Spatial patterns of shiny cowbird brood parasitism on chestnut-capped blackbirds

BRUCE E. LYON

Kananaskis Field Stations, Department of Biological Sciences, University of Calgary

Abstract. Shiny cowbirds, *Molothrus bonariensis*, parasitized a high frequency (average 48%) of nests in five colonies of chestnut-capped blackbirds, *Agelaius ruficapillus*, in Argentina. Two distinct egg morphs occurred, spotted and immaculate white eggs, as well as a few very lightly spotted intermediate eggs. The clear differences between morphs, combined with considerable variation in spotting pattern between spotted morph eggs, made it possible to visually match eggs into groups that were probably laid by single females. Eggs attributed to a single female were more similar in size and shape than eggs attributed to different females. Using egg dimensions and colour patterns to infer spatial patterns of laying by individual females indicated that: (1) individual brood parasites often laid several eggs in the same colony, (2) females also laid eggs in more than one colony, and (3) several females laid eggs in each colony, and often in the same host nests, ruling out the notion that parasites defend exclusive territories with respect to host nests. Multiple cowbird eggs per host nest invariably resulted from several females laying in the same host nest: egg morphs and visual comparisons of spotting patterns at 14 nests indicated that individual females never laid more than a single egg in the same host nest. Experimental parasitism of nests with spotted and white morph eggs, combined with observations of naturally parasitized nests, demonstrated that chestnut-capped blackbirds accept all morphs of cowbird eggs. Since other important host species reject white eggs, however, the adaptive maintenance of the white morph is difficult to explain.

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Obligate brood parasites are completely dependent on other species to raise their offspring. This form of parasitism occurs in 80 species of birds and spans a number of taxonomic groups, including waterfowl, honeyguides, cuckoos, weaver finches and cowbirds (Hamilton & Orians 1965; Lack 1968; Payne 1977; Lyon & Eadie 1991). The close and hostile relationship between obligate parasites and their hosts has made brood parasitism a model system for studying coevolution, particularly in cowbirds and cuckoos (Mason & Rothstein 1986; Brooke & Davies 1989; Rothstein 1990). Owing to the logistical ease of nest-centred studies, however, coupled with the difficulties of monitoring the laying histories and behaviours of individual parasitic females, much more is known about the effect of parasitism on hosts, and the defences hosts adopt to mitigate these costs, than

about the tactics and behaviours of parasites (but see Chance 1940; Davies & Brooke 1988). For example, with respect to spatial patterns of egg laying by individual parasites, it is unclear whether parasitic females defend territories containing host nests. Moreover, the scant information that does exist yields contradictory conclusions (e.g. Dufty 1982; Fleischer 1985). Multiple parasitism, whereby host nests receive several parasitic eggs, is also common in some parasitic species (Friedmann 1929; Payne 1977; Mason 1986b), but in most cases it is unknown whether this reflects single females laying several eggs in the same host nest or multiple females each laying a single egg (but see McGeen & McGeen 1968; Fleischer 1985).

In this study, I examined spatial patterns of egg laying and the basis of multiple parasitism in shiny cowbirds, *Molothrus bonariensis*, parasitizing colonies of chestnut-capped blackbirds, *Agelaius ruficapillus*, in Argentina. Shiny cowbirds are extreme host generalists, with records of

Correspondence: B. E. Lyon, Biology Department, Earth and Marine Sciences Building, University of California, Santa Cruz, CA 95064 (email: lyon@ biology.ucsc.edu).

parasitism for 200 host species, a pattern that is similar to the well-studied brown-headed cowbird, *M. ater* (Friedmann & Kiff 1985). In a few parasitic bird species (some cuckoos), coevolution with the hosts has led to egg rejection by hosts, followed by the subsequent evolution of several gentes of parasites, each laying a different type of mimetic egg (Brooke & Davies 1989). Brown-headed cowbirds do not show this type of mimetic variation, but shiny cowbirds lay two distinct morphs, heavily spotted and immaculate white eggs (Friedmann 1929; Fraga 1978; Mason 1986b), suggesting that they may be in the early stages of host–race specialization. In addition to the possibility of providing insights into the early stages of host–race specialization, this variation in egg features provides a useful tool for inferring spatial patterns of laying by individual females.

I examined spatial patterns of laying as inferred from two levels of variation in egg features; (1) variation in spotting patterns within the spotted morph and (2) variation between morphs. Studies in a variety of taxa have shown that visual comparisons can be used to match eggs laid by the same female with sufficient accuracy to investigate the laying tactics of individual brood parasites (Gibbons 1986; Jackson 1992; Lyon 1993a, b; McRae & Burke 1996) including brown-headed cowbirds (Fleischer 1985). I therefore visually compared spotted and intermediate morph eggs and, based on their markings, placed them into groups where eggs within groups were similar enough to have been laid by a single female. I then compared similarity in egg size and shape, as measured by the Euclidean distance between each pair of eggs (using egg length and width), for eggs that I placed in the same groups and eggs that I placed in different groups. The fact that the Euclidean distances between pairs of eggs were much smaller for eggs in the same groups than for eggs in different groups indicated that it is possible to make inferences about laying patterns of individual females based on egg dimensions. I therefore examined spatial patterns of laying, with respect to Euclidean distances and egg colour patterns, to answer the following questions. (1) Do individual females lay more than one egg in the same blackbird colony? (2) Do individual female cowbirds lay eggs in more than one colony? (3) Does more than one female lay eggs in the same colony? (4) Is multiple parasitism due to several females laying in the same nest, to single females laying several eggs or both?

