ethology international journal of behavioural biology

Ethology

Repeatability of Foreign Egg Rejection: Testing the Assumptions of Co-Evolutionary Theory

Peter Samaš*, Mark E. Hauber†, Phillip Cassey‡ & Tomáš Grim*

* Department of Zoology and Laboratory of Ornithology, Palacký University, Olomouc, Czech Republic
† Department of Psychology, Hunter College of the City University of New York, New York, NY, USA
‡ School of Earth and Environmental Sciences, University of Adelaide, Adelaide, Australia

Correspondence
Tomáš Grim, Department of Zoology and
Laboratory of Ornithology, Palacký University,
tř. Svobody 26, CZ-771 46 Olomouc,
Czech Republic.
E-mail: tomas.grim@upol.cz

Received: March 8, 2011
Initial acceptance: April 4, 2011
Final acceptance: April 6, 2011
(T. Tregenza)

doi: 10.1111/j.1439-0310.2011.01917.x

Abstract

Most theoretical models of coevolution between brood parasites, whether interspecific or conspecific, and their hosts explicitly assume consistent individual behaviour in host egg-rejection responses. Accordingly, hosts cast as acceptors always accept, whereas ejectors always reject parasitic eggs when exposed to stable ecological conditions. To date, only few studies have attempted to test this critical assumption of individual repeatability in egg-rejection responses of hosts. Here, we studied the repeatability of egg rejection in blackbirds (*Turdus merula*) and song thrush (*T. philomelos*), species in which females are reported to reject simulated, non-mimetic foreign eggs at intermediate frequencies at the population level. However, intermediate rates of acceptance and rejection can be consistent with either or both intra- and interindividual variability in rejection behaviours. Our experiments revealed generally high individual consistency in these hosts' responses to experimentally introduced non-mimetic and mimetic model foreign eggs. Individuals also responded faster on average to second than to first trials within the same breeding attempts, but the difference was statistically significant only in blackbirds. These results are consistent with the critical assumption of co-evolutionary models, that statistically egg rejection is mostly individually repeatable, but also reveal that some individuals in both species change their responses even within the short time-window of one breeding attempt. The data suggest that individuals reject foreign eggs faster when perceived parasitism risk is greater because of repeated introductions of experimental parasitic eggs. We provide methodological recommendations to facilitate experimental and meta-analytical studies of individual egg rejection repeatability and discuss how to reduce technical constraints arising from disparate treatments and variable sample sizes for future studies.

Introduction

Avian brood parasitism selects for the evolution of host defences; the rejection of foreign eggs is the most often documented type of defence (Davies 2000). Evolutionary theories of brood parasite–host arms-races explicitly assume that parasitic egg-laying strategies are 'strictly heritable' (Ruxton et al. 2001) in either interspecific (Gibbs et al. 2000) or conspecific parasites (May et al. 1991; Nee & May 1993; Takasu 2004; Shaw & Hauber 2009). Further, several models of interspecific and conspecific parasitism also assume that individual hosts are either rejecters (that always reject) or acceptors (that always accept) in response to parasitism (Yamauchi 1993, 1995; Takasu 1998; Ruxton & Broom 2002; Servedio &

Lande 2003; Servedio & Hauber 2006). Other models permit that rejecter hosts modulate behavioural responses based on experience (Lotem 1993; Lawes & Marthews 2003) and the cost-benefit trade-offs of rejection decisions (Moskát & Hauber 2007); or modulate the perceptual acceptance threshold among own and foreign eggs based on parasitism risks (Davies et al. 1996; Hauber et al. 2006). However, all these models incorporate that selection to reject parasitism produces host individuals which are consistent in their responses to parasitic eggs within the same ecological and perceptual context, otherwise host responses would represent random variation and not the outcome of selection.

Accordingly, all these evolutionary models of brood parasite–host coevolution assume that when a particular host individual rejected a particular foreign egg, it will reject *the same type of model* again when parasitised for the second time. Importantly, such consistency, and the resulting statistical measure of individual repeatability, can be interpreted as a ceiling value of heritability (Bell et al. 2009; Nakagawa & Schielzeth 2010). Thus, estimating repeatability provides a preliminary approach, and also a valuable set of behavioural details, about potential maximum heritability of the response trait (Bell et al. 2009). Violation of the critical assumption of the models that host's egg-rejection response is individually consistent could potentially have a dramatic effect on model predictions, because of the inherent non-linearities in the parasite–host systems dynamics (G. D. Ruxton, pers. comm.) and would call for the need for new theory and for the reassessment of conclusions drawn from data based on previous, theory-driven experiments.

A thorough literature search revealed to us that few studies collected data on individual repeatability of egg rejection and all those studies tested only hosts of interspecific brood parasites. For example, experimentally induced individual repeatability of egg rejection was 'very high' within the same breeding attempt in several species (Honza et al. 2007b; Peer & Rothstein 2010) but very low in common grackles (*Quiscalus quiscula*; Peer & Rothstein 2010). Repeatability between breeding attempts of individual hosts within one breeding season was found to be high in one study (Lotem et al. 1995) but low in another one (Alvarez 1996), when considering different host species of the common cuckoo (*Cuculus canorus*). Finally, repeatability of egg rejection was very low within the same individuals between breeding seasons (Soler et al. 2000; see also Palomino et al. 1998). Additional studies by Hauber et al. (2006) and Vikan et al. (2009) performed repeated experiments, but they used different models in subsequent trials on the same individual hosts, thus, those data could not be used to estimate repeatability.

Some previous studies also included several methodological limitations to allow for explicit testing of individual repeatability. For example, Peer & Rothstein (2010) repeated their experiments only at the nests of rejecters but not at the nests of acceptors, so that repeatability of acceptance/rejection responses could not be estimated. Further, Palomino et al. (1998) pooled host responses across various temporal periods (from within one breeding attempt to between year time scales). However, repeatability depends on the length of time between two consecutive behavioural measurements, irrespective of the outcome (see, e.g. Bell et al. 2009; Weidinger & Kočvara 2010). Egg ejection likely requires sophisticated cognitive abilities that are highly likely affected by memory and experience (e.g. Lotem 1993; Moskát & Hauber 2007; Moskát et al. 2010). Thus, there is good reason to expect that repeatability of egg ejection will be dependent on time-window between successive trials (e.g. naive acceptor will accept in both trials within one breeding attempt, but will reject parasitism in the next breeding attempt; see Lotem et al. 1995). Indeed, Palomino et al. (1998) discuss this potential bias.

Some other previous work also focused on overwhelmingly rejecter (Honza et al. 2007b) or acceptor (Hoover et al. 2006) species. Here, we argue that repeated experiments on *intermediate* rejecters (Røskaft et al. 2002; see also Discussion) provide the most relevant system for the study of consistency and, by proxy, heritability of individual hosts' rejection responses to parasite eggs. This is because strong rejecter species (that always reject, e.g. blackcaps *Sylvia atricapilla*; Honza et al. 2007b) or pure acceptor species (that never reject, e.g. dunnocks *Prunella modularis*; Davies & Brooke 1989) show little or no individual variation in their responses. Such species with invariable behavioural responses are not suitable for measuring repeatability because it can be *a priori* assumed that repeatability (and heritability) would be low (Bell et al. 2009; Nakagawa & Schielzeth 2010).

Quantitatively, meaningful measurement of repeatability also requires some, ideally high, between individual variance (Bell et al. 2009). This is because, conceptually, observations and experimental data revealing very high inter-individual variability at the population level are compatible with mechanisms involving *either* high individual repeatability (i.e. inconsistent behaviour between individuals, consistent behaviour within individuals) or low repeatability (inconsistent behaviour both between and within individuals). Thus, brood parasite host populations showing intermediate egg rejection rates are best suited to test these alternative mechanisms and estimations of individual repeatability of egg-rejection behaviour in the context of brood parasitism (Bell et al. 2009).

