

Foreign egg retention by avian hosts in repeated brood parasitism: why do rejecters accept?

Csaba Moskát · Márk E. Hauber · Zoltán Elek ·
Moniek Gommers · Miklós Bán · Frank Groenewoud ·
Tom S. L. Versluijs · Christiaan W. A. Hoetz · Jan Komdeur

Received: 9 April 2013 / Revised: 11 November 2013 / Accepted: 12 November 2013 / Published online: 4 December 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract Great reed warblers (*Acrocephalus arundinaceus*) are frequently parasitized by egg-mimetic common cuckoos (*Cuculus canorus*) in Hungary, and these hosts reject about a third of parasitic eggs. The timing of parasitism is important, in that the probability of rejection decreases with advancing breeding stages in this host. Also, egg rejection is more common when a clutch is parasitized by a single foreign egg, compared to parasitism by multiple eggs. We repeatedly parasitized great reed warbler clutches with moderately mimetic foreign eggs, either with (1) one foreign egg (single parasitism) and, after 3 days, by all foreign eggs (multiple

parasitism), or (2) all foreign eggs and, 3 days later, by only one foreign egg. Hosts ejected 26–53 % of the experimental parasitic eggs in the first stage of the repeated parasitism, but almost all eggs were accepted in the second stage, irrespective of whether the clutch was singly or multiply parasitized. Video-taping of the behavioural responses of hosts to experimental parasitism revealed no evidence for sensory constraints on foreign-egg recognition, because hosts recognized and pecked the parasitic eggs as frequently in the second stage of repeated parasitism, as they did in the first stage. We suggest that the relative timing of parasitism (laying vs. incubation stage), rather than learning to accept earlier-laid foreign eggs, results in higher acceptance rates of cuckoo eggs in repeated parasitism, because there is decreasing natural cuckoo parasitism on this host species and, hence, less need for antiparasitic defences, with the advancing stages of breeding.

Communicated by M. Soler

Electronic supplementary material The online version of this article (doi:10.1007/s00265-013-1654-y) contains supplementary material, which is available to authorized users.

C. Moskát (✉) · Z. Elek
MTA-ELTE-MTM Ecology Research Group, Hungarian Academy of Sciences, c/o Biological Institute, Eötvös Lóránd University and Hungarian Natural History Museum,
Pázmány Péter sétány 1/C., Budapest H-1117, Hungary
e-mail: moskat@nhmus.hu

M. E. Hauber
Department of Psychology, Hunter College and the Graduate Center of the City University of New York, 695 Park Avenue, New York, NY 10065, USA

M. Gommers · F. Groenewoud · T. S. L. Versluijs · C. W. A. Hoetz · J. Komdeur
Behavioural Ecology and Self-organization, Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 11103, 9700 CC Groningen, The Netherlands

M. Bán
MTA-DE “Lendület” Behavioural Ecology Research Group, Department of Evolutionary Zoology, University of Debrecen, Debrecen 4010, Hungary

Keywords Brood parasitism · Antiparasite defence · Repeated parasitism · Egg recognition · Egg rejection · Egg retention

Introduction

Parental investment is costly (Clutton-Brock 1991), and hosts of obligate brood parasites suffer fitness losses by provisioning unrelated young (Davies 2000, 2011). To avoid detection and rejection by hosts, avian brood parasites may locally specialize on a host species, and evolve eggs or young closely resembling those of the hosts (Kilner and Langmore 2011). Some of the best-known examples of brood parasites' adaptations to hosts can be found in common cuckoos (*Cuculus canorus*; Moksnes and Røskaft 1995; Higuchi 1998; Davies 2000; hereafter: the cuckoo), which lay mimetically coloured and patterned eggs (e.g. Brooke and Davies 1988; Antonov

et al. 2010; Stoddard and Stevens 2010, 2011; Igic et al. 2012; Moskát et al. 2012). In a co-evolutionary arms race, hosts respond to parasitic egg mimicry by evolving ever more fine-tuned abilities of discrimination between own and foreign eggs, to reject parasitic eggs by egg ejection, egg burial, or nest desertion (Rothstein 1975; Davies and Brooke 1988; Moksnes et al. 1991; Lotem et al. 1995; Hosoi and Rothstein 2000; Sealy and Underwood 2012).

Accepting a parasitic egg is particularly costly for hosts of the common cuckoo, because the hatchling parasite evicts all host eggs and nestmates (Anderson et al. 2009), thereby annihilating any fitness benefit gained from that reproductive attempt. Accordingly, the cuckoo egg must be eliminated by the host prior to hatching, either during the egg laying or the incubation period. Despite the clear benefits of rejecting cuckoo eggs, many hosts do not do so; they accept the parasitic egg and eventually face the loss of their reproductive attempt, including cases where the physical characteristics of the eggs prevent rejection by ejection (Antonov et al. 2009; Krüger 2011). More puzzling is the scenario when hosts clearly possess the sensory and morphological adaptations to recognize and remove foreign eggs (Hauber et al. 2006; Moskát and Hauber 2007), yet they do not do so.

Our study aimed to explore the interactions between parasitism pressures, breeding stages, and cognitive dimensions, to examine why hosts do not use their ability to rescue their own breeding attempt by rejecting cuckoo eggs in a heavily parasitized population of the great reed warbler (*Acrocephalus arundinaceus*). We designed a set of treatments to study hosts' responses to parasitism under several conditions, including single, multiple, and repeated parasitism (Hauber et al. 2004; Samas et al. 2011), while also comparing the effects of breeding stage (laying vs. incubation stage) and prior experience with foreign eggs (Hauber et al. 2006). We assessed differences in egg discrimination decisions on the basis of the frequency of egg ejection behaviours in response to our experimental parasitism (e.g. Davies and Brooke 1988; Moksnes et al. 1991; Hale and Briskie 2007; Begum et al. 2012; de la Colina et al. 2012). We also used video recordings of host behaviours at the nest after the experimental manipulation, to record any pecking of eggs by hosts, which can be regarded as an the indicator of foreign egg recognition (Soler et al. 2002; Antonov et al. 2008, 2009; Honza et al. 2007; Pozgayová et al. 2011). We examined the dynamics of the acceptance of the parasitic eggs in great reed warblers, regarding the temporal progress of the breeding cycle (laying vs. incubation nesting stages), and tested which cognitive rules allow or limit foreign egg recognition, by limiting the possibility to use discordancy or template-based recognition (sensu Moskát et al. 2010; Bán et al. 2013).

