

# Nest defenses and egg recognition of yellow-bellied prinia against cuckoo parasitism

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**Abstract** Parasites may, in multi-parasite systems, block the defenses of their hosts and thus thwart host recognition of parasites by frequency-dependent selection. Nest defenses as frontline may block or promote the subsequent stage of defenses such as egg recognition. We conducted comparative studies of the defensive strategies of a host of the Oriental cuckoo *Cuculus optatus*, the yellow-bellied prinia *Prinia flaviventris*, in mainland China with multiple species of cuckoos and in Taiwan with a single cuckoo species. Cuckoo hosts did not exhibit aggression toward cuckoos in the presence of multiple cuckoo species but showed strong aggressive defenses of hosts directed toward cuckoos in Taiwan. Furthermore, the cuckoo host in populations with a single cuckoo species was able to distinguish adults of its brood parasite, the Oriental cuckoo, from adult common cuckoos (*Cuculus canorus*). This represents the first case in which a cuckoo host has been shown to specifically distinguish Oriental cuckoo, from other *Cuculus* species. Hosts ejected eggs at a higher rate in a single cuckoo species system

than in a multi-species cuckoo system, which supports the strategy facilitation hypothesis. Granularity analysis of variation in egg phenotype based on avian vision modeling supported the egg signature hypothesis in hosts because Taiwanese prinias increased consistency in the appearance of their eggs within individual hosts thus favoring efficient discrimination against cuckoo eggs. This study significantly improves our knowledge of intraspecific variation in antiparasitism behavior of hosts between single- and multi-cuckoo systems.

**Keywords** *Cuculus optatus* · Dummy experiment · Egg rejection · *Prinia flaviventris*

## Introduction

Parasitic cuckoos lay their eggs in host nests and thus transfer costly parental care to their hosts, including incubation of eggs and food provisioning for nestlings (Davies 2000). Such interactions will in turn select for host defenses that promote the evolution of further adaptations in the parasite. Thus, the arms race between cuckoo trickeries and host defenses is a model system for studying coevolution and microevolution (Rothstein and Robinson 1998). To counter parasitism, cuckoo hosts have evolved a series of defensive strategies. They can recognize adult cuckoos that approach their nests and direct fatal attacks toward the cuckoo (Bartol et al. 2002; Røskaft et al. 2002). They can also spot cuckoo eggs in their nests and conduct targeted rejection (Rothstein 1975; Davies and Brooke 1989). They can even recognize and reject cuckoo chicks (Langmore et al. 2003; Sato et al. 2010). According to previous studies, attacking cuckoos or rejecting alien eggs is regarded as specific and efficient ways of defense against cuckoo parasitism (Davies 2000; Røskaft et al. 2002). However, most studies of coevolution between cuckoos and

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their hosts took place in Europe, where usually only a single cuckoo species (the common cuckoo *Cuculus canorus*) can be found in any given study area, although some other studies were also conducted in Australia (Langmore et al. 2005, 2008) and Japan (Nakamura et al. 2005). Noteworthy, multiple cuckoo species occur sympatrically in many other parts of the world, raising the question whether the presence of such species affects the evolution of host defenses. However, no study was formerly performed to answer this question specifically.

Both theoretical models and empirical studies suggest that polymorphism may evolve as a result of frequency-dependent selection on the success of different morphs (Kettlewell 1973; Turner and Speed 1999; Yang et al. 2010). A recent study illustrated that plumage polymorphism in common cuckoos combats the socially transmitted defenses of their hosts and thus thwarts host recognition (Thorogood and Davies 2012). Similarly, in a multi-cuckoo system in China, different cuckoo species coexist in sympatry through character displacement in terms of plumage color, color morphs, and body size (Yang et al. 2012). For example, the chestnut-winged cuckoo *Clamator coromandus* has a long black crest, white collar, and bright chestnut wing; the Asian koel *Eudynamis scolopaceus* is glossy black with red iris and light green bill; the Asian emerald cuckoo *Chrysococcyx maculatus* is small and characterized by glossy green from head to back and rump with blackish wing; and the four *Cuculus* cuckoo species are similar in size and plumage, but all have rufous and gray morphs (Fig. 1). Such character diversity would benefit cuckoos in thwarting host recognition in a frequency-dependent manner. Therefore, we propose that cuckoo hosts may not present defenses against adult cuckoos in multi-cuckoo systems because the presence of different cuckoos will render defenses by hosts inefficient. Therefore, we expected that the yellow-bellied prinia *Prinia flaviventris* in a multi-cuckoo population (i.e., in mainland China) would express much weaker defenses against cuckoo dummies than a prinia population that is exposed to parasitism only by a single cuckoo species (i.e., in Taiwan Island).

