

Fixed Rejection Responses to Single and Multiple Experimental Parasitism in Two *Fringilla* Hosts of the Common Cuckoo

Johan R. Vikan*, Bård G. Stokke*, Frode Fossøy*, Craig Jackson*, Esa Huhta†, Jarkko Rutila‡, Arne Moksnes* & Eivin Røskaft*

* Department of Biology, Norwegian University of Science and Technology (NTNU), Realfagbygget, Norway

† Finnish Forest Research Institute, Kolari Research Station, Muoniontie, Kolari, Finland

‡ Department of Biology, University of Joensuu, Joensuu, Finland

Correspondence

Johan Reinert Vikan, Department of Biology, Norwegian University of Science and Technology (NTNU), Realfagbygget, N-7491 Trondheim, Norway.
E-mail: johanrei@bio.ntnu.no

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Abstract

Previous studies have shown that the tendency to reject parasitic eggs among certain hosts is strongly dependent on the degree of similarity with own eggs, whereas other conditional cues do not affect rejection decisions. This paper examines whether two such hosts, the closely related brambling and chaffinch, show a different tendency to reject parasitic eggs if they are multiply parasitized. Some individuals were experimentally parasitized with both a non-mimetic and a low-intermediate contrasting egg in the same breeding attempt. The non-mimetic egg was rejected almost without exception. In chaffinches, the low-intermediate contrasting egg was introduced shortly after rejection of the non-mimetic egg whereas in bramblings, both eggs were introduced simultaneously. A control group consisted of individuals that were parasitized with one low-intermediate contrasting egg. There was no significant difference in the tendency to reject the low-medium contrasting eggs between the experimental and control group in any of the species, implying that the same acceptance threshold is applied to each parasitic egg independently. Moreover, the rejection rate of non-mimetic eggs was high (>90%) regardless of whether the egg was introduced alone or together with a low-medium contrasting egg. The results are discussed in relation to recent studies with great reed warblers *Acrocephalus arundinaceus* that obtained contrasting results in similar experimental designs. The different degrees of flexibility displayed by *Fringilla* and great reed warbler hosts are likely to reflect differences in both the perception and action component of the recognition system.

Introduction

Rejection of parasitic eggs is widespread among actual and potential hosts of avian brood parasites (Davies 2000). There is, however, huge variation in the strength and mode of discrimination (Davies & Brooke 1989; Moksnes et al. 1990). Understanding the basis of this diversity is a major focus for students of brood parasitic interactions (Rothstein & Robinson 1998). A comprehensive understanding requires that sources of variation are examined at multiple levels,

and that both ultimate and proximate issues are addressed (see Fig. 1 in Stokke et al. 2005). Studies that explore the degree of consistency in individual rejection decisions can provide information about constraints relevant to the process of discrimination (Sherman et al. 1997; Liebert & Starks 2004), and are therefore valuable contributions.

For a binary behavioural trait such as egg rejection, the acceptance threshold (*sensu* Reeve 1989) is the degree of dissimilarity between the host's cognitive image of own eggs and a potential parasitic egg,



Fig. 1: Examples of host and parasitic eggs in experiments with chaffinches (rows 1–2) and bramblings (rows 3–4). From left to right: host egg, low–medium contrasting experimental egg, non-mimetic experimental egg.

below which the host incubates the egg, and above which it rejects it. Since recognition systems are intrinsically subject to error, hosts are faced with a risk of committing errors in the process of recognition. This is particularly pertinent when the parasite has evolved mimetic eggs, such as in systems involving the common cuckoo *Cuculus canorus* (Davies et al. 1996; Rodríguez-Girones & Lotem 1999). Some hosts solve this problem by varying their acceptance threshold to optimize the balance between recognition errors and acceptance errors in different contexts (Davies & Brooke 1988; Moksnes et al. 1993, 2000; Alvarez 1996; Davies et al. 1996; Brooke et al. 1998; Øien et al. 1999; Lindholm 2000; Bartol et al. 2002). The response of the host, based on the

perceived dissimilarity between host and the potential parasite egg, constitutes the action component of the host's recognition system (Sherman et al. 1997).

