

ADAPTATIONS BY GREAT REED WARBLERS TO BROOD PARASITISM: A COMPARISON OF POPULATIONS IN SYMPATRY AND ALLOPATRY WITH THE COMMON CUCKOO

by

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Summary

Egg rejection behaviour, as an antiparasite defence against brood parasitism was compared in two great reed warbler *Acrocephalus arundinaceus* populations. In central Hungary the host population lives in sympatry with the common cuckoo *Cuculus canorus*, and it is heavily parasitised (ca 66%). In central Greece the great reed warbler occurs in allopatry with the cuckoo, so it is not parasitised at all. Experimental parasitism with poorly mimetic (often called 'non-mimetic') artificial cuckoo eggs revealed a 100% rejection rate towards the foreign eggs in the Greek population, but the Hungarian hosts accepted 29.4% and rejected 70.6% of the model cuckoo eggs. No other rejection type than ejection was observed. The significantly higher level of rejection in Greece than in Hungary suggests that the Greek great reed warbler population is an abandoned host population of the cuckoo, which kept its egg recognition ability. A computer-based image analysis of host's clutches revealed that interclutch variation in egg colour was greater than intraclutch variation in the Hungarian

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population, but not in the Greek population. Spottedness of eggs were similar in both populations. Generally, a tendency for a higher interclutch variation was found in Hungary. Intraclutch variation was more similar in the two populations, but for the colour variables it showed a lower value in Hungary than in Greece. The heavy cuckoo parasitism in Hungary is suggested to be the main cause for the increased interclutch variation in relation to intraclutch variation in egg appearance among great reed warbler clutches. The high interclutch variation reduces the chance that the parasite egg's appearance matches that of the hosts', which facilitates hosts' egg discrimination.

Keywords: brood parasitism, adaptation, egg rejection, interclutch variation, intraclutch variation, *Cuculus canorus*, *Acrocephalus arundinaceus*.

Introduction

In the sense of the coevolutionary arms race hypothesis of brood parasitism, the host's egg recognition adaptations track the egg mimicry adaptations of the parasite with a time-lag. The result of a series of such of adaptations and counteradaptations, however, is that the parasite has to abandon the host at the end of this coevolutionary process (Dawkins & Krebs, 1979; Davies & Brooke, 1989a, b; Moksnes *et al.*, 1990, Rothstein & Robinson, 1998; Takasu, 1998; Davies, 1999). In the last stages of this coevolutionary process the host's egg recognition is expected to be well-developed, so it is able to reject most of the parasites' eggs. Consequently, the parasite's reproductive success will be low and so it is forced to leave the actual host and switch to a new one. The abandoned host populations may keep their good egg recognition ability for a longer time, probably because of the low cost to preserve the rejecter gene in the absence of parasitism (Briskie *et al.*, 1992; Moksnes & Røskaft, 1992; Sealy & Bazin, 1995; Soler *et al.*, 1999), or they may lose it rapidly if rejection behaviour was mainly based on phenotypic plasticity (Brooke *et al.*, 1998; Lindholm, 2000; Lindholm & Thomas, 2000).

Egg recognition ability of a host population can be examined by direct observation of reactions to natural parasitism, or by experimentally placing real or artificial parasitic eggs into the host's nests (see references in Davies & Brooke, 1998; Rothstein & Robinson, 1998; Stokke *et al.*, 1999; Davies, 2000). The fact that a currently unparasitised host population shows high rejection rate towards parasitic eggs may reflect historical events of interspecific brood parasitism in this population (Davies, 1999). For instance,

presently the red-backed shrike *Lanius collurio* is not parasitised by the common cuckoo *Cuculus canorus* in Hungary, but it is a strong rejecter (Moskát & Fuisz, 1999). There are scarce data that this species used to be parasitised at a low rate until the years of the 1960s, showing that it could have been an important host of the cuckoo earlier in the past (Moskát, unpubl.).

In addition to the well-developed egg recognition and rejection ability, a high variation among clutches in the appearance of host eggs, and a low variation within host clutches are expected to exist, because this pattern of egg appearance variability can easily facilitate the recognition of parasitic eggs by the host and, at the same time, make it more difficult for parasites to achieve perfect mimicry. This phenomenon is hypothesised to be the result of a long term adaptation of the hosts against brood parasitism (Swynnerton, 1918; Davies & Brooke, 1989a, b; Stokke *et al.*, 1999). To investigate this hypothesis, Øien *et al.* (1995) examined egg appearance of 47 suitable and 28 unsuitable host species of the cuckoo. They found strong support for the prediction that interclutch variation was greater in suitable hosts of the cuckoo, but the development of low intraclutch variation in brood parasitism was only partially supported; their study found a statistically significant difference in intraclutch variation between the suitable and unsuitable hosts only if hole-nesters were excluded from the comparison. Soler & Møller (1996) repeated the study with controlling for phylogenetic ancestry of the host species, and found a strong relationship also with intraclutch variation.

