Behavioral Ecology Vol. 12 No. 1: 8–15

Egg discrimination in the Australian reed warbler (*Acrocephalus australis*): rejection response toward model and conspecific eggs depending on timing and mode of artificial parasitism

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In a coevolutionary arms race between an interspecific brood parasite and its host species, both are expected to evolve adaptations and counteradaptations. We studied egg discrimination in the Australian warbler (*Acrocephalus australis*). This species is currently not significantly parasitized by the seven species of cuckoo for which it is a suitable host. However, experimental brood parasitism in the warbler revealed a fine tuned egg discrimination response towards non-mimetic and conspecific eggs, the first such evidence in an Australian passerine: (1) non-mimetic eggs were significantly more often rejected than conspecific eggs; (2) only non-mimetic dummy eggs were rejected selectively, whereas rejection of conspecific eggs entailed a rejection cost; (3) replacement of a host's egg with a conspecific egg during egg laying resulted in a significantly higher rejection rate than after the day of clutch completion; (4) by contrast, rejection rate after addition of a conspecific egg was independent of nest stage; (5) conspecific eggs introduced into a clutch during the egg laying period led to a significantly higher nest desertion rate and a lower egg ejection rate than after the day of clutch completion; and (6) addition of a conspecific egg led to egg ejection while egg replacement with a conspecific egg led to nest desertion. The fact that this species responds differentially toward different modes of artificial parasitism suggests that its egg discrimination has evolved to minimize the costs of rejection and parasitism. The ability to reject highly mimetic conspecific eggs may explain the current paucity of brood parasitism in this species. The significance of this for brood parasite-host coevolution is discussed. *Key words:* egg discrimination, egg rejection, egg ejection, nest desertion, brood parasitism, coevolutionary arms race. *[Behav Ecol 12:8–15 (2001)]*

Parental care is widespread in birds (Lack, 1968) and exploitation of this relatively costly behavior by brood parasites is an evolutionary alternative. Brood parasitism can depress the host's fitness in several different ways (Davies and Brooke, 1988; Payne, 1977; Røskaft et al, 1990; Rothstein, 1975a,b). For example, brood parasites often remove a host egg from the nest they parasitize (Payne, 1977), brood parasitism may reduce hatching success of the host eggs (Røskaft et al., 1990), and parasitic nestlings often eject their foster siblings from the nest (Lack, 1968). These costs of parasitism put a potentially high selection pressure on the host to reduce the extent of parasitism, such as through rejection of the parasitic egg or nestling. The most successful stage of the breeding cycle for rejecting the parasite seems to be at the egg stage (e.g., Moksnes et al., 1990), and many host species are capable of rejecting parasitic eggs (for references see Rothstein, 1990). In turn, these anti-parasite adaptations may then select for counteradaptations in the parasite, such as egg mimicry (Brooke and Davies, 1988). This can initiate a coevolutionary arms race between the host and brood parasite, leading to more and more intricate adaptations and counteradaptations (Davies and Brooke, 1988, 1989b; Dawkins and Krebs, 1979; Payne, 1977; Rothstein, 1990). If parasitism becomes too frequent, the host may become extinct; alternatively, as the host evolves better defense mechanisms, it may benefit the parasite to switch to another species (Davies and Brooke, 1989b).

An important factor in the evolution of egg rejection behavior is the ''rejection costs,'' which involve both a ''recognition cost'' and an ''ejection cost'' (Braa et al., 1992; Davies and Brooke, 1988; Rohwer and Spaw, 1988; Rothstein, 1982a; Spaw and Rowher, 1987). A recognition cost occurs through accidental rejection of the host's own egg instead of the parasitic egg (Davies and Brooke, 1988). Birds are known to make recognition errors (Davies and Brooke, 1988, 1989a; Davies et al., 1996; Marchetti, 1992; Molnar, 1944; Rothstein, 1976, 1982a). An ejection cost occurs through the host accidentally damaging or removing one or more of their own eggs along with the parasite's egg (Davies and Brooke, 1988, 1989a; Moksnes et al., 1991; Rohwer et al., 1989; Rothstein, 1976).

