

No change in common cuckoo *Cuculus canorus* parasitism and great reed warblers' *Acrocephalus arundinaceus* egg rejection after seven decades

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Abstract

The coevolutionary process among avian brood parasites and their hosts involves stepwise changes induced by the antagonistic selection pressures of one on the other. As long-term data on an evolutionary scale is almost impossible to obtain, most studies can only show snapshots of such processes. Information on host behaviour, such as changes in egg rejection rates and the methods of rejection are scarce. In Hungary there is an interesting case between the common cuckoo (*Cuculus canorus*) and the great reed warbler (*Acrocephalus arundinaceus*), where the level of parasitism is unusually high (around 50%). We compared host rejection rates and methods of rejection from within our own project to that of an early study carried out and published almost 70 years ago in the same region. Our comparisons revealed high and stable rates of parasitism (range: 52-64%), and marked fluctuations in the ratio of multiply parasitized nests (range: 24-52%). No difference was revealed in egg rejection rates after 7 decades (34-39%). Linear mixed-effects modelling revealed no year effect on the type host responses toward the parasitic egg(s) during the years of study (categorized as acceptance, ejection, burial, and nest desertion). Cuckoo egg rejection was primarily affected by the type of parasitism, as more cuckoo eggs were rejected during single parasitism than from multiply parasitized nests. Our comparison did not reveal any directional changes in this cuckoo-host relationship, except a slight decrease in the frequency of multiple parasitism, which is likely to be independent from coevolutionary processes.

Keywords Brood parasitism · Arms race · Evolutionary equilibrium · Long-term changes · Adaptations

Avian brood parasitism by the common cuckoo (*Cuculus canorus*; hereafter cuckoo) severely reduces host fitness (Davies 2000, Hauber and Dearborn 2003). A successful parasitic event causes the total loss of the host's current breeding success, as the cuckoo chick evicts the rest of the clutch or brood (Honza et al. 2007, Anderson et al. 2009). Raising the parasite offspring may take up the whole breeding season of the host (Davies 2000), and potentially impose future fitness costs. Within this scenario, an evolutionary arms race may occur (Dawkins and Krebs 1979, Davies and Brooke 1989, Moksnes et al. 1991). Some authors suggest that the arms race could be cyclic, where periods of adaptation and no adaptation may follow each other (Soler et al. 1998). This is facilitated by asymmetries in local selective pressures (Gandon et al 2008), where subpopulations are surrounded by varied environmental factors, thus creating dissimilar sets of adaptive traits among subpopulations. This in turn results in a greater phenotypic and genotypic diversity for the overall population (Thompson et al. 2002). As shown by another study on the great spotted cuckoo (*Clamator glandarius*) and its magpie (*Pica pica*) host at a small geographic scale (Martin-Gálvez et al 2007, Soler et al. 2013), this creates appropriate conditions for mosaic coevolution. This theory predicts that several parameters of brood parasitism, e.g. parasitism rate and host defence (egg rejection), covary with within-plot productivity of hosts, so individuals with highly evolved antiparasite defence ability may occupy the best plots with a high risk of parasitism. In this process, cuckoos abandon a host population where antiparasite defences are well developed, but may recolonize it when antiparasite defences are lowered. During the arms race, the hosts' abilities to reduce the cost of parasitism and the parasites' abilities to trick the host into raising its offspring are competing with each other (Røskft and Moksnes 1998, Takasu 1998, Davies 2000, Krüger 2007), resulting in monotonous gains or oscillating changes in trait values on both sides (Rothstein 1990, Takasu 2003). Others put forward the equilibrium hypothesis, according to which interacting factors are making long-standing coexistence between host and parasite feasible (for an overview see Lotem and Nakamura 1998). Acceptance of the parasite offspring can also be viewed as a consequence of insufficient evolutionary time for a more adaptive response to occur and spread in the host population (the time lag theory: Lotem and Nakamura 1998).