The spatial habitat structure hypothesis suggests that cuckoos only breed in areas where they have access to vantage points in trees, and exploit those host populations quickly, which always breed near trees (Røskaft *et al.*, 2002). Other populations, which breed both near and far from trees give chance for cuckoo-host coevolution, like adaptations in egg appearances. *Acrocephalus* warblers represent a good example of such species, e.g. cuckoos well-adopted to the great reed warbler *Acrocephalus arundinaceus*, which is a widespread migratory bird breeding in the Palearctic (Cramp, 1992). It is a common host of the cuckoo, in many areas in central Europe (Molnár, 1944; Moksnes *et al.*, 1993; Moskát & Honza, 2000, 2002; Edvardsen, 2001; Honza *et al.*, 2001), in east Europe (Malchevsky, 1960), or in Japan (Lotem *et al.*, 1992, 1995; Nakamura *et al.*, 1998). Although the great reed warbler is quite numerous in Greece, there is no evidence that this species has ever been parasitised there by the cuckoo, at least in the central

and southern part of the country, where cuckoos live only in the mountainous areas (Handrinos & Akriotis, 1997).

In this paper we report on a field experiment where we compared egg recognition ability of two populations of great reed warblers, in Greece and Hungary, by using artificial cuckoo eggs. The great reed warbler population in Greece is not parasitised, but in Hungary this species suffers from a heavy (*ca* 66%) cuckoo parasitism (Moskát & Honza, 2000). We also analysed inter- and intraclutch variations in host egg appearance. This is a novel study in the sense that three types of antiparasite adaptations, namely egg rejection, inter- and intraclutch variations are compared in parasitised and non-parasitised populations of a host species of the cuckoo. We hypothesized that the great reed warbler population now existing in Greece has never been parasitised by the cuckoo, and predicted a much higher acceptance rate of the parasitic eggs than in the Hungarian population, and also predicted to have a low interclutch, and a relatively high intraclutch variation in respect to that of the Hungarian host population. Alternatively, if the great reed warbler is an already abandoned host of the cuckoo in Greece, we predict a well-developed egg-recognition ability, and as a consequence, a high rejection rate towards the parasitic eggs, together with high interclutch and low intraclutch variation in hosts' eggs appearance.

Methods

Study areas

The study was carried out in two sites, one in Greece and one in Hungary. The Greek study site was in the Sperchios delta, *ca* 150 km NE from Athens, between the ancient historical site of Thermopylae and the village of Anthili (38°50'N, 22°30'E). The study was conducted between April 25 and May 25, 1998, but experiments were done between May 6 and May 25. Nests of great reed warblers were found in narrow, 3-6 m wide reedbeds, grown over, or at least on both sides of the ditches of the artificial water regime for the rice paddies. These irrigation ditches formed a grid system in the area, dividing the rice fields and the cotton plantations into blocks of pieces. The Hungarian study site was about 930 km NW from the Greek site being situated *ca* 40 km south of Budapest, in the surroundings of Bugyi, Apaj, and Kiskunlacháza (47°09'N, 19°05'E). Here the study was carried out between May 15 and July 15 in 1998, and between May 16 and July 15 in 1999. We studied great reed warbler nests in a habitat similar to in which the species was studied in Greece *i.e.* in narrow reedbeds grown along both sides of flood relief and irrigation channels, crossing through arable fields and grasslands. The great reed warbler is a typical edge species both in Greece and Hungary, preferring the 'open water surface/reedbed'-type edges, and avoids the inner parts of reedbeds (Báldi & Kisbenedek, 1999, 2000). The long strips of reeds in our study areas offered

favourable, edge-like habitat for the great reed warbler, especially in the Hungarian site, where there was a 4–8 m wide water body with open surface between the two channel-side strips of reeds. At this time of the season great reed warblers preferred dry, old reed patches in Greece, but avoided green patches of the short, but rapidly growing new reed. An opposite trend was observed in Hungary, where birds almost totally avoided dry patches of the old reed, but occupied well-developed patches of the new reed. In Greece birds started to breed somewhat earlier than birds in Hungary. In Greece the first egg was found on May 5 whereas in Hungary early breeding females laid their first egg in the middle of May. Note, however, that an exceptionally early case in Hungary was dated back to May 9.

Experimental manipulation

We used artificial cuckoo eggs for our experiments, which were made of plastic (synthetic clay), and painted with acrylic paint. All of the eggs were painted to be similar to a real cuckoo egg found in a great reed warbler nest in Hungary, and stored in the Bird Collection of the Hungarian Natural History Museum. The ground colour was light sepia, somewhat similar to ivory or beige, which was identified as code 614C in the PANTONE (1995) colour code series. Spots were painted using dark brown (code 4625C) and sepia (code 457C). The weight of these plastic eggs (*ca* 3.4 g) was adjusted to be similar to the weight of an average cuckoo egg (2.9–3.8 g; Cramp, 1985). Our model eggs warm up similarly to a real egg (C. Moskát pers. obs.). The coat of the eggs was soft enough to show peck marks of the hosts, if they tried to eject it.

We placed the artificial cuckoo eggs into great reed warbler clutches, and removed one host egg as the laying cuckoo female usually does (Wyllie, 1981). Most of the great reed warblers' nests were found in the nest building stage. The complete clutches of the great reed warblers contained 4–5 (rarely 6) eggs in our study areas, but experiments were carried out during egg laying, including the day of laying the last egg, when clutches had 2–5 host eggs. Hosts' reactions towards the parasitic eggs showed no difference if clutches were incomplete or complete at the beginning of the experiment (chi-square test with Yates correction, $\chi^2 = 0.09$, $df = 1$, $p = 0.770$, for the Hungarian data set, but it could not be computed for the Greek data set because of the absence of acceptance).