Egg rejection behavior is expected to evolve in such a way as to enable a host to minimize the rejection costs (Davies et al., 1996; Moksnes et al., 1991). For example, when hosts perceive that they are parasitized, they can decide either to accept or to reject the parasitic egg. Acceptance of a parasitic egg is adaptive only if the costs of parasitism are low or the rejection costs are high (Rohwer and Spaw, 1988; Røskaft and Moksnes, 1998). This situation may occur if a nest is parasitized during the host's incubation period, in which case the parasite's egg may remain unhatched because it will receive insufficient in-

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Received 15 October 1999; revised 21 February 2000; accepted 10 April 2000.

cubation, or the parasitic chick will be too weak to eject the host's chicks or to compete successfully for parental care. On the other hand, the host should show a rejection response, such as ejection of the parasitic egg or nest desertion, when the parasitic eggs are laid in time to be detrimental to the host's reproductive output or fitness (Moksnes et al., 1990; Røskaft and Moksnes, 1998; Rothstein, 1976). Although ejection of the parasitic egg may seem to be the host's optimal response, hosts may be more likely to accept when recognition costs are high and/or the probability of brood parasitism is low (Davies et al., 1996), or they may be more likely to desert the nest when the ejection costs and/or chances of successful renesting are high (for references Rothstein, 1990).

We investigate egg discrimination behavior in the Australian reed warbler (*Acrocephalus australis*) and the adaptations that have evolved to optimize egg rejection behavior. To our knowledge, intraspecific brood parasitism has not been recorded in this species. Although the Australian reed warbler is a suitable host to at least seven cuckoo species it lives sympatric with, it is currently not a major biological host to any interspecific brood parasite (Brooker and Brooker, 1989). However, it has occasionally been parasitized by at least four species of cuckoo: Fan-tailed cuckoo, *Cuculus pyrrophanus* (two recordings), pallid cuckoo, *Cuculus pallidus* (seven recordings), Horsfield's bronze cuckoo, *Chrysococcyx basalis* (two recordings), and shining bronze cuckoo, *Chrysococcyx lucidus* (two recordings) (collated data from: Brooker and Brooker, 1989; Storr and Johnstone, 1988; White, 1915). These four cuckoo species are all known to remove a biological host's egg when laying their own and their hatchlings evict the remaining offspring from the nest (Brooker and Brooker, 1989). Clearly their parasitism is costly to the host's reproductive success. Therefore, if the Australian reed warbler has been a major biological host in the past it should have evolved the ability to discriminate against brood parasite's eggs. In our study we examine whether the Australian reed warbler is currently suffering from inter- and/or intraspecific brood parasitism. We further examine experimentally whether egg discrimination occurs, and if so, how this behavior is influenced by the various rejection costs and costs of parasitism. To this end, artificial brood parasitism is conducted in which we manipulate the degree of egg mimicry, host egg removal, and the timing in the nesting cycle.

METHODS

Data collection

The study was conducted from September to January 1998, the main breeding season of the Australian warbler, at Koroit Creek, Altona (6 ha, 37°53′ S, 144°48′ E) and Edithvale Wetlands, Edithvale (10 ha, 38°50′ S, 145°80′ E), Australia. Both sites consisted of Australian reed (*Phragmites australis*). To find nests we performed "cold searching" through the reeds using a stick to part the vegetation thus making sure to minimize disturbances to the nests and territories. A total of 102 nests were found and observed regularly to determine laying date, hatching date and brood parasitism (the presence of two or more new eggs in one day [Yom-Tov, 1980]). All eggs were weighed every three days to the nearest 0.1 g, measured (maximum length [L] and width [W]) to the nearest 0.1 mm using vernier calipers, photographed and individually marked with an indelible marker. Egg volume (V) was calculated using the formula $V = 0.51*L*W^2$ (Hoyt, 1979; Preston, 1974). The Australian reed warbler has an average clutch size of 2.8 (SD = 0.4, $n = 95$) spotted or blotched bluish or brownish eggs, with eggs laid on successive days and incubated only by the female. Incubation commences when the last egg is laid (Berg, 1998). Bloodsamples from reed warbler chicks and parents were taken and tissue samples of unhatched embryos were collected for minisatellite DNA-fingerprinting (Berg, 1998). These data were used to assess the occurrence of intraspecific brood parasitism. Cuckoo nestlings could be easily distinguished from warbler nestlings by the presence of an extra backward facing toe.

