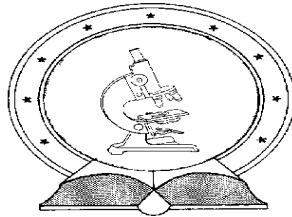


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1949

**KAKUKK PARAZITIZMUS NÁDIRIGÓN: KOEVOLÚCIÓS
ADAPTÁCIÓS MECHANIZMUSOK**

**COMMON CUCKOO PARASITISM ON THE GREAT REED
WARBLER: MECHANISMS OF COEVOLUTIONARY
ADAPTATION**

Egyetemi doktori (PhD) értekezés

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Természettudományi Doktori Tanács
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Debrecen, 201.....

Tanúsítom, hogy Bán Miklós doktorjelölt 2006 - 2009 között a fent megnevezett Doktori Iskola Biodiverzitás programjának keretében irányításommal végezte munkáját. Az értekezésben foglalt eredményekhez a jelölt önálló alkotó tevékenységével meghatározóan hozzájárult. Az értekezés elfogadását javasolom.

Debrecen, 201.....

Kakukk parazitizmus nádirigón: koevolúciós adaptációs mechanizmusok

**Common cuckoo parasitism on the great reed warbler: mechanisms of
coevolutionary adaptations**

Értekezés a doktori (PhD) fokozat megszerzése érdekében
a biológia tudományágban

Írta: Bán Miklós okleveles biológus

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Köszönetnyilvánítás

Szeretnék köszönetet mondani témavezetőmnek Dr. Moskát Csabának. Az ő segítsége és türelme nélkül ez a dolgozat nem jött volna létre. Dr. Barta Zoltán szintén nagyon sokat segített és lehetővé tette, hogy évente több héten át dolgozhassam terepen. Zsoldos István a terepmunkákban segített az utóbbi években nagyon sokat. Micheal G Anderson és a nővérem Bán Helga a kéziratok nyelvi ellenőrzésében segítettek többször. A 2005 óta, mióta a kiskunsági projekteben dolgozom, számos publikáció elkészítése kapcsán az összes társszerzőnek köszönöm az együttműködési lehetőséget és a közös munkát. Ezen idő alatt az alábbi pályázatok jelentettek közvetlen vagy közvetett anyagi forrást a kutatások lebonyolításához: OTKA T48397; OTKA 83217; OTKA K75696; INCORE: FP6-2005- NEST-Path, no. 043318, Integrating Cooperation Research across Europe; Spanyol-Magyar kétoldali együttműködés: HH- 2005-0021/TÉT E-23/2005; TAMOP project (4.2.1./B-09/1/KONV-2010-0007) .

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Bevezetés

Az úgynevezett költésparazita állatok utódaik felnevelését más fajokra bízják és ezzel a nem rokon utódok gondozásának költségeit a gazdára terhelik (Kilner & Langmore 2011). Ez a szociális parazitizmus a gazdára (mostoha szülők) és parazitára egyaránt szelekciós nyomást fejt ki és koevolúciós folyamatot indít be (Rothstein 1990), ami morfológiai, viselkedési és kognitív adaptációk formájában jelentkezik a gazda és a parazita részéről is (Krüger 2007). Ilyen például a parazita tojás felismerése és eltávolítása a gazdák részéről, vagy a tojásméret redukciója a kakukk részéről.

A kakukk (*Cuculus canorus*) obligát költésparazita madárfaj. Fészket nem épít, tojásait más madarak fészkebe rakja, az utódairól a továbbiakban nem gondoskodik. Szaporodási parazitizmusa igen költséges a gazda részére, mivel a sikeresen parazitált fészekben csak a kakukk fiókája nő fel. Emiatt a gazdák többsége védekezik a kakukk parazitizmus ellen, pl. felismeri és eltávolítja a kakukktojást a fészekből. Ez erős szelekciós nyomás, ami miatt a kakukk adaptálódik a gazdához, pl. kialakul és folyamatosan javul a tojásmimikrije. A gazda parazitaellenes viselkedése által szelektált kakukk tulajdonságok visszahatnak a gazdára, ami a kölcsönös adaptációk jól vizsgálható dinamikus „evolúciós versenyfutását” (Dawkins & Krebs 1979) eredményezik. A kölcsönös adaptív válaszok mellett a kakukknak vannak olyan „trükkjei” (Davies 2011), ami a gazda viselkedését specifikusan befolyásolja és a gazdának nem feltétlenül vannak ellene válaszai, mint például a kakukkfióka kérőhangja, ami a szülőket fokozott táplálékfordásra ösztönzi.

A kakukk széles elterjedésű faj Európában szinte mindenfelé előfordul, keleti elterjedési területe Japánig húzódik. Ezen a hatalmas területen számos madárfajjal volt és van kapcsolata. A kakukk különböző gazdákkal kapcsolatban lévő specializálódott rasszait genteknek nevezzük. A kakukk gentek a gazda tojás típusok szerint különböznek egymástól, elterjedésük földrajzi régióként átfed

egymással. A különböző gentek tojástípusai ránézésre elkülöníthetők egymástól. Európában eddig 16 főbb gentet írtak le (Alvarez 1994 , Moksnes & Røskaft 1995). Az egyes gentek fennmaradása nem kizárólag a gazda felismerésén alapul (Lack 1963), hanem a kakukk területhűségének (Seel 1977) és élőhelytípushoz való ragaszkodásának (Moksnes & Røskaft 1995) is van benne szerepe.

A kakukk-gazda kapcsolat egy-egy gazda populáció esetében a sorozatos kölcsönös adaptációk miatt folyamatosan változik (Takasu et al. 1993), aminek különböző kimenetele lehet (Dawkins & Krebs 1979), de a legvalószínűbb az, hogy a gazda a kakukktojást nagyon jól felismeri, ami a kakukk parazitizmus megszűnésével jár (Lovászi & Moskát 2004, Honza et al. 2004 , Stokke et al. 2004, Procházka & Honza 2003). A kakukk-gazda kapcsolat koevolúciós állapotán túl a kakukk parazitizmus mértékét egy gazda populáción belül a helyi élőhelyi jellegzetességek is befolyásolják, mivel nem minden élőhelytípusban képes a kakukk megtalálni a gazda fészket. A legfontosabb a kakukk számára, hogy a gazda kifigyelésére alkalmas fák legyenek a fészkek közelében. Hosszútávú kakukk-gazda kapcsolat olyan élőhelyen tud kialakulni, ahol változatos élőhelyi adottságok vannak a kakukk számára és a gazdapopuláció egy része a kakukk parazitizmustól mentes (Røskaft et al. 2002).

A Magyarországon leggyakoribb kakukk-gazda a nádirigó (*Acrocephalus arundinaceus*) élőhelyein fás és fátlan területek egyaránt vannak. Az általunk vizsgált kiskunsági nádirigók fészkeiben világszinten egyedülállón gyakori a kakukktojás (64% - Moskát & Honza 2002). Ez a magas parazitáltsági szint csak más alacsonyabb parazitáltságú nádirigó populációkból való folyamatos nádirigó betelepüléssel képzelhető el (Barabás et al. 2004). A nádirigók tipikus szegélylakó madarak, nádasok többnyire víz felőli 3-4m-es sávjában találhatóak a fészkei, a víz felett, változatos magasságban. A Kiskunságban többnyire keskeny csatornákat szegélyező 1-3 méter széles nádsávban költenek. A hímek a nád tetejére ülnek ki énekelni. A territóriumok alkalmas nádasban 10-50 méter távolságra vannak egymástól. A fészkeik nádszálakra szőve készülnek, a tojásokon csak a tojó kotlik és a fiókákat mindkét szülő eteti. A fészkek rendben tartását, az idegen tárgyak

eltávolítását csak a tojó végzi, míg a fészkek őrzése elsődlegesen a hímek feladata. Azaz pl. amikor egy kakukk odarepül a fészkekhez, többnyire a hím támad rá először, de amikor kakukktojás kerül a fészkekbe, arra csak a tojó reagál. Ha egy kakukktojásból kikel a kakukkfióka, azt a nádirigó szülők minden esetben felnevelik. Védekező reakciók csak a kakukk tojások és az öreg kakukkok ellen irányulnak. Fióka elutasítás csak más költésparazita madárfajoknál fordul elő és ott sem gyakori (Grim 2006).

A kakukk és gazda koevolúció hosszabb távú együttélés esetén lehetséges, amire jó példa a magyarországi alföldi kakukk és nádirigó példája (Baker 1942, Molnár 1944). A hosszútávú együttélés egyre specializáltabb kognitív mechanizmusok kifejlődését segíti elő adott helyzetben értelmezhető optimalizációs folyamatok által (Reeve 1989). A parazita optimális alkalmazkodása nem feltétlenül a tökéletes mimikri kialakulását jelenti a kakukk részéről és különböző gazda populációk esetében eltérő viselkedések lehetnek dominánsak vagy alakulhatnak ki ami a párhuzamos koevolúcióra jó példa (Nakamura et al 1998). A gazda parazita elutasítási viselkedésének fejlődése szintén összetett folyamat, ami egyrészt különböző populációk esetében hasonló módon zajlik (Davies & Brooke 1989a,b) másrészt gazdapopulációnként egyedileg különböző lehet (például a különböző reakció típusok relatív gyakorisága). Ezek az egyedi különbségek az alapjai a költésparazitizmus általános modelljei (Reeve 1989, Rodriguez-Gironés & Lotem 1999) eltérő támogatottságának (pl.: Stokke et al 1999 vs. Lotem et al. 1995).

Mivel a parazitizmus elutasítása költséges (fészkelhagyás, saját tojás kidobás), ezért beszélhetünk optimális elfogadási szintről a gazdák részéről (Reeve 1989). Az optimális elfogadási szint pedig függhet az egyedek tapasztalatától. A nádirigó alföldi állománya esetében a fejlett felismerési képesség a kiváló mimikrijű kakukktojások mellett viszonylag alacsony kakukktojás elutasítási szintet (34%) eredményez. Egyedi sajátossága a kutatási területünknek a kakukkok nagy gyakorisága, ami miatt a többszörös parazitizmus (több kakukktojó is rak tojást egy gazdafészkekbe) is szokványos jelenség. Emiatt jó lehetőség van ezen a

nádirigó populáción tesztelni a kakukk parazitizmus elutasítás fejlődésének mélyebb részleteit, ami egyrészt korábbi elméleti modellek tesztelésére alkalmas másrészt koevolúciós mechanizmusok általánosabb megértésére.

2005-óta végzek terepi vizsgálatokat a Kiskunlacháza - Kunszentmiklós térségben élő erősen kakukk parazitált nádirigókkal. Dolgozatom témája a kakukk és nádirigó koevolúciós kapcsolatának vizsgálata mindkét faj szempontjából. Az alábbiakban hat tanulmányt mutatok be, amelyekből egyben múzeumi tojásgyűjteményekből származó tojásokról készült fényképeket elemeztem, a többi pedig egy-egy év terepi munkáinak eredményei. Az első kettőben a kakukk adaptációjával kapcsolatos vizsgálatokat, a következőkben pedig a gazda adaptív válaszaival foglalkozó kutatásokat mutatok be.

Módszerek

A gazda és kakukk tojásalakjának összehasonlítása során a Mátra Múzeum tojásgyűjteményében készítettem fényképeket különböző gazdatojásokról ill. a társzerzőktől kaptam terepi munka során készített fényképeket parazitált fészkekről. A fényképek elemzésekor Mónus és Barta (Mónus & Barta 2005) módszerét vettem alapul a tojás alak leírásához és összehasonlításához. A tojás fél körvonalán felvett 9 pontra egy másodfokú polinomot illesztettem. A tojás ív jellemzésére más módszereket is kipróbáltam, de a legegyszerűbb 3 paraméteres polinom bizonyult a legjobban ismételhetőnek és a legjobban értelmezhetőnek. Az analízisek során sokváltozós anovát használtam a három paraméter hatásának az összehasonlítására. A kakukk-gazda összehasonlítás során 1000 ismétlés során válogattam véletlenszerűen gazdatojásokat a páronkénti összehasonlításhoz.

A hangelemzésekkor az egyes nádirigók énekhangjainak összehasonlításához az összes énekhangot felbontottam a legkisebb ismétlődő egységekre (syllabusok). A leggyakoribb, legjobban azonosítható hangjegyeket egyedileg jelöltem minden énekhang felvételen a hangjellemzők összehasonlításához.

