Daurian redstart Phoenicurus auroreus is a host for the common cuckoo Cuculus canorus during the second, but not the first cultch

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Research article

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Abstract

Background: Coevolution in cavity-nesting host-cuckoo systems may differ from those in open-nesting hosts due to unique conditions in cavity nests. We investigated brood parasitism in Daurian redstarts (Phoenicurus auroreus), a regular cavity-nesting host of common cuckoos (Cuculus canorus).

Results: A total of 15.6% (n =358) of host nests were parasitized by cuckoos. Cuckoos were highly successful in parasitizing Daurian redstart nests: nearly all cuckoo eggs were laid in the nest cup, and all cuckoo chicks evicted all host offspring. However, egg ejection by Daurian redstarts was egg morph specific, i.e. hosts laying white eggs ejected most real cuckoo eggs, while hosts laying blue eggs did not eject any. In contrast, hosts ejected most mimetic cuckoo eggs. Moreover, most Daurian redstarts moved to nearby villages during the second breeding attempts, where the risk of cuckoo parasitism was reduced. Parasitism only occurred during the second breeding attempt, since cuckoos had not yet arrived at the breeding grounds when hosts started to lay their first clutches, which may indicate a novel and unique anti-parasite defense, advancing breeding time of hosts.

Conclusions: Our results suggest that Daurian redstarts suffer from high risk of cuckoo parasitism showing more intense egg ejection while building nests closer to human habitation in the second clutch. This suggests that cavity-nesting hosts may show adaptations to brood parasites that differ from those of open-nesting hosts.

Background

Interactions between brood parasitic birds and their hosts are suitable model systems for studying coevolution [1, 2]. Brood parasitism reduces breeding success of the host, and, therefore, it is a major selective force shaping the life history of host [3]. To avoid being parasitized, hosts have evolved a series of strategies, such as habitat selection [4, 5], nest defence [6, 7], egg rejection [8] and nestling discrimination [9]. Moreover, laying eggs before the arrival of cuckoos is a highly efficient strategy to avoid cuckoo parasitism [10].

Coevolution between common cuckoo (Cuculus canorus) and its hosts have been thoroughly studied for many years [1]. However, most studies have been on open-nesting hosts, like great reed warbler (Acrocephalus arundinaceus) [11–14] and ashy-throated parrotbill (Paradoxornis alphonsianus) [15–17]. Few studies focused on cavity-nesting hosts [18, 19]. There are many differences between cavity and open nests such as decreased probability of predation and beneficial microenvironments [20–22]. Studies of the common redstart (Phoenicurus phoenicurus), a cavity-nesting host of the common cuckoo, found many peculiar adaptations. Firstly, the parasitism rate of cuckoos was high, while its effective parasitism rate was low, since most female cuckoos were unable to lay their eggs in the nest cup, laying either on the nest rim or outside the nest [19, 23]. In addition, almost all cuckoo chicks in open-nesting hosts evicted all host offspring, while cuckoo chicks in common redstart nests had low success [18, 19, 23]. This caused some cuckoo chicks to cohabit with host nestlings, which usually led to low fitness for cuckoo chicks [18, 19]. Thus, cavity nesting significantly influenced cuckoo-host interactions potentially shaping the arms-race differently than in open-nesting hosts.

The Daurian redstart (Phoenicurus auroreus) is a small (ca. 17 g), migratory, territorial, socially monogamous and sexually dichromatic passerine with a range extending from Mongolia to the Himalayas [24]. Daurian redstarts lay maculate eggs with two morphs, blue and white (or pink). However, eggs within a clutch have the same color [25]. In our study site, their natural cavity nests are common while the sites chosen are extremely diverse such as holes in trees, rocks or walls, under eaves, and occasionally in relatively open sites. They can easily be attracted to breed in nest-boxes. Here, we present data on cuckoo parasitism of the Daurian redstart, another cavity nesting host of the common cuckoo. This is the first systematic study of common cuckoo parasitism in this species. In addition, we investigated how Daurian redstarts resist cuckoo parasitism by having evolved novel anti-parasite defences.

Results

General Cuckoo Parasitism Characteristics

We followed 426 active Daurian redstart nests in second breeding attempts in 2018 and 2019, but excluded 68 nests, since they failed (deserted or destroyed by humans) before or during the egg-laying stage. In total, we found 49 cuckoo eggs and seven cuckoo chicks in Daurian redstart nests (Table 2) in second breeding attempts of hosts. Thus, 15.6% of Daurian redstart nests were parasitized by common cuckoos (Table 2). Among parasitized nests, one parasite egg was laid during the nest-building stage, which caused nest desertion, one egg was found in the late incubation stage, and one egg was found outside the nest cup, so parasitism rate was 14.8% (Table 2).

Table 2 Total number of nests and number of parasitized nests in the second clutch each year.

Year	Found	Failed[a]	Followed	Parasitized (%)
2018	146	28	118	13 (11.0)
2019	280	40	240	43 (17.9)
		Total	358	56 (15.6)
			Effectively parasitized[b]	53 (14.8)

[a] Nests were depredated, abandoned, or destroyed during the nest-building stage and the egg-laying stage not allowing us to determine parasitism status.

[b] Parasitized nests where the cuckoo egg was found in the nest cup in the egg-laying and the early incubation stage.

