Nest Sanitation Plays a Role in Egg Burial by Yellow Warblers

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Abstract

Some hosts of the brown-headed cowbird (*Molothrus ater*) possess defences that eliminate all or most parasitism costs. Yellow warblers (*Dendroica petechia*) bury cowbird eggs, possibly to clean nests rather than serving strictly as an anti-parasite defence, as non-egg-shaped objects have been ejected, buried, or deserted by other hosts. With two experiments, we tested the 'nest sanitation' hypothesis by recording warblers' responses to objects (1) similar in volume, mass, and colour to cowbird eggs, and (2) half the mass and volume (more easily ejected), placed into nests before and during incubation. We compared outcomes at control nests with responses to objects that were dissimilar (stars) and moderately similar (dumbbells) to eggs, and to real cowbird and warbler eggs. We tested whether rejection (1) declines from stars through dumbbells and real eggs, (2) is similar between stages, and (3) non-egg-shaped objects are ejected because this is the least costly rejection method. Large stars were rejected (most buried) significantly more frequently (43.8%) than cowbird eggs (16.3%) in pre-incubation, suggesting that warblers reject objects shaped unlike their own eggs to rid nests of debris. Objects spent less time in nests the more they diverged from eggs. Warblers rarely rejected large stars and dumbbells, and cowbird eggs during incubation, possibly because burial and desertion are too costly by this time. Responses to small stars and dumbbells, and to foreign yellow warbler eggs did not differ between stages; also warblers rejected stars, mostly by ejection and selective burial, more frequently (28.8%) than dumbbells (1.3%) and warbler eggs (0%). Rejection by yellow warblers, especially burial, may keep nests clean, but also functions in rejecting cowbird eggs.

Introduction

Obligate brood parasites foist their young on other species of birds, which rear the parasitic young, but the hosts often raise fewer or no young of their own. The frequently parasitized yellow warbler (*Dendroica petechia*) is among the hosts whose productivity is reduced (Goossen & Sealy 1982; Lorenzana & Sealy 1999), with up to 40% of all egg losses attributable to parasitism by the brown-headed cowbird (*Molothrus ater*) (Burgham & Picman 1989). Despite similar costs, most hosts accept cowbird eggs, but a

few have evolved defences that minimize or eliminate the costs of parasitism, such as egg ejection (Rothstein 1975; Peer & Sealy 2004), desertion followed by re-location of the replacement nest (Graham 1988; Hosoi & Rothstein 2000), and egg burial at the original nest (Sealy 1995; Moskát et al. 2003). Hosts must discriminate between their own eggs and parasitic eggs before ejection can occur, whereas desertion and burial require only that hosts recognize that their nests have been parasitized (Rothstein 1975; Sealy & Lorenzana 1998; Hosoi & Rothstein 2000).

Only yellow warblers bury cowbird eggs and their own eggs regularly, under new nests built on top of the old nests and their contents (Clark & Robertson 1981; Sealy 1995; Mico 1998). If parasitism occurs early in the laying cycle, about half the females bury, which suggests egg burial is an adaptive response to parasitism (Clark & Robertson 1981; Burgham & Picman 1989; Sealy 1995). The mean success of unparasitized nests vs. nests with buried cowbird eggs is similar (0.80 ± 0.16 vs. 0.78 ± 0.21 fledgling per egg, Mann–Whitney U-test, U for large samples = 0.85, p > 0.05), which suggests nest burial is an effective strategy to minimize costs, despite lost time (Clark & Robertson 1981). Desertion and ejection have been recorded infrequently (Sealy 1995), but because of acceptance of all or most model cowbird eggs at experimentally parasitized nests (approx. 37% burial at naturally parasitized nests vs. approx. 4% at experimental nests, parasitized on the warblers' first laying day), this species has been considered an accepter (Rothstein 1975; see also Sealy 1995). To test whether the stimulus for burial is the cowbird and interactions with it at nests, Sealy (1995) placed a laying female cowbird on the nest at the time of parasitism. Even after warblers had interacted with the models, only approx. 8% of clutches were buried.