The occurrence of two morphs remains an unexplained phenomenon in shiny cowbirds, and the selective factors favouring the maintenance of the white morph have yet to be identified. Specifically, although some hosts reject white morph but accept spotted morph eggs (Fraga 1985; Mason 1986a; Mermoz & Reboreda 1994), there is no evidence for a host species that rejects spotted eggs but accepts white morph eggs. I therefore examined blackbird responses to both egg morphs with natural and experimental parasitisms. Although it has been suspected that females lay only a single egg morph (Fraga 1985), there is also no evidence to support this assumption. I therefore compared similarity of egg size and shape for paired comparisons of same and different morphs as an indirect assessment of whether morphs vary between, but not within, females.

METHODS

I conducted the study from 23 November to 16 December, 1994, on the eastern edge of the Estancia Cari Lauchen near General Lavalle, Buenos Aires province, Argentina. Earlier studies of parasitism by shiny cowbirds have been conducted in this region (Mason 1986a, b; Mermoz & Reboreda 1994). The study area is a flat low marshy region, where roughly half of the area is marshland and the other half is open prairie grazed by livestock. The dominant vegetation in the marshes away from shoreline is *Scirpus californicus*. In some areas the edges of the marshes are dominated by durasnilla, *Solanum glaucum*, a woody shrub. All of the blackbird colonies occurred in this habitat, and nests were invariably built in durasnilla shrubs. The specific habitat used by the blackbirds was both localized and quite open, and I was therefore sure that I had found all potential host nests in each colony. Although other important host species were rare close to the blackbird colonies, large numbers of two other important hosts bred within 5 km of the study area: yellow-winged blackbirds, *A. tilius*, and brown-and-yellow marshbirds, *Pseudoleistes virescens*. Low densities of another important host, the rufous-collared sparrow, *Zonotrichia capensis*, bred in the pastures near the chestnut-capped blackbird colonies. This study therefore concerns spatial patterns of brood parasitism for one host species and not for the entire host

community. Thus, estimates for the minimum number of cowbirds parasitizing host colonies are minimum estimates for the entire area, especially if individual cowbirds are specializing on hosts and females vary in their specialization.

Chestnut-capped blackbirds are colonial and polygynous, but the males build the nests (B. E. Lyon, unpublished data). During nest construction, males sing and display from the nests to attract females. Nests that are not occupied by females within a few days of completion become dishevelled and rapidly deteriorate. I studied parasitism in five blackbird colonies (colonies 1–5), which contained 12, 9, 24, 29 and 6 active blackbird nests, respectively. All colonies were within 2 km of each other. Each colony was visited three times (23 November, 8 or 9 December and 14 or 16 December), and all nests in a colony were checked on each visit. During each census, I numbered all new blackbird and cowbird eggs in each nest with a fine-tipped indelible marker and individually marked nests with numbered flagging tape. Observations revealed that eggs or chicks occasionally disappeared from nests after the first chicks hatched, which could bias the estimate for the frequency of cowbird parasitism if many nests were first found when they contained chicks. Only three of the 80 nests monitored were first found when they contained chicks, however. Moreover, cowbird and blackbird chicks differed in appearance, so it was possible to determine the species identity of the chicks in the three nests first found with chicks. To control for the spurious effects of season when testing for random patterns of parasitism (e.g. Orians et al. 1989), I divided the data into two classes. Early nests were nests initiated no later than 9 December, and Late nests were initiated no earlier than 10 December. This division resulted in nearly equal sample sizes in each category (41 and 39 nests, respectively). Because I visited the colonies only three times, some nests could have been both parasitized and preyed on between visits. Although this would lead to an underestimate of the overall frequency of parasitism, it would not affect the major conclusions of the study.

I examined the spatial patterns of cowbird egg laying, including multiple parasitism of single host nests and a larger scale that involved a comparison of nests within and among colonies, on the basis of egg features like shape and marking type. Cowbird eggs were classified into three 'morphs'

on the basis of their markings: spotted, white and intermediate. Spotted eggs were heavily spotted and looked similar to the eggs of the brown-headed cowbird; intermediate eggs were closer in appearance to white than spotted eggs and had sparse small spots on a white background. I measured the length and width of 38 (52%) of the 73 parasitic eggs that were laid. I also collected 21 spotted or intermediate type eggs and, by visually comparing them on the basis of their spotting patterns, placed them into groups with very similar markings: each group contained eggs probably laid by one female (e.g. Walkinshaw 1949; Dufty 1983; Fleischer 1985; Gibbons 1986; Jackson 1992; Lyon 1993a, b). I used this same technique in an earlier study of brood parasitism in American coots, *Fulica americana*, that involved 400 nests and 600 parasitic eggs (Lyon 1993a). The accuracy of using egg features to match eggs laid by the same female in that study was objectively confirmed with both a discriminant function analysis (Lyon 1993b) and a small-scale DNA fingerprinting study (B. E. Lyon, W. M. Hochachka & J. M. Eadie unpublished data). In the present study, most assignments to a group were clear, but in a few cases, it was unclear whether eggs were similar enough to have been laid by one female or whether more than one female was involved. I considered these eggs as being laid by different females. To estimate differences in size and shape between eggs, I used egg length and width to calculate the Euclidean distance between all possible pairs of eggs. Euclidean distance between two eggs, egg i and egg j, is