Soon after the onset of our study (during the first year), we found that hosts showed almost no rejection toward experimental parasitic eggs in repeated parasitism (i.e., parasitism

with a single egg of the same nest, spaced several days apart; see below). Therefore, we generated two ad hoc hypotheses, and tested their specific predictions, regarding decreasing rejection rates with temporal progress across the breeding stages, from laying to incubation. First, following the hypothesis by Rothstein 1976 (see also Davies and Brooke 1989, and Moksnes and Røskaft 1991), we hypothesized that hosts may modulate the intensity of their egg discrimination in response to changes in the risk of parasitism during the nesting stages. Accordingly, we predicted lower rejection rates with progressing incubation in single parasitism, irrespective of prior experience to parasitism, during periods of lower parasitism pressure and costs, i.e., in the later breeding stages (e.g., few to no cuckoo eggs laid during the incubation vs. more cuckoo eggs laid during the laying stage, Moskát 2005, as later laid cuckoo eggs have a lower chance to hatch, Birkhead et al. 2011). Second, we hypothesized that hosts which did not reject parasitic eggs in the first stage of the experiment, may have learned the attributes of the experimental egg as one of their own eggs, so later these same hosts might consider the second set of foreign egg(s) in the experimental repeated parasitism, as their own, and accept it. Therefore, we predicted lower rejection rates of the foreign eggs in the second stage of the repeated parasitism treatment, compared to rejection rates of foreign eggs in single parasitism during the later stage of the breeding cycle (i.e. incubation).

Methods

Study site and species

The research was performed ca. 50 km south of Budapest, Hungary, in the surroundings of Apaj village (47°07'N; 19°06'E). We searched for the nests of great reed warblers in 2–3 m wide reed beds (*Phragmites australis*) on both sides of narrow irrigation channels, covering the same segments once or twice a week, from mid May until late June in 2010 and 2012. This host species is heavily parasitized by common cuckoos in this area (ca. 40–65 %; Moskát et al. 2012). We used naturally non-parasitized nests for our experiments, and nests that were parasitized during our monitoring period, were excluded from the analyses. At this study site, great reed warblers normally breed once in a season (our unpublished result), but replacement clutches may occur in the same territory, as a consequence of predation. We did not use more than one nest within a territory to avoid pseudo-replication. Individual colour banding of about half of the adult breeding birds in 2010 also helped to avoid pseudo-replication. Only the females are responsible for egg rejection in this species (Pozgayová et al. 2009; Trnka et al. 2012).

In our study area, cuckoo eggs show extensive variation in colour and maculation (e.g., Cherry et al. 2007; Moskát et al. 2012), but the size of a cuckoo egg is similar to a great reed

warbler egg (Hargitai et al. 2010), although they are different in shape (Bán et al. 2011). Therefore, we used painted host eggs for experimental parasitism, instead of introducing model eggs into host nests (Zölei et al. 2012). We used yellow highlighter pens (Schwan Stabilo Boss art No. 7024; for spectral data, see Bán et al. 2013) for dyeing the eggs, causing a green-yellowish coat covering the entire eggs' surface. Although this highlighter's dye was water-soluble, it typically persisted in coating the shells for the full 3- or 6-day monitoring periods. At about 10 % of the nests, small patches of dye were rubbed off the eggshells and made it necessary to repaint during one of the daily nest checks. Great reed warblers rejected this egg type in single experimental parasitism at a similar frequency as real cuckoo eggs in natural parasitism (50 % rejection rate; $n=12$ rejection of yellow dyed eggs in single experimental parasitism; Bán et al. 2013 vs. 40 % of real cuckoo eggs in single parasitism; $n=45$; Moskát et al. 2009).

Experimental treatments

In our experiments, we simulated two types of natural cuckoo parasitism: (1) single parasitism with one foreign egg in a clutch, which can be regarded the most common case of brood parasitism across most species and populations (Davies 2000), or (2) multiple parasitism (Moskát et al. 2009). In theory, multiple parasitism applies for all cases with more than one parasitic egg in a clutch, but in our study, multiple parasitism was simulated with all eggs being parasitic. This experimental design is useful to test the "template-based" recognition mechanism for foreign egg rejection (Moskát et al. 2010; Bán et al. 2013), because the alternative mechanism, "discordancy" (Rensch 1925; but see Rothstein 1974; Sealy and Underwood 2012), cannot be applied by hosts when all eggs in clutch belong to the same, albeit foreign, egg phenotype. This experimental design is also conceptually similar to the case when the hosts' clutch size is only one, and it contains just the parasitic egg (Lahti and Lahti 2002), or to those cases during the early stages of the laying cycle, when the cuckoo lays before the onset of the hosts' incubation (Sealy 1995), or when the parasite replaces the host's first egg in the clutch (Moskát and Hauber 2007). In turn, we called the type of experimental parasitism, when foreign eggs are introduced into the nest at the same point in time, "one-time parasitism"; in turn, when two different parasitism events follow each other at a nest, separated by 1 or more days (e.g., a single parasitism and, after 3 days, multiple parasitism, or vice versa), we call this type of parasitism "repeated parasitism".

Experiment 1 (repeated parasitism)

In this experiment, we studied how the same individuals respond to single and multiple parasitism when the two treatments follow one another (termed "repeated parasitism").

Treatment 1a On the fifth day of laying (denoted by 5d), when the clutch contained five eggs, we chose one host egg randomly from the clutch and dyed it yellow. If clutch size was four, we also started our experiment on the fifth day to parallel the case when clutch size was five, as previous studies revealed no effect of clutch size on great reed warblers' egg rejection (e.g., Moskát et al. 2010). Our manipulation typically altered the original position of all eggs in the clutch (Polaciková et al. 2013). Experimental nests were monitored daily on 3 consecutive days in each categories of Experiment 1. Host response to the foreign egg was categorized as acceptance if the dyed egg was still in the clutch and looked undamaged. If the eggs were cold, and were not rotated for 2 days (for details, see Bán et al. 2013), the hosts' response was categorised as nest desertion. If the eggs were incubated, but the parasitic egg was missing, the outcome of the experiment was considered as egg ejection.

Treatment 1b Three days after treatment 1a began, at 8d, all remaining host eggs were dyed yellow. An ejection was detected if at least one painted egg was missing from the nest.

Treatment 2a As in treatment 1b, but all host eggs in the clutch were dyed yellow at 5d.

Treatment 2b After 3 days of monitoring treatment 2a, at 8d, all dyed host eggs except one was cleaned off of yellow dye, by rinsing the eggs in water, and then one egg was repainted yellow.

Control No eggs in the control nests were dyed, but the eggs were inspected and handled similarly to those in the experimental nests.