Another important aspect of host responses is whether only females (Soler et al. 2002), or both females and males (Honza et al. 2007b), eject parasitic eggs. In the latter case, one member of the pair can pre-empt the other's rejection response by removing the parasitic egg before the other member of the pair has a chance to recognise and reject the egg. Under such conditions, it is impossible to measure repeatability unless nests are video-recorded, but even then all nests where successive parasite eggs were not ejected by the same individual would have to be discarded from analyses because ejections by different individuals cannot be used to estimate individual repeatability (Bell et al. 2009). Thus, we argue that those host species in which *only one sex* (typically the female) ejects parasite eggs provide logistically and heuristically better models to study repeatability.

For this study of the repeatability of host responses to experimental brood parasitism, we selected two appropriate species: the blackbird (*Turdus merula*) and the song thrush (*T. philomelos*). These two species are intermediate rejecters of both dissimilar and similar foreign eggs, with average egg-rejection rates close to 50% (e.g. Grim & Honza 2001). For example, published rejection rates of immaculate blue cuckoo-sized model eggs, the most frequently used non-mimetic model type in studies of brood parasitism in Europe (Davies 2000; Grim et al. 2011), are 48.0 ± 22.6% ($\bar{x}$ ± standard deviation, n = 7 studies) for the song thrush, and 57.6 ± 14.1% (n = 6 studies) for the blackbird (reviewed in Table 1 in Polačiková & Grim 2010). Furthermore, in both of these species, the female is typically the sex that incubates (Cramp 1988; our own unpubl. data). As in other species with female-only incubation (e.g. Amundsen et al. 2002), only females eject eggs in blackbirds (Soler et al. 2002; Weiszensteinová J., Samaš P., Hauber M., Cassey P., Grim T. unpubl. data).

In both thrush species, there are documented cases of brood parasitism both by common cuckoos (Moksnes & Røskaft 1995) and by conspecifics (Grim & Honza 2001; Moskát et al. 2003; Higgins 2006). However, a recent large-scale study (Grim et al. 2011) showed that thrushes (*Turdus* spp.) are unsuitable cuckoo hosts and could not have been involved in long-term coevolution with the cuckoo. Thus, these thrushes' fine-tuned egg-ejection abilities most likely evolved in the context of conspecific brood parasitism (which is known in both thrush species; own unpubl. data), which can also be highly costly to hosts (Lyon 2003; Shizuka & Lyon 2010) and is known to select for anti-parasite adaptations (López-de-Hierro & Moreno-Rueda 2010; Riehl 2010). Thus, the present study is the first to study egg-ejection repeatability in the context of conspecific brood parasitism. However, our results are methodologically and empirically relevant for studies of either interspecific or conspecific parasitism because both types of parasitism create fundamentally similar selection pressures both theoretically (Yamauchi 1995) and empirically (Jackson 1998) and 'it is difficult to separate the effects of the two' (Davies 2000, p. 223).

Moskát et al. (2003) suggested that egg rejection in some potential cuckoo hosts can be explained as an extension of nest sanitation (i.e. removal of non-egg-like objects). Leaving aside theoretical objections (apparent nest sanitation can be simply a by-product of egg-rejection abilities, not *vice versa*), it is highly unlikely that egg rejection in thrushes represents a by-product of nest cleaning. This is because fine-tuned *specific* discrimination abilities (high rates of rejection of conspecific eggs, own unpubl. data) are not likely to be in principle a by-product of general *non-specific* nest cleaning behaviour (see discrimination threshold theory by Reeve 1989).

Repeatability of any trait can be estimated for various temporal scales (Weidinger & Kočvara 2010). For egg rejection, one could estimate repeatability (1) within one breeding attempt, (2) between different breeding attempts within one breeding season, and (3) between breeding seasons. The logical first research step for experimentally estimating repeatability is the short-term consistency in responses: if the short term repeatability were very low, it would follow that the long-term consistency would also be very low, in line with empirical data (Bell et al. 2009). In contrast, high short-term repeatability allows for either high or low long-term repeatability. Thus, we began to study short-term consistency in host responses within one breeding attempt (see also previous studies at the same time scale: Honza et al. 2007b; Peer & Rothstein 2010).

We predicted high repeatability of egg rejection within a single breeding attempt because (1) the shorter the time window between two trials, the higher the behavioural repeatability (Bell et al. 2009), and (2) most birds reject alien eggs at similar frequencies throughout the nesting period, i.e. they do not respond differently between laying and incubation stages (Davies & Brooke 1989; Moksnes et al. 1991; Peer & Rothstein 2010). The latter was reported for blackbirds and song thrush too (Davies & Brooke 1989; Polačiková & Grim 2010; Grim et al. 2011). We also predicted shorter latencies until rejection of the foreign eggs from the first to the second trial in line with both current theoretical models (Stokke et al. 2007) and empirical data (Honza et al. 2007b). Finally, we predicted that responses to mimetic model eggs (conspecific model, see Methods) should be less repeatable than responses to non-mimetic blue model eggs because the higher similarity between recognition cues imposes higher risk of discrimination errors (Reeve 1989).

In any study of brood parasitism, acceptor nests by definition have longer exposure to potential predation than rejecter nests (see Results). As predated nests are excluded from analyses, this methodological constraint might inflate the apparent rejection rates in any brood parasitism study and also egg rejection consistency estimates in studies of repeatability. Therefore, we also re-analysed our data by excluding nests where the host rejected the egg model but the nest was predated within the overall exposure period of acceptor nests (13 d, see Methods). This was to test whether different predation rates at acceptor vs. rejecter nests biased our results. We present both estimates because (1) to our knowledge, almost none of the previous studies made this correction (but see Vikan et al. 2010); thus, to make results comparable across studies we need to present also the uncorrected estimates (this allows inclusion of our data into meta-analyses), and (2) the comparison of corrected and non-corrected estimates may become valuable, especially if these estimates do not match; we propose that (re)analyses with this correction factor of past and ongoing or future studies of brood parasitism will follow this preferable approach.

Methods

We carried out the study in the city of Auckland, New Zealand (36°51′S, 174°46′E) in 2007–2009. Both the blackbird and song thrush are European species that were introduced to New Zealand in late 19th century (Thomson 1922). Both species reject foreign eggs from their nests at similar or even higher frequencies in New Zealand, as in their European populations (Hale & Briskie 2007; Polačiková & Grim 2010) as can be expected if egg rejection is not an evolved response to cuckoos but to conspecific brood parasitism (see Grim et al. 2011).

Model eggs

We tested both species with a plain light blue model egg, which is the most commonly used non-mimetic model in studies of common cuckoo parasitism across Europe (Davies 2000). We are aware of the problem that terms 'mimetic' vs. 'non-mimetic' are confusing and being used inconsistently across various studies (discussed in Grim 2005). This is because these terms dichotomize continuous variation in host–parasite egg similarity (Grim 2005, p. 76). Here, for the readers' convenience, we use terms 'mimetic' (large models painted to resemble respective host eggs) vs. 'non-mimetic' (small models painted to resemble plain blue cuckoo eggs) as done in previous published studies of *Turdus* thrushes, but also present physical reflectance data on the colour similarity of egg models to our model host species' eggs (Fig. 1a,b) and provide illustrations of the models, too (Fig. 2).

Our non-mimetic models were made from polysynthetic material and painted with acrylic paints to resemble eggs laid by the common cuckoo into the nests of the redstart (*Phoenicurus phoenicurus*; Moksnes & Røskaft 1995; Grim et al. 2009b). The size ($\bar{x} \pm$ SD = 22.7 ± 0.54 × 17.4 ± 0.48 mm, n = 10), mass (3.7 ± 0.45 g, n = 10) and the shape of these non-mimetic blue egg models were similar to real, common cuckoo eggs (size range: 20–26 × 15–19 mm, mass range: 2.9–3.8 g, Cramp 1985). Our preliminary experiments showed that blackbirds and song thrushes reject model blue eggs at intermediate frequencies in our New Zealand study populations (see Results).