One host of the common cuckoo (*Cuculus canorus*), the great reed warbler (*Acrocephalus arundinaceus*), is approx. three times larger but is ecologically similar to the yellow warbler (Cramp 1992; Lowther et al. 1999). Great reed warblers eject cuckoo eggs but also bury some eggs and desert some nests (Moskát et al. 2003). A combination of these rejection responses has been recorded after non-egg-shaped objects were placed in their nests, which lead Bártol et al. (2003) and Moskát et al. (2003) to consider that nest sanitation may play a role in egg rejection behaviour. By ridding nests of debris such as leaves, twigs, and faecal sacs, birds reduce their vulnerability to predators and their attraction to arthropods, protect eggs from physical damage, keep nests warm and dry, and remove threats to the health of adults and nestlings (Herrick 1900; Blair & Tucker 1941; Nethersole-Thompson & Nethersole-Thompson 1942; Petit et al. 1989; Moskát et al. 2003). Also, nutritional benefits accrue from eating eggshells and faecal sacs (Nethersole-Thompson & Nethersole-Thompson 1942; Glück 1988). Motor patterns involved in keeping nests clean and ejecting eggs are similar (Rothstein 1975) and would act as anti-parasite defences when released in response to parasitic eggs.

Swynnerton (1918) was the first to draw attention to the similarity between nest sanitation and egg ejection. Several workers have since added non-egg-shaped objects to nests and most have implicated nest sanitation in rejection behaviour (Alvarez et al. 1976; Ortega & Cruz 1988; Ortega et al. 1993; Bártol et al. 2003; Moskát et al. 2003; Underwood & Sealy 2006). Rothstein (1975) hypothesized that egg ejection evolved from nest sanitation, but he and Peer & Sealy (2004) found no relationship between these behaviours. Bártol et al. (2003) and Moskát et al. (2003), however, suggested that rejection is an extension of nest sanitation because great reed warblers rejected more disk- and stick-shaped objects than model cuckoo eggs, and egg-shaped and non-egg-shaped objects were rejected equally before and during incubation, which further supports the hypothesis (Moskát et al. 2003). Debris probably falls into bird nests similarly throughout the pre-nestling stages (Moskát et al. 2003), hence, hosts should clean their nests throughout this period. Moskát et al. (2003:18) concluded that nest sanitation may represent 'an important evolutionary stage towards rejection of parasitic eggs'. Egg rejection should be understood at two levels: (1) all non-egg-shaped objects are rejected within the context of nest sanitation behaviour, and (2) parasitic eggs are rejected after the host has acquired the ability to recognize them (Moskát et al. 2003). A possible exception, however, is the black-billed cuckoo (*Coccyzus erythropthalmus*), an intermediate egg rejecter, in which the removal of eggshells varies from nest to nest (Herrick 1910; Spencer 1943; Rothstein 1975; Peer et al. 2007).

Shape influences egg rejection (Ortega & Cruz 1988; Moskát et al. 2003; Underwood & Sealy 2006). Ortega & Cruz (1988) reported that shape is a component of acceptance because non-egg-shaped objects were ejected by two accepter species, red-winged blackbird (*Agelaius phoeniceus*) and yellow-headed blackbird (*Xanthocephalus xanthocephalus*). Ortega et al. (1993) reported, however, that some non-egg-shaped objects were accepted, possibly because the beads used in an earlier experiment (Ortega & Cruz 1988) had holes through them that may have been perceived as damaged 'eggs' (see Kemal & Rothstein 1988). Hosts may use a 'rule-of-thumb': reject objects dissimilar to their own eggs and accept similar ones (Moskát et al. 2003:18).

Nest Sanitation Hypothesis

We tested the hypothesis that yellow warblers remove objects to clean their nests, behaviour that is both costly and beneficial. Several predictions arise

from the 'nest sanitation' hypothesis: (1) before and during incubation, objects least similar to eggs and, hence, most resembling debris, will be rejected more frequently than moderately similar objects; the latter will be rejected more than egg-shaped objects, (2) non-egg-shaped objects will be rejected at similar frequencies in both nesting stages, and (3) ejection is the most common method of rejection for non-egg-shaped objects throughout because it is less costly than burial and desertion. The thinner non-egg-shaped objects are likely more distinguishable from host eggs and more easily grasped than cowbird and warbler eggs, which would facilitate ejection. Burial and desertion are less costly when they occur before incubation has begun (Clark & Robertson 1981), but delays and increased energy expenditure associated with burial and desertion could be avoided if warblers eject objects during both stages.

