Final Report

"Evolutionary arms race vs. evolutionary equilibrium: the analysis of a host-brood parasite relationship"

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Introduction

We studied co-evolutionary relationships between the common cuckoo (*Cuculus canorus*; hereafter "cuckoo"), an avian brood parasite, and one of its primary hosts, the great reed warbler (*Acrocephalus arundinaceus*). In Hungary parasitism rate is unusually high, it is above 50%, instead of the typically low rates in common cuckoo-songbird host relationships (1-10%, Davies 2000). Other specific characteristic is the high proportion of multiple parasitism, which is a relatively rare phenomenon (Fig. 1). Historical data on this parasitic relationship are also available from central Hungary from the last one hundred years, making it especially suitable for scientific studies of co-evolutionary relationships between brood parasites and their hosts.

Fig. 1 A great reed warbler nest containing 4 cuckoo eggs from different cuckoo females and one host egg (in the top right position of the picture). Multiple parasitism reduces hosts' ability to recognize and reject cuckoo eggs, but it is a selective force for the brood parasite, as only one cuckoo chick could be fledged from a nest.

The aim of the project was to evaluate the state of the great reed warbler-common cuckoo parasitic relationship by comparing recent data on parasitism with historical results, evaluating hosts antiparasitic adaptations (egg discrimination ability), as well as brood parasites' adaptations to the parasitic life form and a well-suitable host species, the great reed warbler. Our project consisted of two main parts:

(1) *Long-term adaptations in cuckoos and counter-adaptations in their hosts*,
and
(2) *Aspects of the adaptation mechanisms in heavy cuckoo parasitism.*

In the present report we discuss our results obtained within the framework of our four-year OTKA grant (No. 83217). In this report we follow the structure of our original research plan.

Main scientific results *(The <u>numbers</u> in this list refer to the numbers in the original research plan)*

(1) *Long-term adaptations in cuckoos and counter-adaptations in their hosts*

(a) Changes in the basic characteristics of cuckoo parasitism (parasitism rate, level of multiple parasitism) and (c) Changes of hosts egg discrimination ability in time (Zölei et al. ms)

We studied the characteristics of cuckoo parasitism on the great reed warbler host population in central Hungary. We evaluated our current data by comparing them with results published previously on the same populations. Although these data are still only snap-shots of the long-term cuckoo-host co-evolution, such data-sets are rare, and these may let us compare our present results with short-term (ca. 10 yrs; Moskát and Honza 2002) and long-term (ca. 70 yrs; Molnár 1944) data seeking for detectable changes in the rate of parasitism or host reactions. We hypothesized an increase in hosts' antiparasitic defence, as a consequence of an escalating co-evolutionary arms race between cuckoos and their hosts, changing hosts from unconditional acceptors in 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 cuckoo-host relationship is at an equilibrium (sensu Brooker and Brooker 1996, Lotem and Nakamura 1998, Takasu 1998).

The main results on recent vs. historical comparison of the basic parameters of this brood parasitic relationship can be consulted in Fig. 2 and Table 1.

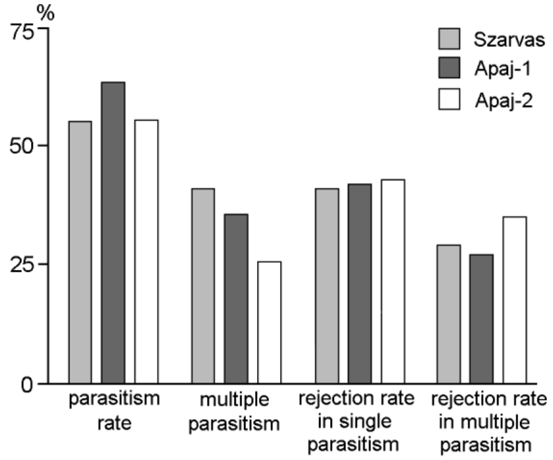

Fig. 2 Changes of parasitism rate, multiple parasitism, and hosts' egg rejection rates in single and multiple parasitism over a 70 years period in central Hungary. Sites: Szarvas (Molnár 1944), Apaj-1 (Moskát and Honza 2002) and Apaj-2 (present study).