A terepi vizsgálatokat minden évben május közepétől június közepéig-végéig végeztem a kísérletek típusától függően. Az első dolog minden évben a nádirigó fészkek megtalálása volt. Ez közös munka volt az éves projektek minden résztvevőjével. A fészkeket a partról, vagy a vízből kerestük a csatornák menti nádsávot átfésülve. Ez évenként 20-30 km hosszú csatorna-menti nádas átnézését jelentette legalább hetente ismételve. A megtalált fészkekhez vezető utat a parton kijelöltük, a fészkek helyének koordinátáit rögzítettük (GPS). A kísérletekben résztvevő fészkeket naponta ellenőriztem. Az ellenőrzések során törekedtem a legkevesebb zavarást okozni, a fészkekbe többnyire csak egy tükör segítségével néztem bele, a tojásokhoz nem nyúltam hozzá. A fészkekellenőrzések során minden adatot rögzítettem. A kísérletek során ahol a tojásokat festettem, a tojásokat egyesével vettem ki a fészkekből és festettem be minden évben azonos típusú tollakat használva. Kísérleteket olyan fészkekben kezdtem amikről biztosan lehetett tudni, hogy nincs és nem is volt benne kakukktojás ill. más kísérletben nem vesz részt. A legtöbb kísérlet maximum hat napig tartott, mivel az inkubáció előrehaladtával a nádirigó reakciója az idegen tojás ellen csökken (Moskát & Honza 2002). A fiatal nádirigók nem területhűek, míg az öreg madarak lehet, hogy legalább részben azok. Öreg nádirigókat egyedileg csak kis számban (~60) jelöltem (színes gyűrűk kombinációja), mivel nagyon nehéz megfogni a költő párokat. Emiatt jelenleg nem tudjuk pontosan, hogy vajon a kor és a korábbi tapasztalatok hogyan befolyásolják a reakciókat az idegen tojások ellen, de egy recens vizsgálat során próbálom kideríteni a kor pontosabb hatását.

Eredmények

A tojásalak

A kakukktojások hasonlósága (mimikrije) a gazda tojásaihoz régóta ismert jelenség és számos tanulmány szól róla (Wyllie 1981, Moksnes & Røskaft, 1995, Davies 2000, Cherry & Bennett 2001). Ezek a vizsgálatok többnyire csak a tojások mintázatával foglalkoztak, a tojás alakját figyelmen kívül hagyták miközben a

tojás alakja fajokra jellemző és feltehetőleg az adott faj átlagos tojásszáma és a fészkek adottságai mellett ideális hőháztartást biztosít a fejlődő embrióknak (Barta & Székely 1994). Az **első fejezetben** bemutatott publikált vizsgálatban magyarországi és japán gazdafajok tojás alakját hasonlítottuk össze a fészkeikben talált kakukktojások alakjával, ill. a különböző földrajzi régiókból származó kakukktojások alakját egymással. Eredményeink szerint a kakukktojások a gazda fajokkal alak tekintetében nem mutat hasonlóságot és földrajzi területenként eltérést mutatnak. Ez az eredmény azt is jelentheti, hogy a kakukk nem tökéletesen alkalmazkodott egy-egy gazdafajhoz, a tojásainak olyan jellegei is vannak, ami a különböző gazdafajok váltása között fontos lehet.

Gazda védekezése és a kakukk választása

A nádirigóknál mindkét szülő részt vesz az utódok gondozásában, viszont egyes feladatokban a két nem eltérő szerepet vállal. A fészket a tojók építik, kotlani is csak a tojó kotlik, viszont a fiókákat mindkét szülő eteti. A fészkek és a territórium védelme leginkább a hímek feladata. A hím nádirigó éneke mint kondíció jelző szignál fontos lehet a tojók számára (Catchpole et al. 1985, Soler et al. 1995, Forstmeier & Leisler 2004, Parejo & Avilés 2007) a fészkek minősége pedig a hím számára jelezheti a tojó szülői képességeit, szándékát (Szentirmai et al. 2005). A nádirigó szülők szülői minősége, etetési aktivitása nem csak a saját fiókái számára alapvetően fontos, hanem az őt kihasználó kakukknak is (Hasselquist et al. 1996) az lehet, mivel a kakukk fióka ugyan egyedül nő föl a fészkekben, de 10 nappal tovább van ott és a kirepülési tömege nagyobb mint egy átlagos nádirigó fészkealj összes fiókájának együttes kirepülési tömege. A **második fejezetben** bemutatott tanulmányban azt vizsgáltuk meg, hogy a gazda párok szexuális szignáljai (hímek éneke) és egyes minőségi paraméterei (etetési aktivitás, fészkekminőség) mutatnak-e összefüggést a kakukktojások elutasításával és a kakukkok fészekválasztásával. Azt találtuk, hogy a kakukk tojók nem használják ezeket az információkat, de a jobb minőségi szignálok erősebb elutasítási

képességekkel párosulnak a gazdák részéről. A kakukk úgy tűnik nem válogat a megtalált fészkek között a vizsgált területen. Ez egyrészt azért is lehet, mert a számára is kedvező gazda-szülői paraméterek hatékonyabb elutasítással párosulnak a gazda részéről, másrészt a vizsgált területen nagyon nagy a kakukkok közötti versengés amit többszörös parazitizmus gyakorisága is jól jelez. Egy szülői minőség vs. kakukk elutasítás optimum modellel és más – kevésbé erősen parazitált területen elvégzett hasonló vizsgálattal ez még tovább vizsgálható kérdés.

Tojásfelismerés és elutasítás

A nádirigó többféle módon tud védekezni a kakukk parazitizmusa ellen. Ezek mindegyike az idegen tojás felismerésével kezdődik, amelyet valamilyen döntés és diszkriminatív esemény követhet. A gazda reakció tipikusan az idegen tojás eltávolítása, a fészkek elhagyása, vagy az idegen tojás fészkekbe építése szokott lenni (Moskát & Honza 2002). Az idegen tojás felismerése nem hiba mentes, legrosszabb esetben irányulhat akár saját tojás ellen is. Pontossága függ a kakukktojás hasonlóságától, a gazda előzetes tapasztalatától a saját és idegen tojásokkal, vagy a parazita tojás fészkekbe kerülésének körülményeitől (Moskát & Hauber 2007). A gazda reakciójának mértéke összefüggést mutat a parazitával való találkozás esélyével és a gazda-parazita tojás eltérésének a nagyságától (Reeve 1989). A **harmadik fejezetben** bemutatott tanulmányban az optimális elfogadási küszöb eltolódását teszteltük a parazita gyakoriság kísérletes növelésével. A nádirigó kiskunsági populációjában az nádirigók idegentojás elutasítási valószínűsége különböző tojás típusonként (mimikri minősége) körülményektől függően változik. Ez azt jelenti, hogy egy költési szezonon belül ugyanaz a nádirigó tojó ugyan olyan típusú kakukktojásra különböző mértékben reagálhat. A kidobás valószínűsége nagyobb, ha előzőleg már találkozott parazita tojással. Azaz az idegen tojás elfogadásának küszöb szintje csökken a parazitizmus valószínűségének növekedésével (Reeve 1989, Bártol et al. 2002). Az

elutasításnak ez az egyedi helyzetfüggő változatossága segíthet egy gazdapopuláció stabilizálódásában egy olyan helyzetben ahol csak jó hasonlóságú kakukktojások vannak jelen.

Diszkriminációs képesség és mechanizmusok

A parazita tojás felismerésének többféle kognitív mechanizmusa ismert. Alapulhat a fészekben levő tojások összehasonlításán, vagy egy memória sablonnal való összevetésen. Az első esetben a diszkrimináció a fészekben a többitől leginkább különböző tojás ellen irányul (*diszkordancia hipotézis*: Rothstein 1974), aminek az evolúciós alapja, hogy a kakukkgazda fajok egy fészekaljon belüli tojásai hasonlítanak egymásra (Davies & Brooke 1988), ami elősegíti az eltérő tojások felismerését (Moskát et al. 2008). Önreferencia alapulhat egyrészt valós idejű összehasonlításon (in situ összehasonlítása a saját és idegen tojásnak) vagy *memórián alapuló idegi folyamatként* (egy belső templátot használva a saját és idegen fenotípusok megkülönböztetésére) (Hauber & Sherman 2001). A **negyedik fejezet** első tanulmányában (4. publikáció) kísérletes úton befolyásoltuk a nádirigók saját tojás tanulását, ami így a memória sablon meghatározott módosítása miatt alkalmas volt a diszkordancián alapuló döntési mechanizmussal való összevetésre. Eredményeink szerint a nádirigónál a diszkordancia és a memória sablon mechanizmusok is szerepet játszanak az idegen tojás felismerésében.

A többszörös parazitizmus olyan eset, amikor egy gazda fészkébe egymástól függetlenül több parazita utód is kerül. A kiskunsági nádirigóknál a magas kakukk gyakoriság miatt rendszeresen előfordul, hogy több kakukktojás is van egy fészekben. A kakukkfiókák egymást is kilökik a fészekből, ami számukra egyértelműen hátrányos a többszörös parazitizmus, de a nádirigó tojásfelismerési hatékonyságát is befolyásolja a több idegen tojás jelenléte a fészekben. A diszkordancián alapuló felismerés hatékonysága ilyen esetekben várhatóan csökken, vagy akár a saját tojások ellen is irányulhat, ha azok vannak

kisebbségben. Ezzel szemben a memória sablon használata esetén nem várunk különbséget többszörös parazitizmusban az egyszereshez képest az idegen tojások elutasításának mértékében. A **negyedik fejezet** második részében (5. publikáció) bemutatott tanulmány eredményei szerint a többszörösen parazitált fészkekből nagyobb eséllyel repültek ki kakukk fiókák, mint az egyszeresen parazitáltakból ami többszörösen parazitált fészkek esetében a diszkordancián alapuló döntési mechanizmus szerepét emeli ki.

A következő tanulmányban (**negyedik fejezet**, 6. publikáció) az idegen tojás elleni diszkrimináció kognitív alapjait vizsgáltuk kísérletesen, úgy hogy az egyszeres és többszörös parazitizmus hatásait együttesen teszteltük olyan esetekben is, ahol kisebbségben van a gazda tojás vagy egyáltalán nincs jelen saját tojás a fészkekben és emiatt sem az *önreferencián*, sem a *diszkordancián* alapuló mechanizmusok nem működhetnek. A kísérletek eredménye szerint a memória sablon használata mindig befolyásolja a nádirigók idegen tojás elutasítási döntését, de a két mechanizmus bizonyos esetekben együttesen határozza meg a gazdák viselkedését. A különböző felismerési mechanizmusok összehasonlítására végzett vizsgálataink eredményei szerint a nádirigók olyan helyzetben is képesek reagálni a parazitizmusra, ha csak idegen tojás van a fészkekben. Ebben a különleges és a természetben ritkán előforduló helyzetben gyakran hagyják el a parazitált fészket. Az olyan helyzetben pedig, ahol a kísérletesen manipulált tojások többségben vannak a fészkekben a saját tojásokkal szemben, az egyébként számukra a legkevésbé elfogadott tojástípust (az egyszeres parazitizmus szerint) utasítják el. Ez a következetesség egy olyan önreferencia jelenlétét feltételezi, amihez bármilyen esetben tud hasonlítani a gazdamadár. Ez a mechanizmus tulajdonképpen megvédi a nádirigókat a saját tojásai elutasításától olyan többszörös parazitizmusban ahol a saját tojásai kisebbségben vannak. Hosszabb távon pedig ennek a különleges gazda-parazita együttélés gazda oldali nyereséggel záruló kimenetelének kulcsa lehet.

Introduction

The so called brood-parasites leave parental care of their offspring to other species thus loading the costs of caring of the non-related young on the host. (Kilner & Langmore 2011). This social parasitism imposes selection pressure both on the hosts (foster parents) and parasites and generates a co-evolutionary process (Rothstein 1990), which will occur in the form of morphological, behavioural and cognitive adaptations (Krüger 2007). For example the recognition and rejection of parasite eggs by the hosts and the egg size reduction by the cuckoos.

Common cuckoos (*Cuculus canorus*) are obligate brood parasites. They do not build a nest, do not spend time with raising the chicks but lay their eggs in the nests of other avian species and let them raise the chicks. Reproduction parasitism is costly for the host, since only the cuckoo chick will grow up in a successfully parasitized clutch. Most of the hosts have evolved different defence mechanisms against cuckoo parasitism, e.g recognition and rejection of the cuckoo egg. This evokes strong selection pressure on the cuckoo, which it tries to overcome by adapting to the host, e.g. its egg mimicry develops and improves continually. Those selected attributes of the cuckoo which effect the hosts' anti-parasite behaviour will in turn influence the host again, and result in a dynamic „evolutionary race” of coevolutionary adaptations that can be studied and analysed on a large scale. (Dawkins & Krebs 1979). Besides the mutually adaptive responses the common cuckoo has evolved some tricks, which will influence host behaviour specifically and on those the host may not have counter-reactions (Davies 2011), for example the begging call of cuckoo nestlings which is intensify the feeding activity.

