



Parasitic egg rejection decisions of chalk-browed mockingbirds *Mimus saturninus* are independent of clutch composition

M. A. de la Colina¹ · L. Pompilio¹ · M. E. Hauber² · J. C. Reboreda¹ · B. Mahler¹ 

Received: 12 September 2017 / Revised: 15 December 2017 / Accepted: 26 December 2017 / Published online: 25 January 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Obligate avian brood parasites lay their eggs in nests of other host species, which assume all the costs of parental care for the foreign eggs and chicks. The most common defensive response to parasitism is the rejection of foreign eggs by hosts. Different cognitive mechanisms and decision-making rules may guide both egg recognition and rejection behaviors. Classical optimization models generally assume that decisions are based on the absolute properties of the options (i.e., absolute valuation). Increasing evidence shows instead that hosts' rejection decisions also depend on the context in which options are presented (i.e., context-dependent valuation). Here we study whether the chalk-browed mockingbird's (*Mimus saturninus*) rejection of parasitic shiny cowbird (*Molothrus bonariensis*) eggs is a fixed behavior or varies with the context of the clutch. We tested three possible context-dependent mechanisms: (1) range effect, (2) habituation to variation, and (3) sensitization to variation. We found that mockingbird rejection of parasitic eggs does not change according to the characteristics of the other eggs in the nest. Thus, rejection decisions may exclusively depend on the objective characteristics of the eggs, meaning that the threshold of acceptance or rejection of a foreign egg is context-independent in this system.

Keywords Brood parasitism · Chalk-browed mockingbird · Cognitive mechanism · Egg rejection · Shiny cowbird

Introduction

Obligate avian brood parasites lay their eggs in nests of other host species, which provide all parental care for the foreign eggs and chicks thereafter (Davies 2000). Brood parasitism exerts strong evolutionary selective pressures on hosts, and rejection of the foreign egg has evolved as the most common response to avian brood parasitism (Davies 2000; Krüger 2007; Stoddard and Hauber 2017).

Different cognitive mechanisms and decision-making rules may guide both egg recognition and rejection behaviors in host species. One possibility is that rejection relies on the comparison among the objective properties of the eggs

such as coloration pattern, size, and shape (Rothstein 1982; Nakamura et al. 1998; Langmore and Spottiswoode 2012). This cognitive process has been termed the “template-based recognition mechanism” (Hauber and Low 2014), whereby the template can be innate, imprinted (Rothstein 1974), or progressively learned (Moksnes 1992; Lotem et al. 1995; Moskát et al. 2014a), and defines the relevant objective dimensions to be compared. Rejection decisions are independent of the context and occur when the rejection threshold is exceeded (Hauber et al. 2006). However, increasing evidence from several host species (e.g., Polacikova et al. 2013; Moskát et al. 2014b) shows that egg rejection decisions are not solely dependent on the objective properties of the options but also on the manner in which the options are presented (“frame”), on the presence of other options, and/or on changes in the motivational state of the individual (compare Tversky and Simonson 1993; Kahneman and Tversky 2003; Pompilio et al. 2006). The hypothesis of context-dependent valuation assumes that the decision to accept or reject an egg depends on the variability of the other eggs in the clutch, and is therefore flexible (Moskát et al. 2010).

The chalk-browed mockingbird (*Mimus saturninus*; hereafter mockingbird) is a partial rejecter of the polymorphic

✉ B. Mahler
bemahler@ege.fcen.uba.ar

¹ Departamento de Ecología, Genética y Evolución and IEGEBA-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

² Department of Animal Biology, School of Integrative Biology, University of Illinois, Urbana-Champaign, IL 61801, USA

eggs laid by the parasitic shiny cowbird (*Molothrus bonariensis*; hereafter cowbird). Cowbirds are generalist brood parasites that lay eggs with variable eggshell patterns, ranging from white to light greenish and brownish backgrounds and from immaculate to heavily spotted maculation patterns (Mahler et al. 2008). Mockingbirds only reject immaculate white eggs, while they accept all spotted ones (Fraga 1985; Sackmann and Reboresda 2003). Previously we have shown that the cognitive rules underlying mockingbird rejection behavior can be explained by a decision-making model in which the level of rejection is inversely related to the number of relevant attributes shared between host and parasite eggs, i.e., presence of spotting, low brightness, and no UV reflectance (de la Colina et al. 2012). Here we analyzed whether this multi-cue decision-making process depends exclusively on the intrinsic characteristics of the foreign egg (as proposed by the hypothesis of absolute valuation) or, instead, depends on the characteristics of the other eggs in the clutch (as proposed by the hypothesis of context-dependent valuation).