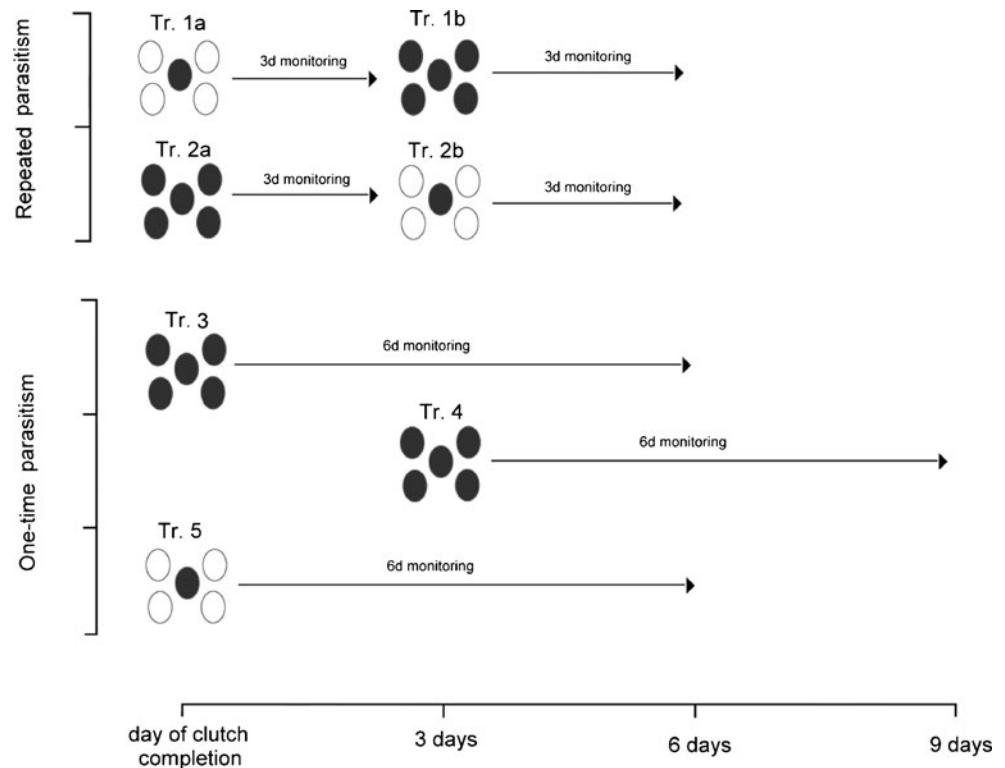
All clutch manipulations in this experiment were carried out in 2010 and 2012. The schematic representation of this experiment's treatments is illustrated in Fig. 1.

Experiment 2 (one-time parasitism)

As Experiment 1 yielded unpredicted results in the second, repeated parasitism portion of our treatments (namely that we detected many fewer rejections toward the foreign eggs in treatments 1b and 2b; see Results for details), we designed and conducted a new experiment to test alternative explanations for the patterns of egg rejection documented in Experiment 1.

Treatment 3 All host eggs were dyed yellow at 5d. This is the same manipulation as in treatment 2a, but the monitoring period was 6 days post manipulation (which is the standard in cuckoo-host egg rejection studies: Grim et al. 2011), instead of 3 days. No other treatment (i.e., repeated parasitism) followed this one.

Fig. 1 The schematic representation of treatments (*Tr.*) applied in repeated parasitisms (Experiment 1) and non-repeated parasitisms (Experiment 2). White eggs denote the host's own eggs, and dark eggs denote experimental parasitic eggs. Monitoring periods after parasitism (3 or 6 days) are also shown, whereas hatching time of host eggs is about 11–12 days after clutch completion (our unpublished result). Clutch size shown in the figure is five, which is the modal clutch size of great reed warblers in our population. However, some clutches contained four or six eggs, which did not affect rejections as our linear model revealed (see Results for more details). On the x-axis, day of clutch completion refers to the fifth day of laying (5d) and 3 days refers to three days later during the incubation period (8d)



Treatment 4 As above in treatment 3, but manipulations were done at 8d. Again, this treatment is similar to treatment 1b, but no manipulation was done preceding this one, and the monitoring period was 6 days post manipulation.

Treatment 5 We used data on one-time parasitism with one host egg dyed yellow, started at 5d, and with a 6-day monitoring period to evaluate to effect of the length of monitoring period (3 or 6 days) on this host's egg rejection rates.

Control As in Experiment 1.

Experiment 2 was carried out in 2012, but for treatment 5 we also included our published results on egg rejection behaviours from recent years (Bán et al. 2013).

Video recordings

In 2012, we documented host behaviours at the nest, immediately after the manipulation of their clutches using five digital camcorders (Samsung HMX-H300 cameras using 32-GB Samsung SDHC memory cards for storage, with the options of 1860/60i resolution and 1920x1080 video quality). We positioned each camera 4–5 m from the host nests on tripods, and left them in place for 15–20 min, before recording started to allow habituation of the parents. To avoid human disturbance, we left the immediate vicinity of the camera (>50 m) to reduce observer disturbance during recording. Recording sessions typically lasted more than 2 h ($2.30 \pm$

0.023 h [mean \pm SE], measured from first arrival of the female bird). We recorded host behaviours in three different treatment categories in 2012: (1) treatment 1b, (2) treatment 3, and (3) control.

As we were interested in host behaviour occurring just after experimental parasitism, we quantified the following elements of host behaviour: (1) "pecking" (number of pecks directed at the clutch/h), (2) "inspection" (Fig. 2; seconds of time spent on looking at the clutch/h); (3) "sitting" (seconds of time spent on sitting on the eggs/h), and (4) "rotation" (seconds of time spent on rotating the eggs/h).

Statistical analysis

Generalized (logistic) linear mixed-effect models (GLMM; Bolker et al. 2009; Nakagawa and Hauber 2011) were used to assess the relationship between explanatory variables and the ejection events of single eggs, using a binary response variable (where 0 or 1 denotes acceptance or rejection, respectively). Nest identity was included as a random effect to account for non-independence of treatments at the same nest in repeated parasitism. We used the following explanatory variables in the models: treatment, start of egg laying (i.e., first egg laying date), and the number of eggs per nest. Two model types were tested, and compared to each other, as well as to the null model, by a log likelihood ratio test and the Akaike Information Criterion (AIC): (1) the simple effect model, where the impact of the explanatory variables was



Fig. 2 A great reed warbler is looking at its clutch (*above*), and ejecting a parasitic egg by puncture ejection (*below*). Both nests were experimentally multiply parasitized by dyeing yellow all of the host's own eggs with a highlighter pen. This host species rejects such yellow-dyed experimental eggs (Bán et al. 2013) at a similar frequency to rejecting real common cuckoo eggs, when the clutch is parasitized in the egg laying stage (Moskát et al. 2009)

considered; (2) the interaction model, where a "number of days" \times treatment interaction term was added to the model. The most parsimonious model was selected to analyze the differences in the responses; the simple effect model provided the better fit to the dataset (for details, see Table I in the [Electronic Supplementary Material](#)).

Differences among the levels of the included explanatory variables were evaluated by multiple comparisons, after first applying a single-argument analysis of variance (ANOVA) for the tested model. The model estimates were obtained using a maximum likelihood method, and the diagnostics included the graphical output for the model residuals. We estimated the model parameters by using the *nlme* (Pinheiro et al. 2012) and *lme4* packages (Zeileis and Hothorn 2002) for likelihood tests in R 2.14.0 (R Development Core Team 2012).