According to the strategy-blocking hypothesis, success in one line of defense will depress selection for defense at another stage (Soler et al. 1999; Planqué et al. 2002; Davies 2011; Feeney et al. 2012). In contrast, the strategy facilitation hypothesis suggests that defense at one stage will promote the defense at another stage (Feeney et al. 2012). The prinia populations of the mainland and Taiwan belong to the same species providing an ideal opportunity to test these two hypotheses by comparing their defensive strategies at different stages of the coevolutionary interactions. Therefore, if hosts differed in nest defenses between single- and multi-cuckoo populations, we predicted differences in egg rejection abilities. Reduction of intraclutch variation (variation within an individual) in egg appearance makes greater variation in the

appearance of parasitic eggs and thus improve the chance of correctly spotting the alien egg hidden among the victim's clutch (Swynnerton 1916; Kilner 2006) while increase of interclutch variation (variation between individuals) in egg appearance favors the individual hosts to identify foreign eggs by producing eggs that look unlike the cuckoo's (Swynnerton 1916; Takasu 2003). Therefore, we finally predicted that an increase in interclutch variation and a decrease in intraclutch variation should be present in the host population with higher rejection ability as a defensive fine-tuning to facilitate egg recognition (Davies 2011). We tested these predictions by conducting studies of the defensive strategies of a host of the Oriental cuckoo *Cuculus optatus*, the yellow-bellied prinia, in single- and multi-cuckoo populations.

