Meadow pipit (*Anthus pratensis*) egg appearance in cuckoo (*Cuculus canorus*) sympatric and allopatric populations

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Received 11 September 2002; accepted for publication 20 January 2003

Host populations tend to show less ability to discriminate against parasites when living in their absence. However, comparison of rejection rates among sympatric and allopatric host populations does not allow determination of whether the greater tolerance in allopatric populations reflects a genetic change or phenotypic plasticity. Here we test the existence of changes in a host's adaptation to brood parasitism in the absence of parasitism by studying intraclutch variation in egg appearance, which is a genetically determined component of host defence favouring discrimination of parasitic eggs. We investigated egg phenotypes of a common host of the European cuckoo, *Cuculus canorus*, in the presence and in the absence of cuckoos. By using objective spectroradiometry techniques of colour assessment we compared intraclutch variation between populations of meadow pipit, *Anthus pratensis*, sympatric (England) and allopatric (Iceland and Faeroe Islands) with *C. canorus*. Allopatric populations of *A. pratensis* showed greater intraclutch variation in egg appearance in the ultraviolet part of the spectrum than did a population sympatric with *C. canorus*. Two possible alternative mechanisms explaining these findings are discussed. © 2003 The Linnean Society of London, Biological Journal of the Linnean Society, 2003, 79, 543–549.


INTRODUCTION

Avian brood parasitism constitutes a well-studied example of the coevolutionary process (Rothstein, 1990; Davies, 2000; Soler & Soler, 2000). Brood parasitism is a form of breeding biology in which certain individuals (parasites) receive parental care from unrelated individuals (hosts). Hosts of brood parasitic species usually suffer reduced reproductive output from a successfully parasitized nest (Røskaft, Orians & Beletsky, 1990; Soler, Martínez & Soler, 1996; Payne, 1997). Therefore, parasitism should favour the evolution of host defences, and of more sophisticated techniques by the cuckoo to overcome them (Davies & Brooke, 1988). This coevolutionary ‘arms race’ leads to intricate adaptations and counter-adaptations by both sides with each party responding to the selective forces imposed by the other (Dawkins & Krebs, 1979; Soler & Møller, 1990). It is assumed that selection of cuckoo eggs to match those of their host requires the hosts to discriminate cuckoo eggs from their own, thus increasing egg mimicry by the cuckoo and egg discrimination by the host (Davies & Brooke, 1988).

The meadow pipit, *Anthus pratensis*, is a small passerine widely distributed across the north Palearctic region (Cramp & Perrins, 1988). It constitutes one of the favourite hosts of the European or common cuckoo, *Cuculus canorus*, in moorland and heathland in Europe (Davies, 2000). *C. canorus* is divided into at least 16 host-specific races or gentes (Wyllie, 1981; Álvarez, 1994; Moksnes & Røskaft, 1995), the females of each race laying a distinctive egg type that tends to match the host's eggs (Gibbs, Brooke & Davies, 1996; Gibbs *et al*., 2000). There is a specific *C. canorus* which specializes in *A. pratensis*, laying a brownish grey
egg, with grey or brown spots, while A. pratensis shows rejection of C. canorus eggs (Davies & Brooke, 1989a; Moksnes, Røskaft & Korsnes, 1993), suggesting that in the coevolutionary arms race between C. canorus and A. pratensis both parties have evolved adaptations and counter-adaptations to enhance their reproductive output.

C. canorus breeds across the Palearctic region, but it is absent from Iceland and the Faeroe Islands, where it is only a rare vagrant that has never been recorded as a breeder (Cramp & Simmons, 1985). Both Iceland and the Faeroe Islands have, however, breeding populations of A. pratensis (Cramp & Perrins, 1988) with evidence of gene flow from Scandinavian and British populations, since neither Iceland nor the Faeroe Islands had breeding populations of A. pratensis during glaciations (Einarsson, 1968). In addition, populations of A. pratensis on Iceland and the Faeroe Islands have not diverged markedly from European populations since they all belong to the same subspecies (Cramp & Perrins, 1988). There is no indication in the literature suggesting that Iceland has been a suitable breeding site for C. canorus since the Viking arrival on the island in 874 AD that led to the devastation of the original forests (Einarsson, 1968). An experimental study by Davies & Brooke (1989a) showed that A. pratensis in Iceland had previously been in contact with C. canorus parasitism since they rejected non-mimetic eggs when artificially parasitized. However, it remains unclear if the persistence of this rejection behaviour is due to C. canorus formerly being a common nester in Iceland or to the existence of gene flow from parasitized A. pratensis populations in other parts of Europe (see discussion in Davies & Brooke, 1989a).