We tested three possible cognitive mechanisms for context-dependent valuation. The “range effect hypothesis” predicts that when the magnitude of the differences in a relevant attribute among options increases, differences between initial options are perceived as smaller (Parducci 1965, 1974). Thus, the presence of eggs which extend the range of differences in the clutch may relax the acceptance threshold, increasing the probability of accepting a foreign egg. On the other hand, mockingbirds’ evaluation mechanisms might be affected by non-associative learning (Groves and Thompson 1970). Egg variability (i.e., the number of different eggs) would be used as a measure of parasitism intensity (multiple parasitism: Bán et al. 2013; Stevens et al. 2013), increasing the likelihood of accepting (“habituation to variation hypothesis”) or rejecting (“sensitization to variation hypothesis”) parasitic eggs.

Materials and methods

Study site and nests

Experiments were carried out at Reserva El Destino, near the town of Magdalena in the Province of Buenos Aires, Argentina (35°08’S, 57°23’W), during the southern breeding season of 2009–2010 (October–January). In an area of 580 ha, we searched for mockingbird breeding pairs and captured and individually color-banded 26 breeding pairs within their territory. Mockingbirds build open nests, egg laying usually begins within 3 days from nest completion, and the modal clutch size is four eggs (range 3–5; Fraga 1985). The natural parasitism rate of mockingbird nests in this area is 61%, with an intensity of 2.2 parasitic eggs per nest (de la Colina et al. 2016).

Experiments

To test whether mockingbirds change rejection decisions with clutch context, we designed eggs to modify both the range and variability of the clutch (Table 1, Fig. 1A). Each treatment consisted of the addition of a pair of cowbird-sized model eggs (24.9 mm × 18.8 mm; de la Colina et al. 2011), molded from plaster of paris, to the host’s clutch (Fig. 1A). Experimental eggs were differentially colored with non-toxic acrylic paint. All treatments contained a brown egg, the focal egg, which generates “intermediate” rejection responses close to 50% (de la Colina et al. 2012) and allows measurement of changes in the threshold of acceptance/rejection (Røskaft et al. 2002). A different experimental egg was added along with the focal egg in each of the three treatments (Fig. 1A), modifying the range, i.e., the magnitude of differences (narrow/wide), and the variability, i.e., the number of eggs perceived as different (low/high), of the clutch, which predicts different changes in rejection of the focal

Table 1 Predicted responses of mockingbird rejection of the focal experimental egg according to four different hypothetical cognitive mechanisms (see text). An intermediate response corresponds to 50% rejection found previously (de la Colina et al. 2012). The features

of the clutch under manipulation between treatments are range (i.e., magnitude of the difference among eggs in the clutch) and variability (i.e., number of different egg types in each clutch)

Hypothesized cognitive mechanism	Treatment		
	1	2	3
Range	Narrow	Wide	Narrow
Variability	Low	High	High
Absolute valuation	Intermediate	Intermediate	Intermediate
Context-dependent			
Range effect	Intermediate	Lower	Intermediate
Habituation to variation	Intermediate	Lower	Lower
Sensibility to variation	Intermediate	Higher	Higher