We compared videotaped host behaviours at nests by analyzing each of the following variables: egg pecking, clutch inspection, sitting and rotation. The ordinary least square mean method was used to estimate the behavioural rates of egg pecking, clutch inspection, sitting and rotation per unit time. These responses were tested between treatments 1b, 3 and the controls by a single-argument ANOVA (permutational ANOVA), where the number of iterations was taken to generate exact P values (Box 1988).

Results

No egg was rejected from any of the control nests which had unmanipulated host eggs ($n=27$ nests). In turn, we found the highest rejection rate (53 %) of the parasitic egg in treatment 5, when single parasitism was started at clutch completion with 6d monitoring period (cf. Fig. 3). Hosts typically ejected the parasitic egg(s) (altogether from 25 nests), but in five cases of three treatments hosts deserted the parasitized clutch (desertions/rejections: 2/9, 1/5 and 2/7 nests in treatments 1a, 2a and 3, respectively). Hosts ejected 1–5 eggs from multiple parasitized nests (mean: $3.25 \text{ eggs} \pm 0.675 \text{ SE}$ in treatments 2a and 3). In one case, in treatment 1a, host ejected the single parasitic egg together with an own natural egg (ejection cost). Latencies of egg rejections were similar in corresponding pairs of treatments regarding the 3d or 6d monitoring periods, both in single and multiple parasitisms (single parasitism: treatment 1a, 1.43 ± 0.202 days [mean \pm SE]; treatment 5, 2.25 ± 0.648 days; multiple parasitism: treatment 2a, 2.50 ± 0.500 days; treatment 3, 1.80 ± 0.837 days). The difference proved to be non-significant both between latencies in single parasitism treatments (Mann–Whitney $U=23.00$, $P=0.613$) and multiple parasitism treatments (Mann–Whitney $U=5.50$, $P=0.286$).

Our linear model (GLMM) for the results from the different treatments in multiple parasitism revealed that the frequency of egg rejections (Fig. 3) was affected by treatment and the onset time (5d vs. 8d) of the experiment (Table 1). Egg rejection was elicited less frequently in treatment 4 than either in treatment 2a or 3 ($B=0.255$, $SE=0.111$, $t_{65}=2.285$, $P=0.023$; $B=0.226$, $SE=0.128$, $t_{65}=1.76$, $P=0.008$, respectively), but was statistically similar to treatment 1b ($B=0.073$, $SE=0.13$, $t_{65}=-0.563$, $P=0.575$). Rejections showed a unimodal pattern according to the calendar date of the onset of laying of experimental nests throughout the breeding season, with a maximum in the middle of the study period and with tails of lower frequencies earlier and later in the breeding season; $B=-0.016$, $SE=0.005$, $t_{65}=-2.836$, $P=0.006$). The number of eggs in clutches had no effect on egg rejections ($B=-0.056$, $SE=0.079$, $t_{65}=-0.716$, $P=0.476$).

Video analyses revealed similar behaviours of hosts related to the different treatments (Fig. 4 and Table 2). We recorded

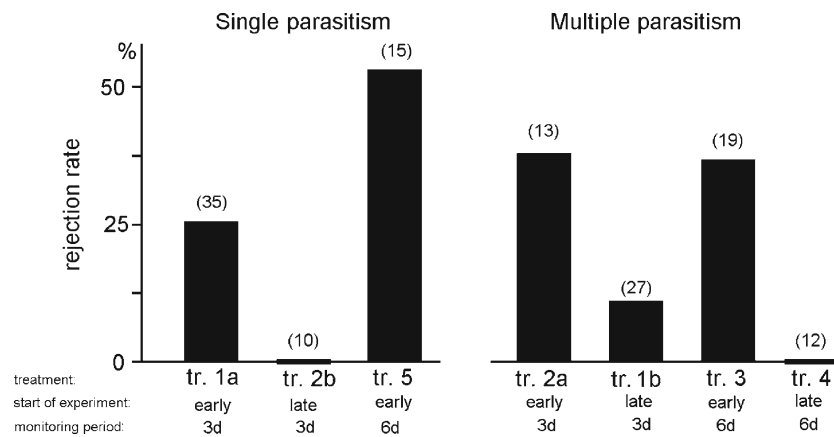


Fig. 3 Rejection rates of experimental eggs in artificial parasitism of the great reed warbler with moderately mimetic yellow-dyed own eggs (*tr. 1a* treatment 1a, *tr. 1b* treatment 1b, *tr. 2a* treatment 2a, *tr. 2b* treatment 2b, *tr. 3* treatment 3, *tr. 4* treatment 4). In single parasitism the clutch contained one experimental egg and four host eggs; in multiple

parasitism the clutch contained five experimental eggs; *early* denotes experimental parasitism on the fifth day of laying/clutch completion (5d); *late* denotes parasitism three days later, during incubation (8d). Nests were monitored either for 3 days (3d) or 6 days (6d) after experiment started

egg pecking at just less than half of the nests (Fig. 4a). It was documented at 30 % of nests with experimental parasitism at 5d (treatment 3; 3/10), and 50 % of nests with parasitism at 8d (in treatment 1b; 4/8). Interestingly, we also found egg pecking behaviour in 38 % of control nests, where no parasitic eggs were present (3/8). There were generally no significant differences in the patterns of the three different aspects of host behaviours between the different treatments and controls (Table 2), except for egg rotation, but that was due to an outlier data point (Grub test: $G=3.6653$, $U=0.441$, $P<0.001$; Fig. I in the [Electronic Supplementary Material](#)); after the removal of the outlier (Grub test: $G=1.919$, $U=0.840$, $P=0.597$), there were no significant differences between the treatments and controls (Table 2). Although egg pecking was not significantly different among the three treatments ($P=0.068$; Table 2), contrary to predictions, the pattern of mean pecking rate showed in fact higher values in late parasitism (treatment 1b) and was 7.58 ± 5.485 , in contrast with lower values in early parasitism (treatment 3: 0.48 ± 0.255) and in the control (0.42 ± 0.264).