Each nest was visited daily for six consecutive days following artificial parasitism to observe the response type of the host. We choose this observation period because Lotem *et al.* (1995) found that 96% of rejections of artificial cuckoo eggs by the great reed warbler occurred within six days of clutch completion (day of the placement of the artificial egg was regarded as day 0, and on day 6 the plastic egg was removed from the nest). If the plastic egg suffered from heavy peck marks, *i.e.* peck marks were observed in the plastic body of the eggs, not only in the coat of the painting, we took it out from nest earlier than the end of the six-day period of control, to avoid damage of the real eggs. We treated this response of the host as an 'ejection', because great reed warblers eject real eggs by puncture ejection (H. Nakamura, unpubl. material, cited in Lotem *et al.*, 1995; M. Honza and C. Moskát, unpubl. video recordings). Nests, which were naturally parasitised by the cuckoo or suffered from predation during the experiment, were not taken into consideration for the present study.

Experiments on 48 great reed warbler clutches were successful, 34 in Hungary, and 14 in Greece. Although the study period was longer in Hungary (from May 15 to July 15) than in Greece (from May 6 to 15), no seasonal effect was shown in acceptance and rejection of the foreign eggs in the Hungarian data. The distribution of these two reactions were tested

along the time scale, started from the day when the first egg was laid (Kolmogorov-Smirnov two-sample test, $D = 0.753$, $p = 0.623$), so we used all Hungarian data for comparison with the data from Greece.

Analysis of egg colours and spottedness

Each clutch together with the plastic Cuckoo eggs was photographed by a Nikon camera on Kodak Grey Card, using a 100 ASA Kodak colour slide film and a Nikon circular flash when clutches were experimentally parasitised. Kodak Colour Control Patches were also placed above the eggs in each photo to help colour adjustment in future analyses. For further investigation slides were scanned into an IBM PC compatible computer by a Nikon LS-2000 35 mm film scanner, using the value 750 for the scale parameter, and the option 300 pixel/inch for the resolution level.

A computer-based technique, image analysis, was carried out on the scanned slides of clutches. For measuring the size parameters of patches the software package Scion Image for Windows was used (Scion Corp., Maryland). First, we transformed each colour picture into black and white for spot size measurements. Then we divided the egg surface into three (bottom, middle and top) parts of equal size along the main axis of the egg, and measured cover of spots in these parts of the images, presenting the spot/egg surface ratio ('spot cover variables'). (Variable names: 'Spot cover (bottom)', 'Spot cover (middle)', and 'Spot cover top)'). Cover values were expressed as percentages, and cover variables were arcsine transformed. As many small spots or a few big spots could result in the same cover value, the average spot size in each of the three parts of the eggs were evaluated ('patch type variables'), and were coded from 1 to 5 (from low to high). (Variable names: 'Patch type (bottom)', 'Patch type (middle)' and 'Patch type (top)').

Colour measurements were made using Adobe Photoshop (Adobe Systems Inc.). We measured colour values in the red, green, and blue channels (R, G, B), making three different colour layers of the picture. Twenty measurements were made with the eyedropper tool of the program, and then averaged for each colour value. All colour measurements were calibrated adjusting to the hues of R, G, B of the Kodak Card, measured on a well-exposed picture. This was used as a standard reference both for the Greek and Hungarian samples. This way we were able to order relative colour values to all pictures, despite the fact that we could not measure absolute colour values. The knowledge of absolute colour values were not necessary for comparison of the two populations. The following 'colour' variables were measured, each in the R, G, B channels: ground, dot and blotch colour. (Variable names: 'Ground colour (R)', 'Blotch colour (R)', 'Dot colour (R)' for the red (R) channel, and the same coding was applied for the green (G) and blue (B) channels, too.) By definition, a dot is a spot less than 1 mm in diameter, and a blotch is more than 1 mm (Nakamura *et al.*, 1998). Altogether 549 measurements were made on each of the 171 egg images, both for colour and spot values, which resulted in a huge number of raw data (over 94,000, plus some more for calibration).

Statistical analyses

All univariate statistical analyses and the stepwise multivariate discriminant analysis (MDA) for comparing egg colour and marking pattern between the two populations were carried out using SPSS (Norris, 1990a, b). In order to compare inter- and intraclutch variations, random effects models of oneway analyses of variance were carried out for each measured

variable with clutches as random factors (Model II ANOVA *sensu* Sokal & Rohlf, 1981). In this ANOVA model the intra- and interclutch variation of egg measurements are estimated by the within group (error) mean square and the between group mean square, respectively. Significant difference means that the eggs differ more between clutches than within clutches. Variance components and their confidence intervals were estimated with the 'nlme' package of the R statistical computing environment (Ihaka & Gentleman, 1996; Pinheiro & Bates 2000). The Wilcoxon signed ranked test was applied for comparison both the between and within clutches variances between the two populations. In multiple tests the sequential Bonferroni correction was applied (Rice, 1989). All statistical tests were two-tailed.

Results

Rejection of artificial cuckoo eggs

Only two response types were observed: (1) acceptance, and (2) ejection of the foreign egg. In the Hungarian study area both acceptance (29.4% of 34) and ejection (70.6%) occurred whereas all parasitic eggs (100% of 14) were ejected in Greece. Heavy peck marks were observed on the artificial eggs and so they were categorised as ejections in 7 cases (20.6% of 34) in Hungary, but no case was observed in Greece (0% of 14). The frequencies of acceptances and rejections between the two populations proved to be statistically significantly different (Fisher's exact test, two-tailed, $p = 0.023$). We observed ejection cost in four cases out of the fourteen clutches in the Greek sample, *i.e.* birds ejected the artificial cuckoo eggs together with one or more of their own eggs. Altogether seven host eggs were lost by this way. In the Hungarian data set we found ejection costs in four clutches out of the 24 ejections (a total of nine mistakenly removed host eggs).

