Testing for correlations between behaviours in a cuckoo host: why do host defences not covary?

Alfréd Trnka [a,*], Tomáš Grim [b]

[a] Department of Biology, University of Trnava, Trnava, Slovakia
[b] Department of Zoology and Laboratory of Ornithology, Palacký University, Olomouc, Czech Republic

Fitness costs associated with brood parasitism have led host species to evolve several lines of defence. The first two lines of defence, aggression against adult parasites and egg rejection, are present at varying levels in almost all hosts. However, it remains unclear how these two fundamental defences covary at host individual level, with previous studies suggesting both positive and negative correlations. A theoretically critical yet empirically untested scenario is that variation in host antiparasite behaviour may relate to individual variation in host behavioural types or personalities. Here we examined whether host aggression against adult brood parasites and egg rejection behaviour were correlated with host behaviours displayed outside the context of brood parasitism. We selected the great reed warbler, *Acrocephalus arundinaceus*, a favourite cuckoo, *Cuculus canorus*, host as a suitable model. Only females reject foreign eggs and show high individual repeatability of both aggression towards cuckoos and nest guarding. We found that female behaviours in different situations (nest guarding, nest defence, handling in the net) were strongly correlated with each other. This is the first empirical evidence on correlation between individually consistent antiparasite adaptation (female nest defence) and behaviours that are not directly related to brood parasitism. In contrast, egg rejection/acceptance responses and latency to these responses did not correlate with any of the female defence/guarding behaviours and behaviour during handling. Proximately, this may be because nest defence and egg recognition represent cognitively and behaviourally completely different tasks. These patterns were not affected by female mating status in this polygynous cuckoo host. We hypothesize that differences in host behavioural types, rather than host egg discrimination ability, may predict host nest defence behaviour against adult brood parasites in general.

© 2014 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Interspecific avian brood parasitism, a peculiar breeding strategy in which parasitic females hand over their progeny to the care of fosterers, represents an important factor that negatively affects host Darwinian fitness (Davies, 2000). To reduce the costs of parasitism, hosts have evolved multiple defensive mechanisms against brood parasite adults, eggs and nestlings (e.g. Grim et al., 2011; Langmore, Cockburn, Russell, & Kilner, 2009; Stokke, Moksnes, & Røskaft, 2002). The most widespread are the first two lines of defence: aggression against adult parasites near the host nest and egg rejection (Davies, 2000). Little is known about how these two fundamental defences covary at the host individual level, and early studies showed mixed results. A positive correlation between host aggression and egg rejection within a species has been found in American yellow warblers, *Setophaga petechia*, parasitized by brown-headed cowbirds, *Molothrus ater* (Guigueno & Sealy, 2011). High-quality host individuals may show higher-quality defences overall, being both bold and more likely to reject parasite eggs (Guigueno & Sealy, 2011). However, Soler, Soler, Pérez-Contreras, Aragón, and Møller (1999) found the opposite pattern in magpies, *Pica pica*, parasitized by great spotted cuckoos, *Clamator glandarius*, and suggested that hosts may modulate their antiparasite behaviour by using one of these two defence tactics based on their associated costs and benefits.

A recent review (Avilés & Parejo, 2011) suggested yet another evolutionary way, based on personality theory, in which host defences may correlate with one another. Accordingly, expression of individual host antiparasite behaviours may relate to individual variation in host behavioural types or personalities (i.e. consistent individual differences in behaviour; Réale, Reader, Sol, McDougall, & Dingemanse, 2007; Sih, Bell, & Johnson, 2004). Behavioural type could influence host defences in multiple ways. Bold

* Correspondence: A. Trnka, Department of Biology, University of Trnava, Priemyselná 4, SK-918 43 Trnava, Slovakia.
E-mail address: atrnka@truni.sk (A. Trnka).

individuals, for example, may be more effective than shy individuals at driving brood-parasitic females away from the nest because of higher levels of their general antipredator aggression (Hollander, Van-Overveld, Tokka, & Matthysen, 2008). On the other hand, given that successful previous lines of host defence may logically decrease positive selection pressure on later lines of defence (Britton, Planqué, & Franks, 2007; Grim, 2006, 2011), bold individuals that are good at keeping brood parasites away may be less efficient at discriminating and rejecting parasitic eggs (Avilés & Parejo, 2011). However, since shy individuals may be less frequently parasitized, for example because of less active and thus less conspicuous behaviour (Banks & Martin, 2001; Gill, Grieef, Staib, & Sealy, 1997), than bold individuals, they may also experience less selection to be good egg discriminators. Finally, given that these two host defence behaviours are based on learning mechanisms (Lotem, Nakamura, & Zahavi, 1995; Thorogood & Davies, 2012), we may also predict a positive relationship between nest defence and egg rejection in hosts with high learning capacities (Avilés & Parejo, 2011). Thus, good learners would better defend their nest and better recognize cuckoo eggs. Therefore, broadening the scope of brood parasitism studies and including host individual differences in behaviour across more situations and contexts, that is, both within and outside the context of brood parasite−host interactions (Réale et al., 2007; Sih et al., 2004) may allow researchers to test whether host behavioural traits (e.g. shyness−boldness) may influence host-specific antiparasite defences (Avilés & Parejo, 2011; Guigueno & Sealy, 2011). Given that individuals expressing a particular behavioural type behave in a consistent way through time and across situations or contexts (Sih & Bell, 2008), studying correlation between host behaviours is a necessary step in testing this hypothesis (Avilés & Parejo, 2011).

Virtually no studies have investigated whether host aggression against adult brood parasites and egg rejection behaviour (i.e. behaviours used by hosts in the context of brood parasitism) covary with other behaviours that are not directly or exclusively related to brood parasitism. We therefore examined the relationship between host-specific antiparasite defences (i.e. nest defence against cuckoos, egg rejection decisions and latency to these decisions), host aggression during handling in a mist net (i.e. behaviour outside the context of brood parasitism) and nest guarding (measured as latency to arrival at the nest), that is, behaviour displayed both within and outside the context of brood parasitism (note that the primary function of nest guarding is antipredator and anticuckoldry, see Davies, Butchart, Burke, Chaline, & Stewart, 2003). These last two types of behaviour, together with host aggression against adult brood parasites, are also commonly accepted as belonging to the major behavioural axis 'shyness−boldness' (Réale et al., 2007; for discussion see Carter, Feeney, Marshall, Cowlishaw, & Heinsohn, 2013) because they involve different levels of predation risk (i.e. handling is more risky because the individual is already captured by a predator/observer whereas nest guarding is comparatively less risky because the individual is not in direct physical contact with the predator/observer).

We selected the great reed warbler, *Acrocephalus arundinaceus*, as a suitable model host species because it is one of the major cuckoo, *Cuculus canorus*, hosts in Europe (Leisler & Schulze-Hagen, 2011) and shows large interindividual variability in both aggression towards adult cuckoo females and responses to natural cuckoo eggs (Požgayová, Procházka, & Honza, 2013; Trnka, Požgayová, Procházka, Prokop, & Honza, 2012). Existence of such variability is an important prerequisite for any test of behavioural correlations (Sih et al., 2004). In this species, the female is solely responsible for recognition and ejection of cuckoo eggs (Požgayová, Procházka, & Honza, 2009) and she also plays a leading role in defending the nest against both predators (Trnka & Prokop, 2010) and parasites

(Trnka & Prokop, 2012; but see Požgayová et al., 2009), independently of male nest defence effort (Trnka & Grim, 2013b). Also, great reed warbler female (but not male) aggression towards cuckoos is highly repeatable (Trnka, Požgayová, Samaš, & Honza, 2013). This suggests that different females may belong to different behavioural types in relation to nest defence. Therefore, we focused on female behaviours.