Further, we tested both thrush species with mimetic model eggs (again made from polysynthetic material and painted with acrylic paints). The 'conspecific model' was painted to replicate appearance of the natural blackbird egg (i.e. greenish-blue background with dense red-brown spotting) and the natural song thrush egg (i.e. blue background with dark spots concentrated at the blunt egg pole), respectively. The size ($\bar{x} \pm$ standard deviation: 29.6 ± 0.26 × 21.5 ± 0.28 mm, n = 10), mass (7.8 ± 0.49 g, n = 10) and shape of blackbird conspecific models were similar to real blackbird egg (size range: 25–35 × 19–24 mm, estimated mass: 7.2 g, Cramp 1988). The size ($\bar{x} \pm$ standard deviation: 25.8 ± 0.40 × 21.0 ± 0.25 mm, n = 10), mass (6.4 ± 0.31 g, n = 10) and shape of song thrush conspecific models were similar to real song thrush egg (size range:

24–31 × 19–22 mm, estimated mass: 6.0 g, Cramp 1988). For representative reflectance spectra of natural and model eggs, see Fig. 1.

Preliminary experiments with single (i.e. non-repeated) introductions of the blue model were performed during the austral summer of 2007–2008. Repeated experiments with conspecific models were carried out in 2008, and repeated experiments with blue models in 2009.

Experimental procedures

Each experiment consisted of two consecutive trials. The nest was first parasitised during the laying stage or in the first 5 d of incubation (we visited nests each day; thus, clutch ages were not estimated but known exactly, assuming one egg laid per day). We did not remove the host's egg, as egg removal has no effect on rejection probability in these species (Davies & Brooke 1989). We decided *a priori* to employ the standard 6-d exposure period of Moksnes et al. (1991) to evaluate host responses. We aimed to check the nests daily until ejection or final acceptance, and despite logistically constraints (e.g. transportation and weather), we were able to check nests on 90% of subsequent days across the two species. Each nest was also checked several hours after the model egg was introduced on the same day. If the model disappeared during the day of the experiment, we assigned the nest latency to rejection of 0. The egg model was assumed to be accepted if it was still in an active nest on 6th day after the start of the experimental parasitism, then the model was removed. Although several previous studies included nest desertions as a specific response to parasite eggs, we did not do so because there were only 2 and 1 desertions (of blue models) in song thrushes and blackbirds, respectively. Thus, we assumed that desertion was not a response to parasitism by the specific egg models in our study population.

Two days after the outcome of the first trial (acceptance or rejection), we placed the same type of model into the nest. Again, we checked the nest daily until ejection or acceptance up to 6 d. Egg laying and incubation periods in both species last approximately 16 d (3–4 d of laying and 13 d of incubation; Higgins 2006) which provided enough time to test repeated acceptors (i.e. individuals that accepted both the first and second experimental egg; 6 + 1 + 6 d). We followed the same procedure in experiments with either blue (non-mimetic) models or with conspecific (mimetic) model eggs. Nests depredated before the first trial finished were excluded from analyses, whereas nests

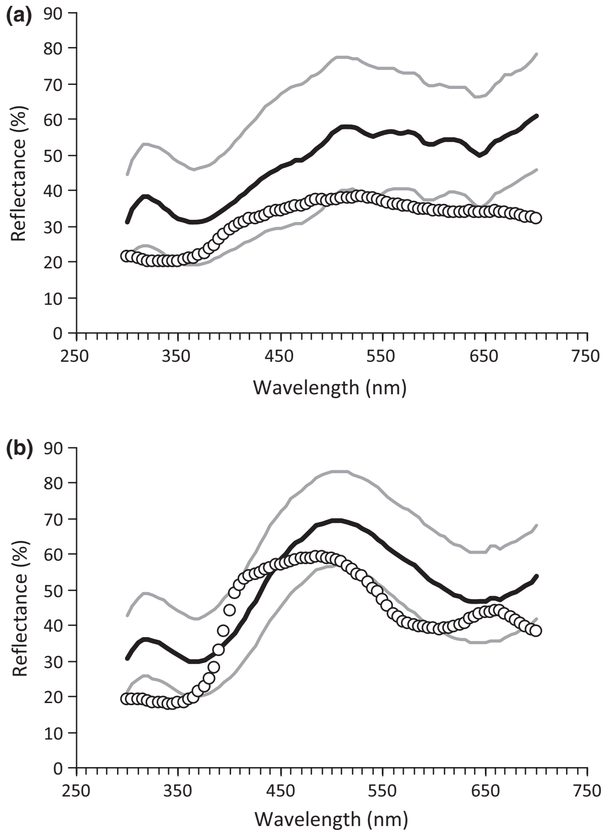

Fig. 1: Average reflectance spectra of the background coloration of natural eggs (black line) and artificial conspecific model eggs (○) for (a) blackbirds *Turdus merula* (n = 108) and (b) song thrushes *T. philomelos* (n = 156) in New Zealand. Spectra of natural eggs are shown with the range of natural variation (5th and 95th percentile ranges, grey lines) and the averages of three measurements are shown for each model egg (n = 5 models per type, because all model eggs were painted identically and variation would represent only measurement errors). In Fig. 1b, the artificial conspecific model reflectance curve refers to the blue background colour of both the conspecific song thrush model and the smaller model resembling the egg laid by the common cuckoo *Cuculus canorus* into redstart *Phoenicurus phoenicurus* nests, as these two types of models differed only in size and the presence of maculation (song thrush-like dark spots) on the conspecific song thrush model. Artificial conspecific models in both blackbirds and song thrushes can be considered highly mimetic because of the extensive overlap between artificial model eggs' reflectance curves and the models' size compared with the range of natural host eggs. The discrepancy between artificial and natural eggs is within the range of differences that had been considered highly mimetic in previous studies (e.g. spectral curves of artificial and natural eggs in Fig. 1 in Honza et al. 2007a). In contrast, artificial redstart-cuckoo (immaculate blue) model eggs are considered non-mimetic, as there is no overlap in size of hosts' eggs and models (see Methods) and coloration differences are striking between natural blackbird eggs and redstart-cuckoo models. Although the latter are similar as for background colour to natural song thrush eggs, the redstart-cuckoo models lack maculation that is used as a discrimination cue in song thrush (Polačiková et al. 2010).

that were depredated only after the first trial successfully ended were used to estimate the population-wide rate of rejection of alien eggs.

Statistical analyses

We applied two approaches to estimate the repeatability of binary egg-rejection (accept or eject) responses. First, we estimated consistencies of individual responses with Spearman's correlation coefficients. This is a statistically relevant approach (Martin & Bateson 2008, p. 74–78) and the resulting r_s-values and p-values are identical as when calculated with Pearson's correlation or phi contingency coefficient (see Weidinger & Kočvara 2010). Yet, approaches based on simple correlations are disadvantageous because they cannot control for possible confounding factors.

Second, to test for the possible confounding effects of covariates, we also estimated the consistency of a binary response between two trials within the nest using Generalised Linear Mixed Models (GLMM) implemented in R 2.12.0 (R Development Core Team 2010: package lme4 v. 0.999375-37; Bates et al. 2008). We used Laplace approximation to estimate the parameters in our GLMM with binary response variable (Bolker et al. 2009). We built four separate models for each species (blackbird, song thrush) and for each egg model type (non-mimetic, mimetic) combination. The global models for blue model eggs included nest identity (random variable) and following explanatory variables: nest age (age of the nest at start of experiment in days, day 0 = start of incubation, negative values denote start of experiment in laying stage), first egg-laying date (including its quadratic term to test for non-linear seasonal trends) and final clutch size. We selected the most parsimonious model based on Akaike information criterion corrected for small sample (AIC_c) (Burnham & Anderson 2002). Statistical models for conspecific model eggs did not include covariates because of low sample sizes so as to avoid overparametrization (Burnham & Anderson 2002).