Davies & Brooke (1989a) compared the rejection behaviour against non-mimetic eggs introduced into A. pratensis nests in Iceland and England to analyze if the duration of sympatry with C. canorus determines changes in host defences. A total of 18% of A. pratensis rejected non-mimetic eggs in Iceland where C. canorus is always absent, while in England, where C. canorus often parasitizes A. pratensis, and there is a race of C. canorus laying eggs mimicking those of A. pratensis, non-mimetic eggs were rejected from 48% of the nests (Davies & Brooke, 1989a). The authors concluded that Iceland was colonized by A. pratensis that had subsequently partially lost defences to parasitism.

The arms-race hypothesis assumes that, after the fixation of rejection behaviour in hosts, one step in the coevolution between cuckoo and host would be selection for a reduction in intracloch variation in egg appearance among hosts (Øien, Moksnes & Røskaft, 1995; Soler & Møller, 1996). This reduction in intracloch variation would favour discrimination of parasitic eggs from own eggs when colour mimicry of the cuckoo’s egg has been perfected (Victoria, 1972; Davies & Brooke, 1989b). Furthermore, host individuals that lay eggs with more extreme phenotypes will less often be subject to successful parasitism, thereby increasing variation between clutches of different host individuals (Soler & Møller, 1996). Most evidence for a relationship between intracloch variation and egg recognition in avian brood parasite systems is based on tests of whether passerine species involved in a coevolutionary arms race with the cuckoo show a low degree of intracloch variation, and, secondly, whether species showing a high frequency of egg rejection have low intracloch variation compared with species with low rejection rates (Øien et al., 1995; Soler & Møller, 1996; Stokke, Moksnes & Røskaft, 2002). The intraspecific approach also supports the relationship between intracloch variation and egg recognition, because rejecter individuals within a host population have less intracloch egg variation in colour than do acceptor individuals (Stokke et al., 1999; Soler, Soler & Møller, 2000), suggesting that differences in intracloch variation within a species might also be relevant in terms of the arms-race hypothesis.

Maintenance of a low level of intracloch variation in a host population might be costly to the host when there is a high risk of mistakenly rejecting one’s own egg instead of the cuckoo egg. In accordance with this idea, Lotem, Nakamura & Zahavi (1992) found that the acceptance of C. canorus eggs by female great reed warblers, Acrocephalus arundinaceus, in a Japanese population occurred mainly among young breeders. The naive first-year breeders need time to learn to recognize their own eggs, and thus do not eject a parasitic egg as fast as do older individuals. Thus a high level of intracloch variation in host egg appearance may cause inexperienced hosts to make recognition errors if they attempt egg rejection.

In this paper we study to what extent the host of an obligate brood parasite such as C. canorus retains defences against parasitism when parasitism is no longer occurring. Host populations tend to show less discriminatory abilities against parasitic eggs when they are isolated from brood parasites (Davies & Brooke, 1989a; Cruz & Wiley, 1989; Soler & Møller, 1990; Briskie, Sealy & Hobson, 1992). However, comparison of rejection rates among populations does not permit identification of whether this greater tolerance reflects a genetic change or phenotypic plasticity. The aim of the present study is to test for a decline of one adaptation by studying a genetically determined component of host defences. We specifically focus on retention of intracloch variation in egg appearance as a defence against C. canorus parasitism. By using objective techniques of colour assessment within clutches we compare intracloch variation in A. pratensis

between populations in England (sympatry) and Iceland and the Faeroe Islands, where C. canorus is absent (allopatry).