Methods

Study Area and Species

We conducted two field experiments, the first in May and June 2006, the second during those months in 2007, in the forested dune ridge that separates Lake Manitoba and Delta Marsh, Manitoba, Canada (50°11′N, 98°23′W), on the properties of the Delta Marsh Field Station (University of Manitoba), Portage Country Club, Delta Waterfowl Foundation, and Bell Estate (study area described in MacKenzie 1982). Yellow warblers and brown-headed cowbirds are common around Delta Marsh and have been recorded in this area since before settlement by Europeans (Thompson 1891). From the 1970s through mid-1990s, the frequency of parasitism on yellow warblers at Delta Marsh averaged 21% (approx. 2000 nests sampled), but has varied from year to year (Sealy 1995). The frequency of parasitism has decreased since, but it is still positively correlated with host abundance (Woolfenden et al. 2004).

Objects

We added wooden stars and dumbbells (Fig. 1) to yellow warbler nests. Ortega et al. (1993) found that more than 91% of star-shaped objects were rejected by red-winged blackbirds. Flat, with many edges, a star's shape is not like an egg's (Fig. 1), thus we expected them to be rejected. We smoothed edges so that stars would not be like broken egg shells because passerine birds, including species that accept
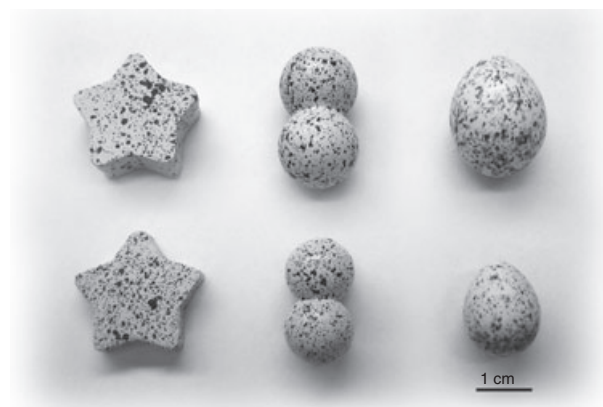

Fig. 1: Experimental objects added to yellow warbler nests. From left to right, top row: large star, large dumbbell, and real cowbird egg. From left to right, bottom row: small star, small dumbbell, and real yellow warbler egg.

cowbird eggs, remove broken eggs (Kemal & Rothstein 1988). This behaviour may be strictly an adaptive response to broken eggs, rather than generalized nest sanitation behaviour (Kemal & Rothstein 1988). Dumbbells were two spheres glued together at their flat ends, which made them look more like an egg than a star (Fig. 1).

In expt 1, we compared responses at nests that received large stars, large dumbbells, and cowbird eggs (treatments 1–3). Stars and dumbbells were the same colour, texture, mass, and volume of real cowbird eggs (Fig. 1; Table 1). A control group (treatment 4) consisted of nests in which warbler eggs were touched daily. Data were available from Sealy (1995) for treatments 3 and 4, for comparison with results of treatments 1 and 2. Sealy (1995) used real cowbird eggs in treatment 3; however, hosts respond similarly to real or model cowbird eggs (Rothstein 1975; Hill & Sealy 1994). Ranges of mass of large stars (3.0–3.2 g) and large dumbbells (2.9–3.2 g) were within that of cowbird eggs (2.1–3.9 g) collected from warbler nests at Delta Marsh (Sealy 1992). Volumes of large stars (2.8–3.2 ml) and large dumbbells (2.8–3.2 ml) were also within the range of cowbird egg volumes (1.9–3.7 ml; Sealy 1992).