Table 1 Results of generalized linear mixed models (GLMM) of rejection rates of experimental eggs by great reed warblers with treatment (treatments 1b, 2a, 3 and 4), timing of experiment, and clutch size as predictor variables

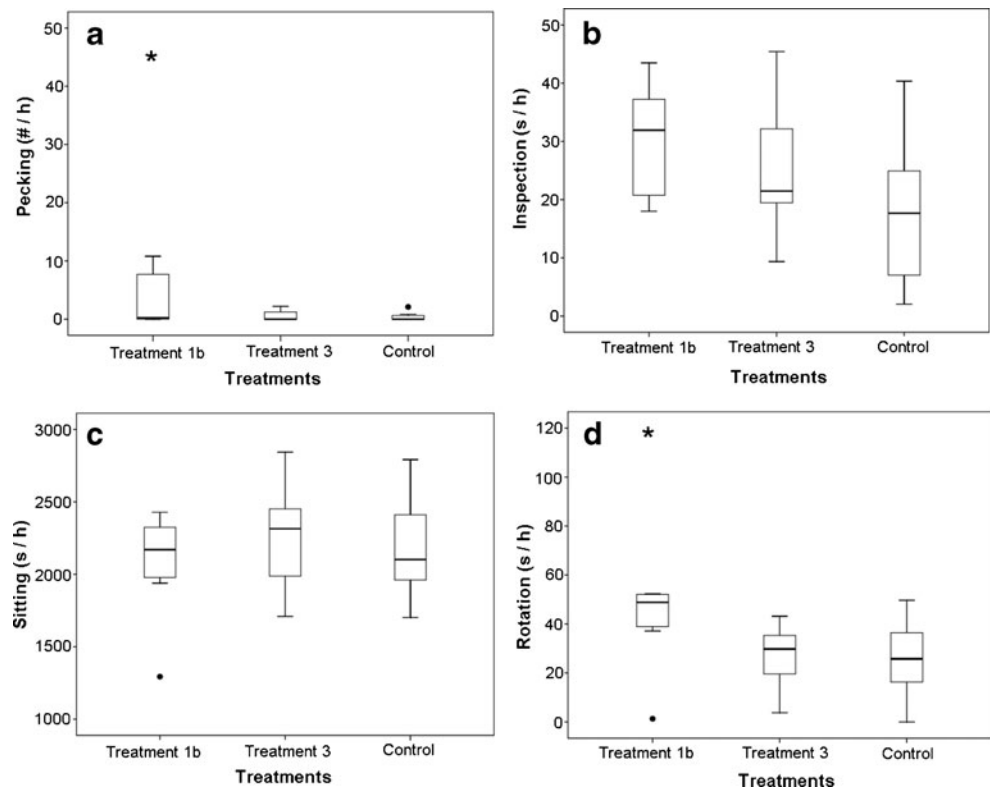
	<i>df</i>	<i>F</i>	<i>P</i>
Intercept	1,65	22.814	<0.0001
Treatment	3,65	3.998	0.011
Timing of exp.	1,65	7.662	0.007
Clutch size	1,65	0.513	0.476
SD (random)			0.125

Discussion

Our results, from repeated experimental parasitism on great reed warblers, revealed an unusual pattern of antiparasitic behaviours: hosts showed intermediate rejection rates (26–53 %) toward one or more parasitic eggs in a clutch at the first stage of parasitism, but typically showed no rejection at the second stage, following a 3-day monitoring period after the first parasitism. Previous studies suggested that hosts may learn their own eggs during their first laying attempts (e.g., Rothstein 1975; Lotem et al. 1995; Stokke et al. 2007; Strausberger and Rothstein 2009), and several theoretical models on egg rejection behaviour also incorporated a learning-based egg recognition component (Rodríguez-Gironés and Lotem 1999; Stokke et al. 2007). However, recent studies demonstrate prominent interannual variation in coloration of eggs laid by the same hosts across different years; consequently, individual hosts should learn their own eggs' phenotype in each year or in each egg laying cycle (Honza et al. 2012; Wheelwright et al. 2012; Soler et al. 2013). Yet, our results did not support this clutch-learning hypothesis (Hauber et al. 2004) whereby hosts may learn the experimental parasitic eggs' phenotype to consider it as their own eggs during the first instance of parasitism, and later they accept it in the second parasitism event. We found the same result of acceptance when experimental parasitism was applied only once, at the later time point. Specifically, these data imply that acceptance of experimental parasitism during the second instance of repeated parasitism is not caused by misimprinting on the parasitic egg(s) as one of the host's own egg(s) during the first instance of the repeated parasitism experiment.

Video-taped evidence on host behaviours at the nest, recorded just after the experimental parasitism, revealed that,

Fig. 4 Hosts' behaviour at nests in three categories (treatment 1b, treatment 3 and control) as video recordings revealed. Measured variables were: **a** pecking — number of egg pecking, **b** inspection — time spent with clutch inspection (seconds), **c** sitting — sitting on the eggs (seconds) and **d** rotation — time spent by rotation of the eggs (seconds). All values are rates and calculated per hour. The box plot shows the median (*central horizontal line*), 75th and 25th percentile (*top and bottom of the box*) and the maximum and minimum values (*top and bottom whisker*), respectively. Minor outliers, observations 1.5 * interquartile range (IQR) outside the central box, are shown by *dots*, and major outliers, observations 3.0 * IQR outside the central box, are indicated by *asterisks*



despite a lack of rejection response, hosts were able to recognize that parasitism took place during incubation. Similar rates of pecking activity were seen in both treatment categories, suggesting that some of the host individuals are capable of detecting foreign eggs, even if they do not reject them (cf. Antonov et al. 2009). Birds in the control group also scanned clutches prior to settling for incubation, most likely to allow the possibility of parasitic vs. own egg discrimination at each

visit to the nest. Although sample sizes in our video recordings were relatively small, these data clearly showed that great reed warblers in later stages of experimental parasitism also inspected their clutches at least as intensively as did birds in earlier parasitism or, at the control nests.

Some of the hosts that were exposed to later parasitism, were able to recognize the foreign eggs clearly, as shown by high pecking rates, but they did not proceed to egg rejection

Table 2 Results from the permutational ANOVA, assessing the effect of treatments 1b, 3, and the control, regarding the frequency of egg pecking, clutch inspection, incubation and rotation of eggs in nests of great reed warblers

Response		<i>df</i>	SS	MS	Iteration no.	<i>P</i>
Egg pecking	Category	2	281.34	140.61	3,893	0.061
	Residuals	23	1,694.35	73.66		
Clutch inspection	Category	2	632.57	316.28	1,071	0.108
	Residuals	23	2,858.92	124.3		
Sitting	Category	2	153,807	76,904	198	0.616
	Residuals	23	2,852,462	124,020		
Rotation	Category	2	3,047	1,523.48	5,000	0.028*
	Residuals	23	10,637	462.26		
Rotation without the outlier	Category	2	926.4	463.22	2264	0.140
	Residuals	22	5,107.6	232.4		

*Non-significant effect when a statistical outlier data point was removed (see section "Rotation without the outlier" in the bottom of the table for details)

during incubation. Earlier studies revealed that mimicry of the parasitic eggs influences host responses to brood parasitism (e.g., Davies 2000; Avilés 2008; Stoddard and Stevens 2010). If mimicry is weak, it causes greater responses (i.e., more rejections) to cuckoo parasitism, but when mimicry is strong, it causes poorer responses in the great reed warbler (e.g., Cherry et al. 2007). Great reed warblers reject highly non-mimetic eggs at the highest rates (close to 100 %) at clutch completion or in the first few days just after completion (Moskát 2005). However, our experimental parasitic eggs could be regarded as moderately mimetic, and their rejection rates are about 30–50 % during the laying stage (Bán et al. 2013). During incubation, the risk of natural cuckoo parasitism is low in this host species (Moskát 2005) and the cost of such late parasitism is also low (because later laid cuckoo eggs are less likely to hatch, Birkhead et al. 2011), so, probably, there is no pressure for hosts to keep egg discrimination responses at high rates. When the hosts do eject a mimetic parasitic egg, there is always a chance that they might mistake their own egg for the foreign egg (ejection error) or damage or destroy one or more own eggs accidentally (ejection cost, see Davies and Brooke 1988; Stokke et al. 2002). In turn, as incubation progresses, the realized fitness value of the clutch increases that compounds the costs of rejection errors and rejection mistakes when hosts damage or destroy their own eggs. Although ejection costs were rare in the present study (see Results), several previous studies on this host population showed ejection errors and costs (e.g., Moskát 2005; Moskát and Hauber 2007), which may select against egg ejection. Together with the decreasing chances of re-nesting before migration, the progress of the breeding season should therefore lower the rate of egg rejection during the later incubation stage of the breeding cycle, compared to the earlier laying stage.