For parasitized nests, 12 were natural nests and 44 were in nests boxes. Among parasitized cuckoo eggs and chicks, nine were in white nests, and 42 in blue nests. We could not assess the morph of the other five parasitized nests, since four were found at the nestling stage, and no host eggs could be found near these nests. Finally, one nest was found at the nest building stage, and that nest was deserted.

By DNA identification, we found that cuckoos were all common cuckoos belonging to two subspecies, *C. canorus bakeri* and *C. canorus canorus*.

Cuckoo Hatching and Success

Among 49 cuckoo eggs, 18 were consumed (by ejection or predation) at the egg experiment stage, 29 hatched, and two were accidentally broken by researchers.

Cuckoo nestlings were immediately replaced into their previous nests after hatching. All cuckoo chicks could evict all host offspring (n = 29). Cuckoo chicks evicted host eggs or chicks by pushing them onto the rim of the nest. The seven cuckoo chicks were also found alone in their nests. Thus 100% cuckoo chicks succeeded in evicting all host young (n = 36). A total of 31 cuckoo chicks fledged, three were depredated, and two were dead in the nest due to difficulties leaving the nest boxes.

Response to Brood Parasitism

Nest desertion

We compared desertion rates of white and blue clutches for different treatments, but no significant difference was found (Fisher's Exact Test, all p > 0.20; Table 3), so we pooled the data for blue and white clutches for later comparisons of nest desertion. There was no difference in desertion rates for control and artificially parasitized nests with mimetic cuckoo eggs both in the first (Fisher's Exact Test, p = 0.07, Cohen's d = 0.51 [95% CI = 0.04 to 0.98]) and second breeding attempts (Fisher's Exact Test, p = 0.07, Cohen's d = 0.52 [95% CI = -0.006 to 1.05]). Desertion rates did not differ significantly between control and naturally parasitized nests (Fisher's Exact Test, p = 0.15, Cohen's d = 0.58 [95% CI = - 0.09 to 1.24]), or between control and artificially parasitized nests with real cuckoo eggs (Fisher's Exact Test, p = 0.26, Cohen's d = -0.67 [95% CI = -1.51 to 0.17]). Therefore, nest desertion is not a response to cuckoo parasitism in Daurian redstarts, and we excluded deserted nests when calculating ejection rates.

Table 3 Deserted nests of the Daurian redstart in different nest categories in 2019.

Category	Egg morph	Deserted	Not deserted	*p*	Cohen's d	95% CI
First clutch						
Control	Blue	0	8	0.53	-0.59	-1.52 to 0.34
	White	3	12			
metic cuckoo egg	Blue	2	35	0.20	0.35	-0.08 to 0.79
	White	0	46			
Second clutch						
Control	Blue	2	7	1.00	-0.06	-1.10 to 0.97
	White	2	6			
metic cuckoo egg	Blue	3	56	0.23	-0.29	-0.73 to 0.15
	White	4	27			
parasitized cuckoo eggs	Blue	0	16	0.27	-0.82	-1.85 to 0.21
	White	1	5			
parasitized cuckoo eggs	White	0	10			

Egg ejection

Ejection rate of artificially parasitized real cuckoo eggs was significantly higher than towards naturally parasitized real cuckoo eggs in white clutches (Fisher's Exact Test, p = 0.008, Cohen's d = 2.16 [95% CI = 0.78 to 3.54]) (Fig. 2). For naturally parasitized cuckoo eggs, 33.3% of white clutches were ejected by Daurian redstarts (n = 6), while none in blue clutches was ejected by hosts (n = 26), so ejection rate towards naturally parasitized real cuckoo eggs in white clutches was significantly higher than that in blue clutches (Fisher's Exact Test, p = 0.03, Cohen's d cannot be reliably estimated due to some treatments showing proportions equal to zero) (Table 4; Fig. 2). Similarly, ejection rate towards mimetic cuckoo eggs in white clutches was significantly higher than that in blue clutches in the first breeding attempt (Table 4; Fig. 3a), and the same pattern was found in the second breeding attempt (Table 4; Fig. 3b). Moreover, ejection rate towards mimetic cuckoo eggs was significantly higher than towards naturally parasitized real cuckoo eggs in blue clutches in second breeding attempts (χ^2 = 16.70, df = 1, p < 0.0001). The same situation was also found in white clutches in the second breeding attempt (Fisher's Exact Test, p = 0.005, Cohen's d = 1.85 [95% CI = 0.82 to 2.88]).

We finally compared ejection rates towards mimetic cuckoo eggs between first and second breeding attempts. And ejection rates in second breeding attempts was significantly higher than in the first breeding attempt, both in blue (χ^2 = 4.42, df = 1, p = 0.04) and white clutches (χ^2 = 10.51, df = 1, p = 0.001) (Fig. 3).