$$\sqrt{((L_i - L_j)^2 + (W_i - W_j)^2)},$$

where L and W are length and width.

To augment information from naturally occurring parasitism on the frequencies of egg-rejection for the two main cowbird egg types, I experimentally added one fresh cowbird egg to six nests (three white morph, three spotted morph) and monitored their subsequent fates a minimum of 8 days later.

RESULTS

Frequency of Parasitism and Egg Morphs

Parasitism by cowbirds was common in the blackbird colonies and almost half the nests were

parasitized (Table I). This may be an underestimate, because egg laying had just begun at some nests during the last nest census and some of these nests could have been parasitized after the end of the study. Seventy-one cowbird eggs were laid in the 77 nests (0.92 eggs/nest) found before the eggs hatched. This number excludes the three nests that contained chicks when first found, because some eggs may have been laid but subsequently disappeared. Spotted eggs were slightly more common than white eggs, and intermediate eggs were the least common (Table I). Although the overall frequency of parasitism was high, parasitism was much more common earlier than later in the season (chi-square test of independence for Early versus Late nests: $\chi^2_1 = 11.3$, $P<0.001$; Table I).

Multiple Parasitism

Multiple parasitism was common: 56% of the 36 parasitized nests found at the egg stage received more than one cowbird egg, and some hosts received up to five eggs (Table II). Comparing the observed frequencies of multiple parasitism with those expected under random laying (Poisson) revealed that the pattern of dispersion deviates from random expectation (chi-square goodness of fit test with the categories three to five eggs per nest combined: $\chi^2_3 = 10.35$, $P=0.015$). The observed pattern is more clumped than expected for random dispersion, with fewer unparasitized nests and more nests with four and five eggs that expected (Table II).

This apparent clumping could be an artefact of seasonal patterns (e.g. Orians et al. 1989). For example, the expected values for random parasitism were based on the overall mean parasitism frequency for the entire season, but this frequency was not constant through the season. Parasitism was more frequent earlier in the season (Table I) and random laying during this period could lead to apparently non-random levels of multiple parasitism in an analysis of the entire season pooled (Orians et al. 1989). To control for such seasonal effects, I separately examined the patterns of egg dispersion for Early and Late nests (Table II). The dispersion of cowbird eggs in Early nests did not differ from random expectation (chi-square goodness of fit test with the categories of three to five eggs per nest combined: $\chi^2_3 = 2.06$, $P=0.56$; Table II). There was a trend towards a clumped pattern of

egg dispersion in Late nests (categories of two to five eggs combined: $\chi^2_2 = 5.18$, $P=0.076$).

The unusual mating system of the hosts, where the male blackbirds build the nests but then abandon them within a few days if they are unable to attract a female to use the nest, could lead to 'dump nests', where several cowbirds lay in inactive nests. This could account for some of the extreme cases of multiple parasitism. Most of the cowbird eggs were laid in active nests, however. Only one case of multiple parasitism, a nest with four eggs, appeared to have been a dump nest, but this could also have been a nest that the host deserted after parasitism. Two other nests with extreme multiple parasitism, with no host eggs and four and five parasitic eggs, respectively, hatched some cowbird chicks; these were therefore neither dump nests nor nests that had been abandoned in response to heavy parasitism. Thus, heavy parasitism coupled with a complete lack of host eggs does not necessarily indicate that a nest was never active.

Do Individual Females Lay Eggs of a Consistent Size and Shape?

The eggs that were visually matched into groups putatively laid by single females, on the basis of their spotting pattern, also appear to cluster together in groups when plotted as a function of their length and width (Fig. 1a). As an objective test of whether the eggs within a group are more similar in dimensions than eggs from between groups, I compared the distances between all pairs of eggs when plotted in length–width space (Euclidean distance; Fig. 2). The distances between pairs of eggs in the same group were significantly smaller (median value=0.51, range=0.10–1.03, $N=25$; Fig. 2a) than distances between pairs of eggs in different groups (median value=1.61, range=0.10–3.02, $N=185$; Mann–Whitney U-test: $z=7.00$, $P<0.001$; Fig. 2b). The maximum Euclidean distance between pairs of eggs within the same group was 1.03, but only 26.5% of the between-group distances were less than this value.