Studies on the effect of nesting stage on egg rejection behaviour in hosts of brood parasites have revealed diverse patterns. For example, five songbird species, including the reed warbler (*Acrocephalus scirpaceus*), rejected the non-mimetic model cuckoo eggs at similar rates, when experimentally parasitized during either laying or incubation (Davies and Brooke 1989). However, the latency of rejection was longer when experimental parasitism took place earlier, at the beginning of laying. In contrast, great reed warblers showed a lower frequency of egg rejection in response to natural parasitism by cuckoos in the incubation stage, relative to the laying stage (Moskát 2005). In a population of the red-backed shrike (*Lanius collurio*), that had been abandoned by the cuckoo, breeding stage had no effect on egg rejections, irrespective of the level of mimicry. However, the method of rejection of mimetic eggs changed from desertion during the laying period, to ejection during the incubation period (Moskát and Fuisz 1999). Cedar waxwings (*Bombycilla cedrorum*) typically ejected foreign eggs in the early stage of breeding (laying),

but showed tendency to accept them during the late stage (incubation) (Rothstein 1976), although this pattern was unusual among several different host species of the brown-headed cowbird (*Molothrus ater*) (Rothstein 1976). Underwood and Sealy (2006) also failed to find a breeding-stage specific effect on egg rejection frequency in the Eastern warbling vireo (*Vireo gilvus*), a common host of the brown-headed cowbird. Some of other cuckoo hosts and potential hosts followed this pattern of egg rejection (Davies and Brooke 1989; Moksnes et al. 1991; Moksnes 1992; Grim et al. 2011), but there are examples when hosts of brood parasites showed reduced rejection in incubation in relation to laying (Moksnes et al. 1991; Lotem 1995), either due to the decrease in the threat of parasitism in this stage or the increased cost of breeding in the later stages (Røskaft and Moksnes 1998).

Hauber et al. (2006) documented that, following the successful ejection of a highly non-mimetic parasitic egg, great reed warblers possess a better egg recognition ability, and eject more mimetic eggs than without this "training" on the parasitic eggs. In our study we did not observe a similar effect, however, our experimental design in the present study differed in several respects from that of applied in Hauber et al. (2006) (e.g. that study varied the type of mimicry, from poor to intermediate, but experimental parasitism was mostly conducted during the laying period). The variation in the avian-perceivable mimicry component of an egg is regarded as a key factor that motivates hosts to engage in egg rejection behaviours (Soler et al. 2012a).

In this study, we discovered that hosts consistently accepted moderately mimetic parasitic eggs in the incubation stage, compared to higher rates of rejection in the laying stage. On the one hand, cuckoos would benefit from this temporally variable pattern of host rejection if they parasitized great reed warbler nests in the incubation stage. On the other hand, such, relatively late, cuckoo parasitism might cause reduced hatchability of cuckoo eggs (Birkhead et al. 2011). Even if the cuckoo egg successfully hatched, there would a risk of unsuccessful eviction of already hatched and larger nestmates, as the younger cuckoo chick might be too weak to displace the hosts' growing chicks from the nest (Molnár 1939; Moskát and Hauber 2010); and so the cuckoo chicks would pay the variable costs of nestmate eviction attempts (Anderson et al. 2009), and of the co-habitation with host nestlings (Hauber and Moskát 2008; Geltsch et al. 2012). Therefore, late cuckoo parasitism should be selected against; indeed, it occurs rarely in natural parasitism: with observations showing that the risk of cuckoo parasitism during the last days of laying is about 30 %, whereas it falls to 4 % during early incubation (Moskát 2005).

Repeated brood parasitism may have cumulative fitness impacts and complex evolutionary effects on hosts of brood parasites, and their anti-parasitic responses. If long-term, repeated risk of parasitism is distributed non-randomly among

hosts, then certain individuals suffer from a high risk of repeated parasitism, while others may serially escape from parasitism (Grim 2002; Hauber et al. 2004; Hoover et al. 2006; Hoover and Hauber 2007; Molina-Morales et al. 2012). In the present study we tested the short-term effects of repeated brood parasitism by field experiments, and revealed reduced egg rejection responses in the incubation stage compared to the egg laying stage. However, our results also showed that the difference in the behavioural outcomes of acceptance or rejection was not related to a lack of opportunity or any cognitive constraint, during repeated brood parasitism. Instead, lack of rejection in repeated parasitism can be regarded as the temporal consequence of later brood parasitism, occurring during the incubation stage. We conclude this because video-records showed that hosts were able to recognize the parasitic eggs in late parasitism, as was indicated by pecking, which did not result in broken eggs (Soler et al. 2002, 2012a; Honza et al. 2007; Antonov et al. 2008, 2009; Pozgayová et al. 2011). Accordingly, our study illustrates an example of the previously hypothesized phenomenon that some hosts may recognize more eggs than they reject (Lyon 2003; Moskát and Hauber 2007; Soler et al. 2012b). We suggest that further field studies on hosts' egg discrimination should focus on how variation in the behavioural display of acceptance vs. rejection, as an evolutionary adaptation, is affected by intrinsic and extrinsic factors of the hosts. Experiments on repeated brood parasitism shall continue to offer valuable tools to reveal the sensory and ecological components of foreign egg recognition behaviour in general (Hauber et al. 2006; Peer and Rothstein 2010; Samas et al. 2011).

Acknowledgments The Hungarian National Science Fund (OTKA, No. 83217 to CM) supported the study. For additional funding, we thank the Human Frontier Science Program and PSC-CUNY (to MEH). István Zsoldos, Nikolettá Geltsch, and Anikó Zólei kindly helped us during the field work.

Ethical standards The Middle- and Lower-Danube-Valley Inspectorates for Environmental Protection, Nature Conservation and Water Management provided permission for research. The authors declare that their experiments performed in this study comply with the laws of Hungary.