The most effective mechanism for host defence is based on egg recognition (Rothstein 1975, Moksnes et al 1991, Hauber and Sherman 2001, Hauber et al. 2006), which, in turn, can propel an increasing level of egg mimicry on behalf of the parasite (Davies 2000). Krüger et al. (2009) showed that speciation in parasitic cuckoos is faster than that of their non-parasitic relatives, but there is no empirical evidence on the speed of coevolution. There are examples of population-level adaptations over relatively short periods of time (Takasu et al. 1993, Avilés et al 2006). If the hosts' evolutionary potential allows for it, hosts' adaptations sooner or later win the arms race and old host species are abandoned. A good example is the red-backed shrike (*Lanius collurio*), which used to be a favoured cuckoo host in Hungary but was abandoned when red-backed shrikes developed such a high-level of egg discrimination ability that parasite eggs were rejected in 93.3% of cases (Lovászi and Moskát 2004; for other cases see Davies and Brooke 1989, Moksnes and Røskft 1992, Honza et al. 2004, Procházka and Honza 2004, Yang et al. 2014a). Egg rejection behaviour may be retained in the absence of parasitism (Lahti 2006), even for millions of years (e.g. Peer et al. 2011), although it may also show deficiency in time in the lack of brood parasitism compared to parasitized populations (Yang et al.

2014b). The speed of coevolution in parasites and their hosts seems to be primarily affected by population dynamics (Soler et al. 1998, Gandon et al. 2008) and habitat structure (Røskoft et al. 2002). New hosts are ‘conquered’ within a few centuries or even decades (Takasu et al. 1993), and parasite egg appearance might be matched relatively quickly to that of the host (Avilés et al. 2006). However, the arms race model is often oversimplified (Lotem and Nakamura 1998) and the lack of historical data makes it difficult to evaluate the different stages on an evolutionary time scale. Consequently, there is the need for long-term field studies, which may provide additional pieces to the coevolutionary puzzle among hosts and brood parasites (Møller and Soler 2012).

Recent studies revealed that cuckoos may know the appearance of their eggs, and females try to increase egg matching with active selection of the host nest (Avilés et al. 2006, Cherry et al. 2007a, Honza et al. 2014, but see Antonov et al. 2012). In contrast, cuckoos do not seem to distinguish between cuckoo and great reed warbler eggs when removing one egg from the host clutch. Cuckoo females randomly take one egg from the nest (Davies and Brooke 1988, Moskát and Honza 2002) and this implies that their offspring may be evicted along with the host’s offspring by another, earlier hatching cuckoo chick. Despite the fact that multiple parasitism is thought to be generally rare in the cuckoo-host system, it can reach high levels in Hungary, as about 36% of the parasitized nests contained 2-4 cuckoo eggs (Moskát and Honza 2002). Multiple parasitism results from repeated parasitic events by different cuckoo females (Moskát et al. 2009).

Egg discrimination ability of hosts is affected by several factors (Davies 2000, Stokke et al. 2007) and may be characteristic of a host population. For example, the duration of parasitism and stage in the arms race (Davies and Brooke 1989, Davies 2000), host age (Lotem et al. 1992, Strausberger and Rotshtein 2009, Molina-Morales et al. 2014, Moskát et al. 2014), stage of breeding (Moskát et al. 2014), stage of season (Lotem et al. 1992), and multiple parasitism (Moskát et al. 2009, Gloag et al. 2014) are such factors. Egg rejection behaviour in cuckoo hosts may have a genetic background (Martin-Gálvez et al. 2006), so hosts may retain egg rejection abilities after parasitism is terminated (e.g. Honza et al. 2004, Lovászi and Moskát 2004, Lahti 2006). Cuckoo hosts may also retain their egg rejection ability when they are introduced into parasite-free areas (Soler and Møller 1990, Yang 2014a). Alternatively, defence can be based on phenotypic plasticity, where hosts may quickly reduce their egg rejection ability when parasitism is declining (Thorogood and Davies 2013). If the distribution of parasitism within a population is non-random, this may affect the hosts’ resistance to parasitism, and host lineages might develop different antiparasite defence levels within the same population (Grim 2002, Hoover et al. 2006, Martín-Gálvez et al. 2007, Soler et al. 2013).