Table 1. Great reed warblers' responses toward cuckoo eggs in single and multiple parasitisms.

	Host responses				
	Acceptance	Rejection*	Ejection	Desertion	Burial
Szarvas (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-1 (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-2					
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.

Only multiple parasitism changed ($\chi^2_6 = 12.943$, $P = 0.044$), all other parameters did not show any significant difference in time. 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 more than one parasitic egg. Year did not affect great reed warblers' egg discrimination ($F_{6,299} = 1.739$, $P=0.112$). The effect of time and type remained similar when we used the dependent variable (rejection categories) separately, i.e. as ejection, desertion and burial (type of parasitism: $F_{1,377} = 8.469$, $P = 0.004$; year: $F_{6,258} =1.378$, $P = 0.224$).

Our results on cuckoo parasitism on great reed warblers after 70 years suggest no change in parasitism rate and host egg rejection. Although 7 decades are not long when measured on the evolutionary time scale, but the scarcity of such type of comparisons, because of the rarity of historical data, our comparisons may well be relevant in host-brood parasite coevolution.

(b) *Changes of cuckoo eggs' appearance (Bán et al. 2011, Igic et al. 2012, Moskát et al. 2012, Geltsch et al. ms)*

We compared the mimicry of cuckoo eggs in respect to eggs of great reed warbler hosts in Hungary in three ways:

(i) We compared cuckoo egg mimicry of the Hungarian population with the Japanese population to a similar *Acrocephalus* host species by perceptual (visual) modeling (Moskát et al. 2012), and for egg shape (Bán et al. 2011). In Japan cuckoos are not so strictly specialized on hosts than in Europe, which was supported by the lower JND ('just noticeable difference') values of chromatic difference in Hungary. This means that recognition of a cuckoo egg is more difficult for the host in Hungary than in Japan. However, hosts showed almost the same rejection rates against cuckoo eggs in the two sites, suggesting that hosts' egg recognition is more developed in Hungary.

(ii) We also compared the changes of spottiness of cuckoo and host eggs along a more than 100 year long period (Geltsch et al. ms). For pattern analyses we used the computer programs developed in MATLAB by Professor Martin Stevens (University of Exeter, U.K.).

We used digital image analysis to compare five variables of eggshell pattern over time (relating to aspects of marking size, diversity, and distribution of spots on the egg surface). Each feature showed different trends in time, and with four of these the changes were significant, and although only two of them were species specific. Specifically, the proportion of the egg surface covered with pattern increased marginally over time, and the distribution of spotting became more even over the egg surface. However, the contrast of the markings decreased and their aggregation became less frequent over the time on the eggs. In general, the magnitude of these changes was greater for great reed warblers than cuckoos. Although brood parasitism appears to be a selective force influencing changes in some egg traits (e.g. pattern coverage and contrast), which could be important in egg recognition by hosts, other characters seem to be less important (for example, marking sizes showed no change during the study period). Our results show how different aspects of egg appearance may change in response to selection from brood parasites.

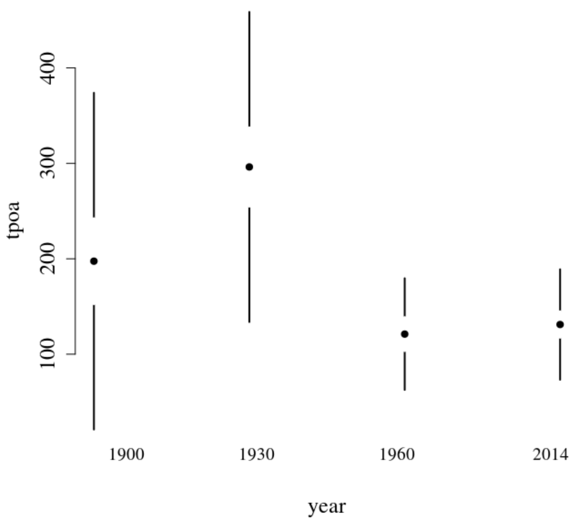

Fig. 3 Changes in eggshell total power average ('tpoa') for mimicry between common cuckoos and great reed warblers over time. (Mimicry was defined as the absolute difference between the two species egg characters.) On the graphs, the full circles shows the mean, while the white space between the circles and the vertical lines represent the standard error for the mean, while the vertical lines denote the standard deviation of the samples.