Analysis of egg colour and spots

Egg morphological variables, including calibrated colour measurements on the ground colour, dots and blotches, all in the R, G, and B channels, as well as spot size and spot ratio variables measured in three parts of the egg (top, middle, and bottom) were analysed by multivariate discriminant analysis. The preliminary analysis of these variables by Wilk's lambda statistic revealed that 11 out of the 15 variables showed significant differences between the populations (Table 1). Eight variables belonged to the group 'colour' variables. From the 4 variables showing non-significant differences,

TABLE 1. *Univariate comparison of morphometrical variables of great reed warbler eggs from Greek and Hungarian populations*

Variable	Wilk's Lambda	<i>F</i>	<i>p</i>	Coefficients for function 1
Ground colour (R)	0.86612	26.12	0.0000***	1.20276
Blotch colour (R)	0.89494	19.84	0.0000***	-0.67355
Dot colour (R)	0.95159	8.598	0.0038**	-0.54186
Ground colour (G)	0.94517	9.803	0.0021*	-0.39440
Blotch colour (G)	0.90517	17.71	0.0000***	not selected by MDA
Dot colour (G)	0.94333	10.15	0.0017**	not selected by MDA
Ground colour (B)	0.91215	16.28	0.0001***	0.34335
Blotch colour (B)	0.97187	4.892	0.0283*†	not selected by MDA
Dot colour (B)	0.99226	1.318	0.2525	0.33361
Patch type (bottom)	0.99311	1.172	0.2805	not selected by MDA
Patch type (middle)	0.98295	2.931	0.0887	-0.30976
Patch type (top)	0.99894	0.1796	0.6723	0.27676
Spot cover (bottom)	0.99540	21.87	0.0000***	0.58030
Spot cover (middle)	0.96294	6.504	0.0116*†	not selected by MDA
Spot cover (top)	0.90280	18.20	0.0000***	-0.38027

Comparison based on Wilk's Lambda statistics, and standardised discriminant function coefficients computed by stepwise multivariate discriminant analysis (MDA). (The original pictures of clutches were divided into three layers, the red, green and blue colour channels by image analysis. The letters R, G and B denotes these colour layers.)

*** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$.

† = After a sequential Bonferroni correction (Rice, 1989) these significances became non-significant at $p = 0.05$ significance level.

3 were 'spottedness' variables, and only one belonged to the group of 'colour' variables. So we found a high level of separation according to the colour variables, and less separation by spottedness.

The stepwise discriminant analysis selected 10 variables from the set of 15 for the separation of the two groups (Table 1). The discriminant function proved to be highly significant (Wilks's Lambda = 0.413, $\chi^2 = 144.89$, $df = 10$, $p < 0.001$). Separation of the 112 Hungarian and 59 Greek eggs showed some overlap along the first discriminant axis (Fig. 1), but classification of MDA results further strengthens the separation between the two populations. 88.4% of the eggs belonged to the Hungarian population were classified correctly, and 89.8% of the Greek eggs were classified correctly to their own population. A randomly chosen individual egg could be classified correctly with high probability ($p = 0.889$).

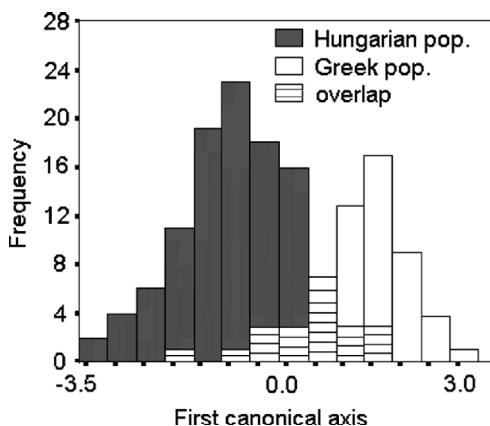


Fig. 1. Distribution of great reed warblers from Hungary and Greece along the first canonical axis, obtained by stepwise discriminant analysis of egg appearance variables.

According to the ANOVAs, the estimated interclutch variation was significantly higher than the intraclutch variation only in the case of the colour variables of the Hungarian population (except blotch and dot colour in the blue channel; Fig. 2). None of the egg measurements varied more between than within the clutches in the Greek population (Fig. 2).

The comparison of the two great reed warbler populations showed that confidence intervals of the between group variances overlapped in 11 variables, but in four cases (Ground colour (R), Blotch colour (R), Spot cover (middle) and Spot cover (top)) interclutch variation was higher in the Hungarian than in the Greek population. No variable showed an opposite relationship for the benefit of the Greek population. This tendency was strengthened by the Wilcoxon test, which showed a statistically significant difference between the two populations when all of the 15 variables were used (mean interclutch variations: 6.909 and 11.178 in Greece and Hungary, respectively; $W = 15$, $p = 0.008$). The same comparison for the within group variances showed that confidence intervals of 10 variables overlapped, but in two cases (Ground colour (G), and Ground colour (B)) intraclutch variation was significantly lower in the Hungarian population than in the Greek population. In the three spot variables (Spot cover (bottom), Spot cover (middle), and Spot cover (top)) it was lower in Greece than in Hungary. Although mean intraclutch variations for the 15 variables was lower in Hungary (5.998) than in Greece (8.162), this difference proved to be not significant when the