References

- Anderson MG, Moskát C, Bán M, Grim T, Cassey P, Hauber MA (2009) Egg eviction imposes a recoverable cost of virulence in chicks of a brood parasite. *PLoS ONE* 4:e7725
- Antonov A, Stokke BG, Moksnes A, Røskaft E (2008) Getting rid of the cuckoo *Cuculus canorus* egg: why do hosts delay rejection? *Behav Ecol* 19:100–107
- Antonov A, Stokke BG, Moksnes A, Røskaft E (2009) Evidence for egg discrimination preceding failed rejection attempts in a small cuckoo host. *Biol Lett* 5:169–171
- Antonov A, Stokke BG, Vikan JR, Fossøy F, Ranke PS, Røskaft E, Moksnes A, Møller AP, Shykoff JA (2010) Egg phenotype differentiation in sympatric cuckoo *Cuculus canorus* genets. *J Evol Biol* 23:1170–1182
- Avilés JM (2008) Egg colour mimicry in the common cuckoo *Cuculus canorus* as revealed by modelling host retinal function. *Proc R Soc Lond B* 275:2345–2352
- Bán M, Barta Z, Munoz AR, Takasu F, Nakamura H, Moskát C (2011) The analysis of common cuckoo's egg shape in relation to its hosts' in two geographically distant areas. *J Zool* 284:77–83
- 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
- Begum S, Moksnes A, Røskaft E, Stokke BG (2012) Responses of potential hosts of Asian cuckoos to experimental parasitism. *Ibis* 154:363–371
- Birkhead TR, Hemmings N, Spottiswoode CN, Mikulica O, Moskát C, Bán M, Schulze-Hagen K (2011) Internal incubation and early hatching in brood parasitic birds. *Proc R Soc Lond B* 278:1019–1024
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–135
- Box G (1988) Signal-to-noise ratios, performance criteria, and transformations. *Technometrics* 30:1–17
- Brooke MdeL, Davies NB (1988) Egg mimicry by cuckoos *Cuculus canorus* in relation to discrimination by hosts. *Nature* 335:630–632
- Cherry MI, Bennett ATD, Moskát C (2007) Host intra-clutch variation, cuckoo egg matching and egg rejection by great reed warblers. *Naturwissenschaften* 94:441–447
- Clutton-Brock TH (1991) The evolution of parental care. Princeton University Press, Princeton
- Davies NB (2000) Cuckoos, cowbirds and other cheats. T and AD Poyser, London
- Davies NB (2011) Cuckoo adaptations: trickery and tuning. *J Zool* 284:1–14
- Davies NB, Brooke MdeL (1988) Cuckoos versus reed warblers: adaptations and counteradaptations. *Anim Behav* 36:262–284
- Davies NB, Brooke MdeL (1989) An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts: I. Host egg discrimination. *J Anim Ecol* 58:207–224
- 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
- Geltsch N, Hauber ME, Anderson MG, Bán M, Moskát C (2012) Competition with a host nestling for parental provisioning imposes recoverable costs on parasitic cuckoo chick's growth. *Behav Process* 90:378–383
- Grim T (2002) Why is mimicry in cuckoo eggs sometimes so poor? *J Avian Biol* 33:302–305
- Grim T, Samas P, Moskát C, Kleven O, Honza M, Moksnes A, Røskaft E, Stokke BG (2011) Constraints on host choice: why do parasitic birds rarely exploit some common potential hosts? *J Anim Ecol* 80:508–518
- Hale K, Briskie JV (2007) Response of introduced European birds in New Zealand to experimental brood parasitism. *J Avian Biol* 38:198–204
- Hargitai R, Moskát C, Bán M, Gil D, López-Rull I, Solymos E (2010) Eggshell characteristics and yolk composition in the common cuckoo *Cuculus canorus*: are they adapted to brood parasitism? *J Avian Biol* 41:177–185
- Hauber ME, Moskát C (2008) Shared parental care is costly for nestlings of common cuckoos and their great reed warbler hosts. *Behav Ecol* 19:79–86