## Materials and methods

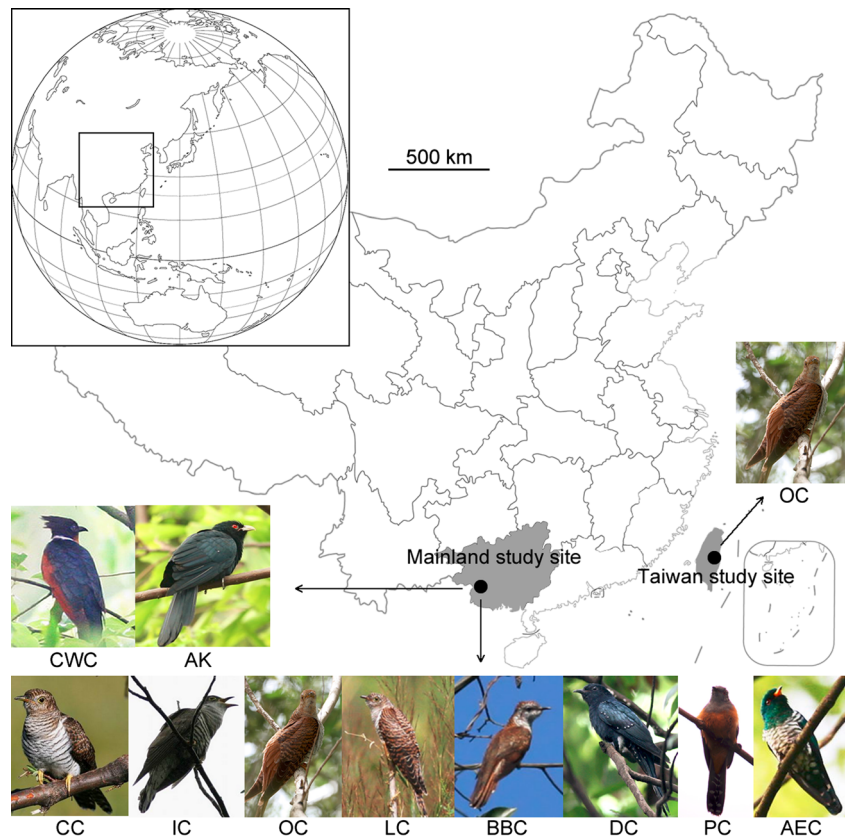
### Study areas and species

The study was performed in mainland China and in Taiwan. In the mainland, fieldwork was carried out in Nonggang National Nature Reserve (23° 39' N, 107° 04' E) at Guangxi from April to July 2011–2013. In Taiwan, experiments were conducted at Shoufeng, Hualien County (23° 51' N, 121° 31' E) from April to July 2010–2011. Guangxi is a province in Southwest China with the highest diversity of cuckoo species in China (up to 13 species, see Yang et al. 2012 for more details), and 10 species of cuckoo were recorded in the study site at Nonggang, including Oriental cuckoo and common cuckoo (Table 1). In contrast, only two cuckoo species are found in Taiwan (Yang et al. 2012), where only the Oriental cuckoo is common throughout the island, and in our study site, only the Oriental cuckoo occurs. These two study sites provided an ideal single- and multi-cuckoo system for our study. Yellow-bellied prinia is a common species that builds nests in grasses and shrubs at a height of ca. 1 m. It is parasitized by the Oriental cuckoo with highly mimetic eggs in both mainland China and Taiwan, and it is also listed as a potential host for several other cuckoo species elsewhere (La Touche 1931–1934; Zhang 1980; Yang et al. 2012). In this study, no cases of parasitism were found in mainland prinias ( $n=232$ ), while the parasitism rate in Taiwan prinias was only 0.91 % ( $n=110$ ). However, this parasitism rate may be considerably underestimated (see “Results and Discussion” section below).

### Cuckoo dummy experiments

Aggressive behavior of hosts toward cuckoos was quantified by using a taxidermist dummy of Oriental cuckoo, which was

**Fig. 1** Cuckoo species in our study sites of mainland China and Taiwan Island. *CWC* chestnut-winged cuckoo (*Clamator coromandus*), *AK* Asian koel (*Eudynamis scolopaceus*), *CC* common cuckoo (*Cuculus canorus*), *IC* Indian cuckoo (*Cuculus micropterus*) *OC* Oriental cuckoo (*Cuculus optatus*), *LC* lesser cuckoo (*Cuculus poliocephalus*), *DC* drongo cuckoo (*Surniculus lugubris*), *BBC* banded bay cuckoo (*Cacomantis sonneratii*), *PC* plaintive cuckoo (*Cacomantis merulinus*), *AEC* Asian emerald cuckoo (*Chrysococcyx maculatus*) (Photograph by Suixing Tian, with permission)



mounted at a distance of 0.5 m from and pointed toward host nests. For each nest, another two dummy species were set up as controls. The long-tailed shrike *Lanius schach* is the most common predator at both study sites. A third dummy was a pale thrush *Turdus pallidus*, which poses no threat to either host parents or nests. The three kinds of dummies were presented at an interval of 1 h between models with a fixed sequence. For each model, two dummy individuals were randomly selected for experiments to avoid pseudo-replication. We found no significant differences in responses

toward the two dummy individuals, and hence, we pooled the data for the subsequent analyses. When a dummy was mounted and the hosts appeared within 5 min, the responses by focal birds were recorded during 15 min. Attack behavior was considered an aggression response to cuckoos. To objectively compare the behavior between the two study sites and better test our predictions, in this dummy experiment, we did not consider any other behavior than attack to avoid any bias in quantification of non-attack behavior. Therefore, the responses by hosts were only classified as attack or no attack