Table 4 Response of Daurian redstarts with different egg morphs to mimetic and real cuckoo eggs.

reatment	Egg morph	Accepted	Ejected (%)	χ^2	df	p	Cohen's d	95% CI
eeding attempt								
.metic egg	White	20	26 (56.5)	9.25	1	0.002	-	-
	Blue	27	8 (22.9)					
reeding attempt								
.metic egg	White	2	25 (92.6)	17.49	1	<0.0001	-	-
	Blue	31	25 (44.6)					
/ parasitized egg	White	4	2 (33.3)	-	-	0.03	-	-
	Blue	26	0 (0.0)					
y parasitized egg	White	0	10 (100.0)	-	-	-	-	-

Proximity to human habitation

We firstly compared rates of white and blue nests that were far from villages among different categories, but no significant difference was found (Pearson chi-square tests and Fisher exact test, all $p > 0.65$) (Table 5). Thus, we pooled data for white and blue clutches in the following comparisons among nest sites. The frequency of nests that was far from villages in parasitized nests was significantly higher than in non-parasitized nests ($\chi^2 = 50.40$, $df = 1$, $p < 0.0001$) (Fig. 4a). The proportion of nests that was near villages in the second breeding attempt was significantly higher than in first breeding attempts ($\chi^2 = 32.89$, $df = 1$, $p < 0.0001$) (Fig. 4b).

Discussion

We found a moderate rate of cuckoo parasitism in our study population of Daurian redstart, with 15.6% of nests parasitized, and the rate was remarkably lower than in common redstarts (more than 20% in any population). Effective parasitism rate in Daurian redstarts (14.8%) was higher than that in common redstarts (11.6% at most), because a large number of cuckoo eggs was laid outside the nest cup in common redstart nests [18, 19, 23]. Still we found a number of adaptations to cuckoo parasitism in Daurian redstarts in terms of rejection of cuckoo eggs from second rather than first clutches. A change in laying date may be an adaptation to parasitism, and a reduction in parasitism rate and a change in nest site towards villages among second compared to first clutches. We will briefly discuss these and novel adaptations to cuckoo parasitism.

Table 5 Daurian redstart nest sites in different categories.

Clutch	Egg morph	Nest distant from villages	Nest near villages	χ^2	p	Cohen's d	95% CI
First clutvh	White	41	59	0.18	0.67	-	-
	Blue	44	56				
Second clutvh							
Non-parasitized nests	White	17	100	0.20	0.65	-	-
	Blue	24	121				
Parasitized nests	White	36	4	-	1	-0.10	-0.81 to 0.60
	Blue	23	19				

Parasitism and Eviction Success

In our study, only one cuckoo egg was found outside the nest cup, and one egg was found during the nest-building stage. In the other word, nearly all cases of cuckoo parasitism were effective and successful. In common redstarts, although higher natural parasitism rates were found (generally more than 30%), less than half of all cuckoo eggs were found inside nest cups, while the remainder were either on the nest rim or on the ground outside nest boxes [18, 19, 23]. This was not caused by ejection by hosts, but rather by mislaying parasites [19, 23]. Different designs of entrances of nest boxes might contribute to this difference [19, 23]. Moreover, this difference in nest hole size might be an efficient defence against cuckoo parasitism for cavity-nesting hosts.

All cuckoo chicks in Daurian redstart nests succeeded in evicting all host eggs or nestlings. Studies of common redstarts showed that cuckoo chicks do not always succeed in evicting all host offspring [18, 19, 23]. The nest structure of the common redstart is a likely explanation, because cuckoo chicks in steeper nest cups showed lower eviction success [27]. Another possible reason is the location of the nest cup relative to the box walls [28]. Sometimes nest cups are built just next to the back wall of the nest box, and there is no space for evicted eggs and chicks, so they fall back into the nest cup again after being evicted by cuckoo chicks [28]. Cohabitation with host nestlings was likely to cause cuckoo chicks to have higher nest mortality [18, 19; but see 23].

In Daurian redstarts, some nest cups were built next to the rear wall or in the corner of the rear or the side wall. According to our observations of eviction behavior of cuckoo chicks, they sometimes failed since host offspring fell back into the nest cup. However, several minutes later, cuckoo chicks showed eviction behavior again, evicting host eggs or chicks to another side. In the end, cuckoo chicks succeeded in evicting all host offspring and they lived solitarily in host nests. That may be the reason why cuckoo chicks in Daurian redstart nests showed high fledging rate with 31 of 36 cuckoo chicks fledging.

Response to Brood Parasitism

Comparisons of nest desertion rates for different categories of nests did not reveal any statistically significant difference between control and (naturally or artificially) parasitized nests. Thus, we suggest that nest desertion is not a specific response to cuckoo parasitism in Daurian redstarts. A similar result was also found in the European common redstart [23, but see 19]. Desertion behavior as a defensive strategy against parasitism has been studied frequently [29, 30]. However, nest desertion often occurs in small-sized hosts such as chipping sparrows *Spizella passerina*, meadow pipits (*Anthus pratensis*), and blue-grey gnatcatchers (*Polioptila caerulea*) that rarely eject parasite eggs [31-33]. They are physically unable to puncture or grasp and finally eject parasitic eggs [34]. However, some medium- and large-sized hosts such as grey-backed thrushes (*Turdus hortulorum*), blackbirds (*Turdus merula*), and common grackles (*Quiscalus quiscula*) do not desert parasitized nests instead of ejecting parasitic eggs [35-37]. Moreover, nest desertion can be triggered by other factors such as parental mortality, human disturbance, or egg loss [38-40]. In fact, human disturbance might be the most obvious explanation for nest desertion in our study, since we hardly found deserted nests with daily checks.