Cuckoo is a widespread species, it can be found everywhere in Europe. Its eastern geographical range reaches to Japan. On this wide geographical range the cuckoo has interactions with many species. Specialized types of cuckoos

related to different hosts are the so-called gentes. cuckoo gentes differ from one another in host egg variants. Their geographical occurrences may overlap. The egg types of different gentes are distinguishable simply by observing. 16 major gentes have so far been described (Alvarez 1994, Moksnes & Røskaft 1995). The existence of a cuckoo gent does not exclusively depend on the ability of recognizing the host (Lack 1963), but also on the cuckoo's fidelity to certain locations (Seel 1977) and habitat types (Moksnes & Røskaft 1995).

The cuckoo-host relationship is changing continuously due to successive coevolutionary adaptations (Takasu et al. 1993). However, this may have different outcomes (Dawkins & Krebs 1979). It is most likely that the host becomes an excellent cuckoo egg recognizer and that will inhibit cuckoo parasitism (Lovász & Moskát 2004, Honza et al. 2004, Stokke et al. 2004, Procházka & Honza 2003). Since Cuckoos may not be able to find the hosts' nest in each habitat type the number of parasitized nests within a host population is not only influenced by the state of development of cuckoo-host relationship but also the characteristics of the local habitat. The most important factor for cuckoos is to find trees in the vicinity of the nests, which will be suitable for observing their hosts. Long-term cuckoo-host relationships can be established in diverse habitats only where at least a proportion of the host population can avoid the parasitism (Røskaft et al. 2002).

There are both wooded and clear areas in the habitats favoured by the most frequent cuckoo host in Hungary, the great reed warbler (*Acrocephalus arundinaceus*). In clutches of the the great reed warblers studied in Hungarian Kiskunság, cuckoo eggs occur in particularly high extent (64% - Moskát & Honza 2002), which is unique in the world. This high rate of parasitism can only be maintained for a longer period if a continuous immigration from a source population with lower parasitism levels exists (Barabás et al. 2004). The great reed warblers typically breed on the water edges, their nests are found in 3-4 meter-wide reedbeds at varied height. In the Kiskunság they mostly breed in 1-3 m wide reedbed along sides of the narrow channels. Male great reed warblers sit on top of

the reed to sing, their territories extending 10-50 ms away from each other. Their nests are weaved and built on reed strips. Only the female incubates and both parents feed the chicks. Females are responsible for cleaning the nests, ejecting foreign objects, whilst males are in charge of guarding the nests. For example, when a cuckoo flies to a nest, mostly the male attacks first. However, when a cuckoo egg gets into the nest it is only the female who will react. Whenever a cuckoo hatches from a cuckoo egg, it will be raised by the great reed warblers by all means. Anti-cuckoo reactions are only taken against eggs and adult cuckoos. Nestling rejection only occurs among other brood parasite systems although it is still uncommon (Grim 2006).

The cuckoo-host coevolution is only possible if species co-occur for a longer period as it can be seen in the example of the cuckoo and great reed warbler populations on the great Plain in Hungary (Baker 1942, Molnár 1944). Long-term coexistence facilitates the development of increasingly complex cognitive mechanisms through local interpretation of optimization processes (Reeve 1989). The optimal adaptation behaviour of the cuckoo may not necessarily result in the development of one perfect mimicry to host species but generating a distinct behavioural pattern or shifting dominances between behaviours for different host populations. This phenomenon is supported by the coevolutionary theory (Nakamura et al 1998). The development of host's anti-parasite behaviour is a complex process which shows strong similarities between populations (Davies & Brooke 1989a,b) but individual patterns within host populations (for example the relative frequency of the distinct rejection types). These individual differences distinctly support (eg.: Stokke et al 1999 vs. Lotem et al. 1995) the general models of brood parasitism (Reeve 1989, Rodríguez-Gironés & Lotem 1999).

Due to the costs (e.g. nest abandonment, own egg ejection) emerging from the rejection of cuckoo parasitism the hypothesis of the existence of an optimal level of acceptance is plausible (Reeve 1989). The optimal acceptance level can depend on the experience of the individuals. Despite the highly developed

recognizing skills of the Great Reed Warblers on the Hungarian Great Plain the perfection of cuckoo's eggs results in a low rejection rate (34%). Multiple parasitism (more than one female cuckoos lay eggs in one host nest) is common at our study site due to the uncommonly high density of cuckoos in the region. These unique characteristics enable us to run detailed tests on the development of the rejection of cuckoo parasitism on great reed warbler populations.

I have been doing field research with great reed warblers since 2005 in the Kiskunlacháza - Kunszentmiklós region; a place of exceptionally high rate of cuckoo-parasitism. My thesis focuses on the coevolutionary relationship between cuckoos and great reed warblers from the point of view of both species. I will present six studies below. One of them based on an image analyses using photos taken of museums' egg collections. The remaining of articles based on the fieldwork was made in several years. The first two studies are investigating the adaptations of cuckoos and the others are research papers focusing on the adaptive answers of hosts.

Methods

Comparison between cuckoo and host egg shapes was made by using images I took in Mátra Museum, Gyöngyös, Hungary and images taken in situ at the site by the co-authors. In order to compare and describe egg shape, my method based on that of Mónus and Barta (2005) was used. I fitted a second order polynom on the nine points that were marked on a half outline of an egg. Although other methods were tested the most simple three-parameter polynom proved to be the best repeatable and also provided biologically interpretable results. Multivariate ANOVA was used to compare the effects of the three parameters. Also, a randomized comparison was repeated 1000 times between host and cuckoo eggs from the same clutch.

In the course of song analysis all recorded songs were split into the smallest repeated pieces (syllables) to compare the calls of great reed warblers. To

compare the song characteristics, I marked the most frequent and most easily identifiable pieces individually on each record.

The field research took place in the Kiskunság region, nearby Apaj village. Depending on the type of experiments we run fieldwork from the middle of May until the middle or end of June every year. The first task in all years was to find the great reed warblers' nests. All participants of the yearly projects were involved at this stage. We were searching for nests from the bank or from the water along the channels. This meant checking a 20-30 km reedbed in consecutive weeks every year. We marked the pathway to the discovered nest on the brink outside and we recorded the gps coordinates to the nest. Those nest that where included in my experiments were checked on a daily basis. I tried to make the least disturbance possible whilst checking the nests by using a mirror to look into the nest and avoiding to touch of the eggs if it was possible. All data collected on the field were recorded. In the course of experiments when and wherever the eggs were dyed, I moved the eggs from nest one by one and used the same type of pens every year. I only conducted experiments with clutches which were free of cuckoo eggs and had not been involved in an other experiments. Since with incubation time running, the great reed warblers' reaction against foreign eggs is getting weaker (Moskát & Honza 2002) most of my experiments took no longer than six days.

The young great reed warblers have no fidelity to sites while the adults probably at least partially do. Only in a smaller sample (~60) did I mark adult great reed warblers individually (with the combination of colourful rings) because it is very difficult to catch the breeding pairs. So currently we don't know whether the age and the previous experiences how influencing the the reactions against the foreign eggs, but in a recent investigation I'm working on to discover the age effect.

Results

The egg shape

The resemblance (mimicry) of the cuckoos' eggs to hosts' eggs has been a well known phenomenon for a long time and several studies have been published about it (Wyllie 1981, Moksnes & Røskoft, 1995, Davies 2000, Cherry & Bennett 2001). However these experiments mostly took into consideration only the eggs' pattern, and ignored the shape of eggs'. The eggs' shape is a unique trait for the species and does not only give information about the average egg quantity and the qualities of clutch aptitudes but presumably also ensures ideal temperature for the embryos. (Barta & Székely 1994), hence these characteristics should also be investigated. In the published study which is presented in the **first chapter** we compared egg shapes of Hungarian and Japanese host species with the eggs' shape of cuckoos found in their nests as well as cuckoo egg shapes from different geographical regions. Findings revealed that cuckoo eggs do not show shape resemblance to host eggs, however, do differ between geographical regions. It means that cuckoos has not specialized on a single host species. Their egg has generalistic characteristics, which might become an important factor for the birds when changing host species.

Host defence and cuckoos' host choice

In case of great reed warblers both parents take part in caring about the nestlings, although with different responsibilities. Nests are built by the females, and it is the female only, who incubates, but feeding is done by both parents. Protection of the clutch and the territory is the task of the males. The song repertoire of a male great reed warbler can be an important condition signal for the females (Catchpole et al. 1985, Soler et al. 1995, Forstmeier & Leisler 2004, Parejo & Avilés 2007). The quality of the nest on the other hand is a marker for males about the females' parental qualities and motivations (Szentirmai et al. 2005). The parental qualities and feeding activity of the male and female great reed warblers are essential not only for their own nestlings but for their cuckoo parasite as well

(Hasselquist et al. 1996). Despite the fact that the chick is raised alone, it stays in the nest for 10 more days and its fledgling weight is more than the combined weight of all great reed warbler fledglings of an average nest. In the study, which was introduced in the **second chapter** we studied whether sexual signals of host couples (e.g. males' song) as well as some of their other quality parameters (feeding activity, nest quality) show correlation with rejection of cuckoo eggs and cuckoos' choice of host clutch. We found that cuckoo females disregard these pieces of information, however better quality-signals are matched with stronger rejection abilities from host's point of view. Apparently, cuckoos were not fastidious about choosing nests in the studied area. The reason could be that favourable host-parent characteristics come with more effective rejection from hosts' side. Strong competition among cuckoos in the studied site might be another explanation marked by the phenomenon of frequent multiple parasitism in several cases. Parental quality vs cuckoo rejection with optimum models and other experiments conducted in less heavily parasitized territories - this might be a field of study to be exploited in the future.

Egg recognition and rejection

The great reed warbler have different defence mechanisms against cuckoo parasitism. Each of these techniques starts with recognition of the foreign egg, which then is followed by some kind of decision and a discriminatory act or event. Ejection of the parasite egg, desertion of the parasitized clutch or building of foreign egg into the nest are the most typical reactions. (Moskát & Honza 2002). The foreign egg recognition is not fail-safe; a wrong decision can result in discriminate own eggs. The accuracy depends on the resemblance of cuckoo eggs, on previous experience of host with parasite and own eggs or on the circumstances how parasite egg got into the hosts' nest (Moskát & Hauber 2007). Hosts' reaction rate correlates with the chance of encountering parasite and host-parasite egg deviation rate. (Reeve 1989). In the study presented in the **third**

chapter we investigated and tested optimal acceptance threshold shifts with increasing experimental parasite-frequency. In the case of the great reed warbler - the most frequent host population in Kiskunság, the cuckoos' eggs rejection likelihood is context dependent according to egg types (mimicry quality). This means that within one breeding season the same great reed warbler female may react to the same type of cuckoo egg in a different extent. Ejection likelihood is higher if host has previously encountered parasite egg (i.e. foreign egg acceptance threshold level decreases with increasing parasitism likelihood Reeve 1989, Bártol et al. 2002). This unique, context dependent variability of rejection may help to maintain a host population where only well-resembling cuckoos are present.

Discrimination ability and mechanisms

There are several cognitive mechanisms of recognizing the parasitic egg. Recognition is either based on comparison of the eggs in clutch or on recognition from a memory template. In the first case the host discriminates the egg which differs most from others in clutch. (*discordancy hypothesis*: Rothstein 1974). The evolutionary background of this act is that the eggs of cuckoo host species within one clutch resemble one another (Davies & Brooke 1988), and this fact facilitates recognition of dissimilar eggs (Moskát et al. 2008). Self-referencing can exist based on online processing (in situ comparison of own vs. foreign eggs) or *memory processing* (using an internal template of own eggs to compare with foreign phenotypes) (Hauber & Sherman 2001). In the first study of the **fourth chapter** (4th publication) we influenced reed warblers' learning experimentally, which due to the defined modification of the memory template proved to be suitable for contrasting with decision mechanism based on discordancy. Our findings suggest that great reed warblers rely on both discordancy and memory template mechanisms in foreign egg recognition.

Multiple parasitism is a phenomenon when more parasite eggs get into the nest of a host, independently of each other. It frequently happens with great reed

warblers of Kiskunság that due to high frequency of cuckoo occurrence that their clutches contain more than one cuckoo eggs. Cuckoo chicks also eject each other from clutches thus for them multiple parasitism is evidently disadvantageous. More alien eggs also influence the efficiency of great reed warblers' egg recognition ability. Discordancy-based recognition efficiency in such cases will presumably decrease, or hosts could end up rejecting their own eggs, once these became the minority in the parasitized clutch. In contrast we do not expect any difference in the rate of alien egg rejection in case of multiple parasitism as opposed to singular parasitism when warblers use templates. Findings of the study presented in the second part of the **fourth chapter** (5th publication) showed that a nest with multiple parasitism had stronger likelihood of releasing cuckoo chicks, than a nest with single parasitism, which emphasizes the role of discordancy-based recognition mechanism in clutches with multiple parasitism.