METHODS

Data on 48 A. pratensis clutches (Faeroe = 19 clutches, Iceland = 10 clutches and England = 19 clutches) were obtained from the egg collections at the Zoological Museum in Copenhagen (Denmark) and the British Museum of Natural History. The clutches of A. pratensis in allopatry with C. canorus had been collected during several expeditions to Iceland and the Faeroe Islands between 1880 and 1920, and the clutches of A. pratensis from sympatric populations, which were from Surrey in England, had been collected between 1900 and 1914.

Previous analyses of intraclutch variation in egg appearance in avian brood parasite system studies have been based on human assessment of host egg similarity within a clutch using an arbitrary scale ranging from 1 to 5 (Lotem, Nakamura & Zahavi, 1995; Øien et al., 1995; Soler & Møller, 1996; Stokke et al., 1999; Stokke et al., 2002), or by using photographs and image analysis (Soler et al., 2000). However, avian colour vision differs substantially from that of humans (Cuthill et al., 2000). In fact, most diurnal birds have at least four kinds of photo pigments in the cones of their eyes (Bowmaker et al., 1997), including a spectrally sensitive peak near the ultraviolet (UV) part of the spectrum (Chen, Collins & Goldsmith, 1984; Cuthill et al., 2000). Furthermore, using UV-visible reflectance spectrophotometry, Cherry & Bennett (2001) recently found that the eggs of the red-chested cuckoo, Cuculus saturatus, and its African hosts were highly matched for chromatic aspects of eggs invisible to humans. Consequently, reflectance spectra in the range 300–700 nm were obtained from all the eggs of the 48 analyzed clutches (Faeroe = 19 clutches, Iceland = 10 clutches and England = 19 clutches) were obtained from the egg collections at the Zoological Museum of Natural History. The clutches of A. pratensis in allopatry with C. canorus had been collected during several expeditions to Iceland and the Faeroe Islands between 1880 and 1920, and the clutches of A. pratensis from sympatric populations, which were from Surrey in England, had been collected between 1900 and 1914.

Colour was measured twice in the same arbitrarily selected area of a single egg and found a high repeatability for all variables except hue (Table 1). Consequently, we excluded hue from subsequent analyses and calculated from the four measurements of each egg one mean value of brightness, UV chroma, blue chroma, green chroma, yellow chroma and red chroma for each egg.

We calculated the coefficient of variation (CV = standard deviation/mean × 100) from mean values per egg

<table>
<thead>
<tr>
<th>Colour characteristic</th>
<th>Same surface</th>
<th>Two different surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brightness</td>
<td>0.98**</td>
<td>0.92**</td>
</tr>
<tr>
<td>Ch_UV</td>
<td>0.95**</td>
<td>0.72**</td>
</tr>
<tr>
<td>Ch_b</td>
<td>0.98**</td>
<td>0.95**</td>
</tr>
<tr>
<td>Ch_y</td>
<td>0.99**</td>
<td>0.94**</td>
</tr>
<tr>
<td>Ch_r</td>
<td>0.97**</td>
<td>0.89**</td>
</tr>
<tr>
<td>Ch_h</td>
<td>0.99**</td>
<td>0.94**</td>
</tr>
<tr>
<td>Hue</td>
<td>0.00 NS</td>
<td>0.04 NS</td>
</tr>
</tbody>
</table>

Two measurements were taken from one randomly selected surface of the egg, and from two randomly selected areas of the same egg.

Ch_UV, Ch_b, Ch_y, Ch_r and Ch_h = estimation of chroma for ultraviolet, blue, green, yellow and red regions, respectively, of the avian visible spectrum. ** = P < 0.001. NS = P > 0.05.
of all colour variables in each clutch as a measure of intraclutch variation.