Hosts may rely on two alternative proximate mechanisms for egg recognition (Rothstein 1974, 1975; Moskát & Hauber 2007; Moskát et al. 2009). True recognition means that the eggs in the clutch are compared with a cognitive image of own eggs. In contrast, recognition by discordancy involves a direct comparison of eggs in the clutch. The process of phenotype matching is part of what is referred to as the perception component of the host's recognition system (Sherman et al. 1997).

Experience with parasitism may affect rejection behaviour in several ways. For example, discriminatory abilities may not be fully developed in naive individuals breeding for their first time. In this case, an individual may respond differently if parasitized at different developmental stages. A frequently cited example is oriental reed warblers *Acrocephalus orientalis* in Japan, where first year breeders go through a prolonged period of learning their own eggs during which their acceptance threshold is relaxed (Lotem et al. 1992, 1995). In addition, parasitism may by itself influence the ontogeny of egg discrimination. For example, if the host is parasitized during learning, miss-imprinting may theoretically increase the probability of acceptance in subsequent parasitic events (Lotem & Nakamura 1998). Experience is also sometimes necessary for proper acquisition and mastering of complex behavioural tasks. Early auditory experience can for example influence the development of auditory discrimination or facilitate rapid development of aurally mediated behaviours (Mateo 1996). Experience with detection and rejection of parasitic eggs could possibly facilitate development of egg rejection behaviour in the same way. Finally, experience with parasitism can provide the host with a clue about the risk of recurrent parasitism which can be used to optimize future decisions (Hauber et al. 2004, 2006).

Recent studies on great reed warblers *Acrocephalus arundinaceus* give striking examples of how an individual's decision to reject can change depending on the pattern of parasitism it experiences. In one study, it was shown that rejection of a non-mimetic egg had a positive effect on the subsequent tendency to reject a moderately mimetic egg (Hauber et al. 2006). In a second study, it was shown that when both parasitic eggs were introduced simultaneously, the tendency to reject the non-mimetic egg decreased (Moskát et al. 2009). These results suggest the presence of a complex recognition system that

both involves a capacity for adaptive adjustments of acceptance thresholds (Hauber et al. 2006) and a mixture of true recognition and recognition by discordancy (Rothstein 1974, 1975; Moskát et al. 2009).

In the present study, we investigate if experience with detection and rejection of a parasitic egg could potentially affect egg discrimination in two passerines which reject non-mimetic eggs at high rates. Bramblings *Fringilla montifringilla* and chaffinches *F. coelebs* possess true egg recognition (Moksnes 1992), and probably obtain a neural template (memory) of their own eggs through imprinting-like learning (Rothstein 1974, 1975; Lotem et al. 1992, 1995). At the population level, experimental eggs are rejected in proportion to the difference in appearance to own eggs, and almost all individuals, regardless of age, reject poorly mimetic eggs (Braa et al. 1992; Stokke et al. 2004). Recognition errors are at best very rare in both species (Stokke et al. 2002a; own data), suggesting that the recognition system efficiently eradicates such errors. In these two respects, bramblings, chaffinches and great reed warblers seem to resemble each other closely (Moskát & Honza 2002; Røskaft et al. 2002). Interestingly, a recent study has shown that chaffinches often accept conspecific eggs that are predicted to be discriminable in terms of the perceptual capacity of the host (own data). However, it is not known if individual bramblings and chaffinches are rigid or flexible with respect to the degree of mimicry required for rejection. We therefore conducted an experiment similar to Hauber et al. (2006), where we parasitized chaffinches twice in the same breeding attempt, first introducing one poorly mimetic egg (with a high probability of being rejected) and subsequently an egg of good or intermediate mimicry. With bramblings we adopted a slightly different design, similar to Moskát et al. (2009), where we introduced both eggs simultaneously, ensuring that the second egg was available for detection and rejection at the same time as the non-mimetic egg was inspected and rejected. If there is an element of discordancy in the recognition mechanism of bramblings, we expect that simultaneous parasitism leads to a lower rejection rate of non-mimetic eggs. This is because the contrast between the two parasitic eggs will often be lower than the contrast between the non-mimetic parasitic egg and the host's own eggs (Moskát et al. 2009). Hence, the discordancy mechanism prescribes that the non-mimetic egg will be perceived as less deviant when a second experimental egg is added to the clutch.