In the next study (**4th chapter**, 6th publication) we investigated the cognitive bases of discrimination against alien eggs experimentally, we tested the effects of single and multiple parasitism jointly and in cases as well, where host eggs are in minority or not present at all thus mechanisms based on self-reference or discordance cannot function. Experiments revealed that memory templates always affect warblers' decision to reject alien egg, however in certain cases, the two mechanisms act together in determining host behaviour. According to findings of experiments, which were conducted in order to compare different recognition mechanisms, great reed warblers are able to react to parasitism even in those cases when there are only alien eggs in their clutches. They often desert parasitized clutch in such peculiar and rare cases, whilst in others, where experimentally manipulated eggs are in majority in the nests, they reject the least acceptable egg types (like in the single parasitism). This consistency assumes the presence of such a self-reference, which the host can use for comparison at any time. The mechanism in fact protects great reed warblers from rejecting their own eggs in multiple parasitized conditions where their own eggs are in minority. But in long term it might also become the clue for host side gainings in

the unique coexistence of host and parasite.

I.

A tojásalak

The egg shape

1.

The analysis of common cuckoo's egg shape in relation to its hosts' in
two geographically distant areas.

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The analysis of common cuckoo's egg shape in relation to its hosts' in two geographically distant areas

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Abstract

Evolutionary adaptations are required by common cuckoos *Cuculus canorus* to match host eggs. Hosts may discriminate against alien eggs; hence, accurate matching of the parasite egg to the hosts' is essential. Egg shape is the least-studied component of egg mimicry, and it may also have other functions: an optimal egg shape is necessary for effective incubation. For this reason, cuckoo eggs may show a wide range of variations in shape to a set of host species. Here, we compare cuckoo and host eggs by using egg shape parameters in two distant areas: from the nests of great reed warblers *Acrocephalus arundinaceus*, robins *Erithacus rubecula* and marsh warblers *Acrocephalus palustris* in Hungary, and oriental reed warblers *Acrocephalus orientalis*, bull-headed shrikes *Lanius bucephalus* and black-faced buntings *Emberiza spodocephala* from Japan. Our results suggest the lack of evolutionary adaptation of different cuckoo gentes to their corresponding hosts in terms of egg shape. However, our analyses revealed that cuckoo eggs showed a geographical difference in egg shape.

Introduction

The common cuckoo *Cuculus canorus* is a brood parasite that exploits many passerine species (Davies, 2000). Cuckoo females lay their eggs into host nests during the hosts' laying period, and cuckoo chicks hatched in parasitized broods typically evict all other eggs or nestlings from the hosts' nests (Wyllie, 1981). This eviction behaviour and the miss-directed parental care clearly reduce hosts' reproductive success (Davies & Brooke, 1988; Kilner, 2005). As a consequence, hosts typically develop antiparasite defence mechanisms against cuckoos and their eggs (for reviews, see Davies, 2000; Krüger, 2007). Hosts' discrimination against parasitic eggs (i.e. their recognition and rejection), in turn, selects for adaptations in the cuckoo to counteract hosts' defences to parasitism, leading to an evolutionary arms race (Dawkins & Krebs, 1979).

Generally, when a cuckoo egg is well matched to its host eggs, it has a greater chance to survive than those with poor matching (Davies & Brooke, 1988; Moksnes *et al.*, 1991; Hauber, Moskát & Bán, 2006; Cherry, Bennett & Moskát, 2007). Other factors, like uniformity of clutches, may also affect hosts' perceptual abilities in egg-rejection behaviour

(Davies & Brooke, 1989; Stokke *et al.*, 1999; Lahti & Lahti, 2002; Moskát *et al.*, 2008a). As a consequence of this evolutionary arms race, cuckoo egg morphs are extremely diverse among geographical regions and host species due to specific mimicry to specific host eggs, leading to the formation of races among cuckoos (the so-called 'gentes', singular 'gens' Baker, 1942; Davies & Brooke, 1989; Moksnes & Røskaft, 1995). Cuckoos' egg mimicry is frequently evaluated on the basis of egg characteristics, like colour (Cherry & Bennett, 2001; Avilés & Møller, 2003, 2004; Takasu *et al.*, 2009; Antonov *et al.*, 2010; Landstrom, Heinsohn & Langmore, 2010) and patterns of spottedness (Moksnes & Røskaft, 1995; Antonov *et al.*, 2010; Poláčiková & Grim, 2010; Stoddard & Stevens, 2010). In several experimental studies, painted model or foreign eggs, instead of real cuckoo eggs, have been used to investigate egg characteristics on egg-rejection behaviour, like the effects of colour (e.g. Honza, Poláčiková & Procházka, 2007; Avilés, 2008; Cassey *et al.*, 2008; Vikan *et al.*, 2009), spottedness (López-de-Hierro & Moreno-Rueda, 2010) or both (Nakamura, Kubota & Suzuki, 1998; Moskát *et al.*, 2008b; Avilés *et al.*, 2010). In spite of the increasing number of such studies, egg shape has attracted little attention (Mason & Rothstein, 1986; Langmore, Hunt & Kilner,

2003) and still remains an often-neglected component in the study of cuckoo–host coevolution.

Avian eggs, nevertheless, vary not only in colour and spottedness but in shape too, which is possibly related to efficient heat transfer during incubation in different-sized clutches (Barta & Székely, 1997). This variation points to the potential importance of egg shape in cuckoo–host coevolution. First, it might facilitate more perfect mimicry. Second, in order to achieve efficient heat transfer, the shape of the cuckoo eggs should match that of the host eggs.

In the present study, we investigated whether cuckoo eggs were similar to host eggs in their shape by comparing the egg shape characteristics of several host species and their corresponding brood parasites. As far as we know, this is the first attempt to examine the role of egg shape in the cuckoo–host relationship. We predict similarity in shape between eggs of a particular host species and its particular cuckoo gens, that is, the cuckoos adapted to host eggs shape in order to ensure more perfect mimicry. We also predict that cuckoos' adaptation to host eggs in shape may vary in geographically distant areas, as cuckoo races specialized on host populations vary in time and space (c.f. Davies, 2000).

Material and methods

We photographed parasitized clutches of great reed warblers *Acrocephalus arundinaceus*, marsh warblers *Acrocephalus palustris* and robins *Erithacus rubecula* in the egg collection of the Mátra Museum, Gyöngyös, Hungary (Fig. 1). These eggs were typically collected in the 1950s, 1960s and 1970s in southern Hungary. We also took pictures in the field on parasitized clutches in two study areas: (1) Kiskunság National Park, Hungary, c. 50 km south of Budapest, around the village Apaj (47°07'N; 19°06'E; great reed warblers), and (2) Nobeyama highlands (35°57'N, 138°28'E; oriental reed warblers *Acrocephalus orientalis*, bull-headed shrikes *Lanius bucephalus* and black-faced buntings *Emberiza spodocephala*, c. 120 km south of Nagano City, central Japan (Fig. 1). All clutches were photographed on a Kodak Grey Card by digital cameras (Olympus Camedia E20P, Olympus Co., Tokyo, Japan and Nikon COOLPIX 995, Nikon Co., Tokyo, Japan). Kodak Colour Control Patches were also placed near the eggs on each photo as a reference. We took the pictures at different

phases of egg laying; therefore, we have no information on the final clutch sizes in most of the cases.

We applied the method originally developed by Barta & Székely (1997) and improved by Mónus & Barta (2005) for measuring egg shape parameters from digital pictures, based on the circle transformation technique by Thompson (1942) and Todd & Smart (1984). To measure the eggs' shape, we used the ImageJ software (Abramoff, Magelhaes & Ram, 2004). We set up mark points around the eggs' edges: two points on the two opposite tips of the eggs, which determined the longitudinal axis of eggs, and seven roughly equally distributed points along each of the two edges of the shape (Fig. 2a). We fitted second-order polynomials to the coordinates of the mark points (Fig. 2b). We used three parameters of the polynomial to describe egg shape. The first (c0) parameter is the compression ratio relative to a circle (Fig. 3); the smaller the c0, the more elongated the egg. The other two parameters describe the level of asymmetry of the eggs (c1, larger c1 means more pointed egg) and roundness of the tips (c2, larger c2 means more blunted tips) (Fig. 3). Consequently, the three polynomial parameters describe egg shape together.

We measured eggs from 135 clutches belonging to six host species (Table 1) in Hungary and Japan. In the analyses, we used one randomly chosen host egg from each clutch to avoid pseudo replication. In order to exclude the possibility that our results arise as the consequence of a particular sampling of the host eggs, we repeated this procedure one thousand times (permutation test) and recorded the number of significant results (this applies only for the host eggs as we have only one cuckoo egg per nest). We combined the two sets of great reed warbler eggs from Hungary, where eggs were collected mainly in the 1950s and 1960s in southern Hungary and field data from Apaj from 1998 to 2004, as no difference was found when we compared these eggs' shape (MANOVA permutation test, $P < 0.05$, 16 cases out of 1000 runs).

All computations and statistical analyses were carried out within the framework of the R interactive statistical environment (R Development Core Team, 2009).

Results

Our analyses revealed that eggs of the different cuckoo genets significantly differed in shape (MANOVA Pillai = 0.388, $F_{15,387} = 3.842$, $P < 0.001$; ANOVA c0: $F_{5,129} = 7.641$,



Figure 1 Cuckoo *Cuculus canorus* (top row) and host (bottom row) eggs from six populations (left to right): black-faced bunting *Emberiza spodocephala* (Japan), bull-headed shrike *Lanius bucephalus* (Japan), oriental reed warbler *Acrocephalus orientalis* (Japan), great reed warbler *Acrocephalus arundinaceus* (Hungary), robin *Erithacus rubecula* (Hungary) and marsh warbler *Acrocephalus palustris* (Hungary).

$P < 0.001$; c1: $F_{5,129} = 4.916$, $P < 0.001$; c2: $F_{5,129} = 0.101$, $P = 0.363$; Fig. 4). Our results also suggested that cuckoo eggs from the two distant geographical areas, that is, Japan and Hungary, differed significantly in terms of egg shape (MANOVA Pillai = 0.256, $F_{3,131} = 15.053$, $P < 0.001$; ANOVA c0: $F_{1,133} = 32.581$, $P < 0.001$; c1: $F_{1,133} = 12.318$, $P < 0.001$; c2: $F_{1,133} = 2.713$, $P = 0.101$; Fig. 4). Cuckoo eggs from Hungary are more elongated than those from Japan (Fig. 4). The cuckoo eggs from different hosts' nests within

these areas, however, did not differ in shape (Hungary: MANOVA Pillai = 0.088, $F_{6,148} = 1.138$, $P = 0.34$; ANOVA c0: $F_{2,75} = 2.25$, $P < 0.112$; c1: $F_{2,75} = 0.968$, $P < 0.384$; c2: $F_{2,75} = 0.461$, $P = 0.632$; Japan: MANOVA Pillai = 0.2, $F_{6,106} = 4.813$, $P = 0.07$; ANOVA c0: $F_{2,54} = 0.657$, $P < 0.522$; c1: $F_{2,54} = 4.649$, $P < 0.013$; c2: $F_{2,54} = 0.809$, $P = 0.45$).

Host eggs from different populations differed from each other in terms of egg shape (MANOVA Pillai = 0.539, $F_{15,387} = 5.655$, $P < 0.001$; ANOVA c0: $F_{5,129} = 12.465$, $P < 0.001$; c1: $F_{5,129} = 1.356$, $P = 0.24$; c2: $F_{5,129} = 6.186$, $P < 0.001$; Fig. 4), and this difference seems not to depend on a particular random sampling of the host eggs (all 1000 repetitions of the MANOVA yielded highly significant results; all $P < 0.001$). Investigating the egg shape parameters separately shows that c0 and c2 differ between hosts species significantly (in all 1000 repetition, $P < 0.008$) while the results for c1 are not so clear ($P < 0.05$ in only 305 cases out of 1000). As there was no common host species in the two geographical areas, we did not analyse geographical differences between the host eggs from the two areas, because the effects of species identity and geographical area cannot be separated.

We found a significant difference between cuckoo and host eggs' shape when we controlled for population effects, that is, for the host races of cuckoos (MANOVA Pillai = 0.148, $F_{3,257} = 14.997$, $P < 0.001$, for all 1000 repetition, $P < 0.001$;

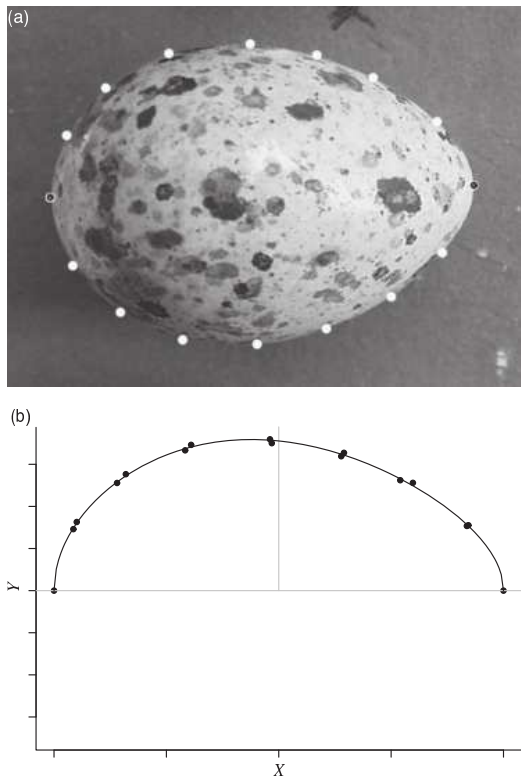


Figure 2 (a) Seven plus seven roughly equally distributed mark points along the two edges of an egg for characterizing egg shape, as demonstrated on a great reed warbler *Acrocephalus arundinaceus* egg. (b) A representation of polynomial curves fitted to the coordinates of the mark points, following Barta & Székely (1997) and Mónus & Barta (2005).