Stars and dumbbells half the mass and volume of cowbird eggs (hereafter, small stars and small dumbbells) were added to nests in expt 2. Responses to these objects were compared to control nests (Sealy 1995) and data from a study of intraspecific brood parasitism in which foreign yellow warbler eggs were added to nests (Sealy et al. 1989). Masses and volumes of small stars (1.4–1.7 g and 1.3–1.6 ml) and dumbbells (1.5–1.8 g and 1.4–1.7 ml) were

Object	n	Length	Width	Volume	Mass
Large five-pointed star	80	21.58 ± 0.03[a]	9.70 ± 0.03[b]	2.91 ± 0.01	3.08 ± 0.01
Large dumbbell	80	26.65 ± 0.02	14.47 ± 0.01[c] 9.17 ± 0.02[d]	2.99 ± 0.01	3.09 ± 0.01
Cowbird egg[e]	77	21.07 ± 0.12	16.36 ± 0.09	2.92 ± 0.04	3.14 ± 0.04
Small five-pointed star	90	21.32 ± 0.03[a]	4.96 ± 0.01[b]	1.41 ± 0.01	1.61 ± 0.01
Small dumbbell	90	21.90 ± 0.02	12.03 ± 0.02[c] 7.55 ± 0.03[d]	1.56 ± 0.01	1.74 ± 0.01
Yellow warbler egg[e]	85	16.80 ± 0.08	12.60 ± 0.04	1.33 ± 0.01	1.43 ± 0.01

Table 1: Mean (± SE) measurements (mm), volume (ml), and mass (g) of non-egg-shaped objects added to yellow warbler nests in 2006 and 2007, and of a sample of cowbird and yellow warbler eggs

[a]Average of the maximum length of each star.
[b]Thickness.
[c]Maximum width.
[d]Minimum width.
[e]Data from Sealy (1992). The exterior volume was calculated using the equation $V = K_v LB^2$ with $K_v = 0.515$ for cowbird eggs (Nolan & Thompson 1978) and $K_v = 0.497$ for yellow warbler eggs (McMaster & Sealy 1997; but see Hoyt 1979), where L = length and B = breath or width.

within the range of half the mass (1.1–2.0 g) and half the volume (1.0–1.9 ml) of cowbird eggs. Although yellow warbler eggs weigh slightly less than half that of cowbird egg models (Table 1), their ranges in mass (1.2–1.7 g) and volume (1.1–1.6 ml) are similar to those of the small models.

All objects were painted with non-toxic, water-resistant acrylic paints (Folk Art Opaque Acrylic Paints by Plaid®; Plaid Enterprises Inc., Norcross, GA, USA; product numbers and colours below) to resemble cowbird eggs. The background of all objects was off-white (Wicker White 1643 mixed with Licorice 1506) and objects had brownish markings (Burnt Umber 1618 mixed with Wicker White). The colours were mixed to resemble closely the colouration of real cowbird eggs (at the same time they also resembled yellow warbler eggs; Fig. 1). Realistic markings were created by spattering spots on the objects with a toothbrush dipped in paint. Volumes of all stars and dumbbells were measured by water displacement and the texture of the dried objects was smooth, as in a real cowbird egg (Ortega & Cruz 1988). Stars and dumbbells, therefore, differed from cowbird and warbler eggs only in shape.

Introduction of Objects

We randomly added stars and dumbbells to nests between 07:00 and 12:00 h (Central Daylight Time) to avoid interfering with laying (cowbirds lay shortly before sunrise, yellow warblers approx. 1 h later; McMaster et al. 2004), in accordance with Sealy's (1995) protocol. We added one object to each nest, used each object only once, tested each nest only once, and placed stars and dumbbells flat in nests. We did not test nests in which naturally laid cowbird

eggs had been buried. We added objects to nests through 15 June. We flagged each nest with numbered tape approx. 1.5 m from the nest (Sealy 1995).