Based on Avilés and Parejo (2011), we predicted that host female aggression towards cuckoos would correlate positively with her aggression during handling and also with her nest guarding. We further explored correlations between classical antiparasite behaviours (aggression towards cuckoos, egg rejection) and other behaviours expressed outside (handling aggression) and both outside and inside the context of brood parasitism (nest guarding) aiming to understand whether host antiparasite behaviours could function as carryover effects of a general behavioural tendency in great reed warblers.

METHODS

General Field Methods

The study was conducted in 2013 at a fish pond system near Štúrovo, southwestern Slovakia (47°51′N, 18°36′E, 115 m above sea level). The overall study area was ca. 45 ha. Great reed warblers breed at this site in narrow (approximately 5−10 m wide) strips of reed, *Phragmites australis*, that border the ponds. The whole study population was individually colour-ringed. All birds were either ringed in previous breeding seasons or mist-netted and colour-ringed shortly after their return from wintering grounds, during territory establishment (see Handling behaviour below). Great reed warblers breed only once per year; renesting in the same season was very rare (2.7%, Trnka, 2011). The great reed warbler has a polygynous mating system (Požgayová et al., 2013; Trnka, Batáry, & Prokop, 2010; Trnka & Prokop, 2010). In the long term, 21−43% of males are polygynous (Trnka et al., 2010). In the year of the study 33% of males ($N = 36$ males) were polygynous. Only the female builds the nest and incubates the clutch. However, both females and males feed and defend the young (Trnka & Grim, 2013b).

Each male was classified as either monogamous or polygynous (Trnka & Prokop, 2011). We considered simultaneous (i.e. not sequential) polygyny: two (or, rarely, three) nests attended by a particular male had to overlap in their laying, incubation and/or nestling period(s) to be classified as polygynous (following Trnka, Požgayová, et al., 2012). Each female mated to a polygynous male was classified as primary, secondary or tertiary based on the order of her egg laying relative to other females mated to the same male (Trnka, Požgayová, et al., 2012). Of 49 tested nests, 25 were monogamous. Of 24 polygynous nests, 10 were primary, 11 were secondary and three were tertiary. The larger sample size for secondary than primary females (see Results) was caused by predation of one primary nest before the experiments were finished (see also Trnka, Požgayová, et al., 2012).

The annual rate of natural cuckoo parasitism in the area (the number of nests in which cuckoo eggs were laid divided by the total number of nests found) ranged from 24% to 37% (Trnka et al., 2013; Trnka, Požgayová, et al., 2012). Grey adult cuckoo females were more common than rufous ones in our study area (60% versus 40%, Trnka & Grim, 2013a). For each great reed warbler female ($N = 49$) we quantified the following behavioural parameters.

Egg Rejection and Latency to Egg Rejection

In the year of study the parasitism rate was 48.5% ($N = 68$), including four nests with multiple parasitism (i.e. two cuckoo eggs

in one host clutch). Therefore, we included two types of parasitized nest. Naturally parasitized nests ($N = 26$) were those in which the parasitic egg was laid directly by a cuckoo female. We used standard procedures for quantification of natural parasitism rate: we checked the nests daily from the start of egg laying until clutch completion, and numbered each egg with a waterproof pen according to the laying sequence. Regular checks enabled us to determine the incidence of cuckoo parasitism reliably (see Trnka, Požgayová, et al., 2012). If the nest was not parasitized naturally before clutch completion, the nest was parasitized experimentally (by A.T.) by introducing a natural cuckoo egg ($N = 23$). Unincubated cuckoo eggs were collected from freshly abandoned ($N = 7$) or multiply parasitized great reed warbler nests ($N = 4$) from the study area and the rest of the experimental eggs were collected from abandoned and multiply parasitized warbler nests found outside the focal study area ($N = 12$). We did not use artificial models which may not match the natural eggs in all potentially relevant discrimination features; see spectral reflectances of natural great reed warbler eggs in Igic et al. (2012) and of model eggs in Samaš, Hauber, Cassey, and Grim (2011).

Nests were checked daily for the standard period of 6 days (Grim et al., 2011). Each host female was scored as 'rejecter' if the cuckoo egg disappeared but the clutch was incubated or 'acceptor' if the egg remained in the nest until the end of the 6-day period. Deserted nests ($N = 7$, not included in the above sample sizes overview) were excluded from the analyses because it is not possible to mist-net a female (see above) at the original nest after she has deserted that nest.

Nest Defence against the Brood Parasite

We tested female aggressive responses during nest defence against parasitic cuckoo dummies. Cuckoo females come in two strikingly different colour morphs (grey and rufous), and great reed warblers in our study population discriminate between them, being more aggressive towards the grey morph (Trnka & Grim, 2013a). Therefore we used three specimens of the grey cuckoo female morph that were randomly presented to nests.

Previously, we have shown that great reed warblers at our study site show fine-tuned ability to discriminate between the cuckoo and several different types of other intruder (Trnka & Grim, 2013a; Trnka & Prokop, 2012; Trnka, Prokop, & Grim, 2012). Importantly, in our study population, specific aggression against cuckoos (i.e. response to cuckoo minus response to innocuous control, see Grim, 2005) correlates strongly with overall aggression against the cuckoo ($r_s = 0.92$, $N = 17$, $P < 0.0001$, data from Trnka, Prokop, et al., 2012). As we were not interested in enemy recognition (Grim, 2005) but in a female's individual ability to defend her nest aggressively, we did not use a control dummy in the present study (see also Grim et al., 2011; Trnka & Grim, 2013b; Trnka & Prokop, 2010).

The dummy experiment was performed by one observer (A.T.). All experiments were consistently done in the earliest part of the egg-laying period, either on the day when the female laid her first egg ($N = 43$) or, in rare cases when it was not logistically possible (inclement weather), on the day when she laid the second egg ($N = 6$). All dummy experiments were also consistently done in the afternoon to reflect natural timing of cuckoo visits and laying at host nests (Moksnes et al., 2000).

We placed the stuffed cuckoo dummy 0.5 m from the focal nest, at the same height above water level as the nest, and facing the nest rim (e.g. Grim et al., 2011). Observations (by A.T.) were done from a blind (a small camouflaged shelter) placed ca. 5 m from the focal nest. Based on our experiences from previous field studies (Trnka et al., 2013; Trnka & Grim, 2013a, 2013b; Trnka, Požgayová, et al., 2012; Trnka, Prokop, et al., 2012), the presence of the shelter did not affect parental behaviour of nest owners. Behaviour of each focal bird (i.e. female and male separately) was recorded for 1 min from the first contact attack or for 5 min from its arrival to the nest when the bird did not make contact with the dummy (following Trnka et al., 2013). Host behaviour was quantified only as the number of contact attacks at the dummy, because this measure showed the highest level of individual variation (0–37, this study; see also Požgayová et al., 2013; Trnka et al., 2013; Trnka, Prokop, et al., 2012). Moreover, this behaviour, in contrast to for example alarm calls, is considered to be the most risky but effective way to drive the cuckoo out of the host nest (Janisch, 1948; Molnár, 1944; Trnka & Grim, 2013a).