Table 1 List of host species, number of host and cuckoo *Cuculus canorus* eggs and location of samples in the present study

Host species	Number of eggs		
	Host	Cuckoo	Country
Black-faced bunting <i>Emberiza spodocephala</i> (field data)	18	6	Japan
Bull-headed shrike <i>Lanius bucephalus</i> (field data)	123	35	Japan
Oriental reed warbler <i>Acrocephalus orientalis</i> (field data)	35	16	Japan
Great reed warbler <i>Acrocephalus arundinaceus</i> (field data, museum coll)	76	25	Hungary
Robin <i>Erithacus rubecula</i> (museum coll)	110	29	Hungary
Marsh warbler <i>Acrocephalus palustris</i> (museum coll)	80	24	Hungary

The number of cuckoo eggs is equal to the number of clutches.



Figure 3 Effects of egg shape parameters (c0, c1 and c2) on the shape of avian eggs. Two extreme and one intermediate values per parameter. The lower the value, the darker the line.

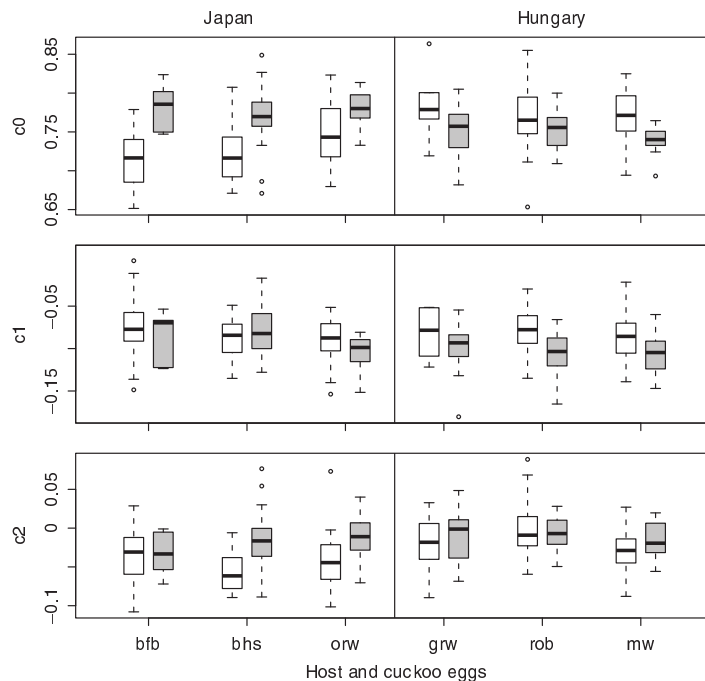


Figure 4 Box-plot diagrams of hosts' (white) and cuckoos' (grey) shape parameters (c_0 , c_1 , c_2). (Hosts: Black-faced bunting *Emberiza spodocephala* 'bfb', bull-headed shrike *Lanius bucephalus* 'bhs', oriental reed warbler *Acrocephalus orientalis* 'orw', great reed warbler *Acrocephalus arundinaceus* 'grw', robin *Erithacus rubecula* 'rob' and marsh warbler *Acrocephalus palustris* 'mw'.)

ANOVA: c_0 : $F_{1,259} = 6.878$, $P = 0.009$; c_1 : $F_{1,259} = 21.246$, $P < 0.001$; c_2 : $F_{1,259} = 27.652$, $P < 0.001$; Fig. 4). The cuckoos' and hosts' eggs differ most in roundness, but this difference is opposite in the two regions. The cuckoo eggs in Japan are more rounded than their hosts while in Hungary the cuckoo eggs more elongated than their hosts.

Discussion

Our results revealed a significant difference in the shape of cuckoo eggs between Japan and Hungary, while no difference was revealed in egg shape between cuckoos parasitizing different hosts within any of these distant areas. Cuckoo parasitism in Japan may have started more recently than in Europe (Nakamura *et al.*, 1998; Takasu *et al.*, 2009), where it has a considerable history (Davies, 2000). The difference in egg shape might be the consequence of geographic isolation in the evolutionary history of cuckoos.

Comparing egg shape in six pairs of host species and their corresponding cuckoos, our results suggest the lack of adaptation by different cuckoo gentes to their specific hosts in terms of egg shape. There are two possible explanations for this finding. On the one hand, hosts' egg recognition mechanisms are not specialized on egg shape as shown in the great reed warbler (Moskát *et al.*, 2003) and also in hosts of the brown-headed cowbird *Molothrus ater* (Rothstein, 1975; Mason & Rothstein, 1986; Ortega & Cruz, 1988; Underwood & Sealy, 2006; Guigueno & Sealy, 2009). Although

some cuckoos are strictly specialized on one host population (Nakamura & Miyazawa, 1997; Honza *et al.*, 2002), others seem to be generalists (Wyllie, 1981); hence, there might be some overlap in host usage by cuckoos (e.g. Edvardsen *et al.*, 2001). Although this might act against the development of mimicry in egg shape, it cannot exploit selection for this, as mimicry in egg colour and pattern could have already been developed in many cuckoo–host relationships (Moksnes & Røskoft, 1995). On the other hand, if heat retention by eggs is the most important selection pressure, the rounded form would be the most desirable option for the shape of a cuckoo egg (Thompson, 1942; Lack, 1968; Drent, 1975; Andersson, 1978; Barta & Székely, 1997). Our results support the latter possibility, as the rounded egg shape seems to be a secondary attribute in the evolution of cuckoo eggs, which is probably more related to efficient heat transfer.

Nevertheless, there could be several other effects, which may affect the adaptations of the egg shape in cuckoos. For example, the symmetry/asymmetry of eggs may have importance in the hatchability of eggs, affected by gas exchange in the air chamber (Mao *et al.*, 2007). Another divergent selection pressure in evictor cuckoos is eggshell strength, as their eggshells are thicker than that of in non-evictor cuckoos, probably developed against host puncture attempts (Hargitai *et al.*, 2010; Spottiswoode, 2010). The puncture resistance hypothesis was also supported in *Acrocephalus* hosts in Bulgaria (Antonov *et al.*, 2006), but thicker eggshells may also protect cuckoo eggs from damage when

they are laid into host nests. García-Navas *et al.* (2011) showed in the blue tit *Cyanistes caeruleus* that supplementation of calcium-rich food resulted in thicker eggshells and shorter embryo-development. Cuckoos lay at every second day (Wyllie, 1981), by retaining their eggs in their oviducts, which results in a 31-h hatching advantage when comparing with host eggs (Birkhead *et al.*, 2010). Our results suggest that egg shape does not play an important role in cuckoo–host coevolution as other eggshell characteristics, for example, colour or pattern (Kilner, 2006; Antonov *et al.*, 2010; Spottiswoode & Stevens, 2010). Eggshell colour components show a wide variety in bird species (Cassey *et al.*, 2010), probably reflecting a long evolutionary history as they can also be found in extinct birds (Igic *et al.*, 2010). Birds have well-developed visual systems, including their sensitivity for the ultraviolet wavelengths in many species (Endler & Mielke, 2005); thus, birds are able to utilize their talent of visual recognition for using visual cues of eggshell as communication signals (Underwood & Sealy, 2002; Cassey *et al.*, 2009; Cherry & Gosler, 2010).

To summarize, our results suggest that the shape of cuckoo eggs did not adapt to host egg shape. When cuckoo eggs from Japan and Hungary were compared, the shape of cuckoo eggs showed significant differences between these geographically distant areas. We suggest further studies on the coevolution of cuckoo–host egg shape in different populations, with variation in the coevolutionary history between cuckoos and their hosts. Marchetti (2000) described a case where the size of the foreign experimental eggs affected hosts' egg discrimination ability. In such cases, we expect a more relevant effect of egg shape and a better adaptation by cuckoos to host eggs than what we have revealed between cuckoos and six populations of hosts in Japan and Hungary.

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II.

Gazda védekezése és a kakukk választása

Host defence and cuckoos' host choice

2.

Common cuckoos (*Cuculus canorus*) Do Not Rely on Indicators of Parental Abilities When Searching for Host Nests: The Importance of Host Defenses.

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COMMON CUCKOOS (*CUCULUS CANORUS*) DO NOT RELY ON INDICATORS OF PARENTAL ABILITIES WHEN SEARCHING FOR HOST NESTS: THE IMPORTANCE OF HOST DEFENSES

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ABSTRACT.—There is widespread evidence that individuals within and among host populations are not evenly parasitized by Common Cuckoos (*Cuculus canorus*). We first investigated whether the song and nest size of a host species, the Great Reed Warbler (*Acrocephalus arundinaceus*), reveal information on parental abilities and level of defense against Common Cuckoos. Second, we analyzed whether female Common Cuckoos' preference for host nests is predicted by the degree of song expression and the nest size of the host. Earlier-breeding hosts built bigger nests, were more active singers, and had less rich syllable repertoires than late breeders. Host nestlings raised in a big nest received more feedings than those raised in a small nest. Host males that were active singers were paired with females that built bigger nests. All host pairs rejected nonmimetic artificial eggs, but those with a big nest were more prone to reject natural Common Cuckoo eggs. Thus, Great Reed Warbler pairs with a big nest were more willing to feed nestlings, but also had higher discriminatory abilities against Common Cuckoo eggs, than those with a small nest. These findings, and female Common Cuckoos' inability to capture the information provided by Great Reed Warblers' sexual signals, may explain why the females followed a simple rule of selecting the more visible host nests in the population. Received 26 August 2008, accepted 8 January 2009.

Key words: *Acrocephalus arundinaceus*, brood parasitism, Common Cuckoo, *Cuculus canorus*, Great Reed Warbler, heterospecific eavesdropping, host quality selection, nest size, parental care, sexual signals.

Cuculus canorus no se Basa en Indicadores de las Habilidades Parentales Cuando Busca Nidos de Hospederos: la Importancia de las Defensas del Hospedero

RESUMEN.—Existe abundante evidencia de que los individuos dentro y entre poblaciones hospederas no son parasitados de manera uniforme por *Cuculus canorus*. Primero, investigamos si el canto y el tamaño del nido de una especie hospedera, *Acrocephalus arundinaceus*, otorga información sobre las habilidades parentales y el nivel de defensa en contra de *C. canorus*. Segundo, analizamos si la preferencia de las hembras de *C. canorus* por los nidos hospederos es predicha por el grado de expresión del canto y el tamaño del nido del hospedero. Los hospederos que criaron más temprano construyeron nidos más grandes, fueron cantantes más activos y presentaron repertorios menos ricos en sílabas que los individuos que criaron más tarde. Los pichones de los hospederos criados en nidos grandes recibieron más alimento que aquellos criados en nidos pequeños. Los machos hospederos que fueron cantantes activos se aparearon con las hembras que construyeron nidos más grandes. Todas las parejas de hospederos rechazaron los huevos artificiales no miméticos, pero aquellas con nidos grandes fueron más propensas a rechazar los huevos naturales de *C. canorus*. Así, las parejas de *A. arundinaceus* con nidos grandes estuvieron más dispuestas a alimentar a los pichones de *C. canorus*, pero también presentaron mayores habilidades para discriminar sus huevos, que aquellas parejas con nidos pequeños. Estos resultados, y la incapacidad de la hembra de *C. canorus* de captar la información suministrada por las señales sexuales de *A. arundinaceus*, pueden explicar por qué las hembras siguieron una regla simple de seleccionar los nidos hospederos más visibles de la población.

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ANIMAL COMMUNICATION USUALLY occurs in a network of several signalers and receivers in which unintended receivers ("eavesdroppers"; *sensu* McGregor and Dabelsteen 1996, McGregor 2005) may process and profit from the information transmitted by signalers (e.g., Grim 2008, Welbergen and Davies 2008). Recently, it has been suggested that the interactions between obligate avian brood parasites and their hosts may represent a particular case of heterospecific eavesdropping on sexually selected signals that reveal the host's parental qualities (Soler et al. 1995, Parejo and Avilés 2007). Obligate avian brood parasites always lay their eggs in the nests of other species that rear their offspring (Davies 2000). Therefore, by eavesdropping on hosts' sexual signals revealing parental qualities or territory quality, obligate brood parasites may obtain information about the prospective survival of their own offspring.