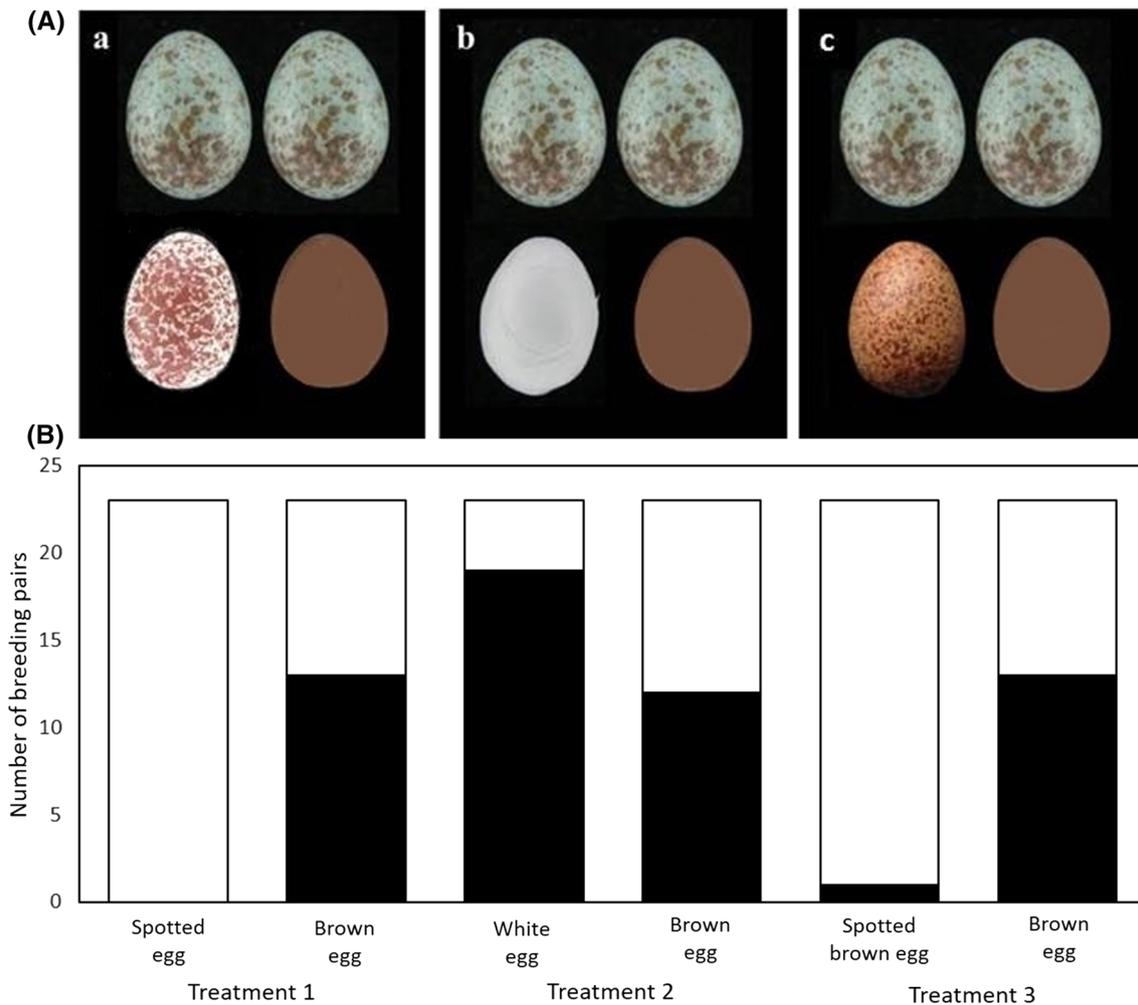


Fig. 1 **A** Experimental eggs added to mockingbird nests (lower line) containing two host eggs (upper line). *a* Treatment 1: narrow range, low variability, *b* treatment 2: wide range, high variability, *c* treat-

ment 3: narrow range, high variability. **B** Breeding pairs rejecting (black bars) and accepting (white bars) each experimental egg

egg according to the cognitive mechanism (Table 1). Treatment narrow/low: spotted egg (0% rejection, de la Colina et al. 2012; Fig. 1a), treatment wide/high: white egg (73% rejection, de la Colina et al. 2012; Fig. 1b), and treatment narrow/high: spotted brown egg (Fig. 1c). We estimate that rejection of this experimental egg falls within the range of acceptance between the focal egg and the spotted egg, as it has both attributes that increase the probability of being accepted (spots and low brightness).