Aggression during Handling

To assess female aggression during handling, the females were mist-netted (by A.T.). The behaviour of the bird when handled by humans is a generally accepted behavioural trait termed 'handling aggression' (Brommer & Kluen, 2012; Class, Kluen, & Brommer, 2014). Mist netting and handling represent a novel and more risky situation than the nest defence experiment for the captured females because of the direct physical contact with a deadly predator (i.e. a human; see also Trnka & Prokop, 2010).

Females were caught consistently on the sixth day of incubation (after the egg rejection experiment was finished); therefore, host female egg rejection could not be confounded by the nest defence experiment, and the potential confound of nest age (or breeding stage) was excluded. The observer monitored the mist net from a hide set 1–2 m from one end of the net. Immediately after the female hit the net the observer approached at a slow walking pace, carefully handled the female for 30 s in the net, and then removed her from the net. After ringing, weighing and measuring the bird, the observer released her back into her territory (e.g. Møller & Ibáñez-Álamo, 2012).

Of all mist-netted females, 33 were not yet ringed at the time of capture (i.e. never mist-netted by us) and thus probably naïve with respect to mist netting. The rest of the females were ringed in previous breeding seasons (10 females in 2012, six females in 2010–2011). However, individual female experience with mist netting and handling by humans (previously caught versus naïve) had no statistically detectable effect on the female's handling aggression rank ($\chi_3^2 = 4.04$, $P = 0.26$).

The female's behaviour fell into several natural categories on the fight–flight continuum (Eilam, 2005) based on the direction of her responses (away or towards the observer), and presence or absence of calls, threatening postures and pecking. Therefore, we used an ordinal scale (1–4; see also Class et al., 2014): 1 = escape behaviour, that is, the female did not produce alarm or warning calls and tried to escape silently away from the approaching observer; 2 = passive behaviour, that is, the female simply lay in the mist net shelf without any vocal or behavioural reaction; 3 = nonoffensive behaviour, that is, the female produced alarm calls and adopted a threatening posture (i.e. spread her wings towards the observer; see p. 533 in Glutz von Blotzheim & Bauer, 1991); 4 = aggressive behaviour, that is, the female produced warning calls and screams ('angry croaking') and pecked the observer's hands.

Nest Guarding

Nest guarding represents spatial activity centred around a nest which is a familiar space for nest owners. Similarly, nest guarding should be the least risky behaviour in comparison with nest defence behaviour and behaviour during handling in the mist net because birds do not expose themselves directly to potential

predators. However, nest guarding is costly because it reduces the time available for feeding and other activities such as attracting other mates for extrapair copulation. The primary function of nest guarding is antipredator and anticuckoldry (Davies et al., 2003) but it may help to detect brood parasites near the nest. Therefore, it could also be additionally selected in the context of brood parasitism.

We used 'latency to arrival' during the dummy experiment as a proxy to quantify nest guarding (Grim, 2005). The latency to arrival was measured from the moment when the observer (A.T.) retreated to the blind (after dummy placement) to the arrival of the nest owner(s) within 2 m of the focal nest. This is a sufficient distance for nest owners to see the dummy. Latency to arrival cannot in principle be part of nest defence behaviour(s) simply because there are no direct cues to nest owners that would reveal to them that an intruder is near the nest. Such cues would need to be acoustic because it is impossible to have visual cues from either a natural cuckoo (which does not call when visiting host nests; Wyllie, 1981) or a cuckoo dummy in very dense reed bed habitat. Indeed, in nest defence studies in general, latency to arrival varies when different dummy types are accompanied by playback (Gill, Neudorf, & Sealy, 1997) but does not vary when playbacks are not used (Duckworth, 1991; Grim, 2005; Honza, Grim, Čapek, Moksnes, & Røskaft, 2004).

Logically, the more intense nest guarding should result in earlier detection of any intruder near the host nest. Empirically, increasing nest guarding (proportion of time spent near the nest) is strongly correlated with decreasing latencies to arrival to the dummy (Davies et al., 2003). This validates the use of latency to arrival as a robust measure of nest guarding. The nest attendance is also individually repeatable (Davies et al., 2003; this study).

Statistical Analyses

To estimate repeatability (*r*) of nest guarding we included latency to arrival for females that were repeatedly tested during nest defence experiments in more than one field season. We used data from previous breeding seasons (2010–2012) when each female ($N = 23$) was tested twice. Repeatability was calculated from variance components (using JMP 8.0.1., SAS Institute Inc., Cary, NC, U.S.A.) with its accompanying 95% confidence intervals, CI (Hopkins, 2009).

Each focal female was subjected to only one experiment of each type (dummy, egg, handling); thus, we used simple general linear models (GLM) to analyse the data (i.e. female ID did not need to be included as a random effect). However, dummy specimen ID could, theoretically, affect host responses. Therefore, we also reanalysed the models (with response being either 'nest guarding' or 'nest defence', see below) with dummy ID as a random effect (categorical with three levels). Similarly to previous studies (e.g. Trnka & Grim, 2013a; Trnka, Prokop, et al., 2012; Welbergen & Davies, 2008) dummy ID did not explain any variation in female aggression (95% CIs widely overlapped zero). Thus, we excluded this redundant random effect (as recommended by Bolker et al., 2009). Furthermore, polygynous males took part in nest defence at their primary, secondary and, rarely, at tertiary nests. However, male ID as a random effect (alone or in combination with specimen ID) did not explain any variation in female aggression (95% CIs widely overlapped zero). Therefore, we excluded this potential confounder.

Other potentially relevant factors that could not be avoided in this study design were included as additional predictors: first egg-laying date (continuous), clutch size (continuous) as a surrogate of nest reproductive value and/or female quality (Campobello & Sealy, 2010), first attacking sex (categorical; male, female, both

at once) and female mating status (categorical: monogamous, primary, secondary, tertiary). All these predictors were directly related to female phenotype. We also included two male traits: male nest guarding (i.e. male latency to arrival) and male nest defence (i.e. male contact attacks). We excluded type of parasitism (natural or experimental) as a predictor because nest defence experiments were done before such parasitism took place (thus, there could be no causal link from parasitism status to nest defence in principle).

The main predictors of interest were egg response (categorical: acceptor, rejecter) and female handling aggression (ordinal: 1–4). Nest defence (number of contact attacks per min) was a continuous response. In a separate analysis we tested whether the same predictors explained variation in nest guarding (latency to arrival).

Before building these models we checked whether there was collinearity (assessed by the variance inflation factor, VIF) between predictors (following procedures recommended by Zuur, Ieno, & Elphick, 2010). We excluded predictors showing VIF larger than 2 (Zuur et al., 2010; see Table 1).

Following standard recommendations we checked the assumption of normality of residual errors, linearity of effect and homogeneity of variances by visual inspection (Grafen & Hails, 2002). Residuals did not deviate from normality for both the female nest-guarding model (Shapiro–Wilk test: $W = 0.98$, $P = 0.72$) and the female nest defence model ($W = 0.98$, $P = 0.39$). Thus, no transformations were needed.

Following recommendations of Forstmeier and Schielzeth (2011) we present full models. However, other authors recommend backward elimination of nonsignificant terms (Grafen & Hails, 2002). When following this alternative approach we reached the same conclusions (results not shown).

Additionally to the GLM models we also used simple univariate analyses to address further questions that were not addressed by GLMs. In these additional analyses we used paired *t* tests, and chi-square tests, depending on the distribution of particular data. Unpaired *t* tests are Welch's *t* tests for unequal variances (as recommended by Ruxton, 2006).