**Table 1** Parasitic cuckoo species at our study sites in mainland China and Taiwan Island

	English name	Latin name	Size (cm)
Mainland China	Chestnut-winged cuckoo	<i>Clamator coromandus</i>	45
	Asian koel	<i>Eudynamis scolopaceus</i>	42
	Common cuckoo	<i>Cuculus canorus</i>	32
	Indian cuckoo	<i>Cuculus micropterus</i>	30
	Oriental cuckoo	<i>Cuculus optatus</i>	26
	Lesser cuckoo	<i>Cuculus poliocephalus</i>	26
	Drongo cuckoo	<i>Surniculus lugubris</i>	23
	Banded bay cuckoo	<i>Cacomantis sonneratii</i>	22
	Plaintive cuckoo	<i>Cacomantis merulinus</i>	21
	Asian emerald cuckoo	<i>Chrysococcyx maculatus</i>	17
Taiwan	Oriental cuckoo	<i>Cuculus optatus</i>	26

without any ambiguous cases. For each observed nest, the experiment was terminated when hosts directly attacked the dummies. Considering that the prinia was also listed as a potential host for the common cuckoo, we also conducted dummy experiments in both mainland and Taiwan populations with a combination of (1) common cuckoo, (2) Oriental turtle dove *Streptopelia orientalis* (a benign control), and (3) Eurasian jay *Garrulus glandarius* (a nest predator).

#### Parasitism experiments

Prinia nests were found by systematic searches in their typical nesting habitats while monitoring their breeding behavior. Active nests with complete clutches with incubation just started were randomly selected for one of the following experimental treatments: (1) model egg treatment in which one non-mimetic white model egg made of polymer clay to match the size and the mass of host eggs was inserted into each focal nest, (2) conspecific egg treatment in which one conspecific egg from another host nest was inserted into each focal nest, and (3) control treatment in which nests were monitored with the same procedure, but not manipulated. For each experimental treatment, prinia nests were monitored for six consecutive days on a daily basis. Responses of hosts were classified as rejection or acceptance, which corresponded to the foreign eggs being incubated and warm or being ejected or left cold, respectively. We also calculated the latency to rejection (time interval from parasitism to rejection) on a daily basis (i.e., days as unit).

#### Quantification of egg appearance

Prinia eggs are densely covered by reddish markings, and we used the recently developed method proposed by Stoddard and Stevens (2010) to quantify and analyze egg appearance according to avian vision (Stoddard and Stevens 2010). Images of all eggs of each clutch were photographed by putting them on a neutral gray card with a fixed distance from eggs to camera lens. The images were subsequently transformed from camera color space to correspond to the relative photon catches of a bird's double cones using the averaged spectral sensitivity of birds (see Stoddard and Stevens 2010 for more details). Granularity analysis, which was recently used for analyzing cuttlefish camouflage markings, was adopted to quantify the pattern sizes of egg markings (Chiao et al. 2009; Stoddard and Stevens 2010). For each image of an egg region, seven new images, which contained information at different spatial scales, were produced by fast Fourier transformation of the original image (Godfrey et al. 1987). In this transformation, seven octave-wide and isotropic band-pass filters were applied (Barbosa et al. 2008). They function like a sieve and capture the information at different spatial scales, with larger filter sizes corresponding to smaller markings and small filter sizes corresponding to larger markings. Finally,

seven granularity bands with normalized energies referring to these seven images were produced to determine the relative contribution of different marking sizes to the overall egg markings (Stoddard and Stevens 2010). In this study, measurements were made at the following filter sizes: 1, 2, 4, 8, 16, 32, and 64.

#### Statistical analysis

We compared the frequency of aggressive responses by prinias to dummy models using Fisher's exact tests. Likewise, we compared rejection rates for different categories of model eggs using Fisher's exact tests.

The normalized energies, which were described above, were computed and used to compare interclutch and intraclutch variation in egg appearance between mainland and Taiwanese prinia populations. For interclutch variation, we calculated the averaged normalized energies of each clutch and compared their standard deviation (SD) as egg variation between mainland and Taiwanese populations by using Levene's test for equality of variances. For intraclutch variation, we calculated the coefficient of variation (CV) within each clutch and compared them between mainland and Taiwanese populations. Analyses of the data from cuckoo dummy and parasite experiments were performed in IBM SPSS 20.0 for Windows (IBM, Inc.), while the egg variance analyses were performed in Matlab R2012a (MathWorks, Inc.). Values were presented as means±SD.