Intraclass correlation coefficients (i.e. repeatability) of egg ejection (binary response variable) were calculated as $r = VA/(VA + VE + \pi^2/3)$, where VA denotes between-nest variance component, VE = component due to overdispersion (Guo & Zhao 2000) and within-nest residual component $\pi^2/3$ represents the distribution-specific variance for the logit GLMM (Nakagawa & Schielzeth 2010), where $\pi = 3.14$. We estimated asymptotic 95% confidence intervals for the correlation coefficients calculated in our GLMM model, using the public calculator http://faculty.vassar.edu/lowry/rho.html. The calculation is based on the Fisher r-to-z transformation according to the formula $z = ½*\ln*(1 + r)/(1–r) = \mathrm{arctanh}(r)$ (where ln is the natural logarithm function and arctanh is the inverse hyperbolic function).

Spearman's correlation coefficients with exact 95% confidence intervals (binary response) were calculated using StatXact 7 (Cytel Inc. 2005). Results are presented as $\bar{x} \pm$ SE.

Individual repeatability of latency to ejection (a continuous response variable) between trials was calculated as $r = VA/(VA + VP)$ (Lessells & Boag 1987), where VA denotes between-nest variance component, and VP = within-nest variance component. Confidence intervals for their intraclass correlation coefficients were calculated according to formula in McGraw & Wong (1996).

Results

Responses of blackbirds to non-mimetic blue egg models

The overall rejection rate of blue egg models by blackbirds in the focal study season (2009) was 71.4% (n = 56, data from nests that were tested only once plus results of first trials from repeatedly tested nests). This is an intermediate rejection rate by definition

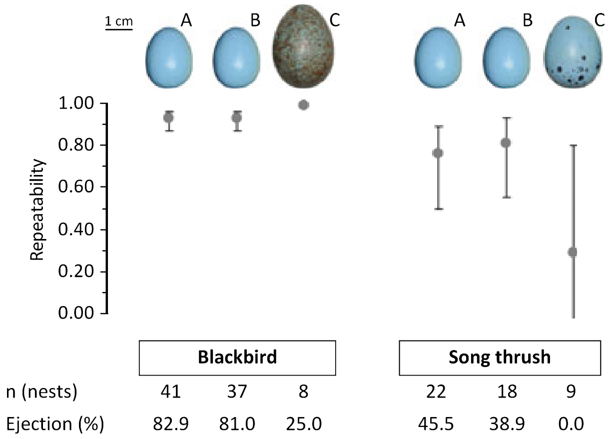

Fig. 2: Repeatability estimates from GLMM (see Methods) with 95% confidence intervals (CIs) of egg rejection in blackbirds and song thrushes. Results shown for (A) non-mimetic blue eggs with all nests included, (B) blue eggs with rejecter nests depredated within 13 d excluded (see Discussion for rationale behind this re-analysis) and (C) mimetic conspecific models. Note that CIs for mimetic conspecific models in the song thrush overlap zero; lower 95% CI (–0.46) is not shown in the graph. Sample sizes and ejection rates from 1st trials are shown below.

Table 1: GLMM models of egg ejection of blue models as the binomial response variable, with NEST = nest identity as random variable and fixed factors of AGE = age of clutch at start of experiment, CLUTCH = final clutch size, SEASON = first egg-laying date and SEASON2 = squared first egg-laying date. Models are ranked from the best to the worst based on Akaike information criteria, corrected for small sample size (AIC_c) (Burnham & Anderson 2002). K = number of parameters in the model. $\Delta_i = AIC_{c(i)} - AIC_{c(min)}$. w_i = Akaike weights

Models	K	AIC_C	Δ_i	w_i
Blackbird (n = 41)				
NEST	2	56.31	0.00	0.29
NEST + CLUTCH	3	57.20	0.89	0.19
NEST + AGE	3	57.41	1.10	0.17
NEST + SEASON	3	58.05	1.73	0.12
NEST + AGE + CLUTCH	4	59.29	2.98	0.07
NEST + CLUTCH + SEASON	4	59.89	3.58	0.05
NEST + AGE + SEASON	4	59.96	3.65	0.05
NEST + SEASON + SEASON2	4	60.66	4.35	0.03
NEST + AGE + SEASON + CLUTCH	5	62.17	5.86	0.02
NEST + SEASON + SEASON2 + CLUTCH	5	62.34	6.03	0.01
NEST + AGE + SEASON + SEASON2	5	63.29	6.98	0.01
NEST + AGE + SEASON + SEASON2 + CLUTCH	6	895.63	839.32	0.00
Song Thrush (n = 22)				
NEST + SEASON	3	50.96	0.00	0.40
NEST + CLUTCH + SEASON	4	53.25	2.30	0.13
NEST + AGE + SEASON	4	53.27	2.31	0.12
NEST + SEASON + SEASON2	4	53.37	2.41	0.12
NEST + CLUTCH	3	54.26	3.30	0.08
NEST + AGE + SEASON + CLUTCH	5	55.68	4.73	0.04
NEST + AGE + SEASON + SEASON2	5	55.81	4.85	0.04
NEST + SEASON + SEASON2 + CLUTCH	5	55.81	4.85	0.04
NEST + AGE + CLUTCH	4	56.57	5.61	0.02
NEST	2	57.67	6.72	0.01
NEST + AGE + SEASON + SEASON2 + CLUTCH	6	58.37	7.42	0.01
NEST + AGE	3	59.94	8.98	0.00

(*sensu* Røskaft et al. 2002; see also Discussion). This rate was statistically similar to rejection rates in previous years at our study site (2007–2008, 54.2%, n = 24; $\chi^2 = 2.24$, df = 1, p = 0.14). All results below refer only to repeatedly tested nests (in 2009).

In the first trials, at 83% tested nests (n = 41), we documented ejection of the model egg. In the second trials, 78% of females ejected the egg in the same sample of nests. Only two females changed their response (both from ejection to acceptance), and the responses of all other females, both acceptors and rejecters, remained identical between the first and the second trial. Repeatability for the binary data (see Methods) showed very high consistency (Spearman's correlation: $r_s = 0.86$, exact 95% CI = 0.67–1.00, n = 41, p < 0.0001).

The most parsimonious GLMM included only random variable nest (Table 1; Fig. 2). However, three other models had $\Delta_i < 2$ (Burnham & Anderson 2002). These models included, additional to nest identity, also final clutch size, nest age, and laying date in the season (Table 1). As 95% confidence intervals for model-averaged parameter estimates of these variables included 0 in all cases, we did not consider these variables further (Burnham & Anderson 2002). The latency to ejection of the model in the second trial was significantly shorter than in the first trial (Fig. 2, paired *t*-test: $t_{32} = 2.43$, p = 0.02).

In the first trials, the probability of egg ejection increased with advancing nest stage (logistic regression: $\chi^2 = 6.88$, df = 1,39, p = 0.009, intercept = −1.75 ± 0.56, slope = 0.69 ± 0.36). Still, this pattern did not confound our results because individual responses remained virtually identical between the first and second trial (39 of 41 females did not change their rejection responses). In the second trials, the egg ejection probability did not covary with nest age (logistic regression: $\chi^2 = 0.41$, df = 1,39, p = 0.52).

Latency to ejection had a tendency to decrease with nest age in the first trials ($F_{1,30} = 4.11$, p = 0.052). In the second trials, latencies to ejection did not correlate with nest age ($F_{1,30} = 0.62$, p = 0.44). Individual repeatability of the latency in egg ejection between trials was $r = 0.46$ (CI = 0.17–0.71), when controlling for nest-age effect.

Responses of blackbirds to mimetic conspecific egg models

In the first trials (2008 breeding season), blackbirds ejected only two and accepted 14 conspecific models. Out of these 16 nests, we successfully completed the second trial at only eight nests (50%). Rejection response in the second trials was identical in all cases. The same two females ejected conspecific egg models, whereas the remaining six females accepted the model eggs, i.e. incubated the model egg successfully for at least 6 d. Non-parametric correlation with the binary data showed high consistency ($r_s = 1.00$, exact 95% CI = 1.00–1.00, n = 8, p = 0.03). GLMM-estimated repeatability for the binary data was quantitatively very similar (Fig. 2).

