, R.M. 2003. Escalation of a coevolutionary arms race through host rejection of brood parasitic young. *Nature* (London), **422**: 157–160. doi:10.1038/nature01460. PMID:12634784.
- Lanyon, S.M. 1992. Interspecific brood parasitism in blackbirds (Icterinae): a phylogenetic perspective. *Science* (Washington, D.C.), **225**: 77–79.
- Lea, S.E.G., and Kattan, G.H. 1998. Reanalysis gives further support to the 'shotgun' model of shiny cowbird parasitism of house wren nests. *Anim. Behav.* **56**: 1571–1573. doi:10.1006/anbe.1998.0925. PMID:9933555.
- Longmire, J.L., Roach, J.L., Maltbie, M., White, P.S., Tatum, O.L., Makova, K.D., and Hahn, D.C. 2001. Tetranucleotide microsatellite markers for the brown-headed cowbird *Molothrus ater*. *J. Avian Biol.* **32**: 76–78. doi:10.1034/j.1600-048X.2001.320111.x.
- Lorenzana, J.C., and Sealy, S.G. 1999. A meta-analysis of the impact of parasitism by the Brown-headed cowbird on its hosts. *Stud. Avian Biol.* **18**: 241–253.
- Lowther, P.E. 1995. Bronzed cowbird (*Molothrus aeneus*). In *The birds of North America*. No. 144. Edited by A. Poole and F. Gill. Academy of Natural Sciences, Philadelphia, Pa., and American Ornithologists' Union, Washington, D.C.
- Lowther, P.E. 2001. New name for the Bolivian blackbird. *Bull. Br. Ornithol. Club*, **121**: 280–281.
- Lowther, P.E. 2005. Host lists for brood parasitic birds of the world. [online]. Available from [http://fm1.fieldmuseum.org/aa/staff\\_page.cgi/staff=lowther&id=417](http://fm1.fieldmuseum.org/aa/staff_page.cgi/staff=lowther&id=417). [Accessed 21 October 2005.]
- Lowther, P.E., and Post, W. 1999. Shiny cowbird (*Molothrus bonariensis*). In *The birds of North America*. No. 399. Edited by A. Poole and F. Gill. Academy of Natural Sciences, Philadelphia, Pa., and American Ornithologists' Union, Washington, D.C.
- Manolis, T.D. 1982. Host relationships and reproductive strategies of the shiny cowbird in Trinidad and Tobago. Ph.D. thesis, Department of Environmental, Population and Organismic Biology, University of Colorado, Boulder.
- Marchetti, K., Nakamura, H., and Gibbs, H.L. 1998. Host-race formation in the common cuckoo. *Science* (Washington, D.C.), **282**: 471–472.
- Marshall, T.C., Slate, J., Kruuk, L.E.B., and Pemberton, J.M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **7**: 639–655. doi:10.1046/j.1365-294x.1998.00374.x. PMID:9633105.
- Mason, P. 1986. Brood parasitism in a host generalist, the shiny cowbird. I. The quality of different species as hosts. *Auk*, **103**: 52–60.
- Massoni, V., and Reboreda, J.C. 2002. A neglected cost of brood parasitism: egg punctures by shiny cowbirds during inspection of potential host nests. *Condor*, **104**: 407–412.
- McLaren, C.M., Woolfenden, B.E., Gibbs, H.L., and Sealy, S.G. 2003. Genetic and temporal patterns of multiple parasitism by brown-headed cowbirds (*Molothrus ater*) on song sparrows (*Melospiza melodia*). *Can. J. Zool.* **81**: 281–286. doi:10.1139/z03-002.
- Mermoz, M.E., and Fernández, G.J. 2003. Breeding success of a specialist brood parasite, the screaming cowbird, parasitizing an alternative host. *Condor*, **105**: 63–72.
- Mermoz, M.E., and Reboreda, J.C. 1996. New host for a specialized brood parasite, the screaming cowbird. *Condor*, **98**: 630–632.