In the above analysis, I included individual eggs many times because Euclidean distances between all pairs of eggs are considered independent. To ensure that the significant difference I found was not due to pseudoreplication, I repeated the analysis using mean values. For each egg, I calculated the mean Euclidean distance between that

Table I. Frequency of shiny cowbird parasitism, by colony and stage of season, and frequency of cowbird egg morphs by colony, for five colonies of chestnut-capped blackbirds

Colony	Number of nests	% Nests parasitized	% Early nests parasitized*	(N)	% Late nests parasitized*	(N)	Number of eggs of each morph		
							Spotted	White	Intermediate
1	12	58.3	60.0	(10)	50.0	(2)	9	5	1
2	9	77.8	100	(2)	71.4	(7)	4	3	2
3	24	41.7	71.4	(14)	0	(10)	11	13	1
4	29	48.3	60.0	(15)	35.7	(14)	10	6	6
5	6	0	—	(0)	0	(6)	0	0	0
All	80	47.5	65.8	(41)	28.2	(39)	34	27	10

*Early nests were initiated no later than 9 December. Late nests began no earlier than 10 December.

Table II. Dispersion of cowbird eggs among host blackbird nests, separately for Early and Late nests, and for all nests combined
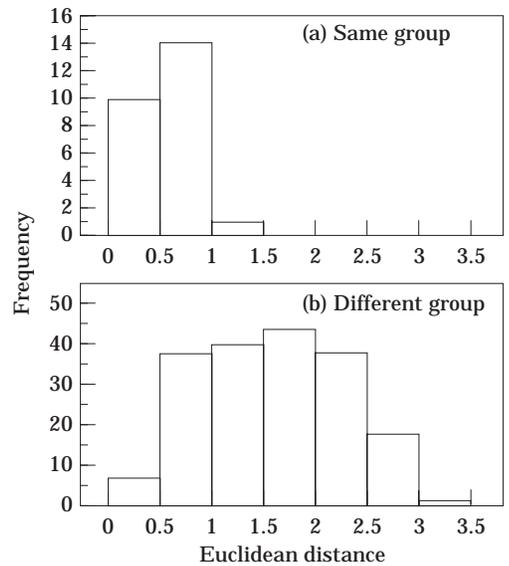
Cowbird eggs/nest	Early nests		Late nests		All nests	
0	14	(10.9)	27	(22.7)	41	(31.0)
1	11	(14.2)	5	(11.1)	16	(28.2)
2	8	(9.2)	4	(2.7)	12	(12.8)
3	4	(4.0)	0	(0.43)	4	(3.9)
4	2	(1.3)	0	(0.05)	2	(0.9)
5	1	(0.4)	1	(0.005)	2	(0.2)
Mean eggs/nest	1.3 eggs/nest		0.49 eggs/nest		0.91 eggs/nest	

Numbers are observed numbers of nests in each category; numbers in parentheses are expected numbers of nests if laying were random and followed a Poisson distribution.
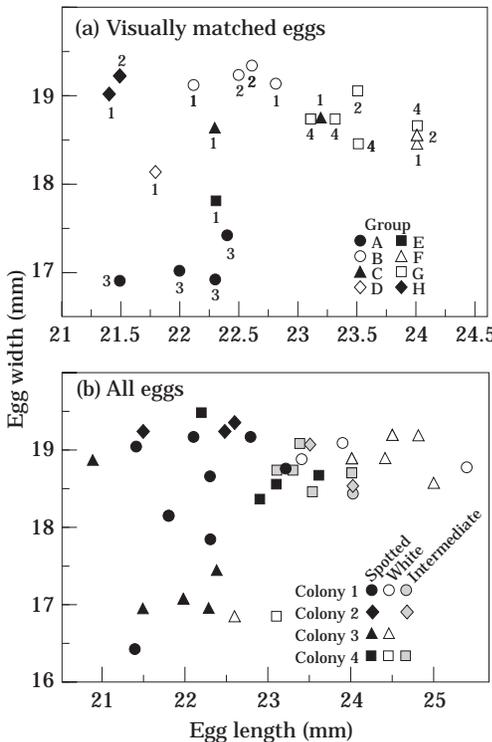
Figure 1. The length and width of (a) shiny cowbird eggs visually matched into groups thought to have been laid by a single female and (b) all measured eggs. (a) For visually matched eggs, each symbol designates a group of eggs thought to have been laid by a single female, based on their spotting pattern, and numbers adjacent to each egg indicate the colony in which it was laid. (b) Symbols indicate both an egg's morph and colony of origin.
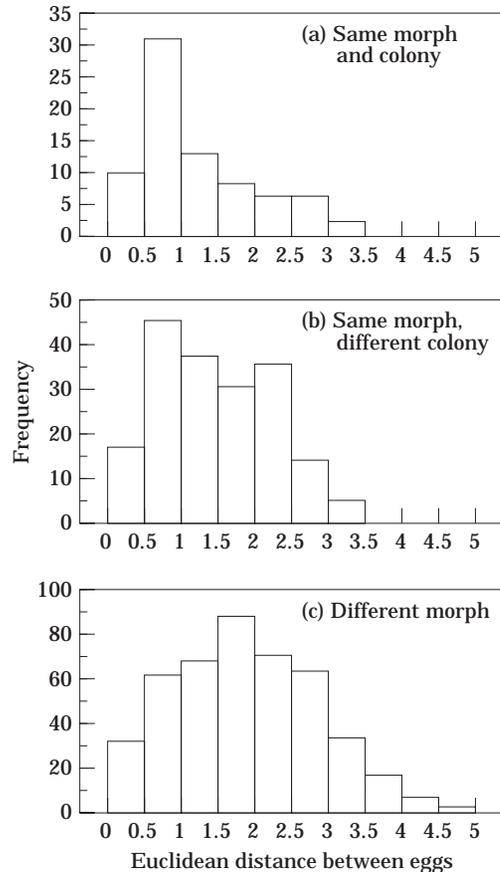