Egg ejection

Egg recognition and rejection are the most common and efficient host defences against brood parasitism [41]. Daurian redstarts eject parasitic eggs, but has specific egg morphs, with hosts laying white clutches showing significantly higher egg ejection rates than those laying blue clutches. Daurian redstarts laying white clutches ejected 33.3% naturally parasitized real cuckoo eggs and all artificially parasitized real cuckoo eggs, while hosts laying blue clutches ejected no real cuckoo eggs (n = 26). However, Daurian redstarts laying blue clutches could eject mimetic cuckoo eggs, which implies that they also have the ability to recognize and eject cuckoo eggs, although their ejection rates were significantly lower than their white counterparts.

We propose the following hypothesis: If Daurian redstarts resist cuckoo parasitism by egg ejection, then egg ejection rates in the second breeding attempt with high risk of parasitism will be higher than in the first breeding attempt, when there is no risk of cuckoo parasitism. From egg experiments, we found that ejection rates of mimetic cuckoo eggs in the second breeding attempt were significantly higher than in the first breeding attempt both in blue and white clutches, which in line with the hypothesis.

Different cuckoo host races are known to differ in timing of breeding, breeding habitat and potentially other phenotypic traits that contribute to isolation among races [10]. Here we have shown that parasites of Daurian redstarts differ in timing of breeding, as expected for isolation by timing of breeding. This finding also suggests that such isolation by timing of breeding may affect the variance in timing of breeding, by common redstart showing greater variance than Daurian redstart. Hence both mean and variance in timing of breeding may contribute to such effects of timing as shown in this study.

Daurian redstarts laying white clutches showed significantly higher egg ejection rates in all categories. A possible explanation is that the classic color of parasitic cuckoo eggs is pale blue, closely matching those of Daurian redstart eggs of the blue morph [25]. This suggests a long co-evolutionary history with strong selection from the host by rejecting non-mimetic eggs [42]. Co-evolutionary interactions between cuckoos and hosts support egg polymorphism [43, 44]. Egg polymorphism in hosts is a specific adaptation to and a defence against cuckoo parasitism, since one female cuckoo is confined to lay one egg morph [45], which can only match its corresponding morph in host nests. Therefore, it favours hosts laying other egg morphs to discriminate and reject matching parasitic eggs [15, 46], which will reduce the rate of successful parasitism in cuckoos. In turn, to increase parasitism success, cuckoos are predicted to increase the frequency of the corresponding egg morph [44]. Most (12 of 13) known species of *Phoenicurus* redstarts lay blue egg morphs, and nearly all (5 of 6) parasitized species of *Phoenicurus* redstarts display white egg morphs [47, 48]. This suggests that a blue redstart egg is the ancestral state, and, likewise, the blue egg morph is an ancient trait in cuckoos [45]. Here, we inferred that white egg morphs are relatively recent in Daurian redstarts, and that it has evolved to cope with cuckoo parasitism. Significantly higher egg rejection rates in white clutches in our study strengthens this idea. Moreover, the large difference in ejection rates of real blue and white cuckoo eggs may be the reason why cuckoos lay eggs mostly in blue clutches [49, but see 50].

In the second breeding attempt in 2018, we found a purple cuckoo egg in a blue clutch, which was laid by a different cuckoo subspecies (see Results). We hypothesized that this may be due to mislaying by the female cuckoo. Alternatively, she may not have been able to wait to find a suitable nest, since after three days when the purple cuckoo egg was found, and the host eggs hatched.

Proximity to human habitation

The rate of parasitism in nests far from villages was significantly higher than that in non-parasitized nests, implying that there is a lower parasitism risk in nests close to villages. That may be the reason why remarkably more redstarts chose to breed near villages in the second than the first breeding attempt with high parasitic risk.

Proximity to human habitation has been proven to be an effective strategy for resisting cuckoo parasitism [5]. In a study of Oriental reed warbler (*Acrocephalus orientalis*), Møller et al. [5] found that cuckoo parasitism rates increased with distance from the nearest human habitation. That is because most parasitic cuckoos tend to avoid close proximity to humans [51, 52], so there is lower risk of cuckoo parasitism in villages. A similar pattern occurs in magpies (*Pica pica*), which tend to choose nest sites with low risk of cuckoo parasitism [53].

We only found parasitized nests in the second breeding attempts, while there were no parasitized nests in the first attempts. This difference between first and second breeding attempts is due to the common cuckoo not having arrived yet when the Daurian redstart has already initiated its first breeding attempt. Thus, we suggest that hosts advancing their timing of breeding can also effectively prevent cuckoo parasitism [10].

Conclusions

In conclusion, breeding success of cuckoos in Daurian redstart nests may be high for three different reasons: (1) Nearly all eggs can be laid in the cup of host nests by female cuckoos; (2) Daurian redstarts laying blue clutches cannot reject cuckoo eggs and nestlings, while most cuckoos choose blue clutches to parasitize; and (3) all cuckoo chicks can evict host progeny successfully. Daurian redstarts may adopt egg ejection (but it has clutch-specific morphs) and proximity to human habitation to efficiently defend their nests against cuckoo parasitism. Moreover, isolation in timing of breeding between the cuckoo and the Daurian redstart may serve as a novel and unique anti-parasite defense, which needs further study. Lastly, several results differed between common and Daurian redstarts [18, 19, 23]. We suggest that more attention should be paid to cavity nesting host-cuckoo systems, a peculiar and unique model system for the study of co-evolution.