We studied the characteristics of cuckoo parasitism on great reed warblers (*Acrocephalus arundinaceus*) in the Hungarian Great Plain. We compared our current data with long-term (< 70 yrs; Molnár 1944) and short-term results (< 10 yrs; Moskát and Honza 2002) published for the same population to seek detectable changes in the rate of parasitism or host reactions. Although these data sets are only snap-shots of the long-term cuckoo-host coevolutionary relationship, such data sets are rare. We hypothesized that an increase in antiparasite defence, as a consequence of an escalating coevolutionary arms race between cuckoos and their hosts, changed hosts from unconditional acceptors in the incidences of new parasitism toward being strong rejecters (c.f. Davies 2000). In this framework,

we predict higher rejection rates of cuckoo eggs in the most recent data set than in the old data. Alternatively, we predict no change in hosts' antiparasitic defence if the cuckoo-host relationship is at equilibrium (sensu Brooker and Brooker 1996, Lotem and Nakamura 1998, Takasu 1998).

Material and methods

We carried out fieldwork in 2006 and 2007, in the surroundings of Kiskunlacháza (47°19'N; 19°05'E) and Apaj (47°07'N; 19°06'E), ca. 50 km south of Budapest, Hungary. Fieldwork started in mid-May and finished in late June, when great reed warblers and cuckoos were still active, but with few new nests being built in the area. Great reed warblers bred in channel-side reed beds, i.e. 2-5 m wide long reed strips (Figure 1). Typically, there are different tree species (mainly *Salix* and *Populus* spp.) along the channels that serve as vantage points for cuckoos, together with pylons and power lines, when searching for nests (Moskát and Honza 2000).

As a fine example of meticulously documented early investigation of the cuckoo-host system, Molnár (1944) started collecting data in the reed beds of the oxbow lakes of the River Körös near the town Szarvas (46°52'N; 20°33'E) in 1935, and worked there until 1944. We also used his original field notes for details. His data set suffered from fluctuating research intensity, and in certain years experiments dominated over observations on the natural host-brood parasite system. For this reason, we selected three years' data between 1940 and 1942, when sample sizes were large and suitable for comparison, i.e. daily nest checking was done on non-experimental nests. Unfortunately, this study site has been mainly degraded, as banks were built-in by summer cottages and the majority of the reed-beds were removed (Lilla Barabás, in litt.). For this reason, we carried out our research at Apaj, and also used results of cuckoo parasitism on great reed warblers at Apaj from the years 1998-99 published by Moskát and Honza (2002). The latter study was carried out ca. 80 km northwest from Molnár's study site, where cuckoos and great reed warblers were also abundant. The 1998-99 sites greatly overlapped with our 2006-2007 study sites, and also included adjacent channels with similar habitats. Such small geographical shifts are necessary because some sections of the habitat suffer from year-to-year perturbations (e.g. poisoning and reed cutting, air pollution caused by heavy traffic, channel dredging). As most of the reed beds are connected or close to each other in Hungary, and there is no geographic barrier among the breeding sites of this species, all great reed warblers can be regarded as members of one interbreeding population. Genetic analysis revealed high similarity of breeding great reed warblers in different parts of the Hungarian Great Plain at large distances (130 km) (Moskát et al. 2008), although the population is not homogeneous (Mátrai et al. 2012). In a previous study at three sites in Hungary similar rejection rates were revealed in great reed warblers' egg rejection (Moskát et al. 2008), suggesting that the two sites included in this study are unlikely to confound the results.

We compared parasitism rate, ratio of multiple parasitism and host responses to parasitism among the datasets. We regarded host response as acceptance if the cuckoo egg remained in the nest for at least six consecutive days. We categorized egg rejection as (a) ejection; (b) desertion of the nest or

(c) burial of the cuckoo egg (when it was built over by the nest owner; for more details see Moskát and Honza 2002). Cuckoo females always remove one egg from the clutch they parasitize and great reed warblers lay one egg each day until clutch completion. This allows the recording of the most likely scenario on the daily visits (i.e. clutch size increased by one host egg: no parasitic event; clutch size remaining constant before clutch completion: parasitic event followed by ejection; clutch size remaining constant with one parasite egg: parasitic event without ejection etc.) A previous study revealed that great reed warblers more readily accept cuckoo eggs in multiply parasitized nests than in cases of single parasitism (Moskát et al. 2009), so we analyzed host responses to parasitism regarding the type of parasitism (single or multiple).