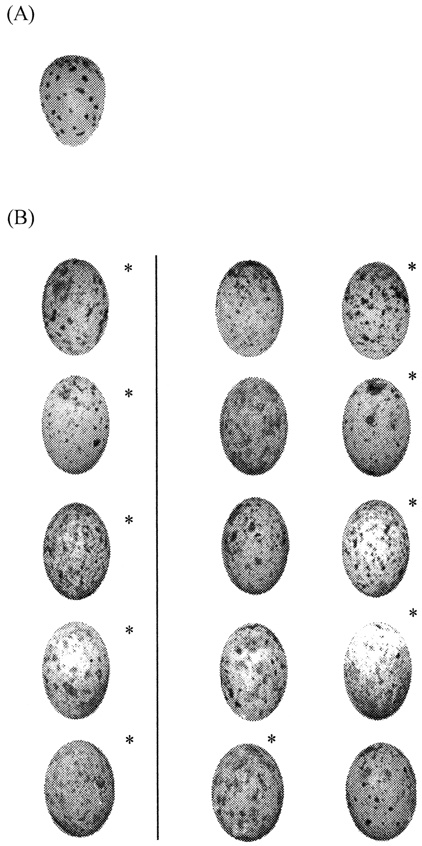

Figure 1
(A) Example of a non-mimetic wax egg; and (B) egg card as shown to the naïve observers, *mark most similar same-clutch eggs (see text).

Experimental brood parasitism

We adopted the experimental procedure first pioneered by Rothstein (1975a) and used extensively by others (Alvarez et al., 1976; Davies and Brooke, 1988, 1989a; Higuchi, 1989; Lawes and Kirkman, 1996; Lotem et al., 1995; Moksnes et al., 1990; Moksnes et al., 1991; Moksnes and Røskaft, 1989) by conducting experiments that mimicked the methods naturally employed by brood parasites. To test for differences in rejection rates according to the degree of mimicry we undertook two experimental treatments. The mimetic treatment consisted of the introduction into a clutch of a single undamaged conspecific egg taken from an active nest in the same study area. The non-mimetic treatment consisted of the introduction into a clutch of a model egg, which was made by pouring white candle wax into a standard mold of plaster-of-paris and then marked with a black indelible marker to approximate the natural pattern of spotting of Australian reed warbler eggs (see Figure 1a). The standard wax-egg measurements (length: 20.0 mm; width: 14.5 mm; mass: 2.0 g) were within the natural range of Australian reed warbler eggs (length: 20.2 ± 0.9 mm, width: 14.6 ± 0.5 mm, $n = 238$; mass: 2.2 ± 0.2 g, $n = 185$).

To test for the effects of the timing in the nesting cycle and host egg removal by the parasite, we used two procedures within each experimental treatment. The ''nest stage procedure'' consisted of the introduction of the experimental egg either during the egg laying period (early) or 1–5 days after the day of clutch completion (late). The egg removal procedure consisted of the addition of the experimental egg either with (replacement) or without (addition) the simultaneous removal of a host egg. Combination of the two procedures resulted in four procedural groups (early replacement, late replacement, early addition, late addition) within each treatment. 3 days after the experimental manipulation the nests were checked for signs of rejection. An experimental conspecific egg and a non-mimetic model egg was considered accepted if it and the rest of the clutch were still present after 3 days without showing any other signs of rejection. If eggs had hatched the nest was excluded from the analysis. Because most rejections (92 %, n = 26) occurred within the 3 day acceptance criterion, using an acceptance criterion of more than 3 days does not affect the overall results. Following Davies and Brooke (1988, 1989a), Lotem et al. (1995) and Rothstein (1975a,b, 1976), we scored the host's response as: (1) rejection by ejection if an egg had disappeared from the nest and the remaining eggs were still being incubated; or (2) rejection by desertion if the nest had been deserted. If all the eggs had disappeared from the clutch, we assumed it had undergone predation and excluded it from the analyses (Rothstein, 1976).

The control nests underwent exactly the same handling as the experimental nest but without the addition of an experimental egg. Control and experimental nests were disturbed in the same frequency (once every 3 days) and for the same duration. Next to the nests, all the eggs were weighed, measured, marked, and some were photographed. After these basic measurements were taken all the eggs were put back with or without the introduction of a foreign egg. Experimental and control treatments were randomly assigned to nests, and the experimental nests and the controls were distributed evenly throughout the study sites to minimize the chance of taking repeated observations of the same individuals.

In a number of experiments employing the introduction of conspecific eggs, we took pictures of the experimental egg together with the host clutch. In the field next to the experimental nest the eggs were laid out on a Kodak© gray card and then photographed with a Fuji© 200 ISO film without a flash. Ten randomly chosen photographs of experimental clutches were scanned using an Agfa DuoScan© scanner. From each photograph the experimental egg and two randomly chosen host eggs were separated from their background using the program Adobe Photoshop 4.0©. The eggs were standardized in size to make sure only egg appearance was a variable. One of the host eggs was used as a reference (placed on the left) to which the other host egg and the experimental egg could be compared (placed in the same row on the right, see Figure 1b). We then asked 28 ''naïve'' people to compare the two eggs next to the reference and rank one of the two as ''most similar'' to the reference.