Methods

Study Site and Study Species

Field work was conducted in a village, Shuang Yu, and surrounding forests in Jilin, Northeast China in 2018-2019. The study area (located at 43º37'19"N and 126º09'54" E) is about 50 ha and has 12 sections surrounded by secondary forest. In the study site, Daurian redstarts mainly breed in or near villages, but also in surrounding forest. We placed 230 nest boxes (140 in the village and 90 in surrounding forest) in 2016 and 2017. In the village, nest boxes were placed on power poles and corn-storing buildings about 2 m above the ground and 50 and 200 m apart. In forests, nest boxes were placed on pine trees about 2 m above the ground and 150 and 350 m apart. Box inner dimensions were 15 × 15 × 22 cm (width, depth and height, respectively) with an oblong entrance 15 and 8 cm in length and width. Common and Indian cuckoos (*Cuculus micropterus*) breed sympatrically with Daurian redstarts in our study site.

General Procedures

Daurian redstarts lay multiple clutches. If the first clutch succeeds, most females will lay a second clutch one to two weeks after hatchlings fledge. If the first clutch fails, the female will lay a replacement clutch. Replacement clutches that are destroyed may be replaced by a third or even a fourth clutch. However, we cannot always assure whether a clutch is the second or the third. Thus, we defined the first clutch as the first breeding attempt, and later clutches as the second breeding attempt. The breeding season of the first breeding attempt of Daurian redstarts was 20 April-11 May in 2018 and 17 April-10 May in 2019, respectively. While common cuckoos arrive at the study site about 10 May (we heard the first call of cuckoos on 7 May 2018, and 13 May 2019), when most hosts had already finished laying.

We searched natural nests every day throughout the breeding season, and we checked empty nest boxes weekly. All natural nests and occupied nest boxes were divided into two groups (more or less than 20 m from the nearest village). To record the laying date, we checked nests (natural nests and occupied nest boxes) in the village every day or two, and checked nests (natural nests and used nest boxes) in forests every two or three days. During the laying period of the second breeding attempt, we checked active nests every day or two, to assess whether nests were parasitized by cuckoos. Cuckoo eggs were easily distinguished from Daurian redstart eggs according to size and colour (Fig. 1).

We checked parasitized nests daily to investigate if hosts rejected cuckoo eggs (ejected cuckoo eggs or deserted nests). To avoid predation, we artificially incubated all cuckoo eggs. After six days of observation, we moved cuckoo eggs from their nests to our laboratory (in the village), candled the eggs and then placed them in incubators (INCUBATOR, LN1-60, China). We set temperature to 37.8ºC and kept humidity about 40-60%. Once hatched, we weighed chicks and returned them to their previous nests immediately (within 2h). We followed all cuckoo chicks to record their eviction or determine whether they fledged. When 10 days old, we took about 100 μL blood from each cuckoo chick for genetic analyses. All birds were sent back to their former nests after measuring and blooding.

Egg Experiments

We performed artificial brood parasitism experiments by adding one real or a mimetic cuckoo egg in 2019. We manufactured mimetic cuckoo eggs from clay and pained them with acrylic colours. Mass and size of artificial eggs were similar to those of real cuckoo eggs. During the laying stage (after three eggs had been laid) of the first and second breeding attempt in 2019, we placed one mimetic cuckoo egg into the focal nest without removing any host eggs (Fig. 1a, b).

From 28 parasitized nests with blue clutches (hereafter, blue clutches) (10 nests in 2018, 18 nests in 2019) (Fig. 1c), we found no cuckoo egg being ejected by hosts. Cuckoos rarely parasitized nests with white clutches (hereafter, white clutches) (see Results), so we moved real cuckoo eggs from blue to white clutches (Fig. 1d). When we found a parasitized blue clutch in a second breeding attempt, we would replace the real cuckoo egg with a mimetic one, and put it into a non-parasitized white nest, which was during the laying or early incubation stage (within 3 days of start of incubation). We also added these failed parasitized cuckoo eggs to suitable white clutches (as above). Each nest and experimental egg was only used once.

If an experimental egg was present in an active nest at least 6 days after it was introduced, we defined the experimental egg as ‘accepted’; if an experimental egg disappeared while the nest was active and host clutches were not reduced, we defined it as ‘ejected’ [19, 23], while within 6 days, the host abandoned the nest and the experimental egg was still there, we defined it as ‘deserted’.

In previous egg experiments with Daurian redstarts, we found that some hosts deserted their nests (unpublished data). Therefore, during the period of egg experiments, we used control nests, and followed similar procedures as for experimental nests (egg measurement, nest checking). Control nests were those found during laying or early incubation (until 3rd day of incubation) [23].