- Mermoz, M.E., and Reboreda, J.C. 2003. Reproductive success of shiny cowbird (*Molothrus bonariensis*) parasitizing the larger brown-and-yellow marshbird (*Pseudoleistes virescens*) in Argentina. *Auk*, **120**: 1128–1139.
- Moksnes, A., and Røskaft, E. 1995. Egg-morphs and host preference in the common cuckoo *Cuculus canorus*: an analysis of cuckoo and host eggs from European museum collections. *J. Zool.* **236**: 625–648.
- Nei, M. 1987. *Molecular evolutionary genetics*. Columbia University Press, New York.
- Ney-Nifle, M., Bernstein, C., Reboreda, J.C., and Kacelnik, A. 2005. Population dynamics and avian brood parasitism: persistence and invasions in a three-species system. *J. Anim. Ecol.* **74**: 274–284. doi:10.1111/j.1365-2656.2005.00921.x.
- Oberholser, H.C. 1974. *The bird life of Texas*. University of Texas Press, Austin.
- Ortega, C.P. 1998. *Cowbirds and other brood parasites*. University of Arizona Press, Tucson.
- Ortega, C.P., Ortega, J.C., and Cruz, A. 1994. Use of artificial brown-headed cowbird eggs as a potential management tool in deterring parasitism. *J. Wildl. Manag.* **58**: 488–492.
- Payne, R.B. 2005. *The cuckoos, bird families of the world*. Oxford University Press, New York.
- Peer, B.D., and Sealy, S.G. 1999a. Parasitism and egg puncture behavior by bronzed and brown-headed cowbirds in sympatry. *Stud. Avian Biol.* **18**: 235–240.
- Peer, B.D., and Sealy, S.G. 1999b. Laying time of the bronzed cowbird. *Wilson Bull.* **111**: 137–139.
- Peer, B.D., and Sealy, S.G. 2004a. Fate of grackle (*Quiscalus* spp.) defenses in the absence of brood parasitism: implications for long-term parasite–host coevolution. *Auk*, **121**: 1172–1186.
- Peer, B.D., and Sealy, S.G. 2004b. Correlates of egg rejection in hosts of the brown-headed cowbird. *Condor*, **106**: 580–599.
- Peer, B.D., Ellison, K.S., and Sealy, S.G. 2002. Intermediate frequencies of egg ejection by northern mockingbirds (*Mimus polyglottos*) sympatric with two cowbird species. *Auk*, **119**: 855–858.
- Post, W., and Wiley, J.W. 1977a. The shiny cowbird in the West Indies. *Condor*, **79**: 119–121.
- Post, W., and Wiley, J.W. 1977b. Reproductive interactions of the shiny cowbird and the yellow-shouldered blackbird. *Condor*, **79**: 176–184.
- Rohwer, S., Spaw, C.D., and Røskaft, E. 1989. Costs to northern orioles of puncture-ejecting parasitic cowbird eggs from their nests. *Auk*, **106**: 734–738.
- Rothstein, S.I. 1990. A model system for coevolution: avian brood parasitism. *Annu. Rev. Ecol. Syst.* **21**: 481–508. doi:10.1146/annurev.es.21.110190.002405.
- Rothstein, S.I. 2001. Relic behaviours, coevolution and the retention versus loss of host defences after episodes of avian brood parasitism. *Anim. Behav.* **61**: 95–107. doi:10.1006/anbe.2000.1570. PMID:11170700.
- Rothstein, S.I., and Robinson, S.K. (Editors). 1998. *The evolution and ecology of avian brood parasitism, an overview*. In *Parasitic birds and their hosts: studies in coevolution*. Oxford University Press, New York. pp. 3–56.
- Rothstein, S.I., Patten, M.A., and Fleischer, R.C. 2002. Phylogeny, specialization, and brood parasite–host coevolution: some possible pitfalls of parsimony. *Behav. Ecol.* **13**: 1–10. doi:10.1093/beheco/13.1.1.
- Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Scott, D.M. 1977. Cowbird parasitism on the gray catbird at London, Ontario. *Auk*, **94**: 18–27.