Statistical analysis

We used logistic regression models to fit proportional data to experimental and correlational variables. Significance of variables was tested by means of the log-likelihood ratio method (McCullagh and Nelder, 1989). It is assumed that the decrease in deviance (ΔDev) caused by addition of a variable to the model is distributed according to an F-distribution with Δdf degrees of freedom in the numerator, where Δdf denotes the change in degrees of freedom by addition of the variable, and df degrees of freedom in the denominator, where df denotes the degrees of freedom in the model with the added variable.

RESULTS

Intra- and interspecific brood parasitism

Four lines of evidence indicate that there was no inter- or intraspecific parasitism in our population: (1) All the observed nestlings were Australian reed warblers (n = 58 nests with 141 nestlings); (2) no more than one egg was laid per day in a single nest (50 nests); (3) no marked eggs ever disappeared with or without being replaced by unmarked egg(s); and (4) DNA minisatellite analysis (Berg, 1998), did not reveal any evidence of intraspecific brood parasitism in 11 broods.

Egg rejection

There was no significant difference between rejection rates across the two sites in the conspecific treatment (Altona, 25%, n = 16; Edithvale, 43%, n = 21, $\chi^2_{\text{yates}} = 0.608$, df = 1, p [two-tailed] = .436) nor in the non-mimetic treatment (Altona, 60 %, n = 5; Edithvale, 100 %, n = 6, Fisher exact test, p [two-tailed] = .363). We therefore pooled the data from both sites. There was no relationship between the number of host eggs in the nest at the time of the experiment (one in 11 cases, two in eight cases, and three in 29 cases) and rejection rate (54.6 %, 62.5 %, and 37.9 rejected, respectively; $\chi^2 = 1.961$, df = 2, p = .375).

The rejection rate from the experimental treatments combined was significantly higher than the control treatment (see Tables 1 and 2), clearly indicating that rejection was a discriminatory response to our artificial parasitism. Both the non-mimetic and the conspecific treatments showed a significantly higher rejection rate than the control treatment (see Tables 1 and 2), indicating that the Australian reed warbler is able not only to discriminate against non-mimetic eggs but against conspecific eggs as well. Additionally, rejection rate was found to be significantly higher in the non-mimetic treatment than in the conspecific treatment (see Tables 1 and 2).

Non-mimetic eggs that were accepted by the hosts were treated similarly by the host as its own eggs: (1) non-mimetic eggs were incubated, turned and moved around the nest during incubation, and (2) in two cases where we swapped a full clutch for an equal number of non-mimetic eggs, one egg was rejected while the other eggs kept on being incubated.

The frequencies of rejection by nest desertion and egg ejection within the conspecific and the non-mimetic treatment are all significantly higher than in the control treatment (see Tables 1 and 2). These findings indicate that both desertion and ejection were rejection responses to our artificial parasitism in the experimental treatments.

Examining these types of rejection within the different treatments more closely we found that in the conspecific treatment the experimental eggs did not have a higher probability of ejection than the host eggs (three experimental eggs ejected out of six [n = 6] ejections (Table 1), all from nests with a total of four [$p = ¼$] eggs, thus $p[X \geq 3, p = ¼, n = 6]$ = .169). This indicates that ejection in response to the introduction of conspecific eggs entails a rejection cost. In all cases either the experimental egg or a host egg was ejected but both types were never ejected from the same nest. Unfortunately too few pictures of experimental clutches from which ejection had taken place were available to relate the probability of (self) ejection to the difference in appearance between experimental and host eggs. In the non-mimetic treatment, experimental eggs did have a higher probability of being ejected than host eggs (four experimental eggs ejected out of four

Table 1

Host rejection responses to control, conspecific and non-mimetic treatments and to egg removal and nest stage procedures

			Rejected				
					Ejected		
Treatment procedure	*N*	Accepted	Total	Deserted	Total	Experimental egg	Own egg
Control							
Early control	17	17	0	0	0	—	—
Late control	21	19	2	1	1	—	—
Total	38	36	2	1	1	—	—
Conspecific							
Early replacement	12	7	5	5	0	0	0
Late replacement	10	10	0	0	0	0	0
Early addition	7	4	3	2	1	1	0
Late addition	8	3	5	0	5	2	3
Total	37	24	13	7	6	3	3
Non-mimetic							
Early replacement	4	1	3	2	1	1	0
Late replacement	4	1	3	2	1	1	0
Early addition	2	0	2	1	1	1	0
Late addition	1	0	1	0	1	1	0
Total	11	2	9	5	4	4	0

ejections (see Table 1), two from nests with a total of three eggs and two from nests with a total of four eggs, thus $p\ (X = 2, p = 1/3, n = 2) * p\ (X = 2, p = \frac{1}{4}, n = 2) = .007$. This indicates that hosts are able to selectively eject a non-mimetic experimental egg.