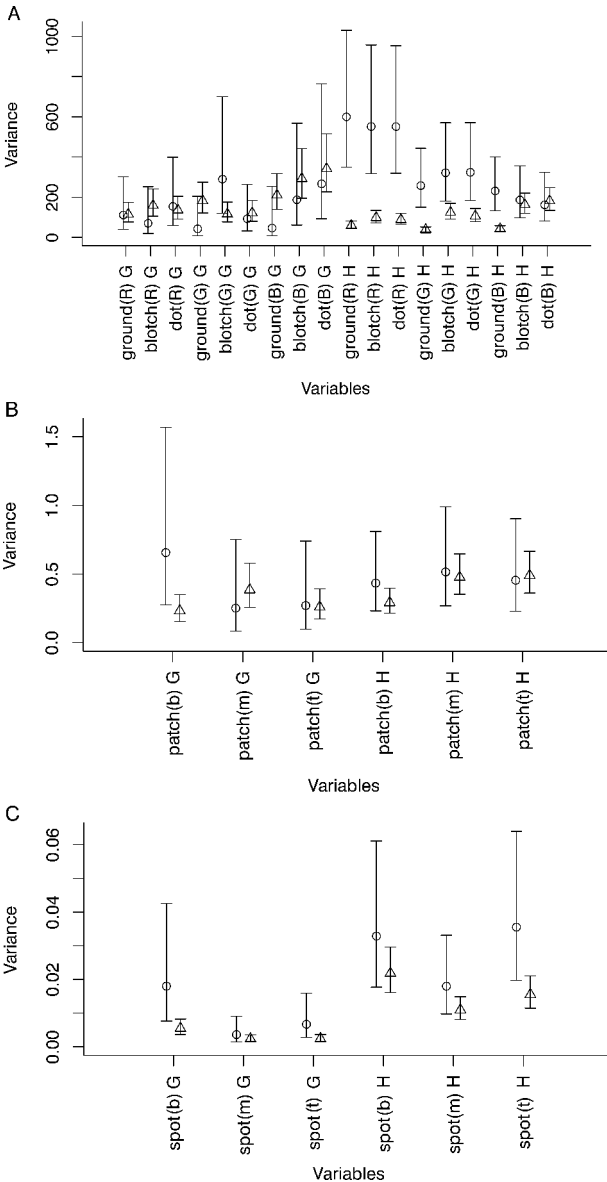


Fig. 2. The estimated variance components for the measured morphometrical variables of great reed warblers from Greece and Hungary. (A): colour variables; (B): patch variables; and (C): spot variables. Circles denote between-clutch variance estimates while triangles mark within clutch variance estimates. Error bars show 95% confidence intervals, between and within estimates differ significantly if they error bars do not overlap. Letters ‘G’ and ‘H’ in variable names identify the Greek and Hungarian populations, respectively.

Wilcoxon test was applied ($W = 92$, $p = 0.073$). If the analysis was carried out on the 'colour' variables, only, *i.e.* the last six variables listed in Table 1 were excluded from the 15 variables, intraclutch variation proved to be significantly lower in the Hungarian population than in the Greek population (means: 9.740 and 13.404 in Hungary and Greece, respectively; $W = 44$, $p = 0.008$).

Discussion

Hosts' response types to parasitism

Our study revealed that great reed warblers in Greece have a well-developed egg recognition ability, so these results did not support the hypothesis that the great reed warbler in Greece still was a naive potential host species. Our findings supported the alternative hypothesis, *i.e.* great reed warblers seemed to be an abandoned host of the cuckoo in Greece. Although the great reed warbler in Greece is not currently a host of the cuckoo, its egg recognition is interestingly better than that of the currently parasitised great reed warbler population in Hungary. Although mimicry of the experimental egg in relation to host eggs may affect rejection rates, and consequently the comparison of the two populations, the 100% rejection rate in Greece is an important indicator of the high egg rejection ability of the Greek population. We think that this result points to an earlier cuckoo parasitism on the great reed warbler in Greece. Theoretically, we cannot exclude another possible explanation, *i.e.* gene-flow transmits the rejecter genes to this population from a nearby parasitised great reed warbler population, such as was found between nearby parasitised and non-parasitised great reed warbler populations in Japan (Nakamura *et al.*, 1998). However, the extremely high ejection rate (100%) makes the former explanation seem much more likely. The great reed warbler population in Hungary is naturally parasitised by the Cuckoo, and it has a moderate egg recognition ability and showed moderate rejection rates against the real cuckoo eggs (34% total rejection rate, including 12% ejection, 20% desertion, and 2% egg burial, $N = 163$; Moskát & Honza, 2002). Although this study on natural parasitism and our study on experimental parasitism were carried out in the same period, hosts' responses to natural parasitism were weaker than responses to artificial cuckoo eggs in the present study (ejection: 71%). A possible explanation is, as our subjective impression

suggests, mimicry of the host eggs was generally better in real cuckoo eggs than those of the artificial ones. In the Hungarian population nest desertion also occurred as a reaction towards the real cuckoo eggs (see above), but we did not find this type of anti-parasite behaviour neither in the Hungarian, nor in the Greek great reed warbler populations when the artificial parasitic eggs were experimentally applied. However, only one nest desertion was observed in the Hungarian study area when great reed warbler clutches were experimentally parasitised with another type of artificial cuckoo eggs (rejection rate: 76%, $N = 42$; Bártol *et al.*, 2002). So great reed warblers more easily eject an artificial cuckoo egg than a more mimetic real cuckoo egg. Another difference in great reed warblers' antiparasite behaviour against artificial and real cuckoo eggs that we did not observe egg burial in our experiments, but 2% of the real cuckoo eggs were buried in the natural cases (see above). Great reed warblers chose this antiparasite defence when cuckoos laid their eggs into empty nests, just before hosts' egg-laying started, so hosts had no risk to bury any of their own eggs together with the parasite egg (Moskát & Honza, 2002). As we experimentally parasitised clutches with the artificial eggs on the day when the fourth host egg was laid into the clutch, the nests were not empty in that time, so hosts did not bury any of these eggs.