Materials and Methods

Experiments and Sample Sizes

All experiments on chaffinches were carried out in two nature reserves in Stjørdal, Central Norway (63°30'N, 11°00'E). The study sites are forested floodplains dominated by grey alder *Alnus incana*. Experiments on bramblings were conducted in Kittilä, Finnish Lapland (67°N, 24°E), Ammarnäs, northwestern Sweden (66°N, 16°E), Tana, north-eastern Norway (70°N, 28°E), and Tydal, Central Norway (63°N, 12°E).

Host clutches were experimentally parasitized using two different foreign eggs (one non-mimetic and one low-intermediate contrasting, see below) within the same breeding attempt (see Fig. 1). With chaffinches, we introduced the low-intermediate contrasting egg shortly after the non-mimetic egg had been rejected. With bramblings, we introduced both parasitic eggs simultaneously. The experimental egg was regarded as accepted if it was still incubated after five days.

To quantify the degree of similarity, or contrast, of parasitic eggs, four experienced persons were used to score the difference in appearance from photographs taken in the field (1 = low contrast, 2 = medium contrast, 3 = high contrast (Braa et al. 1992; Stokke et al. 2004)). All clutches were photographed together with the parasitic egg in a standardized manner on a grey plate using a Canon EOS 30D/350D digital camera and a 100 mm (f 1:2.8) Canon macro lens. This method broadly predicts responses to foreign conspecific eggs in both finches, which makes it useful for our purpose. The pictures were scored blindly, and the persons were highly consistent in their assessments as indicated by high repeatability (Lessells & Boag 1987, $r = 0.79$, $F_{1851,616} = 15.6$). We therefore used mean values of the scores in the analyses. Based on these scores, a non-mimetic egg was defined to correspond to a contrast value ≥ 2.5 , whereas a low-intermediate contrast was defined to correspond to a contrast value < 2.5 (*sensu* Stokke et al. 2004).

A total of 91 chaffinch experiments and 58 brambling experiments were initiated. The non-mimetic egg was either a conspecific egg (45 chaffinch and 38 brambling experiments) or a highly contrasting egg from another passerine species (46 chaffinch and 20 brambling experiments). From previous studies, we know that such eggs are ejected in 80–100% of the cases (Braa et al. 1992; Stokke et al. 2004). In

11 of the initiated experiments (one brambling and 10 chaffinches) the non-mimetic egg was accepted and the experiments were excluded from further analysis. Moreover, in 8 experiments (six bramblings and two chaffinches), the purportedly non-mimetic egg turned out to have a contrast value < 2.5 (i.e. not non-mimetic according to the definition) and these experiments were also excluded. Nineteen initiated experiments (five brambling and 14 chaffinches) were excluded because the response to the low–intermediate contrasting egg could not be evaluated due to nesting failure (predation or desertion). Finally, 11 experiments (three bramblings and eight chaffinches) were excluded because the purportedly low–intermediate contrast egg turned out to have contrast value ≥ 2.5 (i.e. not low–intermediate contrast according to our definition). This left a total of 100 successful double experiments (57 with chaffinches and 43 with bramblings). The low–intermediate contrast egg was either a conspecific egg (57 chaffinch and 40 brambling experiments) or an egg from the congener (three brambling experiments). All double experiments were conducted in 2006–2008.

A control experiment involved replacing a single host egg with a foreign conspecific low–intermediate contrasting egg (in one brambling experiment the experimental egg came from a chaffinch). We also included six cases of natural parasitism of bramblings (from Kittilä) in this group. Thirteen control experiments with chaffinches were scored to have contrast values ≥ 2.5 and were therefore excluded from the analyses. A total of 53 control experiments with bramblings and 32 with chaffinches were included from the period 2006–2008. In addition, we also included 88 control experiments with bramblings from the period 2003–2005, and 53 with chaffinches from the period 1999–2000 to increase the sample size. This was justified by that there is no indication in any of the species that rejection rates of low–intermediate contrast eggs varies across years (logistic regression with year as a two-level category (brambling: 2003–2005 vs. 2006–2008; chaffinch: 1999–2000 vs. 2006–2008) and contrast as covariate: $\chi^2 < 0.17$, $df = 1$, $p > 0.68$). This left a total of 126 successful control experiments (141 with bramblings and 85 with chaffinches).