Neither the nest stage procedure, the egg removal procedure nor their interaction had a significant effect on rejection rate in the experimental treatments combined (see Table 3). The same was true for the effect of these procedures within the non-mimetic treatment (see Table 3). Within the conspecific treatment there was no effect of nest stage and egg removal, however there was a significant interaction between the nest stage and the egg removal procedure (see Table 3). To examine this further we will look at egg rejection in the conspecific treatment in more detail.

Rejection rate in the early replacement group was significantly higher than in the late replacement group (see Tables 1 and 4). In contrast, rejection rates from the early and late addition groups did not differ significantly (see Tables 1and 4). Rejection in the early replacement group did not differ significantly from that in the early addition group, however, rejection in the late replacement group differed significantly

Table 2

Comparisons between treatments for total rejection rates (desertion + ejection), desertion rates, and ejection rates

Term	Deviance	ΔDev	DF	*F*	*p*
Rejection					
Constant	101.84		85		
Control vs. experimental[a]	81.88	19.96	84	20.477	≪.001
Constant	52.19		48		
Control vs. non-mimetic	26.10	26.09	47	46.982	≪.001
Constant	75.06		74		
Control vs. conspecific	63.64	11.42	73	13.100	<.01
Constant	66.21		47		
Conspecific vs. non-mimetic	58.40	7.81	46	6.152	<.017
Desertion					
Constant	36.43		48		
Control vs. non-mimetic	24.41	12.02	47	23.144	≪.001
Constant	50.92		74		
Control vs. conspecific	45.14	5.78	73	9.347	<.004
Ejection					
Constant	32.30		48		
Control vs. non-mimetic	23.67	8.63	47	17.136	<.001
Constant	46.53		74		
Control vs. conspecific	42.05	4.48	73	7.777	<.007

[a] Non-mimetic and conspecific treatments combined.

Table 3

Effect of procedures on rejection rate within the experimental treatments

Treatment	Term	Deviance	ΔDev	DF	*F*	*p*
Experimental	Constant	66.21		47		
	E	63.49	2.72	46	1.971	.167
	E + N	62.53	0.96	45	0.691	.410
	E + N + E * N	60.55	1.98	44	1.439	.237
Non-mimetic	Constant	10.43		10		
	E	9.00	1.43	9	1.430	.259
	E + N	9.00	0.0	8	0	1
	E + N + E * N	9.00	0.0	7	0	1
Conspecific	Constant	47.97		36		
	E	44.31	3.66	35	2.891	.098
	E + N	43.03	1.28	34	1.011	.321
	E + N + E * N	36.45	6.58	33	5.957	.020

E, egg removal procedure; N, nest stage procedure.

from that in the late addition group (see Tables 1and 4). Thus, the warblers showed a different rejection response depending on the time in the nesting cycle the experiment was conducted and whether a conspecific egg was added with or without removal of a host egg.

From the onset of this study we expected that desertion and ejection would be differentially affected by the egg removal and the nest stage procedures. Can this provide an explanation for the apparent effect on rejection rate by the interaction between the egg removal and the nest stage procedure? To answer this we compared differences in type of rejection response within the four procedural groups of the conspecific treatment. If rejection had taken place, both the nest stage procedure and the egg removal procedure had a significant effect on the type of rejection response in the conspecific treatment, however, this was not the case for the interaction between nest stage and egg removal (see Table 5). Thus desertion and ejection rates are differentially affected by the different procedures. Considering desertion rates only, there was no significant effect of the egg removal procedure on desertion rate (see Table 6). There was, however, a significant effect of the nest stage procedure on desertion rate (see Table 6). Considering ejection rates only, both the nest stage and the egg removal procedures had a significant effect on ejection rate (see Table 6). Thus, the warblers showed more desertion and less ejection in response to artificial parasitism during than after the egg laying period. Furthermore, they showed more ejection in response to artificial parasitism by egg addition than by egg replacement.