Each breeding pair was exposed to all treatments sequentially within the same reproductive attempt after the host’s second egg was laid. The design was balanced by experimental egg pair, so as to achieve the same number of breeding pairs exposed to a different sequence of treatment order. The clutch size was fixed at four eggs during the entire experiment; additional mockingbird or cowbird eggs were removed daily. Upon completion of each experiment, host

eggs were returned to their nests. Each pair of experimental eggs was left in the nest for 24 h, the period of time when rejection occurs (de la Colina et al. 2012). Rejection was recorded and accepted experimental eggs were removed and replaced by the ones included in the following treatment. Abandoned nests ($N = 3$) were excluded from the analysis since it was not possible to determine whether the abandonment was a response to treatment or due to natural causes (Hauber et al. 2014). Four nests were excluded because they were victims of natural parasitism during the 3-day experimental protocol, leaving 19 nests for the analyses.

Statistical analysis

Generalized (logistic) linear mixed-effect models (GLMM) were used to assess the relationship between explanatory variables and rejection events using a binary response

variable where 0 or 1 denotes acceptance or rejection, respectively. The models were fitted by the Laplace approximation using the *glmer* function in the *lme4* library (Bates et al. 2014, R version 3.1.2.). Nest identity was included as a random effect to account for the biological and potential statistical non-independence of treatments at the same nest in repeated parasitism. The explanatory variables were clutch treatment (nominal, where 0 or 1 denotes low or high level of range or variability) and treatment order (ordinal: 1, 2, 3). We ran four different models (order, treatment, order + treatment, and order*treatment) and compared AIC values with the null model. Significance of parameters was tested with pairwise ANOVAs between models. We calculated the power of our two-factor model with the *pwr.f2.test* function in the *pwr* library, using the following parameters: $u = 2$, $v = 20$, $f = 0.4$, $P = 0.05$. We also tested whether rejection differed between experimental eggs within treatment with a Wilcoxon signed-rank test using the *mass* library.

Results

Rejection frequencies were different between experimental eggs in all treatments (Fig. 1B): (1) spotted—focal brown egg ($P < 0.001$); (2) white—focal brown egg ($P = 0.01$); and (3) spotted brown egg—focal brown egg ($P < 0.001$). For each type of experimental egg, rejection was similar to those reported in previous, single-egg experimental studies (de la Colina et al. 2012): spotted 0% and white 82%. The spotted brown egg fell within the expected low rejection range (4%). About half of the breeding pairs rejected the focal brown egg in all treatments (52–56%).

Rejection responses to the focal brown egg were consistent in 14 nests (74%, 9 breeding pairs always rejected the focal experimental egg, while 5 breeding pairs accepted it under all treatments). For the 5 nests that changed rejection behavior with treatment, there was no general pattern of change in the response.

Δ AIC values were higher than 2, indicating that no model explained rejection better than the null model (Table 2). Neither the interaction ($P = 0.28$) nor both terms (order $P = 0.91$; treatment $P = 0.87$) were significant. The power of the two-factor analysis was 0.71.

Discussion

Rejection behavior of parasite eggs in mockingbirds did not change according to the characteristics of the other eggs in the nest, thus supporting the hypothesis of absolute valuation in this host's egg rejection decisions. This implies that, unlike in some other host species (Moskát et al. 2014b; Yang et al. 2015), decisions depend exclusively on the objective

Table 2 AIC values of all models and significance values of parameters

Model	AIC	Δ AIC	<i>P</i> value
Null	81.4	–	–
Order	85.1	3.7	0.91
Treatment	85.1	3.7	0.87
Order + treatment	88.9	7.5	–
Order \times treatment	91.9	10.5	0.28

characteristics of the eggs and that the threshold of acceptance or rejection of a particular type of parasitic egg is independent of the clutch context. A context-independent threshold maximizes the rejection of foreign eggs and minimizes the error of rejecting own eggs. Since parasitic eggs do not impose a severe fitness cost to mockingbirds' breeding success (Sackmann and Reboresda 2003), it is expected that evolutionary selective pressures will favor a cognitive mechanism which minimizes the rejection of own eggs, as we found. Only white immaculate cowbird eggs are rejected by mockingbirds, based on a mechanism that relies on several cues to detect a foreign egg (de la Colina et al. 2012). It has been also shown that parasitic eggs in the nest decrease the probability of egg puncture by other parasitizing cowbird females (Gloag et al. 2012), although white eggs make nests more conspicuous and prone to predation (de Mársico et al. 2016). Therefore, rejection of these eggs would be favorable for nest survival in general.