DNA Extraction and Species Identification

We collected samples (blood or tissue) from 38 cuckoo chicks and eggs. We extracted DNA with TIANamp Genomic DNA Kit (TIANGEN). The barcode sequences were amplified with Polymerase Chain Reaction (PCR) using following primers: Bird F1 (TTCTCCAACCACAAAGACATTGGCAC), Bird R1 (ACGTGGGAGATAATTCCAAATCCTG) (for cytochrome oxidase I (COI) sequences, and L14990 (CCATCCAACATCTCAGCATGATGAAA), H16065 (GGAGTCTTCAGTCTCTGGTTTACAAGAC) (for cytochrome *b* (*cytb*) sequences). All reactions were run under the following thermal cycle program: 5 min at 95 °C, followed by 35 cycles of 1 min at 94 °C, 1.5 min at 55 °C, and 1.5 min at 72 °C, and finally 5 min at 72 °C. PCR products were bidirectionally sequenced by company (BGI, Beijing, China). Sequence editing and contig assembly were performed using CodonCode Aligner version 9.0.1 (CodonCode Corp., MA, USA). Identification of tested samples was conducted using BLAST in the National Center for Biotechnology Information database (NCBI) and a local barcode library for selected taxa with a minimum BLAST cut off of 99% identity for a top match.

Statistical Analyses

We used Pearson's chi square tests to investigate how Daurian redstarts responded to cuckoo parasitism. For comparisons of expected numbers close to or less than five, we used Fisher's Exact Tests. In these cases, we reported Cohen's d and its 95% confidence intervals to represent effect size.

At first, we tested if nest desertion is a specific response to parasitism. First, we compared desertion rates of white and blue clutches among different groups, but we did not find any significant differences (see Results). Therefore, we pooled the data of deserted white and blue clutches in the following analysis. Furthermore, we compared desertion rates between control and parasitized nests, and between naturally and artificially parasitized nests.

Next, we investigated if Daurian redstarts adopted egg ejection to cope with cuckoo parasitism. First, we compared egg ejection rates between blue and white clutches if egg ejection was specific for the two morphs of clutches. Second, we compared ejection rates towards real cuckoo eggs between naturally and artificially parasitized nests. Third, we compared ejection rates towards real and mimetic cuckoo eggs. Finally, we compared ejection rates towards mimetic cuckoo eggs between first and second breeding attempt. Since we inferred if egg ejection was a strategy of cuckoo parasitism, we predicted that egg ejection rates should be higher in the second breeding attempt with higher parasitism risk.

Finally, we explored if proximity to human habitation is a strategy for coping with cuckoo parasitism in Daurian redstarts. We did so by comparing the rate of parasitism between parasitized and non-parasitized nests in second breeding attempts. Moreover, we compared rates of nests close to humans between first and second breeding attempts. If hosts resisted cuckoo parasitism by breeding near humans, rates of parasitism should be higher in second breeding attempts with high cuckoo parasitism rat. All analyses were conducted in R 3.4.2 [26]. Alpha level was set at 0.05.

Abbreviations

DNA: Deoxyribonucleic acid; FB: the first breeding attempt; SB: the second breeding attempt.

Declarations

Ethics approval and consent to participate

The experiments comply with the current laws of China. Fieldwork was carried out with permission from Yongji Forestry Bureau, Jilin, China. Experimental procedures were under agreement with the Animal Management Committee at the College of Life Sciences, Beijing Normal University (permit no. CLS-EAW-2018-001).

Consent for publication

Not applicable.

Availability of data and materials

The data sets supporting the results of this article will are available in the Dryad repository (https://doi.org/10.5061/dryad.p8cz8w9kh).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WD, APM and JZ contributed to the design of the research. JZ and DY collected the data. JZ and WD analyzed the data. JZ, APM and WD wrote the paper. All authors read and approved the final manuscript.

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Note Regarding Table 1

Table 1 was omitted by the authors in this version of the paper.

Figures

Figure 1

Daurian redstart nests with real or mimetic cuckoo eggs. (a) One real cuckoo egg in a blue clutch; (b) one real cuckoo egg in a white clutch; (c) one mimetic cuckoo egg in a blue clutch; and (d) one mimetic cuckoo egg in a white clutch.