- Hauber ME, Yeh PJ, Roberts JOL (2004) Patterns and coevolutionary consequences of repeated brood parasitism. *Proc R Soc Lond B* 271: S317–S320
- Hauber ME, Moskát C, Bán M (2006) Experimental shift in hosts' acceptance threshold of inaccurate-mimic brood parasite eggs. *Biol Lett* 2:177–180
- Higuchi H (1998) Host use and egg color of Japanese cuckoos. In: Rothstein SI, Robinson SK (eds) *Parasitic birds and their hosts. Studies in coevolution*. Oxford University Press, New York, pp 80–93
- Honza M, Pozgayová M, Procházka P, Kadlec E (2007) Consistency in egg rejection behaviour: responses to repeated brood parasitism in the blackcap (*Sylvia atricapilla*). *Ethology* 113:344–351
- Honza M, Procházka P, Pozgayová M (2012) Within- and between-season repeatability of eggshell colouration in the great reed warbler *Acrocephalus arundinaceus*. *J Avian Biol* 43:91–96
- Hoover JP, Hauber ME (2007) Individual patterns of habitat and nest-site use by hosts promote transgenerational transmission of avian brood parasitism status. *J Anim Ecol* 76:1208–1214
- Hoover JP, Yasukawa K, Hauber ME (2006) Spatially and temporally structured avian brood parasitism affects the fitness benefits of hosts' rejection strategies. *Anim Behav* 72:881–890
- Hosoi SA, Rothstein SI (2000) Nest desertion and cowbird parasitism: evidence for evolved responses and evolutionary lag. *Anim Behav* 59:823–840
- Igic B, Cassey P, Grim T, Greenwood DR, Moskát C, Rutila J, Hauber ME (2012) A shared chemical basis of avian host–parasite egg colour mimicry. *Proc R Soc Lond B* 279:1068–1076
- Kilner RM, Langmore NE (2011) Cuckoos versus hosts in insects and birds: adaptations, counter-adaptations and outcomes. *Biol Rev* 86: 836–852
- Krüger O (2011) Brood parasitism selects for no defence in a cuckoo host. *Proc R Soc Lond B* 278:2777–2783
- Lahti DC, Lahti AR (2002) How precise is egg discrimination in weaverbirds? *Anim Behav* 63:1135–1142
- Lotem A, Nakamura H, Zahavi A (1995) Constraints on egg discrimination and cuckoo-host co-evolution. *Anim Behav* 49:1185–1209
- Lyon B (2003) Egg recognition and counting reduce costs of avian conspecific brood parasitism. *Nature* 422:495–499
- Moksnes A (1992) Egg recognition in chaffinches and bramblings. *Anim Behav* 44:993–995
- Moksnes A, Røskaft E (1995) Egg-morph and host preferences in the common cuckoo (*Cuculus canorus*): an analysis of cuckoo and host eggs from European museum collections. *J Zool* 236:625–648
- Moksnes A, Røskaft E, Braa AT, Korsnes L, Lampe HM, Pedersen HC (1991) Behavioural responses of potential hosts towards artificial cuckoo eggs and dummies. *Behaviour* 116:64–89
- Molina-Morales M, Martínez JG, Martín-Gálvez D, Dawson DA, Rodríguez-Ruiz J, Burke T, Avilés JA (2012) Evidence of long-term structured cuckoo parasitism on individual magpie hosts. *J Anim Ecol* 42:579–580
- Molnár B (1939) About the cuckoo. Observations and experiments on the eviction instinct of the cuckoo chick. Published by the author, Szarvas, Hungary (In Hungarian)
- Moskát C (2005) Nest defence and egg rejection in great reed warblers over the breeding cycle: are they synchronised with the risk of brood parasitism? *Ann Zool Fenn* 42:579–586
- Moskát C, Fuisz TI (1999) Reactions of red-backed shrikes *Lanius collurio* to artificial cuckoo *Cuculus canorus* eggs. *J Avian Biol* 30:175–181
- Moskát C, Hauber ME (2007) Conflict between egg recognition and egg rejection decisions in common cuckoo (*Cuculus canorus*) hosts. *Anim Cogn* 10:377–386
- Moskát C, Hauber ME (2010) Chick loss from mixed broods reflects severe nestmate competition between an evictor brood parasite and its hosts. *Behav Process* 83:311–314
- Moskát C, Hauber ME, Avilés JA, Bán M, Hargitai R, Honza M (2009) Increased host tolerance of multiple cuckoo eggs leads to higher fledging success of the brood parasite. *Anim Behav* 77:1281–1990
- 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, Takasu F, Munoz AR, Nakamura H, Bán M, Barta Z (2012) Cuckoo parasitism on two closely-related *Acrocephalus* warblers in distant areas: a case of parallel coevolution? *Chin Birds* 3:320–329
- Nakagawa S, Hauber ME (2011) Great challenges with few subjects: statistical strategies for neuroscientists. *Neurosci Biobehav Rev* 35: 462–473
- Peer BD, Rothstein SI (2010) Phenotypic plasticity in common grackles (*Quiscalus quiscula*) in response to repeated brood parasitism. *Auk* 127:293–299
- Pinheiro J, Bates D, DebRoy S, Sarkar D and the R Development Core Team (2012) nlme: linear and nonlinear mixed effects models. R package version 3.1-106 URL <http://CRAN.R-project.org/package=nlme>
- Polaciková L, Takasu F, Stokke BG, Moksnes A, Røskaft 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
- Pozgayová M, Procházka M, Honza M (2009) Sex-specific defence behaviour against brood parasitism in a host with female-only incubation. *Behav Process* 81:34–38
- Pozgayová M, Procházka M, Polaciková L, Honza M (2011) Closer clutch inspection — quicker egg ejection: timing of host responses toward parasitic eggs. *Behav Ecol* 22:46–51
- R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>
- Rensch B (1925) Verhalten von Singvögeln bei Aenderung des Geleges. *Ornithol Monatschr* 33:16–173
- Rodríguez-Girónés MA, Lotem A (1999) How to detect a cuckoo egg: a signal-detection theory model for recognition and learning. *Am Nat* 153:633–648
- Røskaft E, Moksnes A (1998) Coevolution between brood parasites and their hosts: An optimality theory approach. In: Rothstein SI, Robinson SK (eds) *Parasitic birds and their hosts: Studies in coevolution*. Oxford University Press, New York, pp 236–254
- Rothstein SI (1974) Mechanisms of avian egg recognition: possible learned and innate factors. *Auk* 91:796–807
- Rothstein SI (1975) Mechanisms of avian egg recognition: do birds know their own eggs? *Anim Behav* 23:268–278
- Rothstein SI (1976) Experiments on defenses cedar waxwings use against cowbird parasitism. *Auk* 93:675–691
- Samas P, Hauber ME, Cassey P, Grim T (2011) Repeatability of foreign egg rejection: testing the assumptions of co-evolutionary theory. *Ethology* 117:606–619
- Sealy SG (1995) Burial of cowbird eggs by parasitized yellow warblers: an empirical and experimental study. *Anim Behav* 49:877–889
- Sealy SG, Underwood TJ (2012) Egg discrimination by hosts and obligate brood parasites: a historical perspective and new synthesis. *Chin Birds* 3:274–294
- Soler M, Martín-Vivaldi M, Pérez-Contreras T (2002) Identification of the sex responsible for recognition and the method of ejection of parasitic eggs in some potential common cuckoo hosts. *Ethology* 108:1093–1101
- Soler M, Fernández-Morante J, Espinoza F, Martín-Vivaldi M (2012a) Pecking but accepting the parasitic eggs may not reflect ejection failure: the role of motivation. *Ethology* 118:1–11

- Soler M, Martín-Vivaldi M, Fernández-Morante J (2012b) Conditional response by hosts to parasitic eggs: the extreme case of the rufous-tailed scrub robin. *Anim Behav* 84:421–426
- Soler M, Ruiz-Castellano C, Carra LG, Ontanilla J, Martín-Galvez D (2013) Do first-time breeding females imprint on their own eggs? *Proc R Soc Lond B* 280:20122518
- Stoddard MC, Stevens M (2010) Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. *Proc R Soc Lond B* 277:1387–1393
- Stoddard MC, Stevens M (2011) Avian vision and the evolution of egg color mimicry in the common cuckoo. *Evolution* 65:2004–2013
- Stokke BG, Honza M, Moksnes A, Røskaft E, Rudolfsen G (2002) Costs associated with recognition and rejection of parasitic eggs in two European passerines. *Behaviour* 139:629–644
- Stokke BG, Takasu F, Moksnes A, Røskaft E (2007) The importance of clutch characteristics and learning for antiparasite adaptations in hosts of avian brood parasites. *Evolution* 61:2212–2228
- Strausberger BM, Rothstein SI (2009) Parasitic cowbirds may defeat host defense by causing rejecters to misimprint on cowbird eggs. *Behav Ecol* 20:691–699
- Trnka A, Pozgayová M, Procházka P, Prokop P, Honza M (2012) Breeding success of a brood parasite is associated with social mating status of its host. *Behav Ecol Sociobiol* 66:1187–1194
- Underwood TJ, Sealy SG (2006) Parameters of brown-headed cowbird *Molothrus ater* egg discrimination in warbling vireos *Vireo gilvus*. *J Avian Biol* 37:457–466
- Wheelwright NT, Graff ES, Norris DR (2012) Relative consistency in size, shape, and coloration of savannah sparrow eggs within and between breeding seasons. *Condor* 114:412–420
- Zeileis A, Hothorn T (2002) Diagnostic checking in regression relationships. *R News* 2:7–10 URL <http://CRAN.R-project.org/doc/Rnews/>
- Zölei A, Hauber ME, Geltsch N, Moskát C (2012) Asymmetrical signal content of egg shape as predictor of egg rejection by great reed warblers, hosts of the common cuckoo. *Behaviour* 149:391–406