The latency to respond at the two rejecter nests in the first trial was 1 and 3 d respectively. In the second trial, both rejecters responded with 1 d latencies.

Responses of song thrush to non-mimetic blue egg models

The overall rejection rate by song thrush of blue egg models in the focal study season (2009) was 39.5%

(n = 48, data from nests that were tested only once plus results of first trials from repeatedly tested nests). This rate did not differ statistically from the rejection rate in 2007–2008 (21.7%, n = 23, χ^2 = 2.04, df = 1, p = 0.15). All results below refer only to repeatedly tested nests (in 2009).

In the first trials, 45.5% of females (n = 22) ejected the model egg. In the second trials, 50.0% of females ejected the egg in the same sample of nests. Three females changed their response to the repeat trials (one from ejection to acceptance and two from acceptance to ejection) and all other females, both acceptors (n = 10) and rejecters (n = 9), responded identically between the first and the second trial. Repeatability for the binary data showed high consistency (Spearman's correlation: $r_s = 0.73$, exact 95% CI = 0.45–1.00, n = 22, p = 0.002).

The most parsimonious model included, additional to nest identity, also a covariate of laying date (Table 1, Fig. 2). Early in the season more individual rejected our experimental blue eggs, whereas later in the season acceptance was more prevalent (effect size of laying date: intercept = 4.15 ± 1.95, slope = −0.09 ± 0.04; CIs for slope: −0.17 to −0.01). Latencies were similar in the second compared with the first trial (Fig. 3, Wilcoxon matched pairs test: $Z = -3.00$, n = 8, p = 0.25).

In the first trials, the probability of egg ejection did not covary with the nest age (logistic regression: χ^2 = 1.45, df = 1,20, p = 0.23). In contrast, in the second trials, the egg ejection probability decreased with nest age (logistic regression: χ^2 = 5.48, df = 1,20, p = 0.02, intercept = −4.60 ± 2.44, slope = −0.56 ± 0.28).

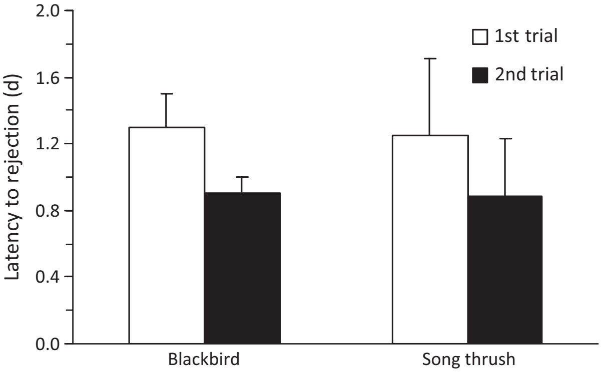

Fig. 3: Latency to rejection ($\bar{x}$ + SE) by blackbirds (n = 32 paired experiments) and song thrushes (n = 8 paired experiments) to experimental parasitism with non-mimetic blue model eggs. Data taken only from rejecters' nests where individuals rejected both in the first and second trial (i.e. nests where there was a non-consistent response to models eggs between the first and the second trial were excluded, see Methods for details).

Latency to ejection decreased with nest age in the first trials ($F_{1,6}$ = 7.99, p = 0.03, latency to ejection = 1.24–0.16*nest age). In the second trials, latencies to ejection did not correlate with nest age ($F_{1,6}$ = 0.42, p = 0.54). Individual repeatability of the latency in egg ejection between trials was r = 0.22 (CI = −0.53–0.77), when controlling for nest age effect.

Responses of song thrush to mimetic conspecific egg models

All song thrush in the focal study season (2008) accepted conspecific models in the first trials (n = 15). We successfully completed the second set of trials within the same breeding attempt for nine song thrushes. In all cases, song thrushes accepted models in both trials. Non-parametric correlation (Spearman's) on the binary data could not be calculated because of the absence of variation in the data. GLMM repeatability for the binary data (see Methods) was, as expected because of the absence of variation in the data, low and CIs included zero (Fig. 2).

Can different exposure of acceptor and rejecter nests bias repeatability estimates?

Importantly, despite the statistical similarity of the data across study years, some of the individual subjects' rejection rates of model eggs detected at repeatedly tested nests might still be inflated in comparison with the overall population rate of that season. This is because repeatedly tested acceptor nests had much longer exposure to potential predation (13 d) than singly tested acceptor nests (6 d) or repeatedly tested rejecters (mostly 3 d, see also latencies to rejection in Fig. 2) (see also Vikan et al. 2010). As predated nests were excluded from our analyses, this methodological constraint could have inflated the apparent rejection rates and possibly also repeatabilities for responses to blue model egg (all rejecter nests survived at least 13 d in conspecific egg treatments). Therefore, we repeated the aforesaid analyses but excluded all rejecter nests that did not survive at least 13 d from the introduction of the first egg model (i.e. from the start of the first trial).

In the first trials, blackbirds ejected the model egg at 81.0% nests (n = 37). In the second trials, 75.7% of females ejected the egg in the same sample of nests (Spearman's correlation: $r_s = 0.85$, exact 95% CI = 0.67–1.00, n = 37, p < 0.0001; GLMM: Fig. 2).

In the first trials, song thrush ejected the model egg at 38.9% nests (n = 18). In the second trials, 38.9% of females ejected the egg in the same sample of nests (Spearman's correlation: $r_s = 0.76$, exact 95% CI = 0.46–1.00, n = 18, p = 0.002; GLMM: Fig. 2). These corrected estimates are statistically identical (see CIs) to those not corrected for different periods of exposure and survival of acceptor or rejecter nests. Furthermore, the results of latencies to ejection remained statistically the same for the blackbirds (shorter for the second trials; paired t-test: $t_{27} = 2.46$, p = 0.02) and the song thrush (similar between first and second trials; Wilcoxon matched pairs test: $Z = 3.00$, n = 5, p = 0.35).

Discussion

Our experiments demonstrate very high levels of individual repeatability of blackbird and song thrush responses towards non-mimetic (both hosts) and mimetic (blackbirds) parasitic eggs. Specifically, 95% blackbirds and 86% song thrush responded identically to parasitic non-mimetic blue eggs (where we reached sufficient sample sizes) in two trials within the same breeding attempt. As documented in only one other species tested thus far (the blackcap, Honza et al. 2007b), in the blackbird, too, the latency to respond to model eggs was shorter between the first and second trials at the same nests.

Individual (in)consistency

Despite high individual repeatability of rejection of non-mimetic blue egg models, 5% blackbirds (n = 41) and 14% song thrush (n = 22) changed their behaviour from rejection to acceptance or *vice versa*. This is unlikely to be explained by altered host experiences (Rodríguez-Gironés & Lotem 1999), female age (Lotem et al. 1995), or light conditions at the nest (Langmore et al. 2005) because individuals experienced only few days of a short time-window between successive trials at the same nest with the same structure of vegetation cover.

Probability of egg rejection depends on a contrast of the parasite egg against host own eggs (e.g. in *Turdus* spp.: Cassey et al. 2008). Such variation of own egg colours, relative to the standardised paint of model eggs, is an inherent feature of any biological system; thus, it cannot be avoided in any experimental study of egg discrimination. However, this relative variation in contrast would also reflect natural variation in similarity between any parasite and any host eggs (see Fig. 3 in Antonov et al. 2010) and must be faced by both the parasites and the hosts, as well as the experimenters. Nevertheless, variation in similarity is not critical for our conclusions as far as host population is sampled randomly and, thus, reflects natural variation in the study system. Therefore, because our approach measured response behaviours within individuals, whose eggs themselves are less likely to vary within individuals than between individuals (Cassey et al. 2009), the resulting tests of repeatability remain valid. Additionally, this confounding effect was minimised in the present study because (1) host eggs remained identical between two within-breeding attempt trials, and (2) model non-mimetic eggs were painted simply (Fig. 2) and thus had highly standardised appearance. Hosts may commit more errors when cues to discriminate are too similar (Reeve 1989), but non-mimetic eggs we used (i.e. small plain blue cuckoo-egg-sized models) are perceivable for discrimination by our subject host species, both theoretically (Cassey et al. 2008) and empirically (Grim et al. 2011; this study). Still, Peer & Rothstein (2010) showed very low egg rejection repeatability in common grackles using a non-mimetic egg model in two trials within one breeding attempt (i.e. same design as our study).