- Scott, D.M. 1991. The time of day of egg laying by the brown-headed cowbird and other icterines. *Can. J. Zool.* **69**: 2093–2099.
- Sealy, S.G., and Lorenzana, J.C. 1997. Feeding of nestling and fledgling brood parasites by individuals other than the foster parents: a review. *Can. J. Zool.* **75**: 1739–1752.
- Sealy, S.G., and Underwood, T.J. 2004. Accepters and rejecters of cowbird parasitism in the New World orioles (*Icterus* spp.). *Ornitol. Neotrop.* **15**: 331–347.
- Sealy, S.G., Sánchez, J.E., Campos, R.G., and Marin, M. 1997. Bronzed cowbird hosts: new records, trends in host use, and cost of parasitism. *Ornitol. Neotrop.* **8**: 175–184.
- Sick, H. 1993. *Birds in Brazil*. Princeton University Press, Princeton, N.J.
- Skjelseth, S., Moksnes, A., Røskaft, E., Gibbs, H.L., Taborsky, M., Taborsky, B., Honza, M., and Kleven, O. 2004. Parentage and host preference in the common cuckoo *Cuculus canorus*. *J. Avian Biol.* **35**: 21–24. doi:10.1111/j.0908-8857.2004.03219.x.
- Smith, J.N.M., and Arcese, P. 1994. Brown-headed cowbirds and an island population of song sparrows: a 16-year study. *Condor*, **96**: 916–934.
- Strausberger, B.M. 1998. Temporal patterns of host availability, brown-headed cowbird brood parasitism, and parasite egg mass. *Oecologia*, **116**: 267–274. doi:10.1007/s004420050588.
- Strausberger, B.M., and Ashley, M.V. 2001. Eggs yield nuclear DNA from egg-laying female cowbirds, their embryos and offspring. *Conserv. Genet.* **2**: 385–390. doi:10.1023/A:1012526315617.
- Strausberger, B.M., and Ashley, M.V. 2005. Host use strategies of individual brown-headed cowbirds *Molothrus ater* in a diverse avian community. *J. Avian Biol.* **36**: 313–321. doi:10.1111/j.0908-8857.2005.03323.x.
- Sturkie, P.D. 1976. *Avian physiology*. 3rd ed. Springer-Verlag, New York.
- Teuschl, Y., Taborsky, B., and Taborsky, M. 1998. How do cuckoos find their hosts? The role of habitat imprinting. *Anim. Behav.* **56**: 1425–1433. doi:10.1006/anbe.1998.0931. PMID:9933539.
- Tewksbury, J.J., Martin, T.E., Hejl, S.J., Kuehn, M.J., and Jenkins, J.W. 2002. Parental care of a cowbird host: caught between the costs of egg-removal and nest predation. *Proc. R. Soc. Lond. B Biol. Sci.* **269**: 423–429.
- Underwood, T.J., Sealy, S.G., and McLaren, C.M. 2004. Experiments on egg discrimination in two North American corvids: further evidence for retention of egg ejection. *Can. J. Zool.* **82**: 1399–1407. doi:10.1139/z04-118.
- Vogl, W., Taborsky, M., Taborsky, B., Teuschl, Y., and Honza, M. 2002. Cuckoo females preferentially use specific habitats when searching for host nests. *Anim. Behav.* **64**: 843–850. doi:10.1006/anbe.2003.1967.
- Weatherhead, P.J. 1989. Sex ratios, host-specific reproductive success, and impact of brown-headed cowbirds. *Auk*, **106**: 358–366.
- Wiley, J.W. 1988. Host selection by the shiny cowbird. *Condor*, **90**: 289–303.
- Woodward, P.W. 1983. Behavioral ecology of fledgling brown-headed cowbirds and their hosts. *Condor*, **85**: 151–163.
- Woolfenden, B.E., Gibbs, H.L., Sealy, S.G., and McMaster, D.G. 2003. Host use and fecundity of individual female brown-headed cowbirds. *Anim. Behav.* **66**: 95–106. doi:10.1006/anbe.2003.2181.