As the rejection frequency of cowbird eggs decreases over the warbler laying cycle (Clark & Robertson 1981; Burgham & Picman 1989; Sealy 1995), the two testing periods (i.e. before and during incubation) in the breeding cycle were separated to increase the probability of detecting differences in rejection frequencies between these periods. Objects for expt 1 were added in pre-incubation when nests contained zero (completed nest), one, or two yellow warbler eggs and an average of 1.2 eggs (± 0.10 SE, n = 40) for stars and 1.1 eggs (± 0.07 SE, n = 40) for dumbbells. Responses to real cowbird eggs during the pre-incubation stage were also noted when there were no eggs in the nest, or one or two warbler eggs (Sealy 1995). For expt 2, all pre-incubation nests contained one warbler egg when parasitized with objects. Data from Sealy et al. (1989) included responses to foreign yellow warbler eggs when nests contained 0, 1, or 2 eggs when parasitized because the significance of results did not change when only responses at the 1-egg stage were included.

Objects introduced during incubation were timed to prevent the 6-d acceptance period (see criteria below) from extending into the nestling stage. The period between laying of the last egg and hatching of the first nestling is approx. 9 d (Goossen & Sealy 1982). Therefore, objects were added 1–3 d after the last eggs were laid. When nest histories were not known, the stages of development of eggs were determined by candling (Lokemoen & Koford 1996) to assign the appropriate time to add the object.

Criteria for Acceptance and Rejection

We considered objects accepted if warbler clutches were completed or incubation continued for 6 d and objects remained in nests during this period (Sealy 1995). This is a trade-off between excluding rejections related to factors other than parasitism and includes rejections delayed due to manipulation. Although there are exceptions (Peer & Sealy 2000), rejection responses after 5 d generally occur <1% of the time (Rothstein 1982). Each nest was checked every 24 h after the object was added (Sealy 1995). If an object was ejected, buried or the nest was deserted, we visited the nest for a few more days to confirm the type of response because sometimes burial takes more than 1 d to complete; a nest may seem deserted 1 d, but the adults may be tending the eggs the next day (M. F. Guigueno & S. G. Sealy, pers. obs.). At the end of the testing period, we removed unburied objects and later looked for peck marks on them under a microscope; peck marks would suggest failed attempts by warblers to puncture-eject (Rothstein 1977). Objects damaged but left in the nest would be considered rejected (e.g. Rothstein 1977; Peer et al. 2000). Some nests in incubation were depredated on day 5 or 6, but were still considered acceptances because this reduced bias towards rejection, and their inclusion did not change the significance of the results.

Desertion was recorded when the parents were no longer observed close to the nest for 3 consecutive days, and the eggs were cold, or if the warbler laid after the interference but never incubated (Sealy 1992, 1995; see also Moskát et al. 2003). We included desertions in the statistical analyses (Sealy 1995). Ejection was recorded if the object was gone but warbler eggs were still tended by the adults (Rothstein 1975). Sealy (1995) did not include ejections in analyses because yellow warblers were assumed to be unable to grasp- or puncture-eject cowbird eggs (e.g. Clark & Robertson 1981; Graham 1988), although grasping them was a possibility (Rothstein 1975), and they can lift them (Sealy & Lorenzana 1998). Stars and dumbbells also are not quite as wide as cowbird eggs (Table 1), and the objects from expt 2 were also half the volume and mass of cowbird eggs, which should increase the ease of ejection. Unlike Sealy (1995), therefore, we included ejections in the statistical analyses.

Statistical Analyses

To detect differences in rejection frequencies between stages, we used two-tailed tests because we predicted no difference between pre-incubation and incubation. If the expected frequency of all cells was more than five, a chi-square test with continuity correction was used, but a Fisher exact test was used when sample sizes were smaller (Zar 1996). To detect differences in rejection method between stages, two-tailed Fisher exact tests were also used.

To compare responses among all objects, 3×2 Fisher exact tests (two-tailed; Grieef & Sealy 2000) or 3×2 chi-square tests were used, depending on the expected frequency of each cell (Zar 1996). To determine precisely which paired comparison was significant, we conducted one-tailed Fisher exact tests because we predicted that stars would be rejected more frequently than dumbbells, which, in turn, would be rejected more frequently than cowbird or warbler eggs (Underwood & Sealy 2006). Multiple pairwise comparisons were conducted on the same set of data (Moksnes & Røskaft 1989); therefore, sequential Bonferroni corrections were used, which have an increased statistical power over the standard Bonferroni correction (Rice 1989). Using Turbo Pascal (Rice 1989), we corrected the p-values from the pairwise comparisons to maintain an alpha at 0.05. For the multiple pairwise comparisons, a corrected $p \leq 0.05$ was considered significant, and for all other tests, $p \leq 0.05$ was significant.