All tests were two tailed. All analyses were done in JMP 8.0.1. All values are mean ± SE.

Ethical Note

We followed the ASAB/ABS guidelines for the treatment of animals in research. Licences and permission to ring and handle the birds and to perform the experiments were provided by the Ministry of Environment of the Slovak Republic, No. 269/132/05-5.1pil and No. 7230/2008-2.1pil. When locating and checking great reed warbler nests we moved slowly through the reeds trying to avoid damaging reed stems and disturbing breeding birds or attracting potential nest predators. Of all tested nests ($N = 57$), one nest was predated and seven naturally parasitized nests (12.3%) were deserted during egg laying, most probably as a response to cuckoo parasitism. Nest desertion is a typical host adaptation against brood parasitism in the great reed warbler and in our study population it averages 15.3% (Trnka, Požgayová, et al., 2012), which is consistent with the desertion rate found in the present study ($\chi^2_1 = 0.3$, $P = 0.58$). None of the remaining nests were abandoned or predated and no egg disappeared during the first 5 days after the experiment, suggesting our research activities did not influence the nesting success of tested birds. Each female was captured in a 10 m long mist net stretched in her territory, but away from the nest so as not to destroy vegetation cover around the nest and disturb her partner. According to our previous experience (e.g. Trnka & Grim, 2013b; Trnka & Prokop, 2010) we are also sure that mist netting

Table 1
Predictors of female great reed warbler nest guarding ('latency to arrival' during nest defence experiment as a proxy) and nest defence (contact attacks/min)

Predictor	df	Nest guarding		Estimate±SE	Nest defence		Estimate±SE
		F	P		F	P	
Intercept				24.07±70.11			4.26±7.34
Female predictors							
Handling aggression ('4')*	3	3.11	0.038	'1': 55.16±22.51 '2': 6.71±14.82 '3': −19.75±13.27	11.26	<0.0001	'1': −8.91±2.47 '2': −3.74±1.56 '3': 2.95±1.40
Nest guarding	1	—	—	NA	6.50	0.015	−0.03±0.01
Nest defence	1	—	—	[VIF=2.70]	—	—	NA
Clutch size	1	0.24	0.63	−6.35±13.04	1.61	0.21	1.73±1.36
Mating status (Tertiary)*	3	1.51	0.23	Monogamous: 9.46±13.81 Primary: 17.82±18.38 Secondary: −29.94±16.48	0.56	0.64	Monogamous: −0.63±1.44 Primary: −0.12±1.94 Secondary: −2.13±1.73
Egg response (Acceptance)*	1	0.12	0.73	Rejection: 3.28±9.47	0.26	0.61	Rejection: 0.51±1.00
First egg date	1	0.55	0.46	0.59±0.80	0.25	0.62	0.04±0.09
1st attacking sex	2	—	—	[VIF=6.25]	—	—	[VIF=2.63]
Male predictors							
Male nest guarding	1	33.62	<0.0001	0.58±0.10	—	—	[VIF=4.07]
Male nest defence	1	7.34	0.01	3.05±1.13	1.71	0.20	−0.12±0.09

Results from a full model. Nest-guarding model: $R^2 = 0.63$, $F_{11,37} = 5.66$, $P < 0.0001$. Nest defence model: $R^2 = 0.69$, $F_{11,37} = 7.43$, $P < 0.0001$. Potential predictors with variance inflation factors (VIF) >2 were excluded (following Zuur et al., 2010). NA: not applicable.

* Reference levels of categorical variables are given in parentheses.

and bird handling did not negatively affect females' nesting behaviour and nest success.

RESULTS

Laying dates by females of varying mating status almost completely overlapped; thus, there was no statistical difference in average laying date (1 May = day 1) among monogamous (28 ± 2 days, $N = 25$), primary (29 ± 4 days, $N = 10$), secondary (32 ± 3 days, $N = 11$) and tertiary females (35 ± 6 days, $N = 3$; $F_{3,45} = 0.69$, $P = 0.56$). Clutch size (number of eggs) varied little among monogamous (4.4 ± 0.1), primary (3.7 ± 0.2), secondary (4.1 ± 0.2) and tertiary females (4.3 ± 0.4; $F_{3,45} = 2.54$, $P = 0.07$). After exclusion of the smallest female category, tertiary females, the differences reached statistical significance ($F_{2,43} = 4.07$, $P = 0.02$): monogamous females had significantly (Tukey HSD: $\alpha = 0.05$) larger clutches than primary females; secondary females did not differ from either monogamous or primary females. Reanalyses of these comparisons with nonparametric Kruskal–Wallis tests led to the same conclusions.

Aggression during Handling

Females showed large variation in aggression when being handled after mist netting. Of all tested females ($N = 49$), some females tried to escape silently (10%), others passively accepted being handled (25%), but the majority showed some level of aggression towards the human observer: some females produced alarm calls and spread their wings towards the observer (i.e. threatening posture; 43%), and some even actively pecked the observer while producing croaking warning calls (22%).

Levels of handling aggression did not covary with female mating status ($\chi^2_9 = 14.7$, $P = 0.10$) or male mating status ($\chi^2_3 = 0.66$, $P = 0.88$). Exclusion of the smallest handling aggression category ('1'; $N = 5$) or mating status category (tertiary females; $N = 3$) did not change the conclusions.

Nest Guarding

We used latency to arrival from the start of the dummy presentation as a proxy for nest guarding. Data from previous

breeding seasons (2010–2012) showed high individual repeatability of female latency to arrival across years ($r = 0.72$, 95% CIs = 0.44–0.87).

In the year of the present study (2013), focal females arrived at their nests very fast (64 ± 11 s). Males arrived significantly later than their partners (101 ± 15 s; paired t test: $t_{48} = 3.02$, $P = 0.004$). At only eight of 49 nests did males arrive before females. Female and male latencies were strongly and positively correlated ($r_s = 0.73$, $N = 49$, $P < 0.0001$). Female latency to arrival did not differ between naturally (67 ± 16 s) and experimentally parasitized nests (61 ± 14 s; $t_{46.5} = 0.31$, $P = 0.76$). Male latency to arrival also did not differ between naturally (104 ± 22 s) and experimentally parasitized nests (98 ± 22 s; $t_{46.8} = 0.21$, $P = 0.83$).

Nest Defence

Female aggression towards the stuffed cuckoo (contact attacks/min) was highly variable, ranging from no attacks at all to 37 contacts within 1 min of dummy exposure. Male aggression was also highly variable (0–32). On average, female aggression (18.4 ± 1.2) did not differ statistically from male aggression (17.9 ± 1.4; paired t test: $t_{48} = 0.32$, $P = 0.75$).

Female contact rate was not correlated with male contact rate ($r_s = 0.12$, $N = 49$, $P = 0.40$). Females at naturally parasitized nests showed statistically the same nest defence levels (17.5 ± 1.7 contacts/min) as those at experimentally parasitized nests (19.4 ± 1.8 contacts/min; $t_{46.34} = 0.78$, $P = 0.44$). Males also did not differ in their nest defence between nests parasitized naturally (17.9 ± 2.0 contacts/min) or experimentally (17.8 ± 1.9 contacts/min; $t_{46.99} = 0.02$, $P = 0.98$).