Species with no parasitic interaction with the cuckoo largely accept unlike eggs, although some cases of rejection may occur. This was shown in populations of the meadow pipit *Anthus pratensis* and pied wagtails *Motacilla alba* in Iceland, where these species are still isolated from the cuckoo (Davies & Brooke, 1989a). In Eastern Austria, a parasitized population of Great Reed Warblers showed high rejections towards artificial non-mimetic cuckoo eggs used for experimental parasitism (Brown *et al.*, 1990). However, its non-parasitised sibling species, the clamorous great reed warbler (= southern great reed warbler) *Acrocephalus stentoreus* in Australia accepted the artificial parasitic eggs (Brown *et al.*, 1990). Another closely related species, the Australian warbler *Acrocephalus australis*, is a good rejecter, which appears to reflect to a potential parasitism in the past with any of the seven cuckoo species living in that area (Welbergen *et al.*, 2001). Presently unparasitised populations with previous contact with the cuckoo may keep their egg discrimination ability for a time if it had already been evolved (Moksnes & Røskoft, 1992; Moskát & Fuisz, 1999), and this ability decreases by geographic distances from the parasitised population (Soler *et al.*, 1999). Over

a longer period, like it was *ca* two hundred years in the case of an isolated population of the village weaver *Ploceus cucullatus*, the hosts may lose their egg recognition ability in the absence of the brood parasite (Cruz & Wiley, 1989). Although rejection behaviour is not costly for the host if parasitism is absent or rare, preserving the rejecter genes when the ranges of the host and the parasite become separated needs a continuous gene flow from parasitised populations (Briskie *et al.*, 1992).

Intra- and interclutch variations in the great reed warbler

Our results show that in Hungary, where the great reed warbler population suffers from heavy cuckoo parasitism, interclutch variation of host eggs' colour variables was significantly greater than intraclutch variation. In Greece, in the absence of parasitism, we did not show this difference neither in the colour, nor in the spottedness variables, although from egg rejection results it is most likely that the studied great reed warbler population is not a naive, (*i.e.* a never parasitised) population, but it is rather an abandoned host. A direct comparison of interclutch variation between the two populations also revealed this trend, *i.e.* higher interclutch variation in Hungary than in Greece, but intraclutch variations proved to be more similar. This is partly an unexpected result in the sense that we expected a high interclutch and probably a low intraclutch variation in egg appearance for an abandoned host. Our interpretation is that in the absence of parasitism natural selection does not favour higher interclutch (and perhaps lower intraclutch variation) any more. We cannot exclude the possibility that great reed warblers in Greece lost some of their adaptive characters in egg appearance in the absence of the brood parasite. Another alternative explanation is that selection from cuckoos most probably directly affects intraclutch variation. A high interclutch variation is a secondary effect, which may depend on the level of parasitism. We argue that preserving the rejecter gene or preserving the pattern of egg appearances are two different problems, as the mechanism how it has evolved, and the speed of its spread or development could also be different. The stability of adaptive patterns of egg characteristics when the parasitic pressure comes to an end is questionable, and should be studied further.

A comparison of several suitable and unsuitable host species revealed that evolution of host egg appearance under parasitic pressure seems to favour greater inter-, and lower intraclutch variation (Øien *et al.*, 1995; Soler &

Møller, 1996). The present study focused on the relative importance of inter- and intraclutch variability, and we were able to show the increased interclutch variation of host eggs in the parasitised population. In Hungary the cuckoo is known to have had developed an almost perfect mimicry in respect to great reed warbler eggs (Southern, 1954), at least in regard to human vision, but the accuracy of mimicry of eggs may depend on the visual system of the host (Cherry & Bennett, 2001). A high interclutch variation in host eggs makes more difficult for the cuckoo to match the parasitic egg with the host's egg type, so a cuckoo egg which mimics perfectly one clutch of host eggs may show only a poor mimicry with another clutch of the host eggs (Moskát & Honza, 2002). Selection for accurately mimicking cuckoo eggs should reflect the appearance of host eggs. Estimates of the number of cuckoo eggs laid by the same female cuckoo in a breeding season varies in a wide range, but usually falls between 10 and 20 (up to 25, Wyllie, 1981), so cuckoos may possibly compensate for the negative effect of high interclutch variation on their fitness by laying higher number of eggs.