In addition, from another study, a total of 72 single experiments with bramblings were available that had contrast values ≥ 2.5 . These experiments were used for a comparison between the rejection rates of non-mimetic eggs in single and double parasitism.

Assessment of Similarity between Host and Parasitic Eggs

Similarity of host and parasitic eggs is the main predictor of host response in chaffinches and bramblings (Braa et al. 1992; Stokke et al. 2004). Moreover, because chaffinch and brambling clutches are highly variable, it is difficult to standardize the similarity between host and parasitic egg. Comparisons of rejection rates between treatment groups should therefore control statistically for potential differences in the distribution of similarity-values. Moreover, since the effect of rejecting a non-mimetic egg need not be equal for all grades of egg similarity, it is also informative to test if there is an interaction between similarity and treatment.

We used two different similarity measures in the analyses. As one of the measures, we used a human-subjective similarity score based on photographs of host and parasitic eggs, as explained above (Stokke et al. 2004). In addition, we also calculated chromatic contrasts (ΔT_C) and achromatic (brightness) contrasts (ΔT_B) using reflectance spectra from the eggs and sensitivity functions for typical passerine cones (Endler & Mielke 2005). These measures allow us to control for possible differences in distribution of egg similarity values as perceived by the birds themselves. Reflectance spectra were available for a subset of the experiments [87 chaffinch experiments (55 experimental and 32 control broods) and 85 brambling experiments (43 experimental and 36 control broods)]. The spectra were collected with a USB2000 spectrophotometer from surfaces illuminated with a deuterium halogen light source (D2-W, mini). We used the OOIBASE32TM operating software, and measurements were taken at a 45° angle to the egg surface, with the spectrophotometer and the light source connected with a coaxial reflectance probe (QR-400-7-UV-vis). Four or eight measurements were taken from each egg by dividing the egg into three sections along the long axis of the egg and measuring in the background (i.e. avoiding areas where spotting is obvious) in each region [one (two) measurements in the distal sections and two (four) measurements in the central section]. All eggs were fresh when measured. To establish whether four spectra per egg are enough to describe egg coloration adequately, we obtained 20 reflectance measurements from each of a total of 16 brambling eggs and four chaffinch eggs, each originating from a different clutch. Firstly, we calculated quantum catch values (see below) for each of these eggs based on the mean of all the 20 spectra. Secondly, for each egg,

we randomly selected four reflectance spectra out of the 20 available reflectance spectra (no replacement) and repeated this procedure 10 000 times. At each step, we calculated a quantum catch value based on the mean of the four spectra. More than 97% of the simulated quantum catch values lied within one SD of the grand quantum catch value in all 20 eggs, suggesting that four spectra per egg are enough to describe egg colouration adequately.

Since chaffinches and bramblings build open and relatively shallow nests, it is reasonable to assume that light conditions meet the requirements of the colour discrimination mechanism. Following Goldsmith (1990) and Stoddard & Prum (2008), we calculated idealized quantum catch values Q_i for each of the four avian cone types:

$$Q_i = \sum_{\lambda=300}^{700} R(\lambda)S_i(\lambda), \quad (1)$$

where $R(\lambda)$ is the mean reflectance spectra for an egg interpolated to a step width of 1 nm between 300 and 700 nm, and $S_i(\lambda)$ is the spectral sensitivity function for cone i . Both S_i and R were normalized to have integrals of one. We used the average of spectral sensitivity curves for UVS-type retinas from Endler & Mielke (2005), available in their supplementary online material. Q_i -values were normalized to sum to one. Goldsmith's (1990) formula treats the irradiance spectrum as a constant at all wavelengths with integral equal to 1. Because colour constancy is a fundamental feature of vertebrate vision in general, and avian colour vision in particular (Vorobyev et al. 1998; Vorobyev 2003), the opportunities for natural variations in the composition of ambient light to affect colour discrimination should be rather limited (Stoddard & Prum 2008). As a matter of form, we also calculated quantum cone catches for eggs viewed under three different ambient light conditions [forest shade, daylight (d 65), and blue sky (Endler 1993)]. We performed the calculations with and without van Kries transformation. The van Kries transformation models light adaptation and colour constancy by normalizing the quantum catch estimate of each cone channel by the quantum catch of a flat achromatic spectrum under the same ambient light [see formula (9) in Endler & Mielke 2005]. The chromatic contrasts (ΔT_C) obtained by each method for each ambient light spectrum were strongly correlated with contrasts based on formula (1) (values based on van Kries transformed quantum catches: $R^2 > 0.98$; values based on Non-Transformed quantum catches: $R^2 > 0.91$, $n = 322$).