Our findings have important implications for those theoretical models of parasite-host coevolution which assume that individuals are fixed in their responses once the foreign egg is perceived to reach an acceptance threshold (e.g. Takasu 1998; Servedio & Hauber 2006) or may change behaviour only when the parasitic egg mimicry is very high (Rodríguez-Gironés & Lotem 1999; Hauber et al. 2006). In turn, although the rates of inconsistency may not seem very large, our finding of 5% and 14% of host individuals changing behaviours between parasitism attempts could potentially have a very dramatic effect on model predictions, because of the inherent non-linearities in the system (G. D. Ruxton, pers. comm.). Our findings provide impetus for more modelling work to address rigorously the question how sensitive the various theoretical models of parasite–host co-evolution are with respect to their implicit and explicit assumptions of consistency and heritability. We provide novel empirical insights for future modelling efforts as the tested individuals in our study and those of Peer & Rothstein (2010) changed their behaviour even when (1) the parasite eggs were non-mimetic (i.e. parasite vs. own egg contrasts in size and colour were high across tested nests), (2) effects of altered experience were minimised, and (3) host clutch appearance remained identical.

We also detected some covariation with breeding stage on egg rejection probability in both blackbirds (only in first trials) and song thrush (only in second trials), despite previous work having showed that neither blackbirds nor song thrush differed in their egg-rejection responses between nesting stages (Davies & Brooke 1989 p. 212; Polačiková & Grim 2010 p. 114). Still, our analyses have shown that this had no statistical effect on estimates of repeatability: the model term nest age was statistically non-significant in all cases and was removed from all models (see Results).

Latency to ejection

Latency to ejection showed moderate repeatability in blackbirds but was low and not significantly different from zero in song thrush. That egg-ejector blackbirds responded faster during their second trials compared with the first ones may seem surprising. At the fitness level, late-laid and, thus, late-hatched interspecific parasites cannot eliminate host offspring (Moskát & Hauber 2007; Grim et al. 2009b), and late-hatched interspecific and conspecific parasites cannot successfully compete with host young (Hauber 2003; Hauber & Moskát 2008; Grim et al. 2009a; Moskát and Hauber 2010; Grim et al. 2011). However, even late parasitism may be costly because of increased costs of incubation and/or reduced hatching success (Siikamäki 1995).

At proximate level, the shifts in the latency of rejection in our results may reflect some combination of more extensive or accurate learning of a females' own eggs during incubation (Lotem et al. 1995; Stokke et al. 2007; Moskát et al. 2010) and/or the shifting of a discrimination threshold because of birds being alerted to a higher risk of parasitism by repeated exposure to foreign eggs, as suggested by both theoretical models (Reeve 1989; Rodríguez-Gironés & Lotem 1999) and experimental data (Davies et al. 1996; Hauber et al. 2006).

Importance on non-zero between-individual variation

We found that blue egg model rejection rates for the blackbird were at the upper limit, whereas those for song thrush were at the lower limit of standard definition of 'intermediate rejection' rates (i.e. 40–80%; Røskaft et al. 2002). Still, we stress that the definition of intermediate rejection should be fluid (E. Røskaft, pers. comm.) and what critically matters is that there is non-zero inter-individual variation in host behaviour (see Bell et al. 2009). This condition was fulfilled in the present study in responses to both non-mimetic models in the blackbirds and song thrush and to mimetic models in the blackbird, but not in the song thrush (although sample sizes were small for the last comparison, see Results).

In contrast, responses to mimetic models in the song thrush demonstrate how no variation in host behaviour affects repeatability estimation: the repeatability measures calculated by GLMM were low and statistically not significantly different from zero (Fig. 2). This inevitably follows from definition of repeatability: 'the proportion of phenotypic variation that can be attributed to between-subject (or between-group) variation' (Nakagawa & Schielzeth 2010, p. 935). Although host responses were seemingly highly consistent (all model eggs accepted in both first and second trials), there was no 'between-subject' variation and repeatability was bound to be statistically equivalent to zero, by definition.

Similarly to some of our data, Honza et al. (2007b) reported that all individuals whose responses were observed in both the first and second trials ejected parasitic eggs. These authors concluded that '[r]epeatability for host responses within the nest was very high' (p. 344). However, there was no 'between-subject' variation in host behaviour in that study. We recalculated repeatability for data from Honza et al. (2007b) and, as expected, repeatability was low and not significantly different from zero ($r = 0.28$; CI = −0.20–0.65; n = 19). This may have been caused by finding that 'one pair member may pre-empt the other's rejection response' (Honza et al. 2007b, p. 349). Accordingly, at some nests, one member of the pair ejected the first parasite model egg, whereas the second member of the pair ejected the second model egg. Although such data may be informative about some issues (e.g. 'do rejecters show assortative mating with rejecters?'), they cannot be used to estimate repeatability of individual behaviour (Bell et al. 2009). After excluding such data (five nests) from data set presented in Honza et al. (2007b), only individuals that ejected in both trials remained in the analysable data set, leading to zero repeatability estimate earlier.

Can differential predation risk at acceptor vs. rejecter nests inflate rejection rates?

Egg-rejection estimates in virtually all studies of brood parasitism may be inflated if there were uneven risks of predation at acceptor vs. rejecter nests (see also Vikan et al. 2010). For example, in many studies, by definition, an acceptor nest must survive at least 6 d to be included in analyses (Davies & Brooke 1989; Moksnes et al. 1991), whereas rejecter nests may be included in analyses after much shorter exposure to potential predation. For example, in the present study, most model eggs were rejected after 1 or 2 d (Fig. 1). Therefore, we reanalysed our data sets including only nests that survived the maximum exposure period of 13 d.

The reanalyses produced the same conclusions and the resulting new parameter estimates (repeatabilities) were statistically identical (based on 95% CIs) to uncorrected estimates. Therefore, biases resulting from differential survival of acceptor and rejecter nests were not detectable in this study. However, this does not exclude the possibility that in other data sets and studies such biases may in fact affect parameter estimates and conclusions. We recommend that future reanalyses and new studies of brood parasitism should explicitly test whether egg-rejection rates are not biased because of varying predation risks at acceptor vs. rejecter nests.

Methodological suggestions for future work

Future studies of individual repeatability of egg-rejection behaviours should focus on host species where (1) solely one sex incubates (and presumably the same sex rejects parasitic eggs), (2) egg-rejection rates are variable between individuals, and (3) egg rejection is by ejection (i.e. deserters cannot be used for estimations of within one breeding attempt repeatability). Also, (4) such studies should use models that are known to (or can be expected to) elicit intermediate rejection rates, and, (5) repeatability studies should use appropriate state of the art statistical tools that can control for possible confounding variables, i.e. GLMM (for details and examples, see Bates et al. 2008; Bolker et al. 2009; Weidinger & Kočvara 2010). Using highly mimetic model eggs leads to a potential outcome that all such models will be accepted, as we have shown with conspecific models in song thrush here, and under such circumstances, estimating repeatability may be unfeasible (Bell et al. 2009).

In any ecological study, authors should strive for representative sample sizes (Taborsky 2010). Although our samples for the mimetic conspecific models were small, we present even these data and the resulting analyses because not presenting collected data as part of a comprehensive experiment increases publication biases. Even small samples should be published because these could be later used in meta-analyses (weighted by sample size) (Johnson 2002; Nakagawa & Hauber 2011).