Egg Rejection and Latency to Egg Rejection

Overall rejection rate of natural cuckoo eggs was 51.0% ($N = 49$). The rejection rate was virtually identical between naturally (53.9%, $N = 26$) and experimentally parasitized nests (47.8%, $N = 23$; $\chi^2_1 = 0.18$, $P = 0.67$). Rejection rates did not differ statistically according to female mating status (monogamous: 52%; primary: 80%; secondary: 27%; tertiary: 33%; $\chi^2_3 = 6.2$, $P = 0.10$). Thus, egg rejection rates by females mated with monogamous (52%) or polygynous males (50%) were the same. There was no

interactive effect of female mating status with natural/experimental type of parasitism on egg rejection response ($\chi_3^2 = 4.5$, $P = 0.21$).

Latency to egg rejection did not differ between naturally parasitized (1.6 ± 0.4 days, $N = 14$) and experimentally parasitized nests (1.2 ± 0.1 days, $N = 11$; $t_{15.3} = 0.93$, $P = 0.39$). Latency to egg rejection did not vary with female ($R^2 = 0.03$, $F_{3,21} = 0.20$, $P = 0.89$) or male mating status ($t_{20.29} = 0.63$, $P = 0.54$).

Exclusion of the smallest mating status category (tertiary females; $N = 3$) did not change any of the conclusions.

Covariation Among Behaviours

Female handling aggression covaried significantly with both nest guarding (negatively; Fig. 1a) and nest defence (positively; Fig. 1b), whereas these patterns were not affected by other female predictors (Table 1). Male nest guarding and defence covaried significantly and positively with female nest guarding (Table 1). Removal of these male predictors had negligible effect on parameter estimates and no effect on our conclusions (results not shown). Egg rejection behaviour did not covary with any other female behaviour. Female acceptors and rejecters did not differ in their nest guarding (Table 1), nest defence (Fig. 2a, Table 1) or handling aggression (Fig. 2b; $\chi_3^2 = 0.65$, $P = 0.88$). Male nest guarding did not differ between nests where the cuckoo egg was accepted
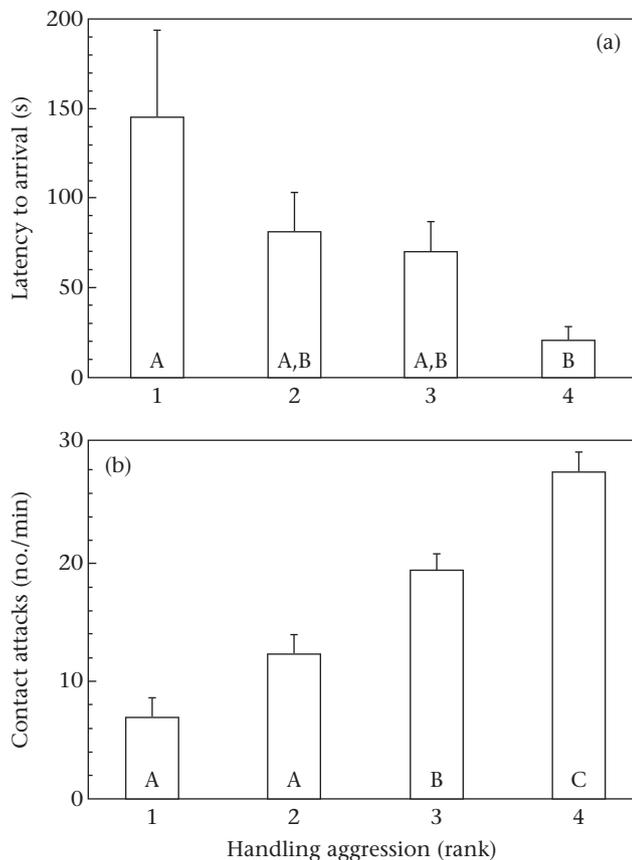
Figure 1. Great reed warbler females' handling aggression in relation to (a) nest guarding ('latency to arrival' during nest defence experiment as a proxy) and (b) nest defence against dummy cuckoos (contact attacks/min). Raw data are shown (mean + SE). Categories with different letters inside bars were significantly different (Tukey HSD post hoc test: $\alpha = 0.01$) according to a GLM (Table 1). Handling aggression categories: 1 = escape behaviour ($N = 5$); 2 = passive behaviour ($N = 12$); 3 = nonoffensive behaviour (alarm calls, threatening posture; $N = 21$); 4 = aggressive behaviour (warning calls, pecking; $N = 11$).

(111.0 ± 22.9 s) or rejected (91.8 ± 21.2 s; $t_{47} = 0.61$, $P = 0.54$). Male nest defence also did not vary between acceptor (18.4 ± 1.8 contacts/min) and rejecter nests (17.4 ± 2.1 contacts/min; $t_{47} = 0.37$, $P = 0.72$). Latency to egg ejection ($N = 25$) did not correlate with female nest guarding ($r_s = 0.10$, $P = 0.63$), nest defence ($r_s = -0.07$, $P = 0.75$) or handling aggression ($r_s = 0.04$, $P = 0.86$).

DISCUSSION

Contrary to our prediction, we found no correlation between egg rejection behaviour of great reed warbler females and other measured behaviours including aggression against adult parasites. This contrasts with previous studies that have reported a strong correlation between these two host defence strategies, either negative (Soler et al., 1999) or positive (Guigueno & Sealy, 2011). On the other hand, a female's aggression towards cuckoos was correlated strongly with her aggression during handling in the net and nest-guarding behaviour. To the best of our knowledge, this is the first empirical evidence on correlation between host antiparasite adaptation (female nest defence) and behaviours that might not be directly related to brood parasitism (handling aggression, nest guarding).

Female nest guarding was significantly predicted by male nest defence traits (latency to arrival and contact attack rate; Table 1). However, it is the female that leads nest defence action (Trnka, Prokop, et al., 2012), and female responses are independent of male presence or absence as shown experimentally (Trnka & Grim, 2013b). Thus, male behaviour covaries with some female behaviours because males adjust their responses to females, but not vice versa (Trnka & Grim, 2013b). This also explains why including or excluding male traits did not alter our conclusions (see Results).

As in any ecological study, additional factors that we did not measure might have introduced noise into our data sets. Degree of matching between host and the parasitic cuckoo egg might have affected host female responses. This potential bias is unlikely in our study because we used naturally available eggs from the same study site and field season; therefore the rejection responses we detected reflect naturally existing variation in cuckoo and host eggs. Importantly, rejection rates were virtually identical between naturally and experimentally parasitized nests. A similar degree of matching of parasitic eggs with host ones has not been done in any previous study of any brood parasite because it is methodologically impossible, at least during the host-laying period (explained in detail in Grim, Samaš, & Hauber, 2014).

Host responses may be tied to spatiotemporal variation in parasitism risk via host phenotypic plasticity (Campobello & Sealy, 2010). However, we believe that local variation in parasitism risk resulting from varying distances of particular host nests from potential cuckoo perches is negligible in our study site which consists of a narrow strip of homogeneous reed beds rimmed with trees running parallel and at an equal distance from the reeds (note that this is a custom-built commercial artificial fish pond system, not a heterogeneous natural lake). Thus, great reed warblers breed here in habitats with similar visibility of the nests from cuckoo perches (Trnka & Prokop, 2011). Similarly, parasitism rates in the area are consistent (i.e. do not differ statistically) across years (Trnka et al., 2013). However, other hosts/populations where risks of cuckoo parasitism vary spatiotemporally (Thorogood & Davies, 2013) would be ideal for testing how parasitism risk, learning (Campobello & Sealy, 2010) and individual reaction norms (Dingemanse, Kazem, Réale, & Wright, 2010) interact to affect covariation between specific antiparasite host defences and general host behaviour.