There is widespread evidence that individuals within and among host populations are not evenly parasitized by cuckoos (Krüger 2007). A few studies have previously analyzed the role of hosts' songs in determining the probability of parasitism by Brown-Headed Cowbirds (*Molothrus ater*) from a location perspective (i.e., higher song activity increases the probability of nest detection by brood parasites; e.g., Clotfelter 1998, Banks and Martin 2001, Grief and Sealy 2000, Garamszegi and Avilés 2005). Also, Soler et al. (1995) found that female Great Spotted Cuckoos (*Clamator glandarius*) in Spain were more prone to parasitize large European Magpie (*Pica pica*) nests, where viability of the parasite offspring was high (but see McLaren and Sealy 2003). No attempt has been made to assess the role of hosts' sexual signals in determining the probability of parasitism by Common Cuckoos (*Cuculus canorus*; hereafter "cuckoos"), despite the fact that this is a very well-known system (Davies 2000).

Great Reed Warblers (*Acrocephalus arundinaceus*) are the most common hosts of cuckoos on the plains of Hungary. Like many other cuckoo hosts, Great Reed Warblers reject many, but not all, cuckoo eggs (Moskát and Honza 2002). Previous studies in the region demonstrated that female cuckoos preferred Great Reed Warbler nests that were near vantage points, more visible as judged by humans, or both (Moskát and Honza 2000). Further, evidence suggests that female cuckoos have evolved finely tuned discriminatory capacities, given that they prefer to parasitize host nests that contain eggs similar in appearance to their own eggs, which hinders the host's discriminatory task (Avilés et al. 2006, Cherry et al. 2007). These studies, however, have neglected the possibility that cuckoos use the sexual signals of Great Reed Warblers as cues, which may have biased the results if the hosts were not evenly distributed, within a population, in relation to their sexual signals.

Male Great Reed Warblers sing highly conspicuous songs, which are subject to a directional female mating preference (e.g., Catchpole et al. 1985, 1986; Catchpole 1986; Hasselquist 1998; Forstmeier and Leisler 2004). More importantly, from our perspective, Hasselquist et al. (1996) found a positive relationship between the viability of the offspring and the repertoire size of the genetic father. Male Great Reed Warblers' songs, therefore, may reveal aspects of offspring viability that could be of interest to eavesdropping cuckoos. In the same vein, once pairing has occurred, female Great Reed Warblers build their nests without

the male's help (Cramp 1998). Nest size (Soler et al. 1998, 2001; De Neve et al. 2004; Szentirmai et al. 2005) and nest-building activities (Soler et al. 1995, Palomino et al. 1998, Szentirmai et al. 2005) have been shown to be postpairing signals that reveal parental quality in European Magpies and other passerine birds (e.g., Soler et al. 1998, Szentirmai et al. 2005). Thus, the nest-building behavior of female Great Reed Warblers, as estimated from nest size, may be related to a willingness to invest in parental care and, thus, may also indirectly assist eavesdropping brood parasites in assessing some aspects of their offspring viability (see also McClaren and Sealy 2003).

We examined the natural variation present in two potential species-specific signals, song and nest size, as well as their information content in terms of parental quality and level of defense against cuckoos in a Great Reed Warbler population heavily parasitized by cuckoos. Second, we analyzed whether female cuckoos' preference for host nests is predicted by the song characteristics and the size of the hosts' nests. We expected that the responses of eavesdropping cuckoos to the signal variation in our host population would depend on parental qualities expressed by the sexual signals of hosts. From a cuckoo's point of view, the suitability of a host may depend on how prone it is to reject cuckoo eggs and its ability to raise parasitic chicks (Davies 2000, Grim et al. 2003, Grim 2007). Therefore, if host song and nest size, as sexual signals, simultaneously reflect the host's ability to raise a chick and its ability to recognize and reject cuckoo eggs, cuckoos may disregard these signals when searching for nests. However, we would expect that cuckoos' selection of host nests is not random, with respect to the signal variation we found in our study population, if signals reflect either the host's ability to raise a chick or its inability to discriminate against cuckoo eggs.

METHODS

Study area.—Field work was conducted around the village of Apaj, Hungary (47°07'N, 19°06'E), in 2006. Great Reed Warblers breed in reed beds along small channels and are parasitized at unusually high frequencies (~65%) by cuckoos (Moskát and Honza 2002). For a detailed description of the study area, see Moskát and Honza (2000). Great Reed Warblers typically arrive at our study area in early May and start breeding in mid-May. From 10 May, we systematically checked the reed beds for nests at least twice a week. Most of the nests were found in the nest-building or early egg-laying stages, which allowed us to monitor the fate of natural cuckoo parasitism in our population (Moskát and Honza 2002). Each nest was checked daily, and all Great Reed Warbler and cuckoo eggs were marked with waterproof pens. As in previous studies, parasitized nests that escaped predation for six days after parasitism were assessed as acceptors or rejectors (egg ejection or nest desertion; Moskát and Honza 2002, Hauber et al. 2006). The inclusion of nest desertion as a form of rejection is justified for this particular system, given that it can affect 15–19% of naturally parasitized nests in Apaj (Bártol et al. 2002, Moskát and Honza 2002), which is almost triple the desertion rate for nonparasitized nests (Bártol et al. 2002).

We also artificially parasitized a randomly selected portion of unparasitized nests in our population to study the relationship

between hosts' discriminatory abilities and the degree of species-specific signaling. Female cuckoos often remove a host egg when laying their own egg (Wyllie 1981), so we simulated parasitism by maintaining the existing clutch size in each artificially parasitized nest. We experimentally parasitized clutches with nonmimetic ($n = 14$ nests) and mimetic eggs ($n = 9$ nests). In the parasitized nonmimetic group of nests, we manipulated one egg in each clutch by dying its entire shell with a transparent yellow highlighter pen (Swan Stabilo Boss art no. 70/24). In addition, we added 12 brown spots with a felt pen (Faber-Castel OHP-Plus 1525 permanent, color code 78). Cuckoo eggs of a yellowish color occur in our study population, though rarely. Therefore, although cuckoo eggs are similar in size to Great Reed Warbler eggs (Török et al. 2004), the use of the term "nonmimetic" is justified in this case of artificial parasitism (see fig. 1 in Moskát et al. 2008a). In the parasitized "mimetic" group of nests, one host egg was manipulated by adding 12 brown spots with a felt pen (Faber-Castel OHP-Plus 1525 permanent, color code 78). The spots were ~4 mm in diameter and corresponded to the maximum size of natural spots on Great Reed Warbler eggs. These spots allowed the egg's background color to be visible, and previous studies in our population have shown that most are accepted by Great Reed Warblers (Hauber et al. 2006, Moskát et al. 2008b), thus justifying the use of the term "mimetic." To simulate natural parasitism (Moskát and Honza 2002), we applied both treatments during egg laying, usually after the fourth egg had been laid (rarely after the third or fifth egg). We monitored nests for six consecutive days after manipulation and report two types of host responses: acceptance or rejection of the parasitic egg (no desertion occurred). Only clutches not parasitized by a cuckoo and not depredated during the experimental period were used for these analyses.

Song recordings.—Male Great Reed Warblers have two different types of songs, which greatly differ in length. Unmated individuals produce long songs that consist of a wide variety of high-amplitude elements, and they switch to short songs that consist of low-amplitude syllables once they have a fertilizable, nest-building (or egg-laying) female to guard (Hasselquist and Bensch 1991). Playback experiments in the field and in the laboratory have demonstrated that long songs are attractive to females (Catchpole 1983, 1986), so eavesdropping cuckoos could target long Great Reed Warbler songs for future brood parasitism. We recorded males that sang a minimum of 30 consecutive strophes of long songs, which is sufficient to determine repertoire size in this species (Forstmeier et al. 2006). Recordings were made between 0500 and 1000 hours with a Marantz PMD670 recorder and a K6ME66 Sennheiser directional microphone and were analyzed with RAVEN, version 1.2 (Cornell Lab of Ornithology, Ithaca, New York). Five of the 22 sampled males were color-ringed before we recorded their songs. Continuous monitoring allowed us to confirm that these five males remained within their territories throughout the breeding season.

To characterize songs, we extracted five parameters from each of the 120 records belonging to the 22 males: (1) song rate, calculated as the proportion of time that a bird was singing as a function of record duration; (2) repertoire size, or the number of different syllable types found in all the strophes of a record (to ensure consistency in classification, all syllables were categorized by the same person [M.B.] on the basis of visual inspection

of spectrograms); (3) strophe length, the average length of all the strophes in a record; (4) percentage performance time (PPT), the average ratio of strophe length divided by the sum of strophe lengths and the subsequent silent period (Forstmeier et al. 2006); and (5) syllable switches, the average number of syllable-type switches within a strophe.

Nest volume.—A nest-volume index was estimated by multiplying nest height (cm), maximum nest width (cm), and minimum nest width (cm) (Moskát and Honza 2000). To ensure consistency, measurements were always taken by the same person (M.B.) while the nests were active. In addition, the number of reed stems to which the nests were attached was recorded. The average number of harnessed stems per nest was 5 (range: 2–11). Larger nests contained more reed stems ($r = 0.26$, $F = 5.12$, $df = 1$ and 67 , $P = 0.02$, $n = 69$ nests), which supports the assumption that nest volume is a good correlate of building effort in Great Reed Warblers.

Parental care.—The rate of nestling provisioning by parents was recorded with two fully compatible digital video cameras (Canon MV500i and 550i with Fuji DX 60-min cassettes) at 18 randomly selected unparasitized nests. This figure represents nearly 50% of the unparasitized nests in our population in which nestling Great Reed Warblers reached five days of age. The cameras were attached to tripods placed in reeds approximately 3–4 m from nests, to record parental feeding. We left cameras for 15 min before recordings started to habituate the birds to their presence. We used provisioning rate (number of feeding visits hour^{-1} , calculated from 1 h of observation nest^{-1}) when nestlings were about five days old (nestling age at the observation: average \pm SD = 5.55 ± 0.92 days) as a measure of parental care.

Distance to vantage points and nest visibility.—A previous study of the same population showed that Great Reed Warbler nests that are close to cuckoos' vantage points and more visible to researchers were more likely to be parasitized by cuckoos (Moskát and Honza 2000). Therefore, we estimated distance (m) to vantage points for every nest with a Garmin 60 geographic positioning system. We defined this as the distance from the nest to the closest tree or electric wire on which a cuckoo could land. Following Moskát and Honza (2000), we evaluated nest visibility from four points (north, east, south, and west) at a distance of 3 m, using a scale of 1–5: (1) bad—the nest is difficult to see from all directions, even from closer than 3 m; (2) intermediate1 (between the states of bad and moderate)—the nest is difficult to see from 3 m, but from a closer distance it can easily be seen, at least from one direction; (3) moderate—the nest can be seen from 3 m in one direction, and from a closer distance it can easily be recognized; (4) intermediate2 (between the states of moderate and good)—the nest is seen relatively well from 3 m, and from a greater distance in one direction; and (5) good—the nest is seen very well from 3 m, and from a greater distance in more than one direction (Moskát and Honza 2000).

Statistical analyses.—Song variables were interrelated, so we performed a principal component analysis (PCA) on the five variables to reduce the number of correlated variables into a single variable summarizing song variation. The first component explained 68.0% of the total variance in song and had high positive loading for song rate (0.89) and negative loadings for repertoire size (–0.76), strophe length (–0.58), PPT (–0.93), and syllable switches (–0.90). Hence, males with high positive scores for this

first component were more active singers, though their syllable repertoires were less rich.

The data complied with normality assumptions, and general linear models (GLM procedure in SAS; SAS Institute 1996) with normal error distribution were used to explore the relationships between likely sexual signals (i.e., nest volume and PC scores on song traits) and parental quality (i.e., provisioning rate). Provisioning rate may be affected by laying date and number of nestlings, given that laying date is an age-dependent feature in Great Reed Warblers (Lotem et al. 1992), and larger numbers of nestlings require higher provisioning rates. Thus, laying date and brood size were entered in the model to control for possible biases.

We used logistic regression models (GENMOD procedure in SAS) for testing the relationship between the fate of natural and artificial cuckoo parasitism in a nest (dependent variable: acceptance vs. rejection) and the expression of sexual signals of the nest owners. We entered laying date as a covariate, because young female Great Reed Warblers often lay in the middle of the breeding season and parasitic egg-recognition abilities are assumed to increase with age (Lotem et al. 1992). We also entered the kind of artificial egg (mimetic vs. nonmimetic) in the model as a fixed factor when dealing with rejection of artificial cuckoo eggs, because nonmimetic eggs are likely to be removed by cuckoo hosts (Davies 2000). Finally, we used a multiple logistic regression model with probability of parasitism as a binomial dependent variable (parasitized vs. nonparasitized) and variables related to sexual signals, and laying date, as independent variables. Nest visibility and perch distance were also entered in the models, because previous work has shown that they are determining factors in predicting the probability of cuckoo parasitism in our population (Moskát and Honza 2000). Finally, to account for the possibility that nest crypsis was a sexually selected trait in Great Reed Warblers, we used Pearson correlations to check for relationships between nest visibility and laying date and number of feedings.