## Results

The mainland population of prinia did not attack the Oriental cuckoo dummy when presented together with long-tailed shrike and pale thrush, as we predicted (Table 2). However, the Taiwanese prinia population exhibited different responses to this combination of models ( $\chi^2_2=15.237$ ,  $P<0.001$ , Fisher's exact test). Prinias in Taiwan attacked Oriental cuckoo dummies more than half of the times (54.5 %, 12/22), which was significantly more aggressive than mainland prinias (0/16,  $\chi^2_1 = 13.804$ ,  $P<0.001$ , Fisher's exact test). For the other two types of dummies, no significant differences were found between these two populations. Similarly, mainland prinias did not attack any dummy in the combination of common cuckoo, Eurasian jay, and Oriental turtle dove. Furthermore, no significant difference was found in response toward common cuckoo between mainland (0/32) and Taiwan populations (7.7 %, 2/26) ( $\chi^2_1 = 2.549$ ,  $P=0.197$ , Fisher's exact test). Surprisingly, the Taiwanese population attacked the Oriental cuckoo (54.5 %, 12/22) more often than the common cuckoo (8.3 %, 2/24) ( $\chi^2_1 = 6.275$ ,  $P=0.015$ , Fisher's exact test).

**Table 2** Response to dummies by priniias in mainland China and Taiwan

	Attack	No attack	Total	$\chi^2$	df	P
Mainland						
<i>Cuculus optatus</i>	0	16	16	–	–	NS
<i>Lanius schach</i>	0	16	16			
<i>Turdus pallidus</i>	0	16	16			
Taiwan						
<i>Cuculus optatus</i>	12	22	34	15.237	2	<0.001***
<i>Lanius schach</i>	0	33	33			
<i>Turdus pallidus</i>	5	30	35			
<i>Cuculus optatus</i>						
Mainland	0	16	16	13.804	1	<0.001***
Taiwan	12	22	34			
<i>Lanius schach</i>						
Mainland	0	16	16	–	–	NS
Taiwan	0	33	33			
<i>Turdus pallidus</i>						
Mainland	0	16	16	2.534	1	0.167 NS
Taiwan	5	30	35			
Mainland						
<i>Cuculus canorus</i>	0	32	32	–	–	NS
<i>Streptopelia orientalis</i>	0	32	32			
<i>Garrulus glandarius</i>	0	32	32			
Taiwan						
<i>Cuculus canorus</i>	2	24	26	0.589	2	1.000 NS
<i>Streptopelia orientalis</i>	1	24	25			
<i>Garrulus glandarius</i>	2	23	25			
<i>Cuculus canorus</i>						
Mainland	0	32	32	2.549	1	0.197 NS
Taiwan	2	24	26			
<i>Streptopelia orientalis</i>						
Mainland	0	32	32	1.303	1	0.439 NS
Taiwan	1	24	25			
<i>Garrulus glandarius</i>						
Mainland	0	32	32	2.653	1	0.188 NS
Taiwan	2	23	25			

Dash denotes that Fisher's exact test was not performed in these cases

NS not statistically significant

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ;

\*\*\* $P < 0.001$

Mainland priniias rejected half of the non-mimetic model eggs (50 %, 9/18) and accepted all conspecific eggs (100 %, 13/13, Table 3). Taiwanese priniias rejected 100 % (13/13) of model eggs, which was a much higher frequency than on the mainland ( $\chi^2_{1,} = 9.159$ ,  $P=0.004$ , Fisher's exact test). They even rejected conspecific eggs on three occasions (16.7 %, 3/18), although in one of them, the host rejected its own egg (Table 3). No rejection was observed in control groups in either population. Furthermore, all rejection in Taiwanese population occurred within 1 day ( $n=13$ ) whereas 33.3 and 11.1 % of mainland population ( $n=9$ ) took 2 and 3 days to reject foreign eggs, respectively. Consequently, the latency to rejection in the mainland population was significantly longer than in the Taiwanese population ( $1.6 \pm 0.7$  vs.  $1 \pm 0$  day,  $n=9$  and  $n=13$ , respectively,  $z=-2.587$ ,  $P=0.01$ , Mann-Whitney  $U$  test).