Nest defence, handling aggression and nest guarding involve different levels of risk as explained above. Thus, covariation
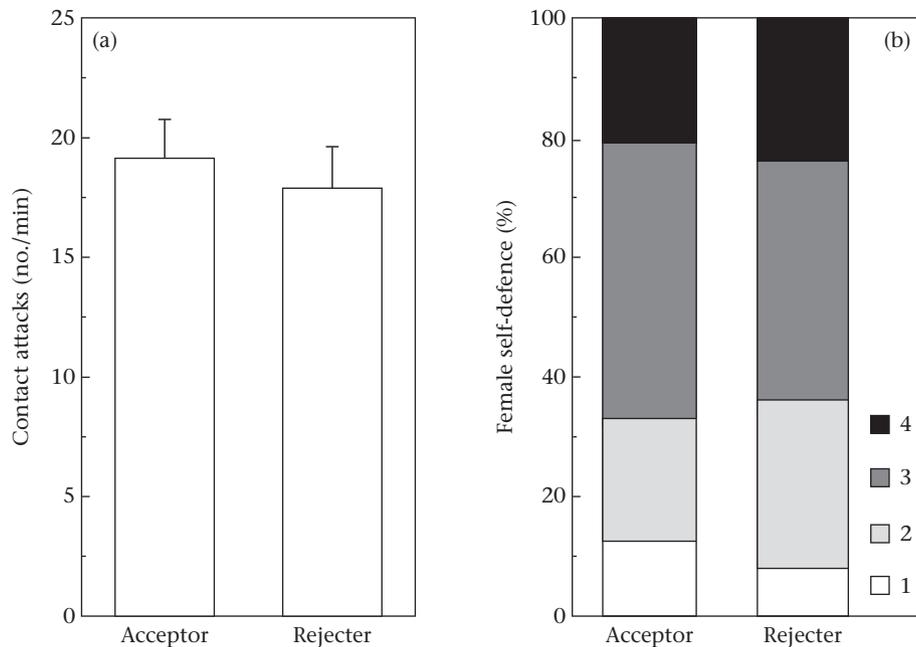
Figure 2. (a) Female acceptors' and rejecters' anticuckoo nest defence (contact attacks/min) and (b) the percentage of females showing a particular level of handling aggression. For definitions of handling aggression ordinal categories see Fig. 1.

between these three types of behaviour suggests consistent behaviour of great reed warbler females across different situations and contexts (Sih et al., 2004). Moreover, females also showed high individual repeatability of both aggression towards cuckoos (Trnka et al., 2013) and nest guarding (this study) in our study population. Therefore, although we do not have data on repeatability of handling aggression (which is considered an important requirement for supporting behavioural type, Sih & Bell, 2008; but see, e.g. Carter, Goldizen, & Tromp, 2010), we hypothesize that differences in host behavioural types (shy–bold continuum), rather than host egg discrimination ability, may predict variation in host nest defence behaviour against adult brood parasites. However, stronger evidence for a behavioural type in terms of additional observations of other behaviours and their consistency outside the context of brood parasitism is needed to test this hypothesis rigorously. Nevertheless, our results raise a crucial question of why nest defence behaviour against adult brood parasites does not covary with egg rejection decisions in our study species as predicted by theoretical models (host personality hypothesis, Avilés & Parejo, 2011).

Why Do Great Reed Warbler Defences Not Covary?

Our findings make biological sense in the light of the ecological setting in which great reed warblers live and the known patterns of personality traits and behavioural types (Réale et al., 2007). Both aggression during handling and nest defence entail relatively similar discrimination tasks, that is, recognition of relatively large, moving and directly dangerous intruders be it cuckoos (Trnka, Prokop, et al., 2012), deadly predators of adult birds (sparrowhawk, *Accipiter nisus*; Trnka & Grim, 2013b) or humans (Trnka & Prokop, 2010). Notably, great reed warblers use the same behavioural repertoire when they are attacking a dummy cuckoo, sparrowhawk or a human observer: close approaches, warning calls, dives and even contact attacks including pecking either on the wing (Trnka & Prokop, 2010) or when being handled by researchers (this study).

In contrast, egg discrimination focuses on small, stationary and not directly dangerous (to the host parent's own survival) objects. Moreover, discrimination of intruders near the nest is extremely fast (in seconds; Trnka, Prokop, et al., 2012) whereas discrimination of intruders inside the nest is much slower (in hours or days; Grim et al., 2011; Grim et al., 2014; Požgayová, Procházka, Polačiková, & Honza, 2011; Samaš et al., 2011). These proximate factors may, at least partly, explain why there was neither a positive correlation nor a trade-off between behaviours performed around the nest (handling aggression, nest defence, nest guarding) versus those performed inside the nest (egg rejection, latency to egg rejection).

An additional explanation for the lack of any covariation between the two lines of defence in the great reed warbler population we studied may be large variation in host success in chasing laying female cuckoos: some cuckoos successfully lay in host nests (see ca. 50% parasitism rate), whereas others are chased away or even killed by nest owners (Trnka & Grim, 2013a). Varying levels of host success in rebutting the parasite may further depend on interactions between nest guarding, adult enemy recognition, nest defence strength and egg recognition. Importantly, female cuckoos sometimes spent a very short (s) or a very long (min) time at the host nests (Moksnes et al., 2000), thus decreasing or increasing the chances that host defence abilities will be effective in the first place: even the strongest nest defence is of no use when nest guarding is weak. Such complex interactions would mask potential covariation between host defences against parasite adults versus eggs. Studying such interactions would require researchers to videorecord natural parasite–host interactions (following Moksnes et al., 2000), quantify host and parasite behaviour during egg laying and record responses to parasite eggs.

Whether responses to adult parasites versus to their eggs should covary will depend on various factors, for example egg mimicry. If mimicry is perfect (from the host's point of view: Igic et al., 2012) then hosts should increase their levels of nest defence no matter whether they are, at least sometimes, able to recognize and eliminate the parasite egg. This would also eliminate any correlation between nest defence and egg rejection.

The traditional view holds that the best way to defend against parasitism is not to become parasitized in the first place (Davies, 2000). However, this may not always be true. Aggressive nest defence is costly in terms of time, energy, risk of injury and risk of attracting other predators and/or parasites (Grim, 2008; Welbergen & Davies, 2008). When the parasite egg mimicry is poor (relative to host egg recognition abilities) then hosts may do better not to attack the laying parasite and later remove the nonmimetic parasite egg but only if the costs of egg removal by the laying parasite (Wyllie, 1981) are smaller than the costs of predation and/parasitism caused by attraction through conspicuous nest defence (Grim, 2008). This host tactic ('do not attack adult parasite; kill parasite embryo later') may be especially adaptive in hosts that are not physically able to prevent the parasite from entering their nest. For example, the strong aggression of great reed warblers is effective in chasing off or even killing cuckoo females (Janisch, 1948; Molnár, 1944; Trnka & Grim, 2013a). In contrast, the weak nest defence (mostly alarm calls) of reed warblers, *Acrocephalus scirpaceus*, is empirically known to be ineffective in chasing off laying cuckoo females (Moksnes et al., 2000; O. Mikulica, personal communication; B. G. Stokke, personal communication). This hypothesis predicts a negative correlation between nest defence and egg rejection, similarly to the 'rarer enemy' hypothesis (Grim, 2006, 2011) and 'strategy-blocking' models (Britton et al., 2007). Thus, it may not be testable in our study system. However, it could be tested in hosts that face multiple sympatric brood-parasitic species varying in body size (e.g. Yang et al., 2013). The same host individual is predicted to use alternative antiparasite strategies, depending on the particular parasite it faces near its nest: hosts should use nest defence against smaller (as for body size) parasites, but egg rejection against larger parasites (other things, like egg mimicry, being equal).