Figure 2. Euclidean distances between pairs of eggs (a) visually matched into groups having similar spotting patterns and presumed to have been laid by the same female and (b) matched into different groups and presumed to have been laid by different females. Euclidean distance is the distance between a pair of eggs when plotted in length–width space.

egg and all eggs classified in the same group and the mean Euclidean distance between the egg and all eggs classified in different groups, and then compared these paired means with a matched-pairs comparison. This analysis confirmed that the Euclidean distances between eggs in the same group were smaller than distances between eggs classified as different (Wilcoxon test: $z= -3.72$, $N=18$ pairs, $P<0.001$). Thus, females appear to lay eggs of consistent dimensions, making it possible to use egg features like length, width and morph to infer spatial patterns of laying by individual parasites.

Spatial Patterns of Parasitism based on Egg Features

A plot of the lengths and widths of all eggs, separately by morph and colony, suggests that there is a tendency for same-morph eggs within a colony to cluster together (Fig. 1b), which would be expected if individual females laid more than one egg per colony. Again, I compared Euclidean distances between pairs of eggs to test objectively for the occurrence of these 'clusters'. I compared three categories of egg pairs: (1) same morph and colony, (2) same morph but different colonies and (3) different morphs (irrespective of colony). The Euclidean distances between these three categories differed significantly (Kruskal–Wallis test: $H_2 = 49.8$, $P < 0.0001$; Fig. 3). Post hoc contrasts with Mann–Whitney U-tests, using error rates adjusted for multiple comparisons (Rice 1989), revealed that all categories differed significantly from each other ($P < 0.01$ in all three pair-wise comparisons). As above, I repeated the analysis using mean distances for each egg, calculating a mean for each of the three categories based on morph and colony. The patterns were identical with this more conservative analysis (Friedman two-way analysis of variance: $\chi_2^2 = 23.3$, $P < 0.001$; post hoc comparisons using Wilcoxon matched-pair comparisons, all $P < 0.01$). Thus, eggs from same morph and colony were more similar in shape and size than were eggs of the same morph but different colonies, and eggs of different morphs are the least similar to each other. These patterns suggest that individual females lay only one egg morph, and that many females lay more than one egg per blackbird colony.

The contention that individual brood parasites laid several eggs in the same colony is further supported by a comparison of two Euclidean distance distributions: the distribution for pairs of 'same morph and colony' eggs (Fig. 3a) versus the distribution for pairs of eggs judged as being laid by different females (Fig. 2b). If females laid only a single egg per colony, all paired comparisons in the 'same morph and colony' sample would involve eggs laid by different females and these two distributions should not differ. They differ strikingly, however (Mann–Whitney U-test: $z = 4.40$, $P < 0.001$). Of the distances for 'same morph and colony' egg pairs, 56.6% were within the cutoff point that included all eggs putatively laid by the same females in the visual comparisons

Figure 3. Euclidean distances between pairs of eggs in three categories: (a) same morph and same blackbird colony, (b) same morph but different blackbird colony and (c) different morphs irrespective of colony location.

(i.e. ≤ 1.03), but only 26.5% of the distances between pairs of eggs attributed to different females were within the cutoff. Thus, some females laid more than one egg per colony. In fact, these data can be used to estimate the proportion of the paired comparisons in the 'same morph and colony' sample that involved eggs from the same female (Appendix). In this case, it is estimated that 41% of the distances between pairs of eggs in the 'same colony and morph' sample involved eggs laid by the same female. Multiplying this value by the average number of eggs of the same morph laid in each colony (3.8 eggs; 38 total eggs in 10 morph/colony combinations) yields a minimum average number of eggs laid per female per colony (1.55). This is a minimum estimate because

only half of all eggs were measured. The visual comparisons, where eggs were visually classified into groups thought to have been laid by one female (Fig. 1a), provide additional evidence that individual cowbirds laid more than one egg per colony. In many cases, two or more eggs classified into the same group were also laid together in the same colony (i.e. eggs with the same symbol and number in Fig. 1a): five of the six groups with more than one egg had multiple eggs in the same colony.

There is also evidence that individual females laid in more than one colony. Based on the visual matching of eggs, four of the six groups with at least two eggs contained eggs from more than one colony (Fig. 1a). Further support comes from the observation that the Euclidean distances of 'same morph but different colony' comparisons (Fig. 3b) were smaller than those of 'different morph' comparisons (Fig. 3c). This pattern is expected if females laid eggs in more than one colony, because some of the same morph/different colony comparisons would involve eggs laid by the same females, but it is almost certain that all comparisons between morphs involved different females. Another factor that could also contribute to the differences in Euclidean distances between these two groups could be consistent differences between morphs in the size or shape of eggs; the morphs differed in egg length (ANOVA, $F_{2,37}=16.86$, $P<0.001$; Fig. 1b).