Although rejection cost (mistakenly ejected or destroyed host egg(s) ejected together with the cuckoo egg) and recognition error (mistakenly ejected host egg(s) with the cuckoo egg remaining in the nest) would be informative on the level of egg rejection ability of the hosts, we did not compare them due to uncertainty in Molnár's data. It was difficult to distinguish recognition errors from partial predation, so we did not find it useful for this purpose. Great reed warblers seem to make these errors rarely, as we found clear cases of rejection costs two times (the costs of one or two great reed warbler eggs), and three times in case of recognition errors (one great reed warbler egg was missing in each case) in the Molnár dataset. For more recent data see Moskát and Honza (2002).

Statistical analyses

We used linear mixed-effects modelling with SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) to evaluate the effects of 'type' (single/multiple parasitism) as a fixed factor on 'host response to parasitism' as a dependent variable. In our models, the dependent variable either was binary (acceptance or rejection of the cuckoo egg) or categorical (after a six-day control period acceptance, ejection, desertion and burial; see Moskát and Honza 2002 for more details of host responses). Linear mixed-effects models were useful to avoid pseudoreplication in data (Bolker et al. 2009, Nakagawa and Hauber 2011). As we analysed host responses to parasitism on the basis of individual cuckoo eggs, we considered nest ID as a random (subject) factor; and cuckoo egg ID as a repetition in the model. We also used SPSS version 17.0 for other statistical comparisons (linear regression, chi-square test).

Results

Parasitism rate was high and stable in all three periods compared (range: 52-64%, $n_{\text{tot}} = 671$ nest; $\chi^2_6 = 11.854$, $P = 0.065$; Table 1; Figure 2). A high proportion of parasitic eggs was found in multiply parasitized nests in this population (range: 45-58%, $n_{\text{tot}} = 541$ cuckoo eggs). Multiple parasitism (range: 24-52%, $n_{\text{tot}} = 368$ parasitized nests; Tables 1 and 2) was slightly lower in the current as compared to the historical samples ($\chi^2_6 = 12.943$, $P = 0.044$). Host responses to parasitism (acceptance/rejection) did not differ significantly among the sampled periods (range of rejection rates: 34-45%, $n_{\text{tot}} = 376$ cuckoo

eggs; Table 3 and Figure 2; for all cases: $\chi^2_3 = 4.324$, $P = 0.115$), neither in single ($\chi^2_3 = 2.439$, $P = 0.295$) or multiple parasitism ($\chi^2_3 = 2.844$, $P = 0.241$).

Linear mixed-effects modelling revealed that host egg rejection behaviour (accept or reject) was affected only by the type of parasitism (single/multiple: $F_{1,388} = 12.470$, $P < 0.001$). This means that hosts are less likely to escape brood parasitism by ejecting the cuckoo egg when the nest contains only one parasitic egg. Year did not affect great reed warblers' egg discrimination ($F_{6,299} = 1.739$, $P = 0.112$). When the interaction of year x type of parasitism was included the interaction term was non-significant ($F_{6,367} = 1.443$, $P = 0.197$), and other effects remained the same (type of parasitism: $F_{1,392} = 5.348$, $P = 0.21$; year: $F_{6,367} = 1.993$, $P = 0.066$). The effect of time and type remained similar when we used the dependent variable (rejection categories) separately, i.e. ejection, desertion and burial (type of parasitism: $F_{1,377} = 8.469$, $P = 0.004$; year: $F_{6,258} = 1.378$, $P = 0.224$).

Discussion

Our results revealed no difference in the rate of parasitism and host responses to cuckoo eggs among the data sets (1940-1942: Molnár 1944, 1998-1999: Moskát and Honza 2002 and 2006-2007: present study). This is surprising, given that hosts may gain high-level adaptations against brood parasitism in relatively short time frames. For example, in central Japan a new cuckoo parasitism started on azure-winged magpies (*Cyanopica cyana*) in the 1970s (Yamagishi and Fujioka 1986), when this host did not show effective rejection toward parasitic eggs, but under the high parasitic pressure their defence developed quickly and they learned to reject eggs of the common cuckoo within two decades from the onset of parasitism (Nakamura et al. 1998; H. Nakamura pers. com.). However, in that population parasite egg mimicry did not change accordingly (Takasu et al. 2009). Soler and Møller (1990) also reported a rapid increase of antiparasite defence in a magpie (*Pica pica*) population in Spain, where the distribution area of the great spotted cuckoo (*Clamator glandarius*) became overlapping with this host species. However, this overlap may also have had occurred previously. In this relationship, antiparasite defence levels increased significantly in both natural and experimental parasitism (Soler et al. 1994, Soler and Soler 2000). The main difference between any of these examples and our case is that we studied an existing host-brood parasite relationship, not a newly formed one. The speed of adaptations may depend on several factors, e.g. the stage of the coevolutionary process (Takasu et al. 2009); the incidence level of parasitism (Røskaft et al. 2002), or gene transfer from nearby populations (Martín-Gálvez et al. 2007, Soler et al. 2001).