Normalized quantum catches for a given egg constitutes a colour vector for that egg in a colour tetrahedron with vertices corresponding to exclusive stimulation of each of the four cone types. We mapped the colour vectors in Cartesian space using the transformation of Endler & Mielke (2005), and thereafter calculated the chromatic contrast ΔT_C of host and parasitic eggs as the euclidean distance between their corresponding vectors. The colour space has the useful property that discriminability of two colours is proportional to ΔT_C . ΔT_C is strongly linearly related to discriminability values calculated by Vorobyev & Osorio's (1998) noise-limited colour opponency model [$R^2 = 0.99$, $n = 322$, assuming blue tit *Cyanistes caeruleus* cone proportions and simple noise structure (e.g. Avilés 2008; Cassey et al. 2008)].

Using non-normalized reflectance spectra, we also calculated quantum catches for double cones, which are assumed to be involved in achromatic discrimination tasks (Osorio & Vorobyev 2005). The spectral sensitivity of double cones was obtained by combining the absorbance spectra of the medium- and long-wavelength-sensitive cones (Gomez & They 2007). The achromatic contrast ΔT_B between two eggs was then calculated as the Euclidean distance between quantum catches of double cones for host and parasitic eggs. ΔT_B is strongly linearly related to values obtained using the double cone sensitivity of the blue tit (Hart et al. 2000, $R^2 = 0.95$, $n = 322$).

Statistical Analyses

Statistical analyses were conducted using the software R 2.8.1 (R Development Core Team 2008). Using all experiments (Table 1), we first ran univariate logistic regressions (binary response: reject = 0, accept = 1) for each species with each measure of egg similarity as the sole explanatory variable. These confirmed that all three similarity measures are significant predictors of rejection behaviour on their own [Chaffinch: Contrast: $\beta(\text{SE}) = -1.89 (0.25)$, $n = 239$; ΔT_C : $\beta(\text{SE}) = -42.5 (8.8)$, $n = 180$; ΔT_B : $\beta(\text{SE}) = -0.09 (0.03)$, $n = 180$; all $p < 0.0001$; Brambling: Contrast: $\beta(\text{SE}) = -2.51 (0.34)$, $n = 255$; ΔT_C : $\beta(\text{SE}) = -38.6 (8.85)$, $n = 142$; ΔT_B : $\beta(\text{SE}) = -0.26 (0.07)$, $n = 142$; all $p < 0.0001$]. The three egg similarity measures were all interrelated (Contrast and ΔT_C : $r_s = 0.64$, $R^2 = 0.38$; Contrast and ΔT_B : $r_s = 0.49$, $R^2 = 0.23$; ΔT_C and ΔT_B : $r_s = 0.34$, $R^2 = 0.05$).

For the brambling, we ran a generalized linear mixed model (binary response: 0 = accept, 1 = reject) (Bates et al. 2008), with treatment (experimental vs. control group), contrast and their interaction as fixed

Table 1: Rates of rejection and latency to rejection of experimental parasitic eggs, and distribution of egg similarity values in experimental groups. Contrast was measured for all experiments whereas chromatic contrasts (ΔT_C) and achromatic contrasts (ΔT_B) were calculated for a smaller subset (see Materials and Methods). Non-mimetic corresponds to contrast value ≥ 2.5 , whereas low contrast corresponds to contrast value < 2.5