Conclusions

Our results showed that the great reed warbler population in central Greece, which is presently not parasitised by the cuckoo, seems to be an already abandoned host. It is unlikely that 100% rejection would occur in a potential host population, which has never been in contact with the cuckoo. It is also unlikely that flow of rejecter genes from adjacent parasitised populations could increase the ratio of rejecters to this high value, because there is no selection pressure against the acceptor gene in the absence of the brood parasite. In contrast with the Greek population, a heavily parasitised population in Hungary showed a significantly lower rejection rate against the same type of artificial cuckoo eggs (*ca* 70%), suggesting an on-going host-parasite arms race. In contrast with these findings, interclutch variation found to be increased in relation to intraclutch variation regarding egg colours only in the presently parasitised population, *i.e.* in the Hungarian study area. We found a tendency for a higher interclutch variation and the ratio of interclutch/intraclutch variations in Hungary than in Greece, but intraclutch variation was more similar in the two study sites. We explain this pattern by the strong effect of natural selection in the Hungarian population, caused

by the high parasitic pressure, *i.e.* clutch parasitism rates of 50-66% in the last more than sixty years. We think that the high interclutch variation in the Hungarian great reed warbler population reduces the chance that the colour and pattern of a cuckoo egg matches that of the hosts', even it shows a perfect mimicry to one type of the hosts' eggs. Our results supported that high interclutch variation among great reed warbler clutches could be regarded as an antiparasite adaptation of the host population against the high parasitic pressure.

References

- Báldi, A. & Kisbenedek, T. (1999). Species-specific distribution of reed-nesting passerine birds across reed-bed edges: effects of spatial scale and edge type. — *Acta Zool. Hung.* 45, p. 97-114.
- — & — — (2000). Bird species numbers in an archipelago of reed at Lake Velence, Hungary. — *Global Ecology & Biogeography* 9, p. 451-461.
- Bártol, I., Karcza, Z., Moskát, C., Røskaft, E. & Kisbenedek, T. (2002). Responses of great reed warblers to experimental brood parasitism: the effects of a cuckoo dummy and egg mimicry. — *J. Avian Biol.* (in press).
- Briskie, J.V., Sealy, S.G. & Hobson, K.A. (1992). Behavioral defenses against brood parasitism in sympatric and allopatric host populations. — *Evolution* 46, p. 334-340.
- Brooke, M. de L., Davies, N.B. & Noble, D.G. (1998). Rapid decline of host defences in response to reduced cuckoo parasitism: behavioural flexibility of reed warblers in a changing world. — *Proc. R. Soc. Lond. B* 265, p. 1277-1282.
- Brown, R.J., Brown, M.N., Brooke, M. de L. and Davies, N.B. (1990). Reactions of parasitized and unparasitized populations of *Acrocephalus* warblers to model cuckoo eggs. — *Ibis* 132, p. 109-111.
- Cherry, I.M. & Bennett, A.T.D. (2001). Egg colour matching in an African cuckoo, as revealed by ultraviolet-visible reflectance spectrophotometry. — *Proc. R. Soc. Lond. B* 268, p. 565-571.
- Cramp, S. (1985). *The birds of the Western Palearctic*. Vol. 4. — Oxford University Press, Oxford.
- — (1992). *The birds of the Western Palearctic*. Vol. 6. — Oxford University Press, Oxford.
- Cruz, A. & Wiley, J.W. (1989). The decline of an adaptation in the absence of a presumed selection pressure. — *Evolution* 43, p. 55-62.
- Davies, N.B. (1999). Cuckoos and cowbirds *versus* hosts: Co-evolutionary lag and equilibrium. — *Ostrich* 70, p. 71-79.
- — (2000). Cuckoos, cowbirds and other cheats. — T&AD Poyser, London.
- — & Brooke, M. de L. (1989a). An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. I. Host egg discrimination. — *J. Anim. Ecol.* 58, p. 207-224.
- — & — — (1989b). An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. II. Host egg markings, chick discrimination and general discussion. — *J. Anim. Ecol.* 58, p. 225-236.