We used LIFETEST procedure in sas (SAS Institute Inc. 1990) to analyse time-to-rejection data and to determine whether survivorship was affected by nesting stage and object shape. Using this analysis, we examined the daily proportion of objects 'alive' (i.e. accepted) over the testing period, and it allowed us to include nests depredated or naturally parasitized before the end of the period. LIFETEST uses two statistical analyses to place weight on the early portion (Wilcoxon-rank test) and later portion (log-rank test) of the survival curve (Underwood & Sealy 2006). Survival curves of object types and nesting stages (pre-incubation and incubation) were compared using these two rank tests.

Results

None of the accepted objects had peck marks. As yellow warblers did not eject non-egg-shaped objects (stars, least egg-shaped and dumbbells, moderately egg-shaped) of the same volume and mass as cowbird eggs, as predicted (expt 1), we conducted another experiment the next year with smaller and lighter objects (expt 2).

Experiment 1

Rejection frequencies differed significantly between stages for stars, dumbbells, and cowbird eggs (Table 2). In pre-incubation, large objects were rejected significantly more frequently at nests receiving a star, dumbbell, or cowbird egg than at control nests (Table 3). Rejection differed significantly among all objects in pre-incubation ($\chi^2 = 11.467$, df = 2, p = 0.003); stars were rejected significantly more frequently than cowbird eggs, but not more than dumbbells (Table 3). Dumbbells were not rejected more frequently than cowbird eggs (Table 3). Few large objects were rejected during incubation (Table 2); rejections were not significantly more frequent at nests that received a star, dumbbell, or cowbird egg, than at control nests (Table 3). Also, rejection did not differ among objects at this stage (Fisher exact test, p = 0.332; Table 3).

Burial was the most common rejection method (65.2%). When nests contained at least one warbler egg at the time of parasitism, yellow warblers always buried the debris-like objects (large stars), large

dumbbells, or cowbird eggs along with their own eggs.

Nest stage influenced survival (i.e. time-to-rejection) of large objects (log-rank test, $\chi^2 = 17.320$, df = 1, p < 0.0001; Wilcoxon-rank test, $\chi^2 = 16.813$, df = 1, p < 0.0001) and, in pre-incubation, as shape increasingly differed from that of an egg, object survival decreased. Thus, shape-influenced survival of large objects in pre-incubation (log-rank test,

Table 3: Corrected p-values for one-tailed Fisher exact tests comparing rejection frequencies between groups

Groups	Large		Small
	Pre-incubation	Incubation	
Control vs.			
Stars	$<1.0 \times 10^{-8}$	0.654	$<1.0 \times 10^{-8}$
Dumbbells	1.8×10^{-5}	1.000	1.000
Eggs	6.8×10^{-7}	1.000	1.000
Stars vs. dumbbells	0.138	1.000	2.1×10^{-6}
Stars vs. eggs	0.004	1.000	1.2×10^{-4}
Dumbbells vs. eggs	0.227	1.000	1.000

Large and small eggs are actual cowbird and yellow warbler eggs, respectively.

	Large			Small		
	Pre-incubation	Incubation	p	Pre-incubation	Incubation	p
Stars						
Rejection (%)	14 (43.8)	2 (5.9)	0.001[b]	10 (30.3)	11 (27.5)	0.994[b]
Burial	12	1		5	0	
Desertion	2	1		1	0	
Ejection	0	0		4	11	
Acceptance	18	32		23	29	
Total nests	32	34		33	40	
Dumbbells						
Rejection (%)	8 (23.5)	0 (0)	0.005	1 (2.8)	0 (0)	0.450
Burial	7	0		0	0	
Desertion	0	0		0	0	
Ejection	1	0		1	0	
Acceptance	26	34		35	44	
Total nests	34	34		36	44	
Eggs[c]						
Rejection (%)	22 (16.3)	0 (0)	0.026	0 (0)	0 (0)	1.000
Burial	10	0		0	0	
Desertion	5	0		0	0	
Ejection	7	0		0	0	
Acceptance	113	25		29	11	
Total nests	135	25		29	11	