Conclusions

Great reed warbler females showed strong covariation between aggression in nest defence against brood parasites and aggression during handling in the mist net and other activities, namely nest guarding. In contrast, neither of these three behaviours covaried with egg rejection decisions or latency to these decisions. We propose that these findings may reflect the differing cognitive nature of the defence versus recognition tasks.

Our work suggests that to understand variation in host defence against parasites (nest defence) we may benefit from considering behaviours unrelated to parasitism per se (handling aggression, nest guarding). Future work should examine how other relevant aspects of avian behavioural and physiological (e.g. stress hormone levels) phenotypes covary with successive lines of host antiparasite defence: habitat selection (Hoover & Hauber, 2007), nest site selection (Hauber & Russo, 2000), nest defence (Feeney, Welbergen, & Langmore, 2012), egg rejection (Požgayová et al., 2011), nest architecture (Grim, Rutila, Cassey, & Hauber, 2009), nestling discrimination (Grim, 2006, 2007; Langmore et al., 2009) or fledgling discrimination (Grim, 2011). Such correlations may have profound effects on spatiotemporal patterns of parasite–host coevolution. For example, a potentially negative correlation between nest site selection (choice of high-quality habitat patches) and host defences (egg rejection) may slow down evolution of both parasite egg mimicry and host defences (Grim, 2002). Nonrandom transgenerational patterns of repeated parasitism (Hoover & Hauber, 2007) coupled with individually repeatable egg rejection decisions (Grim et al., 2014; Samaš et al., 2011) and antiparasite aggression (Trnka et al., 2013) could speed up or slow down (depending on the direction of the correlations) the tempo of parasite–host coevolution. These ideas would be best tested in hosts that show high interpopulation variation in parasitism pressures and antiparasitism defences (Stokke et al., 2008).

References

Avilés, J. M., & Parejo, D. (2011). Host personalities and the evolution of behavioural adaptations in brood parasitic-host systems. *Animal Behaviour, 82*, 613–618.

Banks, A. J., & Martin, T. E. (2001). Host activity and the risk of nest parasitism by brown-headed cowbirds. *Behavioral Ecology, 12*, 31–40.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., et al. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution, 24*, 127–135.

Britton, N. F., Planqué, R., & Franks, N. R. (2007). Evolution of defence portfolios in exploiter-victim systems. *Bulletin of Mathematical Biology, 69*, 957–988.

Brommer, J. E., & Kluen, E. (2012). Exploring the genetics of nestling personality traits in a wild passerine bird: testing the phenotypic gambit. *Ecology and Evolution, 2*, 3032–3044.

Campobello, D., & Sealy, S. G. (2010). Enemy recognition of reed warblers (*Acrocephalus scirpaceus*): threats and reproductive value act independently in nest defence modulation. *Ethology, 115*, 498–508.

Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlishaw, G., & Heinsohn, R. (2013). Animal personality: what are behavioural ecologists measuring? *Biological Reviews, 88*, 465–475.

Carter, A. J., Goldizen, A. W., & Tromp, S. A. (2010). Agamas exhibit behavioral syndromes: bolder males bask and feed more but may suffer higher predation. *Behavioral Ecology, 21*, 655–661.

Class, B., Kluen, E., & Brommer, J. E. (2014). Evolutionary quantitative genetics of behavioral responses to handling in a wild passerine. *Ecology and Evolution, 4*, 427–440.

Davies, N. B. (2000). *Cuckoos, cowbirds and other cheats*. London, U.K.: T. & A. D. Poyser.

Davies, N. B., Butchart, S. H. M., Burke, T. A., Chaline, N., & Stewart, I. R. K. (2003). Reed warblers guard against cuckoos and cuckoldry. *Animal Behaviour, 65*, 285–295.

Dingemanse, N. J., Kazem, A. J. N., Réale, D., & Wright, J. (2010). Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology & Evolution, 25*, 81–89.

Duckworth, J. W. (1991). Responses of breeding reed warblers *Acrocephalus scirpaceus* to mounts of sparrowhawk *Accipiter nisus*, cuckoo *Cuculus canorus* and jay *Garrulus glandarius*. *Ibis, 133*, 68–74.

Eilam, D. (2005). Die hard: A blend of freezing and fleeing as a dynamic defense—implications for the control of defensive behavior. *Neuroscience and Biobehavioral Reviews, 29*, 1181–1191.

Feeney, W. E., Welbergen, J. A., & Langmore, N. E. (2012). The frontline of avian brood parasite-host coevolution. *Animal Behaviour, 84*, 3–12.

Forstmeier, W., & Schielzeth, H. (2011). Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner's curse. *Behavioral Ecology and Sociobiology, 65*, 47–55.

Gill, S. A., Grieef, P. M., Staib, L. M., & Sealy, S. G. (1997). Does nest defence deter or facilitate cowbird parasitism? A test of the nesting-cue hypothesis. *Ethology, 103*, 56–71.

Gill, S. A., Neudorf, D. L., & Sealy, S. G. (1997). Host responses to cowbirds near the nest: cues for recognition. *Animal Behaviour, 53*, 1287–1293.

Glutz von Blotzheim, U. N., & Bauer, K. (1991). *Handbuch der Vögel Mitteleuropas. Band 12/1. Passeriformes (3. Teil). Sylviidae*. Wiesbaden, Germany: Aula Verlag.

Grafen, A., & Hails, R. (2002). *Modern statistics for the life sciences*. Oxford, U.K.: Oxford University Press.

Grim, T. (2002). Why is mimicry in cuckoo eggs sometimes so poor? *Journal of Avian Biology, 33*, 302–305.

Grim, T. (2005). Host recognition of brood parasites: implications for methodology in studies of enemy recognition. *Auk, 122*, 530–543.

Grim, T. (2006). The evolution of nestling discrimination by hosts of parasitic birds: why is rejection so rare? *Evolutionary Ecology Research, 8*, 785–802.

Grim, T. (2007). Experimental evidence for chick discrimination without recognition in a brood parasite host. *Proceedings of the Royal Society B: Biological Sciences, 274*, 373–381.

Grim, T. (2008). Are blackcaps (*Sylvia atricapilla*) defending their nests also calling for help from their neighbours? *Journal of Ornithology, 149*, 169–180.

Grim, T. (2011). Ejecting chick cheats: a changing paradigm? *Frontiers in Zoology, 8*(6), 14.

Grim, T., Rutila, J., Cassey, P., & Hauber, M. E. (2009). The cost of virulence: an experimental study of egg eviction by brood parasitic chicks. *Behavioral Ecology, 20*, 1138–1146.

Grim, T., Samaš, P., & Hauber, M. E. (2014). The repeatability of avian egg ejection behaviors across different temporal scales, breeding stages, female ages and experiences. *Behavioral Ecology and Sociobiology, 68*, 749–759.

Grim, T., Samaš, P., Moskát, C., Kleven, O., Honza, M., Moksnes, A., et al. (2011). Constraints on host choice: why do parasitic birds rarely exploit some common potential hosts? *Journal of Animal Ecology, 80*, 508–518.