Egg variability

As there were no differences between the sites in Altona and Edithvale in egg lengths, widths, and volumes (Welbergen, 1997), these results were pooled for further analysis. The greatest source of variance in egg sizes was at the between-clutch level (length: $F_{2,94} = 8.84$, $p < .0001$, width: $F_{2,94} = 9.20$, $p < .0001$, volumes: $F_{2,94} = 10.03$, $p < .0001$), suggesting that egg-size could be used as a cue to parasitism with conspecific eggs. The difference between the average volume of the host eggs and the volume of the rejected experimental eggs was the same as the difference between the average volume of the host eggs and the volume of the accepted experimental eggs ($t = 0.859$, df = 19, p (two-tailed) = .401).

In response to the egg cards (as shown in Figure 1b) the observers correctly assigned the host egg as most similar to the reference egg at an average rate of 84% in the 10 experimental clutches (right vs. wrong, 236 vs. 44, p (X > 235, p = 0.5, n = 280) = .0001). In 8 of the 10 experimental clutches (p (X > 7, p = 0.5, n = 10) = .044), more than 22 of the 28 naive observers assigned the host egg as "most similar" to the reference egg (p (X > 22, p = 0.5, n = 28) < .001). This suggests that egg appearance could potentially be used as a reliable cue to parasitism with conspecific eggs.

DISCUSSION

As far as we know, these are the first data to show that an Australian passerine rejects eggs. The fact that most of the experimental eggs (81%) from the non-mimetic treatment were rejected by the warblers, indicates that the majority of warblers have at least some form of egg rejection behavior. Furthermore, there is strong evidence that the Australian reed warbler discriminates against conspecific eggs. This ability has only rarely been reported for passerine species, although some species are known to reject conspecific eggs before laying but accept them once laying has begun (Davies and Brooke, 1989a; Emlen and Wrege, 1986; Mumme et al., 1983;

Table 4

Comparisons between rejection rates from the procedural groups

Term	Deviance	ΔDev	DF	*F*	*p*
Constant	23.58		21		
Early replacement vs. late replacement	16.30	7.28	20	8.933	0.007
Constant	20.73		14		
Early addition vs. late addition	20.15	0.58	13	0.374	0.551
Constant	25.86		18		
Early replacement vs. early addition	25.86	0.0	17	0	1
Constant	21.27		17		
Late replacement vs. late addition	10.59	10.68	16	16.136	<0.001

Table 5

Effect of procedures on type of rejection response in the rejected cases of the conspecific treatment

Term	Deviance	ΔDev	DF	*F*	*p*
Constant	17.94		12		
E	9.00	8.92	11	10.902	<.006
E + N	3.82	5.18	10	13.560	<.004
E + N + E * N	3.82	0	9	0	1

Møller, 1987); only the weaver bird (*Ploceus cucculatus*; Victoria, 1972), the brambling, (*Fringilla montifringilla*) and the chaffinch (*Fringilla coelebs*) (Braa et al., 1992, Moksnes, 1992) are known to reject conspecific eggs once laying has begun. Although we did not find any evidence for intraspecific brood parasitism in our study species, we cannot completely exclude this as a possible factor to account for the rejection of conspecific eggs. However, because this species is a suitable host to at least seven cuckoo species it lives sympatric with, and because there is evidence from the literature for the occurrence of interspecific brood parasitism in this species, our most parsimonious explanation for its ability to discriminate against conspecific eggs is that the Australian reed warbler has been adapted to an interspecific brood parasite with a very high degree of egg mimicry.

Because we can exclude the possibility that egg dimensions are used as a cue to parasitism, the only alternative possibility is that the warblers use egg appearance (i.e., spotting pattern, background color) to discriminate between their own and conspecific eggs. In this study we found that the interclutch and intraclutch variation in egg appearance were such (for a discussion of the evolution of interclutch and intraclutch variation in response to brood parasitism see Øien, et al., 1995; Soler and Møller, 1996; Stokke et al., 1999), that they enabled human observers to differentiate significantly between host and experimental conspecific eggs. Therefore, egg appearance can potentially be used as a cue to parasitism by the warblers even if brood parasites produced eggs with the same high degree of mimicry as conspecific eggs naturally have.

Mimetic eggs were significantly less often rejected than non-mimetic eggs, suggesting that the warbler is more tolerant towards smaller contrasts between the appearance of its own eggs and that of parasitic eggs. This tendency has been found in several other studies (Brooke and Davies, 1988; Davies and Brooke, 1988; Higuchi, 1989; Lotem et al., 1995; Rothstein, 1982b). We also found that non-mimetic eggs were rejected selectively, where the introduction of (highly mimetic) conspecific eggs led to rejection costs through the accidental ejection of the host's own egg instead of the conspecific egg. These findings all account for the adaptiveness of egg mimicry by brood parasites.