Table 2: Responses to single objects added experimentally to yellow warbler nests at different nesting stages[a] and p-values of two-tailed Fisher exact tests analysing differences in rejection frequencies between stages

[a]Two (1.1%) of 192 control nests (Sealy 1995) were deserted.
[b]p-value for chi-square test with continuity correction (large: $\chi_1^2 = 10.892$; small: $\chi_1^2 = 5.064 \times 10^{-5}$).
[c]Data on large (cowbird) and small (yellow warbler) eggs from Sealy (1995) and Sealy et al. (1989), respectively.

$\chi^2 = 12.185$, df = 2, p = 0.002; Wilcoxon-rank test, $\chi^2 = 12.747$, df = 2, p = 0.002), but not during incubation (log-rank test, $\chi^2 = 3.352$, df = 2, p = 0.187; Wilcoxon-rank test, $\chi^2 = 3.348$, df = 2, p = 0.188).

Experiment 2

Rejection frequencies did not differ between stages for stars, dumbbells, or yellow warbler eggs when using small objects (Table 2), hence, data were pooled and rejection frequencies were compared between groups. Only rejection of stars was significantly greater than at control nests. Among objects, rejection differed significantly (Fisher exact test, p = 7.962×10^{-9}), with stars rejected more frequently than dumbbells and warbler eggs (Table 3). Dumbbells were not rejected more frequently than yellow warbler eggs (Table 3).

For small objects, nest stage did not affect the survival of objects (log-rank test, $\chi^2 = 0.003$, df = 1, p = 0.954; Wilcoxon-rank test, $\chi^2 = 0.008$, df = 1, p = 0.929), whereas the shape did (log-rank test, $\chi^2 = 31.851$, df = 2, p < 0.0001; Wilcoxon-rank test, $\chi^2 = 32.346$, df = 2, p < 0.0001). Stars remained in nests for less time than dumbbells and warbler eggs.

Methods of rejection of small stars differed significantly between stages (Fisher exact test, two-tailed, p = 0.004); burials, ejections and one desertion were reported for pre-incubation, whereas only ejections were recorded during incubation (Table 2). Of the five small stars buried before incubation, three were buried, but the warbler eggs remained on top of the lining and thus the clutch remained intact, the latter being similar to ejections. By comparing desertions and burials to ejections and 'selective burials,' the methods of rejection no longer differed significantly between stages for small stars (Fisher exact test,

Fig. 2: Female yellow warbler grasp-ejecting a small star by its point.

two-tailed, p = 0.090). To confirm ejections, we re-parasitized with stars and video-recorded in one morning three nests at which ejections had been recorded previously. Two successive grasp-ejections were recorded at one of these nests: the first one occurring 25 min 51 s after the female first peered into the nest after parasitism, and the second ejection occurred 23 s later. For both ejections, the female sat on its clutch before removing the small star. It grasped the star by one point during the first ejection (Fig. 2) and between two points during the second.

Discussion

Our results suggest that nest sanitation is important in the yellow warbler's egg rejection behaviour, especially burial. Small and large stars, which most resembled debris, were rejected more frequently than eggs, and the frequencies did not differ between stages in the nesting cycle when the cost of rejection was constant. When costs differed, however, time and energy investments apparently determined whether objects were rejected, especially large ones.

Most rejected large stars were buried along with the hosts' own eggs, but this may have been a general response. Yellow warblers bury experimentally added model eggs of black-billed cuckoo and real eggs of American robin (*Turdus migratorius*), both of which are larger and heavier than cowbird and warbler eggs (Rothstein 1982; Stewart 2003). Bártol et al. (2003) recorded burial of round, flattened objects (coins) by great reed warblers. In our study, burial of large stars may have been for sanitation because stars were not round and other cues, such as the sight of a cowbird at the nest, were absent.