Model selection was performed by removing, one by one, the effects that were farthest from statistical significance. Because of low sample size, we did not test interactive effects between independent variables.

RESULTS

Expression of Potential Sexual Signals and Parental Quality in Great Reed Warblers

Nest volume.—In our study population, Great Reed Warblers built nests ($n = 116$) with an average (\pm SD) volume of 972.83 ± 224.13 cm³. Variation in volume between nests was perceptible: the smallest nest found was about a third the volume of the biggest one (range: 448–1,700 cm³). Early breeders produced nests with larger volume than late breeders ($r = -0.38$, $F = 13.02$, $df = 1$ and 77 , $P = 0.0005$; Fig. 1). Video recordings revealed that variation in the rate of nestling provisioning by adult Great Reed Warblers was marginally explained by nest size (volume effect: $r = 0.43$, $F = 3.80$, $df = 1$ and 16 , $P = 0.06$; Fig. 2), but not by laying date (laying-date effect: $F = 0.51$, $df = 1$ and 15 , $P = 0.48$) or the number of fledglings at the nests (number-of-fledglings effect: $F = 0.21$, $df = 1$ and 14 , $P = 0.65$).

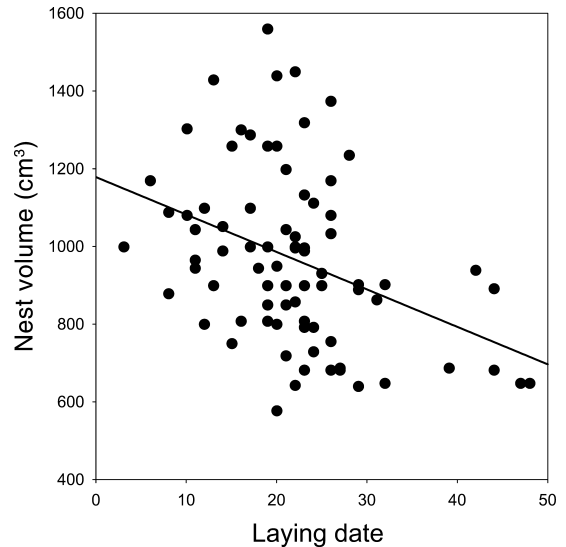


FIG. 1. Relation between nest volume and laying date (1 = 1 May) in Great Reed Warblers ($n = 79$ nests).

Song.—Early breeders were marginally significantly more active singers and had smaller syllable repertoires (i.e., higher PC1 scores) than late breeders in Apaj (Spearman correlation: $r_s = -0.40$, $P = 0.06$, $n = 21$; Fig. 3). Unfortunately, we were able to videotape only three nests in which male songs had been recorded,

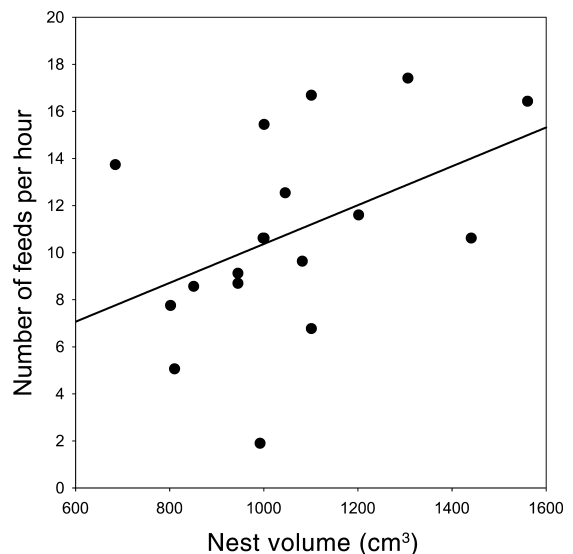


FIG. 2. Relation between number of feedings in 1 h and nest volume in Great Reed Warblers ($n = 18$ nests).

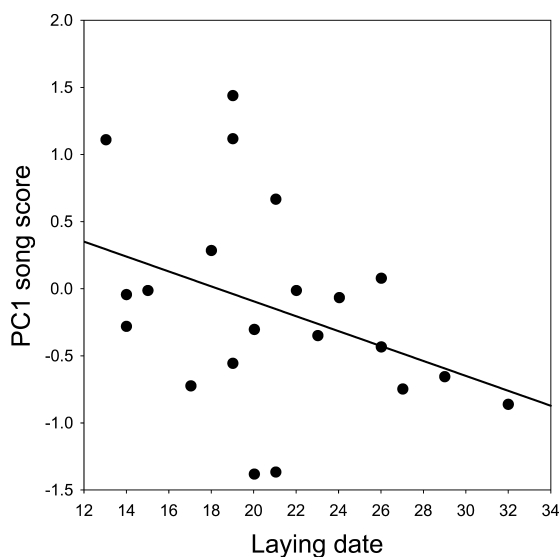


FIG. 3. Relation between song scores and laying date (1 = 1 May) in Great Reed Warblers ($n = 21$ nests).

which precluded a direct test of male song as an indicator of parental quality at the nests. However, characteristics of male songs were related to nest volume (Spearman correlation: $r_s = 0.42$, $P = 0.04$, $n = 22$). Active male singers (i.e., those with high PC1 scores) were mated with females that built nests of larger volume (Fig. 4).

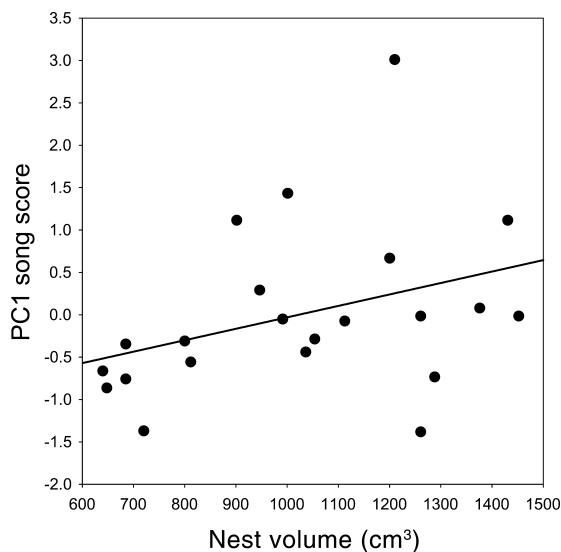


FIG. 4. Relation between song scores and nest volume in Great Reed Warblers ($n = 22$ nests).

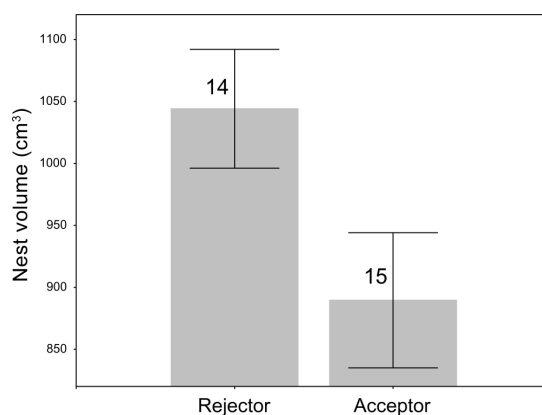


FIG. 5. Volume (means \pm SE) of Great Reed Warbler nests in relation to the occurrence of rejection of natural Common Cuckoo eggs. Sample sizes are on mean bars.

This association was likely mediated by laying date, because nest volume and PC1 scores were unrelated when laying date was considered in the analysis (volume effect: $F = 0.82$, $df = 1$ and 18 , $P = 0.37$; laying-date effect: $F = 1.36$, $df = 1$ and 18 , $P = 0.25$).

Expression of Potential Sexual Signals and Level of Defense against Cuckoos

Thirty-eight of the 60 Great Reed Warbler nests found during the nest-building stage were parasitized by cuckoos (parasitism frequency = 63.3%). We could not ascertain the fate of cuckoo eggs in eight nests because they were depredated promptly after being parasitized. Great Reed Warblers rejected at least one cuckoo egg in 14 of the remaining 30 nests (rejection rate of natural cuckoo eggs = 46.7%).

Interestingly, rejection of natural cuckoo eggs was related to nest size (volume effect: $\chi^2 = 4.39$, $df = 1$, $P = 0.03$) once we controlled for the effect of laying date (laying-date effect: $\chi^2 = 3.61$, $df = 1$, $P = 0.057$). Pairs with a big nest were more prone to reject natural cuckoo eggs than those with a small nest (Fig. 5). The probability of rejection of natural cuckoo eggs, however, was unrelated to song characteristics (song effect: $\chi^2 = 0.09$, $df = 1$, $P = 0.76$).

Nest size, however, did not explain rejection among the artificially parasitized Great Reed Warbler nests (volume effect: $\chi^2 = 1.76$, $df = 1$, $P = 0.18$). Also, laying-date effects on rejection of artificial cuckoo eggs were negligible (laying-date effect: $\chi^2 = 0.60$, $df = 1$, $P = 0.18$). Because of low sample size, we could not test for a relationship between song and host response to cuckoo parasitism. Rejection in this group of nests was influenced only by the level of egg mimicry (mimicry effect: $\chi^2 = 14.95$, $df = 1$, $P < 0.0001$), with mimetic eggs (33.3%) less prone to being rejected than nonmimetic eggs (100%).

Cuckoo Nest Selection and Host Sexual Signals

A multivariate analysis in which all factors likely to affect cuckoo parasitism were simultaneously considered revealed that the probability of cuckoo parasitism was unrelated to Great Reed Warbler

TABLE 1. Results of a logistic regression testing for the effect of Great Reed Warbler song, nest size, perch proximity, laying date, and nest visibility on the probability of parasitism by Common Cuckoos. Independent effects are presented in the order in which they were removed (see text). The number (*n*) of nests in which measurements were taken for each independent factor is given in parentheses.

Source	df	χ^2	<i>P</i>
PC1 scores (<i>n</i> = 20 nests)	1 and 17	0.01	0.91
Perch distance (<i>n</i> = 74 nests)	1 and 47	0.03	0.85
Nest volume (<i>n</i> = 70 nests)	1 and 48	0.11	0.73
Laying date (<i>n</i> = 56 nests)	1 and 50	0.25	0.62
Variables retained in the model:			
Nest visibility (<i>n</i> = 70 nests)	1 and 68	3.83	0.050

song and nest size (Table 1). In addition, laying date and proximity to a perch site did not predict cuckoo parasitism (Table 1). Instead, the probability of cuckoo parasitism was significantly influenced by the visibility of hosts' nests: parasitized nests were more visible (Fig. 6) than unparasitized nests (Table 1). Nest visibility was not significantly correlated with number of feedings ($r = -0.23$, $P = 0.40$, $n = 17$) or laying date ($r = 0.24$, $P = 0.09$, $n = 53$).

DISCUSSION

The present study confirms the suggestions of previous investigators that the expression of host signals near host nests might reveal aspects of hosts' parental capacities that are critical for parasitic cuckoos in selecting individuals to parasitize (Soler et al. 1995, Parejo and Avilés 2007). We found that nest size and singing activity of male hosts declined seasonally and that hosts' nestlings raised in larger nests received more feedings than those raised in a small nest, though this result was barely significant ($P = 0.06$). Most active singers in the host population also were mated with females that built bigger nests. A multivariate logistic analysis,

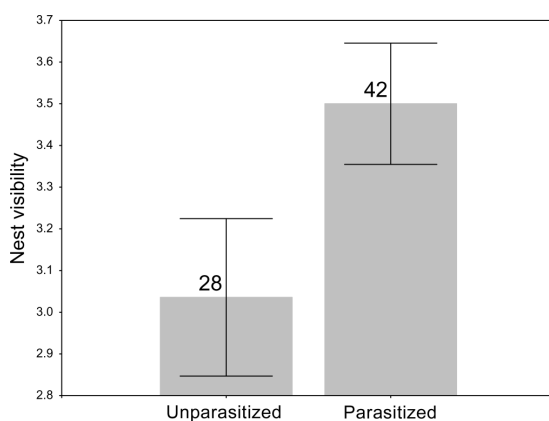


FIG. 6. Nest visibility (means \pm SE) of Great Reed Warbler nests in relation to the occurrence of parasitism by Common Cuckoos. Sample sizes are on mean bars.

in which all factors that likely influence the probability of cuckoo parasitism were simultaneously considered, revealed that more visible nests were more prone to be selected by female cuckoos. Neither nest size nor song output—variables that likely reflect the host's parental abilities—predicted the probability of cuckoo parasitism. Thus, our results do not indicate that cuckoos eavesdrop on Great Reed Warblers' sexual signals; rather, female cuckoos may follow a simple rule of parasitizing the most accessible Great Reed Warbler nests in the population.