The final, large-scale spatial issue of interest concerns the number of female cowbirds that lay eggs in a given colony. Based on the assumption that individual females can lay only a single morph of egg, the fact that two or three morphs were laid in each colony indicates that at least two or three females laid in each colony (Fig. 1b). This is an underestimate, because the visual comparisons suggest that within each morph type, up to six females can lay in a colony (i.e. eggs in different groups but same colony in Fig. 1a). Given that distances between eggs of greater than 1.03 are likely to indicate eggs laid by different females, an inspection of the distances between eggs of the same morph in Fig. 1 provides additional evidence that, in several cases, more than one female of a morph type laid in the same colonies. Overall, combining these various forms of evidence (morphs, visual groups and Euclidean distances between eggs) yields the following rough minimum estimate for the total number of females

laying per colony: eight females in colony 1, four in colony 2, two in colony 3 and two in colony 4. These are minimum estimates because only half of all eggs were measured and less than a third were visually compared.

Multiple Parasitism and the Number of Females Laying per Nest

In the visual comparisons, where eggs were matched into groups (Fig. 1a), there were no cases where two eggs from the same group had been laid in the same host nest. Combining these data with information from egg morphs revealed that in the 14 nests where I was able to determine the total number of females involved (Table III), there was not a single case where the same female laid more than one egg per nest. Thus, most, if not all, cases of multiple parasitism resulted from several females laying in the same nest, not from single females laying several eggs.

Host Responses to Different Egg Morphs

Both the observational and experimental data indicate that chestnut-capped blackbirds accept all morphs of shiny cowbird eggs. For natural parasitisms, no intact cowbird eggs were ever observed rejected from an active host nest during laying or incubation, although one white egg that had a peck hole subsequently disappeared from the host nest. The lack of rejection for natural parasitisms was not due to insufficient observations, because 14 cowbird eggs (five white, seven spotted, two intermediate) remained in host nests for at least a week. All of the six experimental eggs added to nests, three white and three spotted eggs, were still in the nests when last checked 8 days after they had been added.

DISCUSSION

Spatial Patterns of Parasitism

In this study, visual comparisons of egg spotting patterns, the spatial distribution of egg morphs and the degree of similarity in the dimensions of eggs all provided evidence about the spatial laying patterns of individual female shiny cowbirds. Females often laid several eggs in the same colonies, which makes sense if females were

Table III. The minimum number of individual brood parasites that laid in each multiply parsitized nest, based on the number of egg morphs in the nest and, for spotted and intermediate morph eggs, the number of females based on visual comparison of eggs

Host nest	Egg morph			Number of females based on		
	S	W	I	Egg morphs	Visual groups*	Total
Two eggs						
6	1	1	—	2		2
12	1	1	—	2		2
15	1	1	—	2		2
18	1	—	1	2		2
25	2	—	—	≥ 1	?	≥ 1
35	1	1	—	2		2
39	2	—	—	≥ 1	2	2
42	2	—	—	≥ 1	2	2
60	—	1	1	2		2
61	1	—	1	2		2
62	1	1	—	2		2
80	1	—	1	2		2
Three eggs						
9	1	2	—	≥ 2	?	≥ 2
19	1	2	—	≥ 2	?	≥ 2
26	1	1	1	3		3
29	1	1	1	3		3
Four eggs						
10	3	1	—	≥ 2	?	≥ 2
17	1	3	—	≥ 2	?	≥ 2
Five eggs						
13	1	4	—	≥ 2	?	≥ 2
74	3	1	1	≥ 3	5	5

*Question marks indicate nests containing multiple eggs of the same morph where visual comparisons were not done.

monitoring several nests to assess their stage. My observations also rule out the hypothesis that individual shiny cowbirds defended territories to gain exclusive access to blackbird nests. Based on egg morphs and visual comparisons, up to eight females laid eggs in the same colony and up to five females laid eggs in the same nests. This lack of monopolization of areas containing host nests by individual females mirrors earlier observations of shiny cowbirds parasitizing rufous-collared sparrows (Friedmann 1929; Fraga 1978) and chalk-browed mockingbirds, *Mimus saturinus* (Fraga 1985), except that I have more quantitative evidence rather than simply stating that visual comparisons of eggs in the field indicate broad spatial overlap of females. Conclusions from studies of brown-headed cowbirds have varied. Studies based on female locations indicated that females probably defend territories or at least distinct 'domains' (Dufty 1982; Rothstein et al. 1984);

those based on patterns of egg dispersion showed little evidence for territoriality (McGeen & McGeen 1968; Fleischer 1985). These differences suggest that behavioural observations of females are not sufficient to deduce laying patterns.