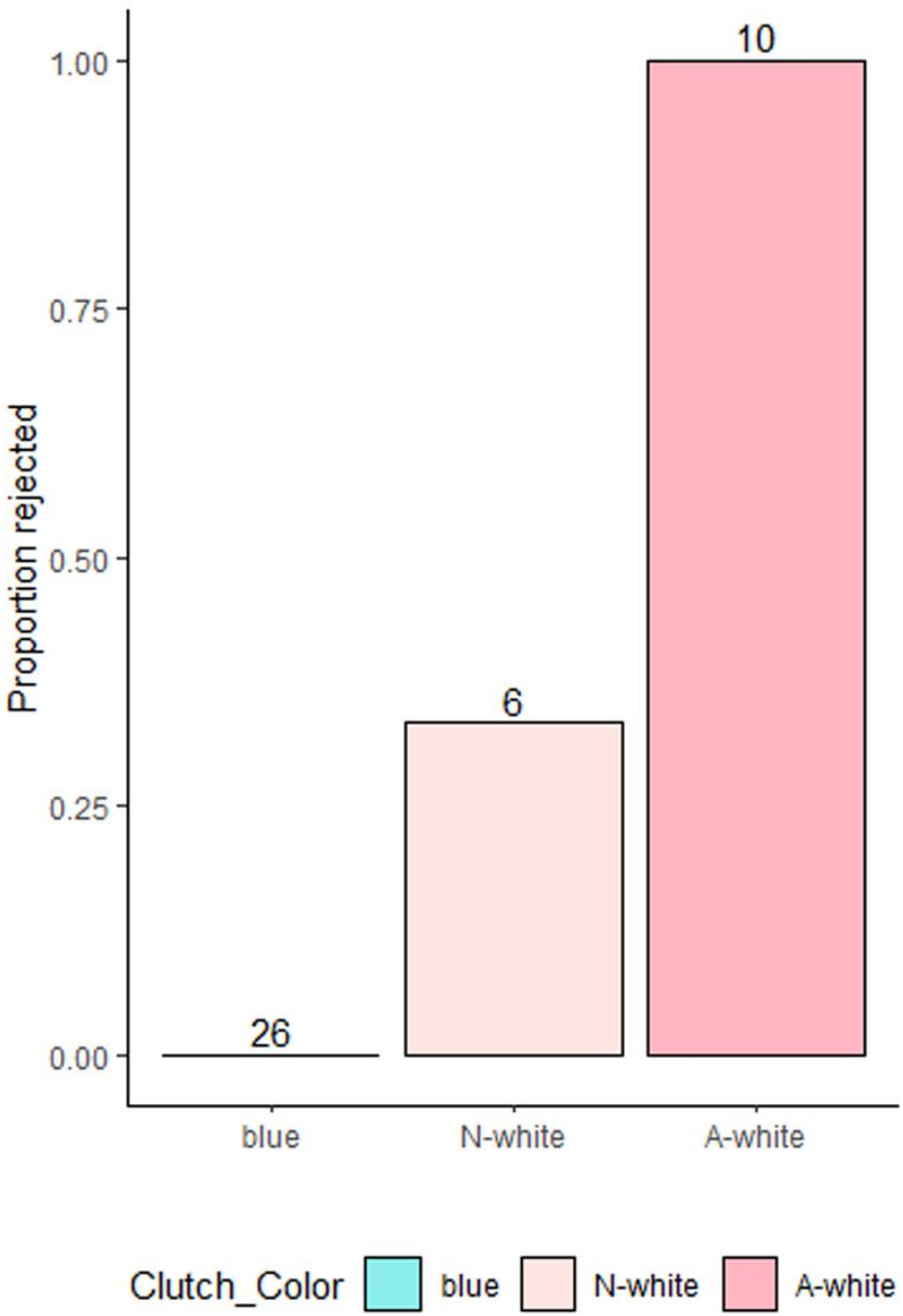

Figure 2

Rejection rates of real cuckoo eggs. N-white and A-white refer to naturally and artificially parasitized real cuckoo eggs in white clutches, respectively. Numbers on top of bars are sample sizes.