No statistical differences were found in interclutch variation in egg phenotype between mainland and Taiwan populations of prinia for all normalized energies of the seven filter sizes (Table 4;  $P > 0.05$  for all,  $df=1, 57$ , Levene's test for equality of variances). For intraclutch variation in egg phenotype, the normalized energies of the mainland population in 4, 8, and 16 filter sizes that represented the medium-sized markings were significantly larger than for the island population (filter 4,  $0.529 \pm 0.232$  vs.  $0.367 \pm 0.177$ ,  $F_{1,57}=9.125$ ,  $P=0.004$ ; filter 8,  $0.552 \pm 0.212$  vs.  $0.372 \pm 0.191$ ,  $F_{1,57}=11.663$ ,  $P=0.001$ ; filter 16,  $0.550 \pm 0.210$  vs.  $0.415 \pm 0.202$ ,  $F_{1,57}=6.321$ ,  $P=0.015$ ).

No cases of parasitism were found in mainland priniias ( $n=232$ ), while the parasitism rate in Taiwan priniias was only 0.91 % ( $n=110$ ). The clutch size in the Taiwanese prinia population ( $3.14 \pm 0.59$ ,  $n=30$ ) was significantly smaller than

**Table 3** Comparison of egg rejection behavior of prinias between mainland China and Taiwan

	Rejection	Acceptance	Total	Rejection lag (days)
Model eggs				
Mainland China	9 (50)	9	18	<1.6±0.7 (n=9)
Taiwan	13 (100)	0	13	<1±0 (n=13)
Conspecific eggs				
Mainland China	0 (0)	13	13	–
Taiwan	3 (16.7)	15	18	<1±0 (n=3) <sup>a</sup>
Control				
Mainland China	0 (0)	15	15	–
Taiwan	0 (0)	15	15	–

Numbers in parentheses refer to the percentage of rejection

<sup>a</sup> In one case the host rejected its own egg

in the mainland population (4.43±0.63, n=28; z=-5.644, P<0.000001).

## Discussion

While the population of prinias in mainland China did not directly attack Oriental cuckoo and common cuckoo, the prinia population in Taiwan recognized and strongly attacked cuckoo models. They even attacked Oriental cuckoos more frequently than common cuckoos, revealing that prinias in Taiwan that were parasitized by Oriental cuckoos were able to spot the subtle differences between Oriental and common cuckoos. Our study constitutes the first empirical evidence suggesting that the aggressive defenses of hosts directed toward cuckoos may have been blocked by the presence of

multiple cuckoo species, although historical contact between cuckoo and host may also have effect to some extent. In this case, an increase in the local frequency of one cuckoo morph altered host defenses specifically to that morph and decreased its success, thus benefiting other cuckoo morphs as a result of frequency-dependent selection. In other words, cuckoo diversity in areas of sympatry with the host may combat the socially transmitted defenses of their hosts and thus thwart host recognition. Our results also represent the first case in which a cuckoo host has been shown to specifically distinguish its parasite, the Oriental cuckoo, from other cuckoo species belonging to the genus *Cuculus*, even though they are very similar in phenotype to each other and almost impossible to distinguish for humans (MacKinnon and Phillipps 1999).

The strategy-blocking hypothesis posits that success in one line of defense will depress selection for defense at other stages (Soler et al. 1999; Planqué et al. 2002; Davies 2011; Feeney et al. 2012). This hypothesis was not supported in our study. In fact, we found results opposite to it in mainland populations, where the host was an intermediate rejecter of non-mimetic eggs, while showing no aggression directed toward cuckoos, whereas the Taiwanese host population rejected 100 % of non-mimetic eggs and strongly attacked the cuckoo models. According to the strategy-blocking hypothesis, success in one line of defense will depress selection for defense at another stage, producing a negative relationship between the defensive strategies among different stages such as nest defense and egg rejection. However, in our study, both defensive strategies of these two stages were significantly stronger in Taiwan than in mainland populations (Fig. 2). Therefore, our results support the strategy facilitation hypothesis suggesting that the frontline of nest defense promotes the subsequent evolution of egg recognition. This also explains