Guigueno, M. F., & Sealy, S. G. (2011). Aggression towards egg-removing cowbird elicits clutch abandonment in parasitized yellow warblers, *Dendroica petechia*. *Animal Behaviour, 81*, 211–218.

Hauber, M. E., & Russo, S. A. (2000). Perch proximity correlates with higher rates of cowbird parasitism of ground nesting song sparrows. *Wilson Bulletin, 112*, 150–153.

Hollander, F. A., Van-Overveld, T., Tokka, I., & Matthysen, E. (2008). Personality and nest defence in the great tit (*Parus major*). *Ethology, 114*, 405–412.

Honza, M., Grim, T., Čapek, M., Moksnes, A., & Røskaft, E. (2004). Nest defence, enemy recognition and nest inspection behaviour of experimentally parasitised reed warblers *Acrocephalus scirpaceus*. *Bird Study, 51*, 256–263.

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. *Journal of Animal Ecology, 76*, 1208–1214.

Hopkins, W. G. (2009). *Calculating the reliability intraclass correlation coefficient and its confidence limits* (Excel spreadsheet) newstats.org/xICC.xls.

Igic, B., Cassey, P., Grim, T., Greenwood, D. R., Moskát, C., Rutila, J., et al. (2012). A shared chemical basis of avian host–parasite egg colour mimicry. *Proceedings of the Royal Society B: Biological Sciences, 279*, 1068–1076.

Janisch, M. (1948). Fight between *Cuculus c. canorus* L. – cuckoo – and *Acrocephalus a. arundinaceus* L. – great reed warbler. *Aquila, 55*, 291.

Langmore, N. E., Cockburn, A., Russell, A. F., & Kilner, R. M. (2009). Flexible cuckoo chick-rejection rules in the superb fairy-wren. *Behavioral Ecology, 20*, 978–984.

Leisler, B., & Schulze-Hagen, K. (2011). *The Reed Warblers. Diversity in a uniform bird family*. Zeist: KNNV Publishing.

Lotem, A., Nakamura, H., & Zahavi, A. (1995). Constraints on egg discrimination and cuckoo–host co-evolution. *Animal Behaviour, 49*, 1185–1209.

Moksnes, A., Røskaft, E., Hagen, L. G., Honza, M., Mork, C., & Olsen, P. H. (2000). Common cuckoo *Cuculus canorus* and host behaviour at reed warbler *Acrocephalus scirpaceus* nests. *Ibis, 142*, 247–258.

Møller, A. P., & Ibáñez-Álamo, J. D. (2012). Escape behaviour of birds provides evidence of predation being involved in urbanization. *Animal Behaviour, 84*, 341–348.

Molnár, B. (1944). The cuckoo in the Hungarian Plain. *Aquila, 51*, 100–112.

Požgayová, M., Procházka, P., & Honza, M. (2009). Sex-specific defence behaviour against brood parasitism in a host with female-only incubation. *Behavioural Processes, 81*, 34–38.

Požgayová, M., Procházka, P., & Honza, M. (2013). Is shared male assistance with antiparasitic nest defence costly in the polygynous great reed warbler? *Animal Behaviour, 85*, 615–621.

Požgayová, M., Procházka, P., Polačiková, L., & Honza, M. (2011). Closer clutch inspection—quicker egg ejection: timing of host responses toward parasitic eggs. *Behavioral Ecology, 22*, 46–51.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews, 82*, 291–318.

Ruxton, G. D. (2006). The unequal variance *t* test is an underused alternative to Student's t test and the Mann–Whitney *U* test. *Behavioral Ecology, 17*, 688–690.

Samaš, P., Hauber, M. E., Cassey, P., & Grim, T. (2011). Repeatability of foreign egg rejection: testing the assumptions of co-evolutionary theory. *Ethology, 117*, 606–619.

Sih, A., & Bell, A. M. (2008). Insights for behavioral ecology from behavioral syndromes. *Advances in the Study of Behavior, 38*, 227–281.

Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution, 19*, 372–378.

Soler, J. J., Soler, M., Pérez-Contreras, T., Aragón, S., & Møller, A. P. (1999). Antagonistic antiparasite defenses: nest defense and egg rejection in the magpie host of the great spotted cuckoo. *Behavioral Ecology, 10*, 707–713.

Stokke, B. G., Hafstad, I., Rudolfsen, G., Moksnes, A., Møller, A. P., Røskaft, E., et al. (2008). Predictors of resistance to brood parasitism within and among reed warbler populations. *Behavioral Ecology, 19*, 612–620.

Stokke, B. G., Moksnes, A., & Røskaft, E. (2002). Obligate brood parasites as selective agents for evolution of egg appearance in passerine birds. *Evolution, 56*, 199–205.

Thorogood, R., & Davies, N. B. (2012). Cuckoos combat socially transmitted defenses of reed warbler hosts with a plumage polymorphism. *Science, 337*, 578–580.

Thorogood, R., & Davies, N. B. (2013). Reed warbler hosts fine-tune their defences to track three decades of cuckoo decline. *Evolution, 67*, 3545–3555.

Trnka, A. (2011). On the second breeding of the great reed warbler (*Acrocephalus arundinaceus*) in the same season in Slovakia. *Tichodroma, 23*, 42–44.

Trnka, A., Batáry, P., & Prokop, P. (2010). Infanticide or interference: does great reed warbler selectively destroy eggs? *Annales Zoologici Fennici, 47*, 272–277.

Trnka, A., & Grim, T. (2013a). Color plumage polymorphism and predator mimicry in brood parasites. *Frontiers in Zoology, 10*, 25.

Trnka, A., & Grim, T. (2013b). To compensate or not to compensate: testing the negotiation model in the context of nest defence. *Behavioral Ecology, 24*, 223–228.

Trnka, A., Požgayová, M., Procházka, P., Prokop, P., & Honza, M. (2012). Breeding success of a brood parasite is associated with social mating status of its host. *Behavioral Ecology and Sociobiology, 66*, 1187–1194.

Trnka, A., Požgayová, M., Samaš, P., & Honza, M. (2013). Repeatability of host female and male aggression towards a brood parasite. *Ethology, 119*, 907–917.

Trnka, A., & Prokop, P. (2010). Does social mating system influence nest defence behaviour in great reed warbler (*Acrocephalus arundinaceus*) males? *Ethology, 116*, 1075–1083.

Trnka, A., & Prokop, P. (2011). Polygynous great reed warblers *Acrocephalus arundinaceus* suffer more cuckoo *Cuculus canorus* parasitism than monogamous pairs. *Journal of Avian Biology, 42*, 192–195.

Trnka, A., & Prokop, P. (2012). The effectiveness of hawk mimicry in protecting the cuckoos from aggressive hosts. *Animal Behaviour, 83*, 263–268.

Trnka, A., Prokop, P., & Grim, T. (2012). Uncovering dangerous cheats: how do avian hosts recognize adult brood parasites? *PLoS ONE, 7*, e37445.

Welbergen, J. A., & Davies, N. B. (2008). Reed warblers discriminate cuckoos from sparrowhawks with graded alarm signals that attract mates and neighbours. *Animal Behaviour, 76*, 811–822.

Wyllie, I. (1981). *The cuckoo*. London, U.K.: Batsford.

Yang, C., Stokke, B. G., Antonov, A., Cai, Y., Shi, S., Moksnes, A., et al. (2013). Host selection in parasitic birds: are open-cup nesting insectivorous passerines always suitable cuckoo hosts? *Journal of Avian Biology, 44*, 216–220.

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution, 1*, 3–14.