Egg discrimination is an important antiparasite defence mechanism in the great reed warbler (Moskát et al. 2009, Pozgayová et al. 2011). In several cuckoo host species nest guarding may also be a defence mechanism (e.g. Welbergen and Davies 2009). Experimental studies revealed that great reed warblers frequently attack stuffed cuckoos (Bártol et al. 2002) and model cuckoos (Honza et al. 2006) at their nests in our study area. However, the efficiency of this frontline defence is questionable, as we observed several times that when parasitizing cuckoos were driven away by hosts, they came back to the same nest several times within the next 24 hours and so the attempts eventually resulted in successful parasitism (C. Moskát, unpublished). Interestingly, egg discrimination and nest defence did not correlate in the great reed warbler (Trnka and Grim 2014), as different cognitive mechanisms are

responsible for these two types of defence. Unfortunately, we had no quantitative data on hosts' aggression toward cuckoos from the 1940-1942 period to compare with recent data, but Molnár (1944) observed several cases when great reed warblers attacked cuckoos heavily.

Cuckoo parasitism is costly for the hosts, as the young cuckoo chicks evict all host eggs or hatchlings from the nest (Honza et al. 2007). Our great reed warbler host population is likely to be a sink under such a high parasitic pressure. A well-connected metapopulation structure of the host species with a high carrying capacity of brood parasitism (potentially including allopatry with the cuckoo) could be one of the possible solutions to stabilize this system. Simulations revealed that if naïve individuals with poor egg discrimination ability immigrate every year, it may slow down the antiparasite adaptation of the host population (Barabás et al. 2004). The immigration of hosts seems to be important in stabilizing our host-brood parasite relationship that would go extinct quickly without this process as the consequence of the heavy brood parasitism. The immigration of naïve individuals into our study area may slow down the evolution of adaptations. As shown by another study on the great spotted cuckoo and its magpie host at a similar geographic scale (Martin-Gálvez et al 2007, Soler et al. 2013), this creates appropriate conditions for mosaic coevolution. It was also suggested that rejection and acceptance are better explained by genetic rather than by geographical distances among subpopulations (Soler et al. 2001).

As female cuckoos lay one egg per host nest (Moskát and Honza 2009), multiple parasitism only occurs when the availability of appropriate nests is low. The unusually high parasitism rate with high proportions of multiply parasitized nests is a unique characteristic of the Hungarian cuckoo-great reed warbler relationship. As our study revealed, about half of the cuckoo eggs can be found in multiply parasitized nests. Even though we cannot exclude the possibility that this reflects an adaptation in cuckoos, the decrease in multiple parasitism in 70 years can signal a decrease in 'parasite load' which might be independent from co-evolutionary processes linking hosts and brood parasites. It may simply be the consequence of changes in the habitat resulting in differential dispersion of hosts and/or cuckoos, or changes in cuckoo population density for reasons so far unbeknownst to us.

Clutch characteristics are expected to show lower variation in an advanced stage of the arms race than in undeveloped stages (Moksnes and Røskaft 1995, Øien et al. 1995, Stokke et al. 1999, 2005), as rejection is driving intra-clutch variation down to a very low level. As appropriate data on clutch appearances from the studied historical period are lacking, we could not evaluate whether intra-clutch variation changed in the last 70 years in our host-brood parasite relationship. Recent spectrophotometric studies in single parasitism revealed that although mimicry is an important factor in egg discrimination (Igic et al. 2012, Moskát et al. 2012), higher, not lower, intra-clutch variation facilitated egg rejection in our hosts (Cherry et al. 2007b).