- — & — — (1998). Cuckoos *versus* hosts. — In: Parasitic birds and their hosts: studies in coevolution (S.I. Rothstein & S.K. Robinson, eds). Oxford University Press, New York, p. 59-79.
- Dawkins, N.B. & Krebs, J.R. (1979). Arms races between and within species. — Proc. R. Soc. Lond. B 205, p. 489-511.
- Edvardsen, E., Moksnes, A., Røskaft, E., Øien, I.J. & Honza, M. (2001). Egg mimicry in cuckoos parasitizing four sympatric species of *Acrocephalus* warblers. — Condor 103, p. 829-837.
- Handrinos, G. & Akriotis, T. (1997). The birds of Greece. — Christopher Helm, London.
- Honza, M., Moksnes, A., Røskaft, E. & Stokke, B. (2001). How are different common cuckoo *Cuculus canorus* egg morphs maintained? An evaluation of different hypotheses. — Ardea 89, p. 341-352.
- Ihaka, R. & Gentleman, R. (1996). R: A language for data analysis and graphics. — J. Computational and Graphical Stats 5, p. 299-314.
- Lindholm, A.K. (2000). Tests of phenotypic plasticity in reed warbler defences against cuckoo parasitism. — Behaviour 137, p. 43-60.
- — & Thomas, R.J. (2000). Differences between populations of reed warblers in defences against brood parasitism. — Behaviour 137, p. 25-42.
- Lotem, A., Nakamura, H. & Zahavi, A. (1992). Rejection of cuckoo eggs in relation to host age: a possible evolutionary equilibrium. — Behav. Ecol. 3, p. 128-132.
- —, — — & — — (1995). Constraints on egg discrimination and cuckoo-host coevolution. — Anim. Behav. 49, p. 1185-1209.
- Malchevsky, A.S. (1960). On the biological races of the common cuckoo (*Cuculus canorus*) in the territory of the European Part of the USSR. — Proc. XII. Int. Orn. Congress, p. 464-470.
- Moksnes, A., Røskaft, E., Braa, A.T., Korsnes, L., Lampe, H.M. & Pedersen, H.C. (1990). Behavioural response of potential hosts towards artificial cuckoo eggs and dummies. — Behaviour 116, p. 64-89.
- — & — — (1992). Responses of some rare cuckoo hosts to mimetic model cuckoo eggs and to foreign conspecific eggs. — Ornis Scand. 23, p. 17-23.
- —, — —, Bicík, V., Honza, M. & Øien, I.J. (1993). Cuckoo *Cuculus canorus* parasitism on *Acrocephalus* warblers in Southern Moravia in the Czech Republic. — J. Orn. 134, p. 425-434.
- Molnár, B. (1944). The cuckoo in the Hungarian Plain. — Aquila 51, p. 100-112.
- Moskát, C. & Fuisz, T. (1999). Reactions of red-backed shrikes *Lanius collurio* to artificial cuckoo *Cuculus canorus* eggs. — J. Avian Biol. 30, p. 175-181.
- — & Honza, M. (2000). Effect of nest and nest site characteristics on the risk of cuckoo (*Cuculus canorus*) parasitism in the great reed warbler (*Acrocephalus arundinaceus*). — Ecology 23, p. 335-341.
- — & — — (2002). Cuckoo *Cuculus canorus* parasitism and host's rejection behaviour in a heavily parasitized great reed warbler *Acrocephalus arundinaceus* population. — Ibis 144, p. 614-622.
- Nakamura, H., Kubota, S. & Suzuki, R. (1998). Coevolution between the common cuckoo and its major hosts in Japan. — In: Parasitic birds and their hosts: studies in coevolution. (S.I. Rothstein & S.K. Robinson, eds). Oxford University Press, New York, p. 94-112.
- Norusis, M.J. (1990a). SPSS/PC+ 4.0 Base Manual for the IBM PC/XT/AT. — SPSS Inc., Chicago.

- — (1990b). SPSS/PC+. Advanced Statistics 4.0 for the IBM PC/XT/AT. — SPSS Inc., Chicago.
- Øien, I.J., Moksnes, A. & Røskaft (1995). Evolution of variation in egg color and marking pattern in European passerines: adaptations in a coevolutionary arms race with the cuckoo, *Cuculus canorus*. — Behav. Ecol. 6, p. 166-174.
- PANTONE (1995). Color formula guide 1000. — Pantone Inc., New Jersey.
- Pinheiro, J.C. & Bates, D.M. (2000). Mixed-effects models in S and S-PLUS. — Springer, New York.
- Rice, W.R. (1989). Analyzing tables of statistical tests. — Evolution 43, p. 223-225.
- Røskaft, E., Moksnes, A., Stokke, B.G., Moskát, C. & Honza, M. (2002). The spatial habitat structure of host populations explains the pattern of rejection behavior in hosts and parasitic adaptations in cuckoos. — Behav. Ecol. 13, p. 163-168.
- Rothstein, S.I. & Robinson, S.K. (1998). The evolution and ecology of avian brood parasitism. — In: Parasitic birds and their hosts: studies in coevolution (S.I. Rothstein & S.K. Robinson, eds). Oxford University Press, New York, p. 3-56.
- Sealy, S.G. & Bazin, R.C. (1995). Low frequency of observed cowbird parasitism on eastern kingbirds: host rejection, effective nest defense, or parasite avoidance? — Behav. Ecol. 6, p. 140-145.
- Sokal, R.R. & Rohlf, F.J. (1981). Biometry: The principles and practice of statistics in biological research. — Second edition. W.H. Freeman & Co., New York.
- Soler, J.J. & Møller, A.P. (1996). A comparative analysis of the evolution of variation in appearance of eggs of European passerines in relation to brood parasitism. — Behav. Ecol. 7, p. 89-94.
- —, Martínez, J.G., Soler, M. & Møller, A.P. (1999). Genetic and geographic variation in rejection behavior of cuckoo eggs by European magpie populations: an experimental test of rejecter-gene flow. — Evolution 53, p. 947-956.
- Southern, H.N. (1954). Mimicry in cuckoos' eggs. — Evolution as a process (J. Huxley, A.C. Hardy & E.B. Ford, eds). George Allen & Unwin, London, p. 219-232.
- Stokke, B.G., Moksnes, A., Røskaft, E., Rudolfsen, G. & Honza, M. (1999). Rejection of artificial cuckoo (*Cuculus canorus*) eggs in relation to variation in egg appearance among reed warblers (*Acrocephalus scirpaceus*). — Proc. R. Soc. Lond. B 266, p. 1483-1488.
- Swynnerton, C.F.M. (1918). Rejections by birds of eggs unlike their own: with remarks on some of the cuckoo problems. — Ibis 60, p. 127-154.
- Takasu, F. (1998). Modelling the arms race in avian brood parasitism. — Evol. Ecol. 12, p. 969-987.
- Welbergen, J., Komdeur, J., Kats, R. & Berg, M. (2001). Egg discrimination in the Australian reed warbler (*Acrocephalus australis*): rejection response toward model and conspecific eggs depending on timing and mode of artificial parasitism. — Behav. Ecol. 12, p. 8-15.
- Wyllie, I. (1981). The cuckoo. — Batsford, London.