We used permutational ANOVA for the comparison of different time classes of variables, and also used time series analysis for prediction. For prediction we used the method ARIMA (autoregressive integrated moving average), which is the generalization of the autoregressive moving average (ARMA) models. From the several results obtained by the analyses we show an example here, the changes of egg mimicry over time (Fig. 3). We concluded that most of the different eggshell spotting characters evolved independently from each other, which agrees with a recent similar study (Spottiswoode and Stevens 2012).

(iii) We had the possibility to join an international study on cuckoo egg mimicry, which revealed the chemical components of mimicry in the common cuckoo and their redstart (*Pheonicurus phoenicurus*), reed warbler (*Acrocephalus scripaceus*) and great reed warbler (*A. arundinaceus*) hosts in Finland, Czech and Hungary, respectively (Igic et al. 2012). We tested the alternative scenarios that (i) cuckoos replicate host egg pigment chemistry, or (ii) cuckoos use alternative mechanisms to produce a similar perceptual effect to mimic host egg appearance. In parallel with patterns of similarity in avian-perceived colour mimicry, the concentrations of the two key eggshell pigments, biliverdin and protoporphyrin, were most similar between the cuckoo host-races and their respective hosts. Thus, the chemical basis of egg colour mimicry between avian brood parasites and their hosts is evolutionarily conserved, but also intraspecifically flexible.

(2) *Aspects of the adaptation mechanisms in heavy cuckoo parasitism*

(a) Mechanisms of egg discrimination (Zölei et al. 2012, Bán et al. 2013, Moskát et al. 2014a,b,c, Hauber et al. 2015)

To answer the question 'How hosts can recognize and reject the parasitic eggs from their nests?' is a great challenge for researchers working on brood parasitism. Our previous studies have already revealed that great reed warblers use two main mechanisms, they reject the odd-looking egg ('discordancy') and they also have an inner representation of their eggs ('template-based recognition') (Moskát and Hauber 2007, Moskát et al. 2010). In the present project we revealed several new details how these mechanisms work. For example, we showed that host egg is not necessary to be presented in a nest for successful ejection of the parasitic eggs (Bán et al. 2013; see also Yang et al. 2014). We revealed the so-called 'discordancy effect', when the phenotypic difference between the egg types in the minority and majority affect egg recognition (Moskát et al. 2014b). We also revealed that nesting stage affect rejection rates, as recognition of foreign eggs is more frequent in the stages when cuckoo parasitism naturally occurs (Moskát et al. 2014c). Comparing first-time breeding young hosts and experienced hosts at least two years old we showed that older hosts had better egg rejection ability. During egg laying older hosts can learn again their present egg phenotype, which ensures some flexibility to the slightly variable egg phenotypes. We called this phenomenon as 'fine tuning' of egg recognition (Moskát et al. 2014a). We experimentally proved that egg shape affects egg recognition when we placed special model eggs made of plastic into great reed warbler nests (normal egg, egg with two blunt poles, and eggs with two sharp poles). Artificial eggs with two blunt poles were rejected significantly more often than those with a single blunt pole or two sharp poles (Zölei et al. 2012). We also had the chance to take part in an opinion paper on experimental egg rejection studies in the framework of an international cooperation (Hauber et al. 2015).