Variable	Experimental group				Control group			
	Non-mimetic egg		Low contrast egg		Non-mimetic egg		Low contrast egg	
	n	$\bar{x} \pm SD$	n	$\bar{x} \pm SD$	n	$\bar{x} \pm SD$	n	$\bar{x} \pm SD$
Chaffinch								
Proportion rejected (%)	89	0.89	57	0.40			85	0.39
Latency to rejection (days)	83	2.3 \pm 1.1	19	2.6 \pm 1.7			25	3.2 \pm 1.4
Contrast	89	2.9 \pm 0.1	57	1.7 \pm 0.4			85	1.6 \pm 0.4
ΔT_C	85	0.065 \pm 0.028	55	0.029 \pm 0.015			32	0.036 \pm 0.016
ΔT_B	85	11.86 \pm 7.29	55	4.92 \pm 3.25			32	4.51 \pm 3.11
Brambling								
Proportion rejected (%)	52	0.98	43	0.49	72	0.88	141	0.44
Latency to rejection	25	1.7 \pm 1.0	12	3.3 \pm 2.0	53	2.2 \pm 1.2	44	2.9 \pm 1.6
Contrast	52	3.0 \pm 0.1	43	1.7 \pm 0.4	72	2.8 \pm 0.2	141	1.6 \pm 0.4
ΔT_C	52	0.08 \pm 0.03	43	0.037 \pm 0.021			36	0.036 \pm 0.019
ΔT_B	52	10.20 \pm 7.77	43	4.50 \pm 2.61			36	3.75 \pm 2.85

explanatory variables, and population as a random factor. For the chaffinch, we ran an ordinary logistic regression with treatment, contrast and their interaction as explanatory variables. Using the subset of experiments for which eggshell reflectance was available, we also ran models with contrast, ΔT_C , and ΔT_B and their interactions with treatment as explanatory variables. Significance of the variables was evaluated by stepwise backward deletion using log likelihood ratio tests to compare models with and without the parameter in question (Crawley 2007).

Results

Chaffinches and bramblings rejected the non-mimetic egg in 89% (79/89) and 98% (51/52) of the cases, respectively (Table 1). In experiments where both eggs were introduced simultaneously (brambling), the low-medium contrasting egg was never rejected before the high contrast egg. Among chaffinches, 40% of the individuals in the experimental group rejected the low-intermediate contrasting egg compared to 39% in the control group. Among bramblings, 49% of the individuals in the experimental group rejected the low-intermediate contrasting egg compared to 44% in the control group. Thus, there was no indication that the tendency to reject the low-intermediate contrasting egg was different for individuals in the two treatment groups (Tables 1 and 2). Moreover, there was no

Table 2: Results of logistic regressions on the probability of rejecting a foreign low-intermediate contrasting egg (reject = 0, accept = 1) for individuals with and without recent experience (treatment) in rejecting a non-mimetic parasitic egg. Minimal adequate models are in bold. See Materials and Methods for explanation of sample sizes

Variable	N	df	Deviance	p
Chaffinch				
Model 1				
Contrast	142	1	13.5	0.0002
Treatment		1	0.17	0.68
Contrast \times Treatment		1	0.82	0.37
Model 2				
Contrast	87	1	12.4	0.0004
Treatment		1	0.89	0.35
ΔT_B		1	0.25	0.62
ΔT_C		1	0.22	0.64
Contrast \times Treatment		1	1.04	0.31
$\Delta T_C \times$ Treatment		1	0.51	0.47
$\Delta T_B \times$ Treatment		1	0.24	0.62
Brambling				
Model 1				
Contrast	184	1	24.4	<0.0001
Treatment		1	0.05	0.82
Contrast \times Treatment		1	1.26	0.26
Model 2				
Contrast	79	1	3.43	0.06
ΔT_B		1	1.35	0.24
ΔT_C		1	0.07	0.80
Treatment		1	0.06	0.81
$\Delta T_C \times$ Treatment		1	1.63	0.20
$\Delta T_B \times$ Treatment		1	1.29	0.26
Contrast \times Treatment		1	<0.01	0.97

effect of treatment on the relationship between contrast and rejection probability (Table 2). For both species, the minimal adequate model explaining rejection/acceptance contained contrast as the only explanatory variable (Table 2). This justifies using contrast as the only egg similarity measure when analysing the larger sample of experiments.