**Table 4** Comparison of differences in variation in egg phenotypes of prinia populations between mainland China (n=29 clutches) and Taiwan (n=30 clutches)

	Normalized energies in seven filter sizes						n	
	1	2	4	8	16	32		64
Interclutch variation (values in SD)								
Mainland	11.625	12.068	8.641	4.705	2.505	1.429	1.068	29
Taiwan	6.348	10.055	9.737	6.465	2.435	1.159	0.848	30
F <sub>1,57</sub>	0.731	0.758	0.013	0.901	0.028	2.088	1.322	
P	0.396	0.388	0.910	0.347	0.869	0.154	0.255	
Intraclutch variation (values in CV)								
Mainland	0.431±0.177	0.444±0.196	0.529±0.232	0.552±0.212	0.550±0.210	0.415±0.164	0.384±0.149	29
Taiwan	0.434±0.291	0.387±0.216	0.367±0.177	0.372±0.191	0.415±0.202	0.405±0.184	0.329±0.147	30
F <sub>1,57</sub>	0.001	1.128	9.125	11.663	6.321	0.047	2.054	
P	0.972	0.293	0.004**	0.001**	0.015*	0.829	0.157	

Clutches for egg variation analysis were randomly selected from all observed nests

\*P<0.05; \*\*P<0.01

**Fig. 2** An illustration summarizing differences in the defensive behavior of prinias between mainland China and Taiwan. For more details on data, see Tables 2, 3, and 4

<b>Mainland China (multiple cuckoos)</b>	do not attack cuckoos	low intraclutch consistency	reject half non-mimetic eggs but no conspecific eggs  slow rejection
	attack cuckoos and distinguish <i>Cuculus optatus</i> from <i>Cuculus canorus</i>	high intraclutch consistency	reject all non-mimetic eggs and some conspecific eggs  quick rejection
	nest defense	egg variation	egg rejection

the specialized aggression directed toward Oriental cuckoo in the island population. Differences in intensity of selection from cuckoo parasitism between these two populations may be one possible explanation. Cuckoo hosts could benefit from interspecific competition among different cuckoo species, and such competition may be intense in multi-cuckoo systems thereby reducing population densities of the many sympatric cuckoo species, including those of the Oriental cuckoo. The low parasitism rate of prinias implied that prinias might be a rare host of cuckoo (Moksnes and Røskaft 1992) or have experienced a long-term coevolution with cuckoo (Peer and Sealy 2004). But considering that the Taiwan prinias rejected 100 % of non-mimetic eggs and 16.7 % of conspecific eggs, their parasitism rate is likely to be considerably underestimated because many cuckoo eggs could have been rejected before our nest inspections. We found that the clutch size in the Taiwanese prinia population was significantly smaller than that in the mainland population, and thus, this finding is consistent with the suggestion that rejection of cuckoo eggs by hosts occurs before our nest visits.

Finally, our study indicated that consistency in egg appearance within clutches in the Taiwanese host population was higher than in the mainland population, although no difference was found in phenotypic variation in eggs between individuals. Therefore, our results support the egg signature hypothesis (Swynnerton 1916; Takasu 2003; Davies 2011) proposing that higher consistency in egg appearance within clutches favors host individuals that recognize foreign eggs. This may explain the considerably higher egg rejection rate (100 %) of non-mimetic eggs and 16.7 % rejection of highly mimetic conspecific eggs by Taiwanese prinias.

In summary, the aggressive defenses of hosts directed toward cuckoos were blocked in the presence of multiple cuckoo species, although cuckoo hosts were specifically able to distinguish its parasite, the Oriental cuckoo, from other very similar species belonging to the same genus *Cuculus*. Furthermore, our study supported the strategy facilitation hypothesis suggesting that the frontline of nest defense promotes the subsequent evolution of egg recognition. This study significantly improved our knowledge of intraspecific variation in antiparasitic behavior of hosts between single- and multi-cuckoo systems and provided a novel hypothesis for empirical testing.

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**Ethical standards** The studies reported in this paper conform to the laws in the countries in which they were performed.

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