(b) Physiological adaptations, hormones and antioxidants (Geltsch et al. 2012, Hargitai et al. 2012)

Great reed warblers are relatively large-sized *Acrocephalus* hosts, consequently, they rear cuckoo chicks in good condition (Kleven et al. 1999). Experimentally induced cohabitation of host and parasitic chicks temporarily slows down cuckoo chick growth, which is compensated after the host nestlings are fledged (Geltsch et al. 2012).

We examined whether the cuckoo nestling’s plasma oxidative status was related to prior effort in eviction and quantified variation in the level of reactive oxygen metabolites, of nonenzymatic antioxidant capacity, and of testosterone concentration in plasma at various phases of the cuckoo’s development. Levels of both reactive oxygen metabolites and antioxidant capacity were greater in older than in younger nestlings, suggesting that younger nestlings effectively counterbalance their increased production of free radicals, whereas, near fledging, levels of reactive oxygen metabolites increase despite improved antioxidant capacity. Possibly, overall energy expenditure increases with age and elevates the production of reactive oxygen species to a rate higher than what the antioxidant system could eliminate. Plasma testosterone level was the highest at nestlings’ intermediate phase of growth. High levels of testosterone may be required during the period of fastest growth, and when the growth rate levels off near fledging, testosterone levels may also decline. Cuckoo chicks that evicted more host eggs from steeper nests had higher plasma levels of reactive oxygen metabolites shortly after the eviction period, suggesting that eviction is costly in terms of an increased level of oxidative stress (Hargitai et al. 2012).

(c) Modeling of the cuckoo-host population dynamics in relation to habitat patchiness (Bán et al. ms)

The high rate of cuckoo parasitism (50-60%) on the great reed warbler population reduces hosts' own reproduction, so the host population is supposed to be a "sink" population. Our earlier metapopulation model suggested that immigration of great reed warblers from less or non-parasized populations may help in their survival (Barabás et al. 2004; see also Martin-Gálvez et al. 2007, Soler et al. 1998). However, habitat structure affects parasitism rate locally. If there are trees usable as vantage points for cuckoos, cuckoos can parasitize great reed warbler nests, but where trees are missing, they cannot (Moskát and Honza 2000). Our present modeling investigates if habitat structure could substitute immigration effect, so what ratio of treeless and woodland type habitat patches is necessary for hosts' survival. Our model (Table 2) revealed that even at high parasitism rate, the ratio of woodland/treeless patches should be lower than 0.53 to ensure hosts' survival. It is an interesting result, suggesting that immigration from nearby subpopulations is not a key factor in the maintenance of high parasitism rate in our study area (Bán et al. ms).

Table 2 Model parameters	*Parasitism rate* (p_c)*; Ejection rate* (p_e)
Host species: great reed warbler	*Desertion rate* (p_a)*; Predation rate* (p_p)
Percent tree cover (p_w)*; Clutch size* (N_E)	*Recurrence rate of juveniles*(M_{juv})
Fledging success (in the lack of parasitism) (S_{chick})	*Recurrence rate of adults* (M_{ad})

The current tree cover (~80%) is much higher than the calculated value (52.8%) for maintaining a stable population beside the mean values of the other field parameters. This high value of tree cover causes a reduced growth rate of the model great read warbler population (91-94%). We found that 5.6 eggs/nest/pair/year would be necessary for maintaining a stable population with the currently observed habitat structure (percent tree cover).

(d) Others

In the orginal research plan we mentioned that beside the main points of our plan we would like to check the reason why pale and darker host eggs are found in nest sometimes among the seemingly normally coloured eggs. We planned to carry out DNA sequencing to reveal if these eggs are irregularly coloured own eggs, probably caused by extreme weather or food, or the result of intraspecific parasitism. Unfortunately, we did not find such eggs in the study period, so we could not do this extra task. Another problem with this conditional topic that Ágnes Major (Genetic Lab, Hungarian Natural History Museum), who agreed with doing DNA sequencing for parentage analysis, deceased unfortunately. Therefore, instead of the original idea, we started to study eggshell bacteria on cuckoo and host eggs, using commercial sequencing. Now we are just about to finish DNA sequencing, and start to write our paper.

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