When cowbird densities are high, extensive overlap of areas used by individual female cowbirds may result in high levels of multiple parasitism, a pattern that could lead to competition between cowbird chicks for host parental care. In the blackbird colonies I studied, most cowbird eggs (77%) ended up in host nests with at least one other cowbird egg. Female cowbirds could reduce potential costs incurred from multiple parasitism in two ways: by laying a single egg per host nest or by avoiding laying eggs in nests where other cowbirds have already laid. I have presented evidence that females laid only a single egg in each host nest (Table III), but there is no evidence that cowbirds avoided already parasitized nests. In
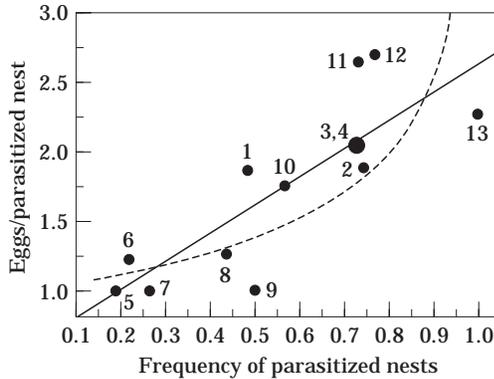
Figure 4. Relation between the intensity of parasitism (mean number of eggs/parasitized nest) and the frequency of parasitized nests for 11 species of hosts parasitized by shiny cowbirds in Buenos Aires province, Argentina. Two species are represented by two studies, and for each species from Mason's (1986b) study, I pooled his two sites for a single overall value. The solid line is the observed linear regression, the dashed line is the expected intensity of parasitism based on random parasitism (Poisson) as a function of the frequency of parasitized nests. Numbers indicate species identity: (1) *Agelaius ruficapillus* (this study); (2) *Pseudoleistes virescens* (Mermoz & Reboreda 1994); (3) *Zonotrichia capensis* (Fraga 1985); (4) *Mimus saturninus* (Fraga 1985). All remaining species are from Mason 1986b; (5) *Furnarius rufus*; (6) *Zonotrichia capensis*; (7) *Molothrus badius*; (8) *Tachycineta leucorrhoa*; (9) *Satrapa icterophrys*; (10) *Tyrannus savana*; (11) *Mimus saturninus*; (12) *Troglodytes aedon*; (13) *Turdus rufiventris*.

fact, an overall comparison indicated an excess of multiple parasitism, but this disappeared once the confounding influence of seasonal variation in parasitism frequency was taken into account (e.g. Orians et al. 1989); females appear to have laid their eggs randomly with respect of other cowbirds. Because parasitism occurred at a high frequency, random laying resulted in a high incidence of multiple parasitism.

Friedmann (1929) also noted that multiple parasitism is common in many hosts parasitized by shiny cowbirds and he suggested that multiple parasitism could be a inadvertent consequence of a high parasitism frequency. To test this idea, I examined the relation between the frequency of parasitism and levels of multiple parasitism with data collected from the literature for 11 hosts parasitized by shiny cowbirds (Fig. 4). Following Mason (1986b), the intensity of multiple

parasitism was expressed in terms of the average number of parasitic eggs per parasitized nest. As Friedmann (1929) suggested, there was a strong relation between frequency of parasitism and intensity of multiple parasitism (linear regression: $F_{1,11} = 23.9$, $P = 0.005$, $R^2 = 68.5$; Fig. 4). Five intensity values were lower than those predicted by random (Poisson) laying (dashed line; Fig. 4) and eight values were greater (Binomial test: two-tailed $P = 0.58$), suggesting no overall tendency for either overdispersion or underdispersion of eggs. Thus, the high levels of multiple parasitism in hosts parasitized by shiny cowbirds can apparently be explained by a high frequency of parasitism coupled with random laying by females. Others studying multiple parasitism in brown-headed cowbirds also concluded that the observed levels of multiple parasitism resulted from random laying (Preston 1948; Mayfield 1965; Orians et al. 1989). In a study of shiny cowbirds in the Caribbean, Wiley (1985) found that high-quality hosts were more heavily parasitized and had more multiple parasitism than low-quality hosts, but he did not attribute high levels of multiple parasitism to a high frequency of parasitism.

My investigation of spatial patterns of parasitic egg laying by female shiny cowbirds was based on indirect evidence, egg features. Although powerful molecular techniques (e.g. Burke & Bruford 1987) have the potential to reveal the laying behaviour of individual females more directly and accurately, that approach is not always feasible, because it is expensive and time consuming. The techniques I have used in this study provide an alternative in such cases, and can also provide useful additional information even in studies where molecular techniques are applied (e.g. McRae & Burke 1996).