**STATISTICAL ANALYSES**

We tested whether intraclutch variation of the colour variables of *A. pratensis* eggs were normally distributed. Transformation of variables was not required because all intraclutch colour variables fitted normality assumptions (Kolmogorov–Smirnov tests for continuous variables, $P > 0.20$ in all six cases). Because colour variables are typically interrelated (Endler, 1990), we used multivariate analysis of variance (MANOVA) as an a priori test (Scheiner, 2001) to analyze differences in egg appearance between populations of *A. pratensis* sympatric and allopatric with *C. canorus*. *A. pratensis* intraclutch variation in egg appearance did not significantly vary between the two allopatric populations in Iceland and the Faeroe Islands (Wilks’ lambda = 0.78, d.f.1 = 6, d.f.2 = 22, $P = 0.43$), and consequently we pooled the Iceland and Faeroe Island clutches. Two tailed $t$-tests were used for post hoc testing. To avoid type-I errors we applied Bonferroni correction for multiple comparisons with the adjusted level of significance at 10% being 0.02 (Chandler, 1995). In all analyses equality of variances was checked using Levene’s test.

**RESULTS**

Intraclutch variation in egg appearance differed between sympatric and allopatric populations of *A. pratensis* (Wilks’ lambda = 0.68, d.f.1 = 6, d.f.2 = 41, $P = 0.012$). In accordance with the hypothetical relaxation of host defences to parasitism, intraclutch variation in UV chroma was significantly higher in Iceland and the Faeroe Islands than in England where *C. canorus* still parasitizes *A. pratensis* (Table 2, Fig. 1). Variation within clutches for the remaining colour components (blue chroma, green chroma, yellow chroma, red chroma and brightness) did not vary significantly between sympatric and allopatric populations of *A. pratensis* (Table 2, Fig. 1).

**DISCUSSION**

Previous results showing a decline in rejection rates in the absence of parasitism in avian brood parasite studies do not allow identification of whether this greater tolerance reflects a genetic change or phenotypic flexibility (Rothstein, 2001). As far as we know our study constitutes the first test of the loss of a host defence in the absence of parasitism in an avian brood parasite for a genetically determined component of host defence (Victoria, 1972; Collias, 1984, 1993). Our results provide direct evidence supporting a lower level of host defences in *A. pratensis* in the absence of *C. canorus* parasitism. We measured, using spectro-radiometry techniques considering the complete avian range of vision (300–700 nm), the appearance of host eggs within clutches of *A. pratensis* in allopatry and in sympatry with *C. canorus*. We detected larger intraclutch variation in colour appearance in the UV part of the spectrum in populations of *A. pratensis* allopatric to *C. canorus* populations compared with that of a sympatric population ($r^2 = 11.21\%$, intermediate effect size according to Cohen (1988)).

Table 2. Analyses of effects of sympatry vs. allopatry with *C. canorus* on egg colour appearance within clutches of *A. pratensis*

<table>
<thead>
<tr>
<th>Colour characteristic</th>
<th>$t$</th>
<th>d.f.</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brightness</td>
<td>1.39</td>
<td>46</td>
<td>0.16</td>
</tr>
<tr>
<td>ChUV</td>
<td>2.41</td>
<td>46</td>
<td>0.02*</td>
</tr>
<tr>
<td>ChB</td>
<td>0.02</td>
<td>46</td>
<td>0.98</td>
</tr>
<tr>
<td>ChG</td>
<td>0.42</td>
<td>46</td>
<td>0.67</td>
</tr>
<tr>
<td>ChY</td>
<td>0.50</td>
<td>46</td>
<td>0.61</td>
</tr>
<tr>
<td>ChR</td>
<td>0.06</td>
<td>46</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*Significantly different with sequential Bonferroni adjustment. ChUV, ChB, ChG, ChY and ChR = estimation of chroma for ultraviolet, blue, green, yellow and red regions, respectively, of the avian visible spectrum.

Figure 1. Mean (± standard deviation) variation in ultraviolet chroma (□), blue chroma (●), green chroma (■), yellow chroma (●●), red chroma (○) and brightness (○) within clutches in *A. pratensis* populations sympatric and allopatric with *C. canorus*. 

Previous studies compared the rejection behaviour against non-mimetic eggs in A. pratensis nests in Iceland and England to analyze if time of sympathy with C. canorus affected the level of host defences (Davies & Brooke, 1989a). However, the comparison of rejection rates from the two populations may not be the best way to detect a change of host defences since variation in host response to different levels of cuckoo parasitism may exist (Brooke, Davies & Noble, 1998). The experiments in Iceland were made in the absence of C. canorus, while in England A. pratensis had encountered C. canorus during the breeding season. Moreover, Moksnes et al. (1993) found that rejection rates of A. pratensis in Norway of both mimetic and non-mimetic eggs increased to more than 32.0% with a C. canorus dummy mounted beside the nest. Consequently, the detected decrease of host defences to C. canorus parasitism in Iceland by Davies & Brooke (1989a) might be explained by phenotypic plasticity.