If the rejection costs have been a factor in the coevolution between egg discrimination of the warbler and egg mimicry of its brood parasite(s), we can expect the warbler to have evolved adaptations that minimize these costs. Provided there are costs of rejection, it will only pay the host to reject a parasitic egg that is laid in time to be detrimental to the host's reproductive output. To our knowledge, only two species, the waxwing (*Bombycilla cedrorum*; Rothstein, 1976) and the bluethroat (*Luscinia svecica*; Moksnes et al., 1990), show a higher rejection rate towards model eggs introduced early than those introduced late in the nest cycle. In our study we found clear evidence for such a decline in rejection rate within the replacement group. Since the replacement procedure mimics the natural egg replacement behavior of the cuckoo species that are known to occasionally have parasitized the Australian reed warbler, this response can be reasonably considered representative of the warbler's natural reaction to brood parasitism. But why did we not find the same pattern of response in the addition procedure? The addition procedure may have an additional effect on the host's reproductive output that overrides the effect of nest stage. If the clutch and the brood patch of the Australian reed warbler have a certain optimal size-ratio, it may be that enlarged clutches, such as those caused by the addition procedure, receive less efficient incubation. For this same reason, in some species enlarged clutches have a greater incidence of unhatched eggs (Heg and Treuren, 1998; Hills, 1980; Lerkelund et al., 1993; Wiklund, 1985). In this case it would still be costly, in terms of reduced hatchability of the host's eggs, to refrain from rejection even though the parasitic egg will hatch too late or will not hatch at all. To examine this we compared the survival rates of the accepted clutches from the early replacement group with those from the early addition group. Of the clutches followed beyond hatching, the proportion of broods that contained one or more unhatched original eggs in the early addition group (3/4) is significantly greater than in the early replacement group (0/7) (Fisher exact test: $p = .024$), sup-

Table 6

Effect of procedures on desertion and ejection rates within the conspecific treatment

Term	Deviance	ΔDev	DF	*F*	*p*
Desertion					
Constant	35.89		36		
E	35.36	0.53	35	0.525	.474
E + N	24.68	10.68	34	14.713	<.001
E + N + E * N	24.68	0	33	0	1
Ejection					
Constant	32.80		36		
E	20.19	12.51	35	21.860	≪.001
E + N	16.33	3.86	34	8.037	<.008
E + N + E * N	16.33	0	33	0	1

porting this prediction. This result is in line with the ''host incubation limit hypothesis,'' developed by Davies and Brooke (1988) to explain the still puzzling egg removal behavior of many cuckoo species. According to their hypothesis, it may pay a cuckoo to remove an egg because simple addition of an egg to the host's clutch would lead to less efficient incubation and a concomitant reduction in the hatchability of the cuckoo egg. Provided that a reduction in incubation efficiency affects cuckoo and warbler eggs similarly, our result indirectly verifies their hypothesis.

Considering the effect of the nest stage and the egg removal procedures on the type of rejection response in the conspecific treatment, the rejection responses have the following intricate features: (1) more desertion during than after egg-laying; (2) less ejection during than after egg-laying; and (3) addition leads mainly to ejection whereas replacement leads to desertion. Because the further a host is in the nesting cycle the more investment has been put into the clutch in terms of time, energy and reduced chances of predation and, presumably, reduced chances of successful re-nesting, the overall ''reproductive value'' of a nest will rise according to nest stage. When the overall reproductive value of the nest increases, the benefit of desertion will decline relative to the benefit of ejection. We would therefore expect less desertion late in the nesting cycle than early and conversely, less ejection early in the nesting cycle than late. This is in line with our findings. As demonstrated in other bird species (Davies and Brooke, 1988; Lawes and Kirkman, 1996; Moksnes and Røskaft, 1989; Rothstein, 1975b), the Australian reed warbler did not show a difference in rejection rate when eggs were replaced or when eggs were added to a clutch. However, we found that the type of rejection response was affected by the egg removal procedure. In the Australian reed warbler, with an average clutch size of 2.8, egg replacement by an interspecific brood parasite entails an initial expected reduction of 36% ($1/2.78 = 0.36$) of the warbler's clutch. This initial cost cannot be avoided by ejection. In such a case, given that re-nesting occurs regularly, the warbler will actually gain from desertion; this is in line with our findings. Simple addition of an egg does not entail an initial cost; if the warbler is able to eject the parasitic egg successfully, it will still have the same number of eggs as before it was parasitized. Here we would expect relatively more ejections to occur, this is also in line with our findings.