Inferences about the laying patterns of specific females, based on egg features, have also been an important part of previous studies of both conspecific brood parasitism (Gibbons 1986; Jackson 1993; Lyon 1993a; McRae & Burke 1996) and interspecific brood parasitism (Friedmann 1929; Walkinshaw 1949; McGeen & McGeen 1968; Fleischer 1985). In some species with unmarked eggs, egg dimensions alone can be useful for discriminating eggs laid by different females and making inferences about patterns of parasitic laying (Eadie 1989). All of these studies assumed that eggs laid by the same female are more similar in features than eggs laid by different females. This

assumption has been shown to be valid in two studies of brown-headed cowbirds. Dufty (1983) collected eggs from known captive cowbirds and found that naive observers could successfully match eggs laid by the same female far more than expected by chance. Fleischer (1985) collected eggs from host nests, used protein electrophoresis to identify eggs laid by the same female and then showed that egg features like spotting pattern, size and shape could be used successfully to identify eggs laid by the same female. Whether this assumption holds for shiny cowbirds remains to be rigorously confirmed, but the observation that eggs with similar spotting patterns also tend to be more similar in dimensions (Figs 1a, 2a) supports the assumption. This same concordance between visually matched eggs and egg dimensions has also been noted for brown-headed cowbirds (Walkinshaw 1949; McGeen & McGeen 1968).

For shiny cowbirds, the existence of distinctive morphs adds another way to help identify eggs that were probably laid by different females, assuming that each female lays a consistent morph (Fraga 1985). Given the normal range of variation within a female's clutch in most birds (Gibbons 1986; Lyon 1993a), even in species with extremely variable eggs (Collias 1993), this seems like a robust assumption. The observation that Euclidean distances between pairs of different morph eggs were greater than between pairs of same morph eggs also supports this assumption. The presence of distinctive morphs thus provides a useful tool for examining the minimum number of female cowbirds laying in an area, or even in a single nest.

The Maintenance of Two Egg Morphs

The egg polymorphism in shiny cowbirds occurs in only part of the species' range, occurring in eastern Argentina, Uruguay, and southeastern Brazil (Friedmann 1929). The existence of two distinct egg morphs in these areas is intriguing, particularly since hostile coevolution with hosts has led to the evolution of clear egg morphs in other brood parasitic taxa (Brooke & Davies 1989). Whether coevolution with hosts can account for the maintenance of the egg polymorphism in shiny cowbirds remains to be demonstrated, however. Although the polymorphism seems likely to have some genetic basis, rather than an environmental basis like female age, this

has yet to be verified. From an ecological perspective, we have yet to identify a host that provides natural selection favouring the white morph through rejection behaviour. Some hosts reject both morphs, some hosts reject only the white morph, but no host is known to accept white morph eggs but reject spotted morph eggs (Fraga 1985; Mason 1986a; Mermoz & Reboreda 1994; J. Reboreda, personal communication). Alternatively, the white egg morph could be favoured if females that laid white eggs specialized on hosts that do not differentiate between the morphs, and the fitness gained from reduced investment in egg pigments exceeded the fitness costs of reduced host availability. This possibility is unlikely, however, because the fitness costs of manufacturing pigments would be small, and host specialization would have to be virtually complete. Moreover, such restricted host specialization would incur a considerable cost of reduced host availability, because some geographically widespread, important hosts reject white morph eggs (Fraga 1985, Mason 1986a).

Given that previous adaptive explanations apparently do not account for the maintenance of white morph eggs, I propose an alternative explanation that is not based on selection favouring the white morph, but instead involves a combination of historical and genetic factors. White morphs could result from the 'hybridization' of previously allopatric populations that diverged in the genetic basis of egg pigmentation, either due to genetic drift or selection for specific spotting patterns through partial egg rejection. The appearance of spotted eggs is remarkably variable (Friedmann 1929), and some important hosts do show partial rejection of spotted morph eggs (Mason 1986a). If genetic divergence in allopatry were sufficient, 'hybrid' females that resulted once sympatry reoccurred might be incapable of producing egg pigmentation, due to incompatible alleles at loci coding for egg pigmentation, and thus lay white eggs. Given the difficulties of reinforcement selection to reduce 'hybrid' matings (Spencer et al. 1986), the maintenance of hybrid-type females, and thus white morph eggs, could persist for a long time even in the face of strong selection against white eggs. The ratio of egg morphs appears to have remained stable for least a century (Friedmann 1929), despite a selective disadvantage for the white morph in some regions (Mason 1986a). The explanation I propose could

also explain why the egg polymorphism occurs only in part of the cowbird's range and predicts current, geographical variation in natural selection for specific spotting patterns. A captive breeding program could elucidate the genetic basis of this interesting egg polymorphism.

APPENDIX

Based on the information from the visual comparisons (Fig. 2), it is possible to estimate the proportion of paired comparisons (=distances) in an unknown sample that involve the same female. Assume an unknown sample of eggs, where s is the proportion of same female comparisons, d is the proportion of different female comparisons, and x is the proportion of distances that are no more than 1.03, the cutoff identified in the visual comparisons for eggs laid by the same female. Given that 100% of distances between pairs of eggs classified as being laid by the same female were no more than 1.03, compared with 26.5% of the distances between eggs classified as being laid by different females,

$$s + 0.265d = x, \text{ and since } s + d = 1, \text{ then}$$
$$s + 0.265\,(1 - s) = x, \text{ and}$$
$$s = (x - 0.265)/0.735$$

In the sample of 'same morph and colony' comparisons shown in Fig. 3, $x = 0.566$, and therefore $s = 0.409$. Thus, 41% of the paired comparisons are estimated to be between eggs laid by the same female.

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