Additionally, a context-independent threshold might be an advantageous mechanism in cases of multiple parasitism with polymorphic parasite eggs. Indeed, multiple parasitism is found in 61% of the nests in this area (de la Colina et al. 2016), which can receive up to 11 cowbird eggs (Gloag et al. 2012). In this scenario, rejection would be very unpredictable if it relied on a context-dependent mechanism.

We showed that the cognitive basis of egg rejection decisions in mockingbirds is insensitive to changes in variability (number of eggs perceived as different) and magnitude of differences (range) among eggs in a clutch, as was found in the American robins (*Turdus migratorius*; Croston and Hauber 2015). These results are therefore compatible with the idea that this host compares each egg in the clutch against a template and rejects dissimilar eggs.

Acknowledgements We thank Fundación Elsa Shaw de Pearson for allowing us to conduct this study at “Reserva El Destino.” All authors declare that they have no conflict of interest and are grateful to following institutions for funding: Royal Society of New Zealand's ISAT grant program, Universidad de Buenos Aires, Agencia Nacional de Promoción Científica y Tecnológica de Argentina, and Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina. D. Allen provided helpful comments on the English text of an early manuscript draft. MAC dedicates this work to Carmen *Mausy* Tornow and deeply appreciates her collaboration in manufacturing all experimental eggs.

Compliance with ethical standards

Human and animal rights statement Experiments were carried out with governmental approval (Organismo Provincial para el Desarrollo Sostenible de la Provincia de Buenos Aires, OPDS Disp# 202/2012) and the ASAB/ABS guidelines for the use of animals (2012) were followed.