Intraspecific differences in rejection rate

Inter- and intraspecific differences in egg discrimination have been found within the *Acrocephalus* genus (see Brooke et al., 1998; Brown et al., 1990; Davies and Brooke, 1988, 1989a; Gärtner, 1982). It is reasonable to expect that these differences reflect dissimilar current or past parasitism rates. In another study, Brown et al. (1990) compared egg rejection rates between the reed warbler (*Acrocephalus scirpaceus*) and the Australian reed warbler. They found a higher proportion of desertions in response to addition of non-mimetic and mimetic model eggs in their experimental group (4/34) than in the control group (6 % * 448 = 27/448), but this difference was not significant (Fisher exact test, $p = .166$), suggesting that the desertion was not a response to their models. In contrast to their results, we found clear evidence for desertion in response to artificial parasitism. Because they used model eggs of a different type from our study we cannot compare their results with those from the addition procedure in the non-mimetic treatment in our study. We can, however, compare their results with our results from the addition procedure in the conspecific treatment because it is reasonable to expect that the warblers have more difficulty discriminating against conspecific eggs than any kind of model egg. Comparing their collated results (rejections/total; 4/34) to ours (8/15, Table 1), we found their rejection rate to be significantly lower (Fisher exact test, $p = .0036$). Thus, the result is exactly opposite to the predicted direction: their model eggs are rejected significantly less often than our conspecific eggs. This indicates that there is an inherent difference between the rejection rates of these two geographically separated populations.

The study of Brown et al. was conducted in Middlesex, Western Australia, more than 3500 kilometers away from our study sites. Middlesex and our study sites are separated by a very arid landscape. This explains why the Australian reed warbler and the four cuckoo species that are known to occasionally have parasitized the Australian reed warbler all show a discontinuous distribution between the two sites (Brooker and Brooker, 1989). Additionally, these cuckoo species also show a different host preference in the two regions where the experiments were conducted (Brooker and Brooker, 1989). We suspect that there is little if any gene-flow between both populations of warblers. Therefore, it is likely that these populations display adaptations in response to differential selective pressure from brood parasites.

Recognition versus mimicry

We found that Australian reed warblers are able to discriminate against conspecific eggs that, by definition, represent the upper limit of egg mimicry. Additionally, we have shown that egg appearance can be used by the Australian reed warblers to reliably differentiate between their own and conspecific eggs. This indicates that Australian reed warblers have both the ability and the cues to reliably detect brood parasitism involving parasitic eggs representing the upper limit of mimicry. This seems to be the most likely reason for the fact that the Australian reed warbler is currently not a major biological host to any cuckoo species. This suggests that recognition can evolve beyond mimicry and that hosts may therefore have an inherent advantage in the coevolutionary arms race.

Since the Australian reed warbler does not seem to be parasitized at this point in time and since egg mimicry generally has to coevolve with egg discrimination (Davies and Brooke, 1988; Rothstein, 1990) does this mean that there is no possibility of the Australian reed warbler ever to be parasitized again in the future? In a population that is not parasitized but that shows egg discrimination there may be selection against egg rejection due to occasional recognition errors (Davies and Brooke 1989b). However, even if there were no selection against egg discrimination due to occasional recognition errors, egg discrimination as a neutral trait may still be lost in a population due to random drift. Either way, the result would be that a previously parasitized host now becomes susceptible to new brood parasitism. In this scenario, brood parasites could go shopping among an array of susceptible species before returning to a previous host that has now become susceptible again. The host will be involved in an ''ever oscillating coevolutionary arms race'' with a brood parasite (see also Soler et al., 1998), with both egg-mimicry and egg discrimination being recent adaptations to an ancient evolutionary struggle.

The manuscript benefited greatly from comments and advice provided by Ido Pen, Joost Tinbergen, Rudi Drent, and Claudio Carera (Department of Zoology, University of Groningen, and The Netherlands) and three anonymous referees. We are indebted to Rebecca McIntosh, Iain Woxvold, Michael Magrath (Department of Zoology, University of Melbourne, Australia), and Floor Hallema for their help in the field, and we thank the people from the Department of Zoology, University of Groningen for participating in the assessment of the egg photos. We thank Melbourne Water for allowing us to work on their grounds. Funds were provided by the Australian Research Council (LARC: S19711564) allocated to Jan Komdeur.

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