We used artificial model eggs whose colours can be perceived differently from natural eggs by the avian sensory system (Cassey et al. 2008). However, we did not specifically investigate whether and how individual birds perceived the eggs in our study. This is because we presented them with two identical stimuli which is the most important prerequisite for reliable estimates of repeatability (Martin & Bateson 2008).

Overall, our data imply that egg rejection decisions are highly consistent for individual female blackbirds and song thrush within the same breeding attempt. It remains to be seen in future studies whether, and how, consistent egg rejection decisions are across breeding attempts of a single individual and across generations of acceptor or rejecter females (Hauber et al. 2004; Hoover & Hauber 2007). The implications of these results are that a critical assumption of the evolvability of egg-rejection behaviours has been roughly met in both *Turdus* species. Also, contrary to assumptions of theoretical models, we confirmed that some individuals are flexible in egg-rejection behaviours even in the short time-window of one breeding attempt and may change their responses between subsequent exposures to brood parasitism. Thus, these extensive experimental data provide empirical impetus to assess rigorously how sensitive various theoretical models of parasite-host co-evolution are to their assumptions.

Acknowledgements

Experiments were carried out with permission from local authorities and institutional animal ethics committees in New Zealand. We are grateful to Michael Anderson, Alena Dvorská, Branislav Igic, Stefanie Ismar, Lenka Polačiková, Lucia Turčoková, and Zuzana Strachoňová for their help in the field and the School of Biological Sciences, University of Auckland, for logistic support. The study was supported by a Human Frontier Science Program award (to TG, PC, and MEH), the student project 2010/027 fund of Palacky University (to PS and TG), the PSC-CUNY fund (to MEH), and MSM6198959212 (to TG).

Literature Cited

Alvarez, F. 1996: Model cuckoo *Cuculus canorus* eggs accepted by rufous bush chats *Cercotrichas galactotes* during the parasite's absence from the breeding area. Ibis **138**, 340—342.

Amundsen, T., Bobakken, B. T., Moksnes, A. & Røskaft, E. 2002: Rejection of common cuckoo *Cuculus canorus* eggs in relation to female age in the bluethroat *Luscinia svecica*. J. Avian Biol. **33**, 366—370.

Antonov, A., Stokke, B. G., Vikan, J. R., Fossøy, F., Ranke, P. S., Røskaft, E., Moksnes, A., Møller, A. P. & Shykoff, J. A. 2010: Egg phenotype differentiation in sympatric cuckoo *Cuculus canorus* gentes. J. Evol. Biol. **23**, 1170—1182.

Bates, D., Maechler, M. & Dai, B. 2008: lme4:linear mixed-effects models using S4 classes. R package version 0.999375-28. [Online.] Available at http://lme4.r-forge.r-project.org/.

Bell, A. M., Hankison, S. J. & Laskowski, K. L. 2009: The repeatability of behaviour: a meta-analysis. Anim. Behav. **77**, 771—783.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H. & White, J. S. S. 2009: Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. **24**, 127—135.

Burnham, K. P. & Anderson, D. R. 2002: Model Selection and Multimodel Inference: a Practical Information-Theoretic Approach. Springer Verlag, New York.

Cassey, P., Honza, M., Grim, T. & Hauber, M. E. 2008: The modelling of avian visual perception predicts behavioural rejection responses to variable egg colours. Biol. Lett. **4**, 515—517.

Cassey, P., Ewen, J. G., Marshall, N. J., Vorobyev, M., Blackburn, T. M. & Hauber, M. E. 2009: Are avian eggshell colours effective intraspecific communication signals in the Muscicapoidea? A perceptual modelling approach. Ibis **151**, 689—698.

Cramp, S. 1985: The Birds of the Western Palearctic. Volume IV (Terns to Woodpeckers). Oxford Univ. Press, Oxford, UK.

Cramp, S. 1988: The Birds of the Western Palearctic. Volume V (Tyrant Flycatchers to Thrushes). Oxford Univ. Press, Oxford, UK.

Cytel Inc. 2005: StatXact 7. Statistical Software for Exact Nonparametric Inference. Cytel Inc., Cambridge, USA.

Davies, N. B. 2000: Cuckoos, Cowbirds and Other Cheats. T & AD Poyser, London, UK.

Davies, N. B. & Brooke, M. L. 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.

Davies, N. B., Brooke, M. L. & Kacelnik, A. 1996: Recognition errors and probability of parasitism determine whether reed warblers should accept or reject mimetic cuckoo eggs. Proc. R. Soc. B **263**, 925—931.

Gibbs, H. L., Sorenson, M. D., Marchetti, K., Brooke, M. L., Davies, N. B. & Nakamura, H. 2000: Genetic evidence for female host-specific races of the common cuckoo. Nature **407**, 183—186.

Grim, T. 2005: Mimicry vs. similarity: which resemblances between brood parasites and their hosts are mimetic and which are not? Biol. J. Lin. Soc. **84**, 69—78.

Grim, T. & Honza, M. 2001: Differences in behaviour of closely related thrushes (*Turdus philomelos* and *T. merula*) to experimental parasitism by the common cuckoo *Cuculus canorus*. Biologia **56**, 549—556.

Grim, T., Rutila, J., Cassey, P. & Hauber, M. E. 2009a: Experimentally constrained virulence is costly for common cuckoo chicks. Ethology **115**, 14—22.

Grim, T., Rutila, J., Cassey, P. & Hauber, M. E. 2009b: The cost of virulence: an experimental study of egg eviction by brood parasitic chicks. Behav. Ecol. **20**, 1138—1146.

Grim, T., Samaš, P., Moskát, C., Kleven, O., Honza, M., Moksnes, A., Røskaft, E. & Stokke, B. G. 2011: Constraints on host choice: why do parasitic birds rarely exploit some common potential hosts? J. Anim. Ecol. **80**, 508—518.

Guo, G. & Zhao, H. 2000: Multilevel modelling for binary data. Annu. Rev. Sociol. **26**, 441—462.

Hale, K. & Briskie, J. V. 2007: Response of introduced European birds in New Zealand to experimental brood parasitism. J. Avian Biol. **38**, 198—204.

Hauber, M. E. 2003: Interspecific brood parasitism and the evolution of host clutch sizes. Evol. Ecol. Res. **5**, 559—570.

Hauber, M. E. & 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, M. E., Yeh, P. J. & Roberts, J. O. L. 2004: Patterns and coevolutionary consequences of repeated brood parasitism. Proc. R. Soc. B **271**, S317—S320.

Hauber, M. E., Moskát, C. & Bán, M. 2006: Experimental shift in hosts' acceptance threshold of inaccurate-mimic brood parasite eggs. Biol. Lett. **2**, 177—180.

Higgins, P. J. 2006: Handbook of Australian, New Zealand and Antarctic Birds. Vol. 7B (Boatbill to Starlings). Oxford Univ. Press, Melbourne, Australia.

Honza, M., Polačiková, L. & Procházka, P. 2007a: Ultraviolet and green parts of the colour spectrum affect egg rejection in the song thrush (*Turdus philomelos*). Biol. J. Linn. Soc. **92**, 269—276.

Honza, M., Požgayová, M., Procházka, P. & Tkadlec, M. 2007b: Consistency in egg rejection behaviour: responses to repeated brood parasitism in the blackcap (*Sylvia atricapilla*). Ethology **113**, 344—351.

Hoover, J. P. & Hauber, M. E. 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, J. P., Yasukawa, K. & Hauber, M. E. 2006: Spatially and temporally structured avian brood parasitism affects the fitness benefits of hosts' rejection strategies. Anim. Behav. **72**, 881—890.

Jackson, W. M. 1998: Egg discrimination and egg-color variability in the northern masked weaver: the importance of conspecific versus interspecific parasitism. In: Parasitic Birds and Their Hosts (Rothstein, S. I. & Robinson, S. K., eds). Oxford Univ. Press, New York, pp. 407—416.

Johnson, D. H. 2002: The importance of replication in wildlife research. J. Wildl. Manag. **66**, 919—932.