Great reed warblers deserted more nests during pre-incubation than incubation, whereas ejection increased later (Moskát et al. 2003), probably because the costs of desertion and burial became too great. Great reed warblers could have ejected earlier in the nesting stage and avoided all of the costs of burial. Unable to manipulate large stars and dumbbells, yellow warblers apparently were left with two options, the more costly burial or desertion (Ortega & Cruz 1988). Before incubation, costs of rejection of objects by burial and desertion were outweighed by costs of not cleaning the nest.

In expt 2, yellow warblers rejected only smaller stars, possibly because they differed enough from warbler eggs. A tactile cue seems necessary for rejection by yellow warblers because, in both

grasp-ejections we video-recorded, the female sat on the clutch before ejection. Although yellow warblers apparently do not recognize their own eggs visually (Sealy & Lorenzana 1998), they may recognize them tactilely. Non-egg-shaped objects may have provided the cue for discrimination between the warblers' own eggs and the foreign objects.

We cannot conclude, however, that selection from parasitism has not affected the egg rejection behaviour of the yellow warbler, because there is strong evidence for its influence on the evolution of egg rejection (Rothstein & Robinson 1998). During laying, yellow warblers respond most aggressively to female brown-headed cowbirds, which suggests the selection for the evolution of certain anti-parasite behaviours (Briskie et al. 1992; Gill & Sealy 2004). Because the frequency of egg rejection is much higher at naturally parasitized nests than at experimentally parasitized nests, yellow warblers apparently respond to something besides a cowbird egg in their nests. Perhaps this includes the presence of a live cowbird (Sealy 1995; see also Hosoi & Rothstein 2000). Egg rejection in the yellow warbler may be more than just an adaptation, but an exaptation, a behaviour that evolved from benefits accrued by cleaning the nest, but this behaviour is now also adaptive in the context of brood parasitism (Mumme & Koenig 1991).

Peer et al. (2007) presented nest sanitation behaviour as an alternative explanation for the maintenance of egg rejection in island scrub-jay (*Aphelocoma insularis*) and western scrub-jay (*Aphelocoma californica*) populations that are not and were not sympatric with cowbirds. These authors deemed this possibility unlikely, however, because nearly all North American passerines practice nest sanitation, but relatively few reject eggs. Therefore, nest sanitation behaviour alone cannot explain egg rejection behaviour.

Implications for Other Hosts

Results from this study have implications for other host species. Species that usually eject cowbird eggs should eject non-egg-shaped objects of the same volume and mass similarly between pre-nestling stages, as their bill size would not impose the use of a more costly method of rejection, such as burial. The least costly form of rejection, therefore, should always be used. Underwood & Sealy (2006) reported gray catbirds (*Dumetella carolinensis*) and American robins rejected non-egg-shaped objects at similar frequencies during laying and incubation. Ejection was the more common method (95.4% for robins, 97.1% for

catbirds; Underwood & Sealy 2006). Similar results would be expected with other ejecter species such as the eastern kingbird (*Tyrannus tyrannus*). We would predict a higher rejection frequency of debris-like objects compared with egg-shaped objects throughout the pre-nestling stages. American robins and gray catbirds ejected more non-egg-shaped objects, and sooner, than those shaped like eggs (Underwood & Sealy 2006). Eurasian magpies (*Pica pica*) ejected cubes sooner than egg-shaped objects (Alvarez et al. 1976).

An increased ability to detect foreign objects could be acquired by stronger selection for improved egg recognition (Moskát et al. 2003; Underwood & Sealy 2006). Therefore, additional experiments should provide more insight into the relationship between anti-parasite behaviours and nest sanitation in yellow warblers and other host species. Our experiments should be repeated in an unparasitized population of yellow warblers, to compare results to those from the present study to determine whether the rejection frequencies of non-egg-shaped objects have increased in the Delta Marsh population as a side effect of pressure to combat the costs of parasitism. Similarly, comparison of responses of a closely related species-pair (one species an accepter, the other a rejecter) should be conducted to determine whether the presence of anti-parasite defences has an effect on the rejection frequency of non-egg-shaped objects.

Acknowledgements

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