The higher intraclutch variation in egg appearance in A. pratensis populations allopatric with C. canorus might be the consequence of the loss of defence in the absence of C. canorus parasitism. Theoretical studies have proposed that in the absence of parasitism the costs of recognition errors are the only ones that may counteract the benefits of rejection and this may explain the loss of a defence (Lotem et al., 1992, 1995). However, Rothstein (2001) stated that no clear evidence exists of recognition errors at unparasitized nests in avian brood parasite studies, suggesting that available data from the literature are consistent with the maintenance of host defences. Moksnes et al. (1993) studied the rejection behaviour of unparasitized A. pratensis nests in Norway in the presence and absence of a C. canorus dummy and failed to find differences in the rejection rate. This would suggest that recognition errors, if they exist, should be negligible in unparasitized A. pratensis nests. However, we can not preclude this possibility since differences among populations in the level of recognition errors may exist and no information on the occurrence of such costs exists for the British A. pratensis population.

An alternative explanation is that patterning of A. pratensis eggs in allopatry with C. canorus populations has not changed from the ancestral state, and that higher intraclutch variation in the islands is a result of genetic drift in the absence of C. canorus parasitism maintaining this trait favoured by a very low level of gene flow between populations. Information about the level of mimicry between C. canorus eggs and those of the ancestors of current A. pratensis populations in Iceland and the Faeroes is lacking. Thus, according to this idea, a reduction in intraclutch variation has never been selected for in island A. pratensis populations because there was a bad match between the appearance of the C. canorus eggs and the appearance of the eggs of the ancestors of the current A. pratensis on the islands. In accordance with this, Davies & Brooke (1989a) did not find evidence of gene flow between Iceland and continental populations of A. pratensis after analyzing the Iceland ringing recoveries for the species. Moreover, measurements of Icelandic and British A. pratensis in museums also showed significant differences in size, which may indicate genetic differences (Davies & Brooke, 1989a).

Nonetheless, A. pratensis still rejects in areas of allopatry with C. canorus even when no recent history of co-occurrence is known (Davies & Brooke, 1989a). Interestingly, they rejected five of the ten redstart type models introduced into their nests by Davies & Brooke (1989a) in Iceland. Theoretical models have shown that a rejection rate of about 50% is sufficiently strong to result in the evolution of mimicry (Kelly, 1987). Thus, it is likely that C. canorus mimicry of the eggs of the ancestors of current A. pratensis populations had already evolved, and therefore that a reduction of intraclutch variation in the appearance of A. pratensis eggs was advantageous for discriminating against C. canorus eggs.

We found differences within the part of the spectrum not visible to the human eye in intraclutch variation in egg appearance between populations of A. pratensis sympatric and allopatric with C. canorus. When in allopatry with C. canorus, A. pratensis had a higher intraclutch variation in UV chroma than it had when in sympatry. Therefore, our results suggest that methods of assessment of egg appearance within clutches based on human vision might not be sufficiently sensitive to assess variation in certain colour components that could be crucial for host discrimination of parasite eggs.

In summary, our study is the first documenting intraspecific evolutionary changes in a genetically determined component of host defence to brood parasitism. Further work reporting the real occurrence of recognition errors in A. pratensis populations in allopatry and sympathy with C. canorus is needed, because these data are crucial for explaining the evolution of host defences in the absence of C. canorus parasitism.

ACKNOWLEDGEMENTS

We thank J. Fjeldså and J. Bolding of the ornithological section of the Zoological Museum of the University of Copenhagen and R. Præss-Jones and especially M. Walters of the British Museum (Natural History), Tring, for help and facilities during data collection. D. Parejo, P. Ninni and L. Garamszegi made useful suggestions during the preparation of the paper. We also thank one anonymous referee for their comments on the paper. This research was funded by a European
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