References

- ASAB, ABS (2012) Guidelines for the treatment of animals in behavioural research and teaching. *Anim Beh* 83:301–309
- Bán M, Moskát C, Barta Z, Hauber ME (2013) Simultaneous viewing of own and parasitic eggs is not required for egg rejection by a cuckoo host. *Behav Ecol* 24:1014–1021
- Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. R package vers 1:1–23
- Croston R, Hauber ME (2015) Experimental shifts in intraclutch egg color variation do not affect egg rejection in a host of a non-egg-mimetic avian brood parasite. *PLoS ONE* 10:e0121213
- Davies NB (2000) Cuckoos, cowbirds and other cheats. T and AD Poyser, London
- de la Colina MA, Mahler B, Reboreda JC (2011) Differences in morphology and colour pattern of shiny cowbird (*Molothrus bonariensis*) eggs found in nests of two hosts. *Biol J Linn Soc* 102:838–845
- de la Colina MA, Pompilio L, Hauber ME, Reboreda JC, Mahler B (2012) Different recognition cues reveal the decision rules used for egg rejection by hosts of a variably mimetic avian brood parasite. *Anim Cogn* 15:881–889
- de la Colina MA, Hauber ME, Strausberger BM, Reboreda JC, Mahler B (2016) Molecular tracking of individual host use in the Shiny Cowbird, a generalist brood parasite. *Ecol Evol* 6:4684–4696
- De Mársico MC, Ursino CA, Reboreda JC (2016) Experimental evidence for an antipredatory function of egg rejection behaviour in a common host of the brood-parasitic shiny cowbird. *Behav Ecol Sociobiol* 70:1689–1697
- Fraga RM (1985) Host-parasite interactions between chalk-browed mockingbirds and shiny cowbirds. *Ornithol Monogr* 36:829–844
- Gloag R, Fiorini VD, Reboreda JC, Kacelnik A (2012) Brood parasite eggs enhance egg survivorship in a multiply parasitized host. *Proc R Soc Lond B* 279:1831–1839
- Groves PM, Thompson RF (1970) Habituation: a dual-process theory. *Psychol Rev* 77:419–450
- Hauber ME, Low J (2014) Ch. 9: Avian brood parasitism: how to spot a foreign egg in the nest? In: Yasukawa K (ed) *Animal behavior*, volume 3. Integration and application with case studies. Praeger Press, Santa Barbara, pp 249–268
- Hauber ME, Moskát C, Bán M (2006) Experimental shift in hosts' acceptance threshold of inaccurate-mimic brood parasite eggs. *Biol Lett* 2:177–180
- Hauber ME, Samaš P, Anderson MG, Rutila J, Low J, Cassey P, Grim T (2014) Life-history theory predicts host behavioural responses to experimental brood parasitism. *Ethol Ecol Evol* 26:349–364
- Kahneman D, Tversky A (2003) The rational choice, values and frames. *Psikhol Zhurnal* 24:31–42
- Krüger O (2007) Cuckoos, cowbirds and hosts: adaptations, trade-offs and constraints. *Phil Trans R Soc B Biol Sci* 362:1873–1886
- Langmore NE, Spottiswoode CN (2012) Visual trickery in avian brood parasites. In: Hughes DP, Brodeur J, Thomas F (eds) *Host manipulation by parasites*. Oxford University Press, Oxford, pp 95–118
- Lotem A, Nakamura H, Zahavi A (1995) Constraints on egg discrimination and cuckoo-host co-evolution. *Anim Behav* 49:1185–1209
- Mahler B, Confalonieri VA, Lovette IJ, Reboreda JC (2008) Eggshell spotting in brood parasitic shiny cowbirds (*Molothrus bonariensis*) is not linked to the female sex chromosome. *Behav Ecol Sociobiol* 62:1193–1199
- Moksnes A (1992) Egg recognition in chaffinches and bramblings. *Anim Behav* 44:993–995
- Moskát C, Bán M, Székely T, Komdeur J, Lucassen RWG, van Boheemen AL, Hauber ME (2010) Discordancy or template based recognition? Dissecting the cognitive basis of the rejection of foreign eggs in hosts of avian brood parasites. *J Exp Biol* 213:1976–1983
- Moskát C, Ban M, Hauber ME (2014a) Naïve hosts of avian brood parasites accept foreign eggs, whereas older hosts fine-tune foreign egg discrimination during laying. *Front Zool* 11:45
- Moskát C, Zölei A, Ban M, Elek Z, Tong L, Geltsch N, Hauber ME (2014b) How to spot a stranger's egg? A mimicry-specific discordancy effect in the recognition of parasitic eggs. *Ethology* 120:616–626
- Nakamura H, Kubota S, Suzuki R (1998) Coevolution between the common cuckoo and its major hosts in Japan. In: Rothstein SI, Robinson SK (eds) *Parasitic birds and their hosts: studies in coevolution*. Oxford University Press, New York, pp 94–112
- Parducci A (1965) Category judgement: a range-frequency model. *Psychol Rev* 72:407–418
- Parducci A (1974) Category judgment: a range-frequency analysis. In: Carterette EC, Friedman MP (eds) *Handbook of perception*, vol 11. Academic Press, New York
- Polacikova L, Takasu F, Stokke BG, Moksnes A, Roskaft E, Cassey P, Hauber ME, Grim T (2013) Egg arrangement in avian clutches covaries with the rejection of foreign eggs. *Anim Cogn* 16:819–828
- Pompilio L, Kacelnik A, Behmer S (2006) State-dependent learned valuation drives choice in an invertebrate. *Science* 311:1613–1615
- Røskaft E, Moksnes A, Meilvang D, Bick D, Jemelkova J, Honza M (2002) No evidence for recognition errors in *Acrocephalus* warblers. *J Avian Biol* 33:31–38
- Rothstein SI (1974) Mechanisms of avian egg recognition: possible learned and innate factors. *Auk* 91:796–807
- Rothstein SI (1982) Mechanisms of avian egg recognition: which egg parameters elicit responses by rejecter species? *Behav Ecol Sociobiol* 11:229–239
- Sackmann P, Reboreda JC (2003) A comparative study of shiny cowbird parasitism in two large hosts: chalk-browed mockingbird and rufous-bellied thrush. *Condor* 105:728–736
- Stevens M, Troscianko J, Spottiswoode CN (2013) Repeated targeting of the same hosts by a brood parasite compromises host egg rejection. *Nat Commun* 4:2475
- Stoddard MC, Hauber ME (2017) Colour, vision and coevolution in avian brood parasitism. *Phil Trans R Soc B* 371:20160339
- Tversky A, Simonson I (1993) Context-dependent preferences. *Manag Sci* 39:10
- Yang C, Chen M, Wang L, Liang W, Møller AP (2015) Nest sanitation elicits egg discrimination in cuckoo hosts. *Anim Cogn* 18:1373–1377