Langmore, N. E., Kilner, R. M., Butchart, S. H. M., Maurer, G., Davies, N. B., Cockburn, A., Macgregor, N. A., Peters, A., Magrath, M. J. L. & Dowling, D. K. 2005: The evolution of egg rejection by cuckoo hosts in Australia and Europe. Behav. Ecol. **16**, 686—692.

Lawes, M. J. & Marthews, T. R. 2003: When will rejection of parasite nestlings by hosts on non-evicting avian brood parasites be favored? A misimprinting-equilibrium model. Behav. Ecol. **14**, 757—770.

Lessells, C. M. & Boag, P. T. 1987: Unrepeatable repeatabilities: a common mistake. Auk **104**, 116—121.

López-de-Hierro, M. D. G. & Moreno-Rueda, G. 2010: Egg-spot pattern rather than egg colour affects conspecific egg rejection in the house sparrow (*Passer domesticus*). Behav. Ecol. Sociobiol. **64**, 317—324.

Lotem, A. 1993: Learning to recognize nestlings is maladaptive for cuckoo *Cuculus canorus* hosts. Nature **362**, 743—745.

Lotem, A., Nakamura, H. & Zahavi, A. 1995: Constraints on egg discrimination and cuckoo-host co-evolution. Anim. Behav. **49**, 1185—1209.

Lyon, B. E. 2003: Egg recognition and counting reduce costs of avian conspecific brood parasitism. Nature **422**, 495—499.

Martin, P. & Bateson, P. 2008: Measuring Behaviour. Cambridge Univ. Press, Cambridge, UK.

May, R. M., Nee, S. & Watts, C. 1991: Could intraspecific brood parasitism cause population cycles? Proc. Int. Ornithol. Congr. **20**, 1012—1022.

McGraw, K. O. & Wong, S. P. 1996: Forming inferences about some intraclass correlation coefficients. Psychol. Methods **1**, 30—46.

Moksnes, A. & Røskaft, E. 1995: Egg-morphs and host preference 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, A. T., Korsnes, L., Lampe, H. M. & Pedersen, H. C. 1991: Behavioural responses of potential hosts towards artificial cuckoo eggs and dummies. Behaviour **116**, 64—89.

Moskát, C. & Hauber, M. E. 2007: Conflict between egg recognition and egg rejection decisions in common cuckoo (*Cuculus canorus*) hosts. Anim. Cogn. **10**, 377—386.

Moskát, C. & Hauber, M. E. 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., Karcza, Z. & Csörgö, T. 2003: Egg rejection in European blackbirds (*Turdus merula*): the effect of mimicry. Ornis Fennica **80**, 86—91.

Moskát, C., Bán, M., Székely, T., Komdeur, T., Lucassen, R. W. G., van Boheemen, L. & Hauber, M. E. 2010: Discordancy or learning? Dissecting the cognitive basis of the recognition of foreign eggs in hosts of avian brood parasites. J. Exp. Biol. **213**, 1976—1983.

Nakagawa, S. & Hauber, M. E. 2011: Great challenges with few subjects: Statistical strategies for neuroscientists. Neurosci. Biobehav. Rev. **35**, 462—473.

Nakagawa, S. & Schielzeth, H. 2010: Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol. Rev. **85**, 935—956.

Nee, S. & May, R. M. 1993: Population-level consequences of conspecific brood parasitism in birds and insects. J. Theor. Biol. **161**, 95—109.

Palomino, J. J., Martín-Vivaldi, M., Soler, M. & Soler, J. J. 1998: Females are responsible for ejection of cuckoo eggs in the rufous bush robin. Anim. Behav. **56**, 131—136.

Peer, B. D. & Rothstein, S. I. 2010: Phenotypic plasticity in common grackles (*Quiscalus quiscula*) in response to repeated brood parasitism. Auk **127**, 293—299.

Polačiková, L. & Grim, T. 2010: Blunt egg pole holds cues for alien egg discrimination: experimental evidence. J. Avian Biol. **41**, 111—116.

Polačiková, L., Stokke, B. G., Procházka, P., Honza, M., Moksnes, A. & Røskaft, E. 2010: The role of blunt egg pole characteristics for recognition of eggs in the song thrush (*Turdus philomelos*). Behaviour **147**, 465—478.

R Development Core Team. 2010: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [Online] Available at: http://www.R-project.org.

Reeve, H. K. 1989: The evolution of conspecific acceptance thresholds. Am. Nat. **133**, 407—435.

Riehl, C. 2010: A simple rule reduces costs of extragroup parasitism in a communally breeding bird. Curr. Biol. **20**, 1830—1833.

Rodríguez-Gironés, M. A. & 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., Meilvang, D., Bičík, V., Jemelíková, J. & Honza, M. 2002: No evidence for recognition errors in *Acrocephalus* warblers. J. Avian Biol. **33**, 31—38.

Ruxton, G. D. & Broom, M. 2002: Intraspecific brood parasitism can increase the number of eggs that an individual lays in its own nest. Proc. R. Soc. B **269**, 1989—1992.

Ruxton, G. D., Broom, M. & Colegrave, N. 2001: Are unusually colored eggs a signal to potential conspecific brood parasites? Am. Nat. **157**, 451—458.

Servedio, M. R. & Hauber, M. E. 2006: To eject or to abandon? Life history traits of hosts and parasites interact to influence the fitness payoffs of alternative anti-parasite strategies. J. Evolution. Biol. **19**, 1585—1594.

Servedio, M. R. & Lande, R. 2003: Coevolution of an avian host and its parasitic cuckoo. Evolution **57**, 1164—1175.

Shaw, R. C. & Hauber, M. E. 2009: Experimental support for the role of nest predation in the evolution of brood parasitism. J. Evolution. Biol. **22**, 1354—1358.

Shizuka, D. & Lyon, B. E. 2010: Coots use hatch order to learn to recognize and reject conspecific brood parasitic chicks. Nature **463**, 223—226.

Siikamäki, P. 1995: Are large clutches costly to incubate – the case of pied flycatcher. J. Avian Biol. **26**, 76—80.

Soler, M., Palomino, J. J., Martín-Vivaldi, M. & Soler, J. J. 2000: Lack of consistency in the response of rufous-tailed scrub robins *Cercotrichas galactotes*. Ibis **142**, 151—154.

Soler, M., Martín-Vivaldi, M. & Perez-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.

Stokke, B. G., 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.

Taborsky, M. 2010: Sample size in the study of behaviour. Ethology **116**, 185—202.

Takasu, F. 1998: Why do all host species not show defense against avian brood parasitism: evolutionary lag or equilibrium? Am. Nat. **151**, 193—205.

Takasu, F. 2004: How many eggs should be laid in one's own nest and others' in intra-specific brood parasitism? Popul. Ecol. **46**, 221—229.

Thomson, G. M. 1922: The Naturalisation of Plants and Animals in New Zealand. Cambridge Univ. Press, Cambridge, UK.

Vikan, J. R., Stokke, B. G., Fossøy, F., Jackson, C., Huhta, E., Rutila, J., Moksnes, A. & Røskaft, E. 2009: Fixed rejection responses to single and multiple experimental parasitism in two *Fringilla* hosts of the common cuckoo. Ethology **115**, 840—850.

Vikan, J. R., Stokke, B. G., Rutila, J., Huhta, E., Moksnes, A. & Røskaft, E. 2010: Evolution of defences against cuckoo (*Cuculus canorus*) parasitism in bramblings (*Fringilla montifringilla*): a comparison of four populations in Fennoscandia. Evol. Ecol. **24**, 1141—1157.

Weidinger, K. & Kočvara, R. 2010: Repeatability of nest predation in passerines depends on predator species and time scale. Oikos **119**, 138—146.

Yamauchi, A. 1993: Theory of intraspecific nest parasitism in birds. Anim. Behav. **46**, 335—345.

Yamauchi, A. 1995: Theory of evolution of nest parasitism in birds. Am. Nat. **145**, 434—456.