For bramblings, we also compared the rejection rates of non-mimetic eggs from single control experiments (contrast values ≥ 2.5) with double experiments (single: 88%, $n = 72$; double: 98%, $n = 57$). The rejection rates are not directly comparable because the experimental eggs tended to have different contrast values in the two groups (Table 1). If we restrict the comparison to experimental eggs of maximum contrast (i.e. contrast = 3.0), there is no significant difference in rejection rate between the two groups (single parasitism: 92%, $n = 37$; double parasitism: 98%, $n = 43$, fisher's exact probabilities test, $p = 0.33$). By and large, there is no strong indication that the tendency to reject non-mimetic eggs is affected by simultaneous presence of a low-intermediate contrasting egg. The similarity between the non-mimetic and low-intermediate contrast experimental eggs tended to be higher than the similarity between the non-mimetic egg and the host eggs (ΔT_C was on average 0.013 units lower (paired t-test: $t = 3.00$, $df = 42$, $p = 0.005$) and ΔT_B was on average 1.5 units lower (paired t-test: $t = 2.08$, $df = 42$, $p = 0.03$).

The latency to rejection did also not differ significantly between the experimental and control group in any of the two species (Table 1, Wilcoxon tests: $W = 169.5$, $p = 0.11$ and $W = 291.5$, $p = 0.59$ for chaffinch and bramblings respectively). For these analyses we only included nests that were visited at least every second day after initiation of the experiment. For those cases where the exact day of rejection was not known, rejection was defined to occur mid between the day rejection was confirmed and the day of the preceding nest visit.

Discussion

In the present study, we have shown that both rejection rate and latency to rejection of low-medium contrasting parasitic eggs is unaffected by recent experience with rejection of experimental parasitism in chaffinches and bramblings. This suggests that acceptance thresholds are relatively rigid, which agrees well with previous studies where stimulation with cuckoo dummies (simulating high risk of parasitism) failed to trigger increased rejection of experi-

mental parasitic eggs or rejection of own eggs from unparasitized nests (Braa et al. 1992; Stokke et al. 2002a, 2004).

Why do chaffinches and bramblings operate with a fixed acceptance threshold? One general explanation is that components of the recognition system (Sherman et al. 1997) have evolved so that use of conditional stimuli have become superfluous in the decision-making process, thereby favouring the loss of flexibility (Stokke et al. 2005). It is widely approved that the optimal acceptance threshold of the host depends critically on the distributions of cuckoo and host egg phenotypes (Rodriguez-Girones & Lotem 1999; Servedio & Lande 2003; Stokke et al. 2007b). Both chaffinches and bramblings are known to have comparatively low intra- and high inter-clutch variation in egg appearance (Øien et al. 1995; Stokke et al. 2002b). These characteristics contribute to reduce the overlap between host and parasite egg phenotypes, which in turn reduce the risk of committing both acceptance and recognition errors (Sherman et al. 1997). This reduces the influence of parasitism risk on the position of the optimal acceptance threshold, which in turn means that conditional adjustments becomes increasingly redundant (Liebert & Starks 2004). The rejection decision of chaffinches and bramblings appear to be less flexible than for example those of great reed warblers, reed warblers *Acrocephalus scirpaceus*, and meadow pipits *Anthus pratensis*. These species react to the presence of cuckoos at the nest by increasing their subsequent tendency to reject (Davies & Brooke 1988; Moksnes et al. 1993, 2000; Bartol et al. 2002). In these cases, the use and disuse of conditional cues broadly agrees with expectations based on relative values of the species intra- and interclutch variation (Øien et al. 1995; Stokke et al. 2002b, 2005, 2007b).

In contrast to our experiments, Hauber et al. (2006) found a positive effect of recent experience on rejection of inaccurate mimetic eggs in great reed warblers. This could reflect adaptive adjustments of acceptance thresholds. The predictability of parasitism may increase when distribution of parasitism-risk among individuals is non-random (e.g. Hauber et al. 2004; Hoover et al. 2006). In such cases, individuals might benefit from adjusting their threshold accordingly. In the great reed warbler case, it was suggested that an unusually high rate of multiple parasitism increases the predictability of recurrent parasitism (Hauber et al. 2006). Although the explanation is intuitive, it is worth clarifying whether it is necessarily the rate of multiple parasitism *per se* that is important in this scenario. The