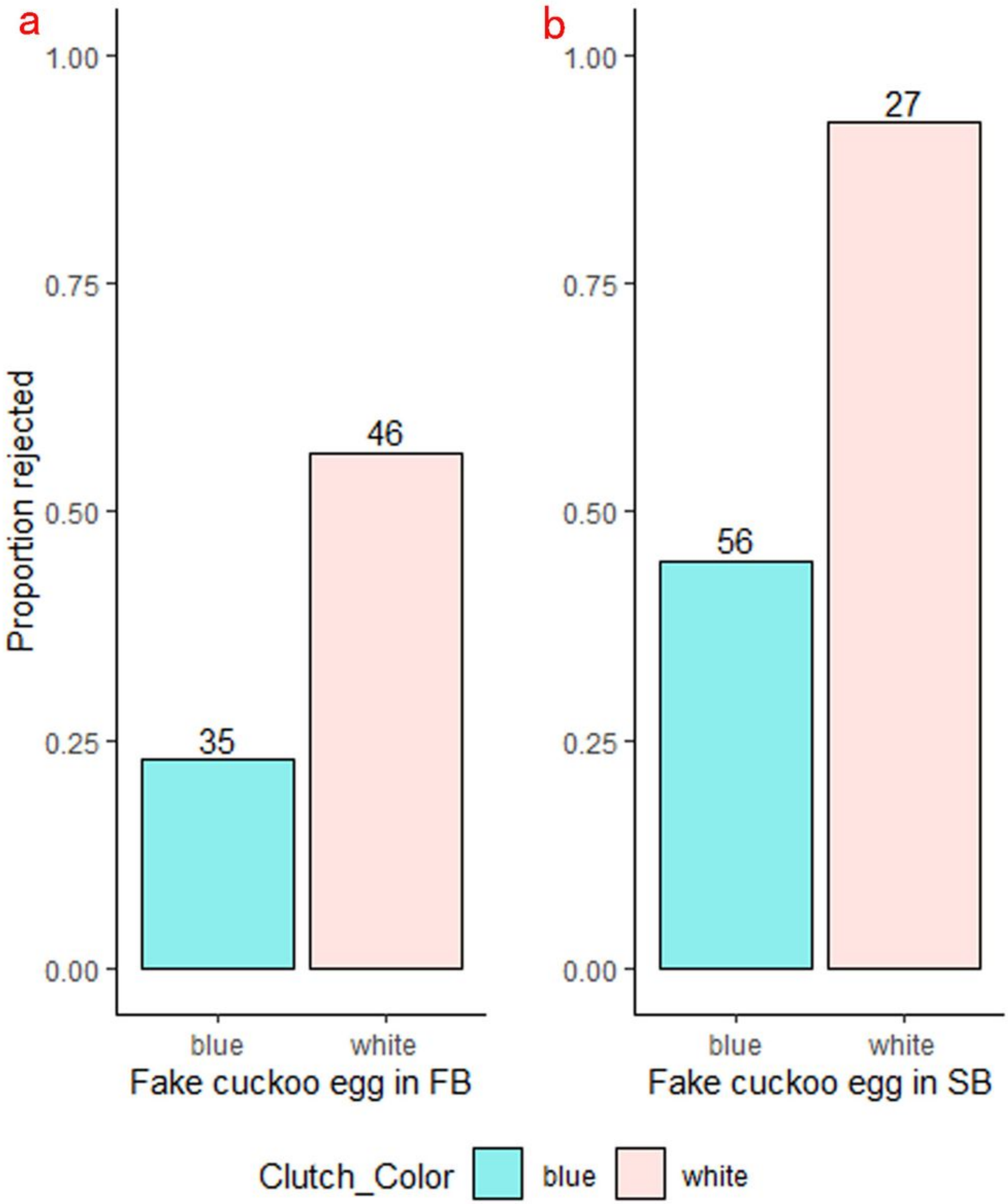

Figure 3

Rejection rates of model cuckoo eggs in (a) first breeding attempts (FB) and (b) second breeding attempt (SB). Numbers on the top of bars are sample sizes.

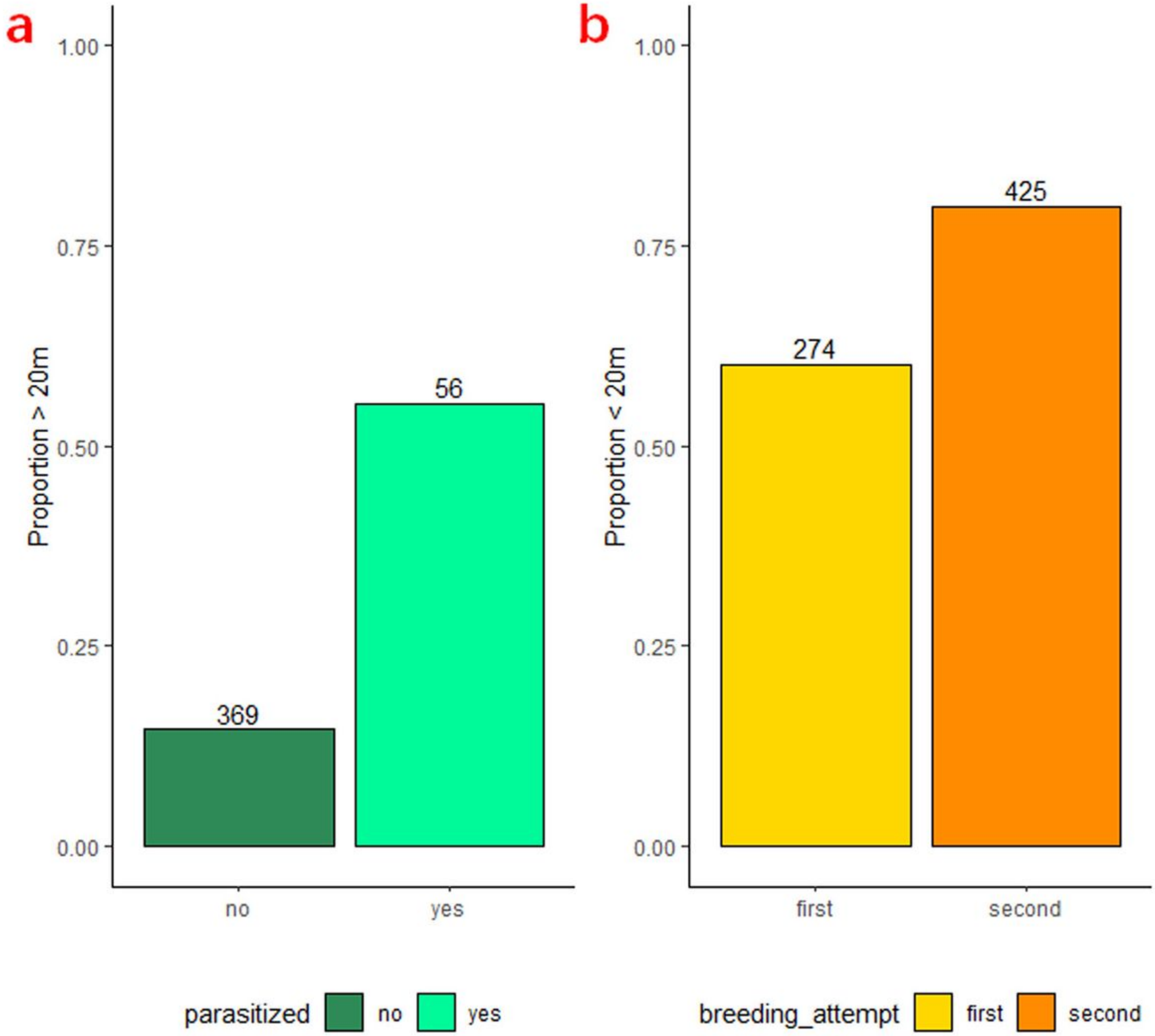

Figure 4

Distance of nest sites from villages in (a) parasitized or unparasitized nests and (b) first and second clutches. Numbers on top of bars are sample sizes.