Compared to generalist brood parasites, a more advanced stage in the arms race model is expected to be found among the specialist cuckoos and their hosts (e.g. Dawkins and Krebs 1979, Davies and Brooke 1989, Moksnes et al. 1991, Davies 2000, Takasu 1998, Krüger 2007). For the brown-headed cowbird, another well-studied brood parasite of the Northern hemisphere, the time lag and evolutionary equilibrium hypotheses seem to be more generally suited (Lotem and Nakamura

1998, Rothstein and Robinson 1998). However, egg discrimination in the generalist cowbird system has developed in only a small number of host species than among cuckoo hosts (Rothstein and Robinson 1998, Davies 2000).

We compared basic characteristics of cuckoo parasitism on great reed warblers in a long-term (about seven decades) and a short-term (almost one decade) view and the most important antiparasitic defence adaptation of hosts, the egg rejection behaviour. However, a future study should investigate the changes of cuckoo egg mimicry in the last seven or more decades. Although we had no exact data for several decades from the intervening period, ornithological notes on the continuously high cuckoo parasitism rate on this host (Moskát and Honza 2002, Egon Schmidt in litt.) is suggestive of a temporary equilibrium instead of a time lag with a stable rate of parasitism and hosts' antiparasite level.

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Tables

Table 1. Parasitism rates and the relative frequency of multiple parasitism in the great reed warbler.

Study years	Total no. of nests	Unparasitized nests	Parasitized nests (total)	Multiple parasitism*	Source
1940	231	120 (47%)	111 (53%)	32 (29%)	Molnár 1944
1941	89	43 (48%)	46 (52%)	24 (52%)	Molnár 1944
1942	54	21 (39%)	33 (61%)	14 (42%)	Molnár 1944
1998	103	38 (37%)	65 (63%)	27 (42%)	Moskát & Honza 2002
1999	90	32 (36%)	58 (64%)	17 (29%)	Moskát & Honza 2002
2006	54	26 (46%)	29 (54%)	7 (24%)	present study
2007	50	20 (43%)	26 (57%)	7 (27%)	present study

* = multiply parasitized nests are expressed as percent of all parasitized nests

Table 2. Number of non-parasitized, single and multiple parasitized nests. Multiple parasitism is shown in categories of cuckoo eggs in clutch. (Please take note of the uneven sizes of the study areas in the Apaj site, related to reed quality and intensity of the study.)

No. of cuckoo eggs per clutch	Szarvas 1940-1942 (Molnár 1944)	Apaj 1998-1999 (Moskát & Honza 2002)	Apaj 2006-2007 (present study)
0	114 (37.4%)	70 (36.3%)	50 (48.1%)
1	121 (39.7%)	79 (40.9%)	40 (38.5%)
2	50 (16.4%)	28 (14.5%)	10 (9.6%)
3	17 (5.6%)	12 (6.2%)	3 (2.9%)
4	2 (0.6%)	4 (2.1%)	1 (0.9%)
5	1 (0.3 %)	0 (0%)	0 (0%)

Table 3. Great reed warblers' responses toward cuckoo eggs in instances of single and multiple parasitism.

	Host responses				
	Acceptance	Rejection*	Ejection	Desertion	Burial
Szarvas 1940-1942 (Molnár 1944)					
Single	33 (59%)	23 (41%)	8	7	8
Multiple	68 (71%)	28 (29%)	4	15	9
All	101 (66%)	51 (34%)	12	223	17
Apaj 1998-1999 (Moskát & Honza 2002)					
Single	41 (58%)	30 (42%)	13	14	3
Multiple	67 (73%)	25 (27%)	6	18	1
All	108 (66%)	55 (34%)	19	32	4
Apaj 2006-2007 (present study)					
Single	20 (57%)	15 (43%)	11	3	1
Multiple	17 (65%)	9 (35%)	3	6	0
All	37 (61%)	24 (39%)	14	9	1

* Including egg ejections, nest desertions and egg burials.

Figure Legends

Figure 1 Mean values of common cuckoo parasitism on great reed warblers in three study periods (1940-42, 1998-99, and 2006-2007) in Hungary. (Parasitism rate = percent of parasitized nests, multiple parasitism = percent of parasitized nests which contained more than one cuckoo egg, rejection rate in single parasitism = percent of cuckoo eggs rejected by egg ejection, nest desertion or egg burial, and rejection rate in multiple parasitism = percent of cuckoo eggs rejected by ejection, desertion or egg burial.)