central question facing decision making hosts is whether the future predictability of parasitism is lower if it has not yet been parasitized than if it has already been parasitized once (and detected the egg). In order to make a flexible strategy superior (relative to a fixed threshold), the probability of a female being parasitized at least once (which equals the population parasitism rate) should be lower than the conditional probability of a female being parasitized given that she has already been parasitized (the proportion of parasitized nests that are multiply parasitized). In the great reed warbler population, the situation was the opposite during the years of study (Moskát & Honza 2002; Hauber et al. 2006). It could therefore be a better strategy to apply the more restrictive threshold regardless of context, or instead adjust the threshold to the perceived parasitism rate of the population (Davies et al. 1996)). When there is high spatiotemporal variation in parasitism risk (Lindholm 1999; Stokke et al. 2007a; Moskát et al. 2008), it could still be valuable for the host to know whether it has settled to breed in a parasitized population, and thereafter adjust its threshold (Davies 2000). Experience with recognition and rejection of a poorly mimetic cuckoo egg would be a reliable cue in this respect. In comparison, cuckoo density is not always a good indicator of parasitism risk because the cuckoo may primarily be utilizing other host species in a given area (Davies 2000).

However, over a longer time period, it is possible that cuckoo density may be sufficiently high and parasitism risk sufficiently skewed to create a disproportionately high probability of recurrent parasitism in some great reed warbler populations. In contrast, bramblings and chaffinches are currently parasitized at low rates (bramblings in Kittilä) or not regularly parasitized at all (bramblings in Ammarnäs, Tydal and Tana; chaffinches in general). Thus, in these species, the probability of being parasitized a second time in the same breeding bout or breeding season is extremely low. One could therefore propose that these hosts should adopt a more permissive acceptance threshold if they used recent experience with parasitism to estimate the probability of recurrent parasitism. Since they show the same tendency to reject regardless of experience, they appear to apply the same acceptance threshold regardless of parasitism risk.

Interestingly, in a recent study where great reed warblers were experimentally parasitized simultaneously with a non-mimetic and a mimetic egg (i.e. similar to our experiments with bramblings), a 40%

reduction in the rejection rate of non-mimetic eggs was observed (the same result was observed for naturally parasitized nests, Moskát et al. 2009). Since the non-mimetic experimental egg tends to be more similar to the mimetic experimental egg than the hosts' own eggs, this result suggests that recognition is partly based on comparison of the most deviant egg in the nest with the next most deviant egg (i.e. recognition by discordancy, Moskát et al. 2009). Bramblings, in contrast, showed an equally strong tendency to reject the non-mimetic egg when it was introduced together with a low-intermediate contrasting egg. Although the difference in background colour between the two parasitic eggs was not much lower than the difference between the non-mimetic egg and the host eggs, this result corroborates previous work (Moksnes 1992) in showing that recognition is predominantly based on memory templates (i.e. true recognition) with no element of discordancy. Another study has indicated that great reed warblers may also use a memory based template which they update during egg laying when they acquire additional information about its own eggs (Moskát & Hauber 2007). In contrast, it has been argued that the best strategy for bramblings and chaffinches is to imprint on the first egg they lay, and that this is made possible because of the extraordinarily low intraclutch variation displayed by these species (Stokke et al. 2004, 2007b). Thus, differences seem to exist between the great reed warbler and the *Fringilla* finches in both the perception (Mateo 2004) and action (Liebert & Starks 2004) component of recognition, and each may contribute to the contrasting degree of observed flexibility.

Another situation where rejection might have a short-time effect on the subsequent tendency to reject is if there are carry-over effects related to motivational state (Rothstein 1974). If such effects were important, one could predict that simultaneous introduction of both parasitic eggs would trigger an increased rate of rejection. This is because the motivation to reject is arguably present (the non-mimetic egg is rejected) at the same time as the eggs in the nest (including the second parasitic egg) presumably are inspected. However, no increase in rate of rejection was observed in the present study.

In conclusion, bramblings and chaffinches give examples of a simple but yet advanced recognition system which is characterized by a relatively rigid acceptance threshold and a recognition mechanism based exclusively on a memory based template.

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