Early passerine nests are more successful (Perrins 1970). Because most of the Great Reed Warblers in our study population were unmarked, we could not ascertain whether the late breeders that built smaller nests and sang less were young, inexperienced individuals or low-quality adults. In any case, both young, inexperienced and low-quality adult pairs are expected to give parasite offspring less care than experienced, high-quality pairs. Thus, nest size and song outcome could be used by cuckoos as reliable indicators of the rearing capacities of a particular Great Reed Warbler pair. However, if cuckoos parasitize only hosts with elaborate songs, this might result in strong selection against elaborate songs in the host, considering the extremely high frequency of cuckoo parasitism in this area. This would be consistent with the results of a study of cowbird parasitism in which host species had smaller song repertoires than non-host species (Garamszegi and Avilés 2005). Also, it may explain why early-breeding (presumably older) males in the present study had marginally smaller repertoires than late breeders, which contrasts with results from other populations of Great Reed Warblers (e.g., Catchpole et al. 1986, Hasselquist et al. 1996, Forstmeier and Leisler 2004).

Particularly novel is the finding that the expression of sexual signals by individual hosts may reflect discriminatory abilities against parasitism by cuckoos. Indeed, all the Great Reed Warbler pairs we tested rejected the nonmimetic eggs we introduced, but pairs with big nests recognized a cuckoo egg better than those with a small nest. It seems that the informative value of nest size as a predictor of discriminatory abilities is evident only at naturally parasitized nests. This is clearly relevant to understanding cuckoos, which have evolved a moderately good (sometimes "perfect") level of mimicry of Great Reed Warbler eggs in Apaj (Moskát and Honza 2002, Cherry et al. 2007). By relying on Great Reed Warbler nest size, a female cuckoo may obtain information on the probability that her eggs would be evicted from a particular nest. That is, the benefits for a female cuckoo, in terms of parental care, of selecting a big nest are counterbalanced by the costs of egg rejection. The association between rejection of cuckoo eggs and parental abilities may be attributable to these two behavioral traits being age-dependent in Great Reed Warblers. Previous studies have shown that discrimination of cuckoo eggs by Great Reed Warblers is an age-dependent behavior (Lotem et al. 1995). Older male Great Reed Warblers also sing more elaborate songs (Hasselquist et al. 1996, Hasselquist 1998; but see Forstmeier et al. 2006) and may have different habitat preferences (Grim 2002). Alternatively, individual Great Reed Warblers may simultaneously exhibit high proficiency in feeding and in avoiding cuckoo parasitism, because these two behavioral tasks are governed by a common cognitive capacity (Sih et al. 2004). Finally, in this population, >60% of Great Reed Warbler nests receive at least one cuckoo egg, and multiple parasitism is frequent (Moskát and Honza 2002). Thus, a preference for

high-quality hosts in this cuckoo population may be “punished” by the exceptionally high frequency of parasitism, which increases the chance that prospecting female cuckoos will find already-parasitized nests. Multiple parasitism is especially costly for brood parasites whose chicks evict all eggs and nestmates (Davies 2000).

Female cuckoos parasitized more visible than nonvisible nests in Apaj. It could be argued that host-nest crypsis better predicted an individual's parental quality than its signaling. However, neither laying date nor number of feedings was related to nest visibility in our population. Visible nests probably allow female cuckoos to more effectively inspect a nest before laying and to parasitize the nest more quickly, thus diminishing the risk of being chased by hosts (Wyllie 1981). Also, the high risk of multiple parasitism and the high density of cuckoos in the study population (see above) may have facilitated nest selection based on simple rules. This finding only partially confirms previous findings by Moskát and Honza (2000) in the same population. In that study, perch distance, as well as nest visibility, mainly explained the probability of cuckoo parasitism. Our study was conducted in the same geographic area but, because of habitat degradation, we sampled reed beds situated in channels adjacent to those sampled by Moskát and Honza (2000). In the present study, tree lines followed the channel more continuously than in the earlier study, where trees were more patchily distributed in relation to the reed-bed line. Differences in habitat structure between the two studies may explain the difference between the two results regarding the importance of cuckoos' vantage points.

In conclusion, we found that nest size was positively related to the quality of parental care in Great Reed Warblers, as well as to their capacity to reject cuckoo eggs. Therefore, nest size may reveal multiple but contradictory aspects of host suitability, and cuckoos may favor a simple nest-visibility rule when searching for host nests. This may help to explain why we did not find support for the suggestion that cuckoos eavesdrop on the sexual signals of Great Reed Warblers, which is consistent with previous negative results in studies of exploitation of sexual signals by cuckoos. Before stronger inferences can be made, however, this multiple-signaling approach needs to be replicated in a multi-year study that includes other host behaviors that may affect brood parasites, such as those related to the expression of hosts' sexual ornaments.

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III.

Tojásfelismerés és elutasítás

Egg recognition and rejection

3.

Experimental shift in hosts' acceptance threshold of inaccurate-mimic brood parasite eggs.

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Experimental shift in hosts' acceptance threshold of inaccurate-mimic brood parasite eggs

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Hosts are expected to evolve resistance strategies that efficiently detect and resist exposure to virulent parasites and pathogens. When recognition is not error-proof, the acceptance threshold used by hosts to recognize parasites should be context dependent and become more restrictive with increasing predictability of parasitism. Here, we demonstrate that decisions of great reed warblers *Acrocephalus arundinaceus* to reject parasitism by the common cuckoo *Cuculus canorus* vary adaptively within a single egg-laying bout. Hosts typically accept one of their own eggs with experimentally added spots and the background colour left visible. In contrast, hosts reject such spotted eggs when individuals had been previously exposed to and rejected one of their own eggs whose background colour had been entirely masked. These results support patterns of adaptive modulation of antiparasitic strategies through shifts in the acceptance threshold of hosts and suggest a critical role for experience in the discrimination decisions between inaccurate-mimic parasite eggs and hosts' own eggs.

Keywords: brood parasitism; Darwinian algorithms; optimal conspecific acceptance threshold; template

1. INTRODUCTION

Many species have evolved accurate and efficient mechanisms that are used to resist or limit exposure to parasites and pathogens. Coevolved hosts of brood parasitic birds, for example, may reject parasitic eggs by ejection or nest desertion (e.g. Davies & Brooke 1988; Moksnes *et al.* 1990). Parasite rejection, however, is not absolute (Hauber *et al.* 2004), as it shows extensive variability between different populations, years, stages of breeding cycle, extents of egg mimicry and adult parasites' presence near nests, and states of host–brood parasite coevolution (Rothstein & Robinson 1998; Davies 2000). What explains this variation in the propensity to respond to brood parasitism among different hosts at the fitness (ultimate) and cognitive (proximate) levels?

Optimal conspecific acceptance threshold theory (Reeve 1989) provides an integrative explanation for variability of discrimination decisions, including rejection of parasites, through cognitive mechanisms that evaluate host–parasite trait dissimilarity. For example, hosts of cuckoos may reject inaccurate-mimic parasite eggs because of differences in size (Langmore *et al.* 2003) or in UV-inclusive colour-reflectance of host eggs versus parasite eggs (Cherry & Bennett 2001; Aviles *et al.* 2004). The acceptance threshold (figure 1a) is then set flexibly along the trait-dissimilarity dimension to maximize the fitness payoff between the benefits of rejecting parasite eggs and the costs of erroneously rejecting own eggs (Stokke *et al.* 2002), especially when the appearance of the hosts' own eggs are variable within and between clutches (Stokke *et al.* 1999). This theory is thus best applied to host taxa whose rejection decisions appear to be flexible (i.e. intermediate rejecters: Stokke *et al.* 2005). A specific prediction of acceptance threshold theory is that, when the frequency or future predictability of parasitism increases, the acceptance threshold should become more restrictive (Reeve 1989; Davies *et al.* 1996; Rodríguez-Gironés & Lotem 1999; figure 1b).

Here, we examine the hypothesis that parasite-rejection decisions of great reed warbler *Acrocephalus arundinaceus* hosts of common cuckoos *Cuculus canorus* show adaptive plasticity at the level of individual experience. We capitalized on consistently high rates of multiple cuckoo eggs laid in host clutches at our study site (Moskát & Honza 2002). Accordingly, when a warbler nest is parasitized once, this demonstrates that the nest can be located and is accessible for the same or other cuckoos on subsequent days. We, therefore, expected that experimental parasitism causes a shift in the rejection decisions of hosts towards a more restrictive acceptance threshold.

2. MATERIAL AND METHODS

Great reed warblers are similar to several other common cuckoo host species in that they reject many, but not all, cuckoo eggs. Egg rejection decisions in this species do not depend on the extent of intraclutch variability of eggs (Lotem *et al.* 1995; Karcza *et al.* 2003), suggesting that other factors, including mimicry of the parasite eggs, may influence the hosts' antiparasite behaviours. At our study site near Apaj (47°07' N; 19°06' E), Hungary, the rejection rate is 34% of natural cuckoo eggs (Moskát & Honza 2002) and 71% of artificial non-mimicking eggs (Moskát *et al.* 2002). Cuckoo eggs at this site show variable appearance, and typically have a light bluish, almost white, background colour with brown spots, resembling closely the host eggs as judged by human observers (Moskát & Honza 2002).

We used data from 1998 to 2005 on natural instances of single and multiple parasitism to describe characteristics of multiple cuckoo parasitism in our population. For extensive details about the habitat and general methods, see Bártl *et al.* (2002). Great reed warblers generally lay 4–5 eggs per nest (modal clutch size: five eggs, C. Moskát, unpublished data from 1998–2003) and all the experiments took place before or on the day of completion of warbler clutches. Individual hosts are not banded at our study site and we assumed that each host nest was attended by the same pair during a single reproductive bout. Only nests that were not depredated or naturally parasitized during the experiments were included in the analyses.

Cuckoos typically remove one host egg when laying a parasite egg (Wyllie 1981), and we simulated cuckoo parasitism by maintaining current clutch size in each nest. Nests during the 2002, 2003 and 2005 breeding seasons were assigned to three experimental treatments: in all nests, including control nests, warbler eggs were removed, handled and numbered at their blunt

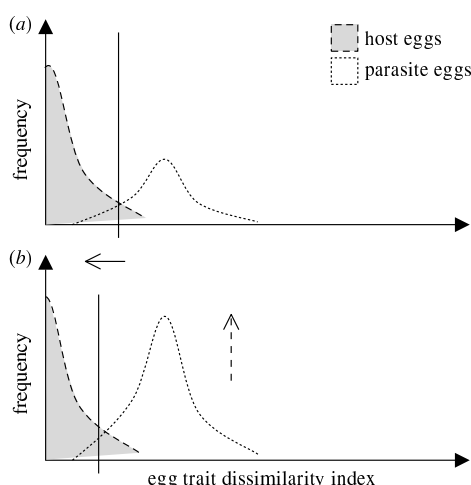


Figure 1. A graphical explanation of a shift towards more restrictive optimal acceptance threshold in the context of an increase in the frequency of brood parasitism: (a) low-frequency parasitism, (b) higher frequency of parasitism. Modified from Reeve (1989) and Liebert & Starks (2004).

end with a waterproof pen. In Treatment 1 (T1) nests, on laying day 2 or 3, the phenotype of a single warbler egg was manipulated by adding 20 brown spots with a felt-pen (Faber-Castel OHP-Plus 1525 permanent; figure 2). These spots were approximately 5 mm in diameter and were distributed throughout the entire egg surface; the spots did not cover the entire egg surface and allowed the eggshell base colour to remain visible (figure 2). In preliminary tests these eggs were mostly accepted by great reed warblers (*C. Moskat et al.*, unpublished data from 2002, also see §3). Following this single manipulation, we then determined whether hosts rejected each spotted egg either by the method of ejection or nest desertion.

In treatment 2 (T2) nests, on laying day 2 or 3 a single warbler egg was manipulated by covering the entire surface of the egg with the brown pen (figure 2). In preliminary tests, these covered eggs were consistently rejected by warblers (*C. Moskat et al.* unpublished data, also see §3). In those T2 nests, where experimentally painted brown eggs disappeared within 1–2 days (i.e. still during the laying stage), we manipulated an additional warbler egg by adding 20 spots as described above. No change was detected in rejection rates of hosts during the breeding season (*Moskat et al.* 2002) and by chance the difference in the rank-order of the laying dates for nests from the different treatments were similar (Mann-Whitney $U_{17,12} = 99.5$, $p > 0.9$). Hence, we assumed no consistent age-differences between female hosts in the different treatments (cf. Lotem *et al.* 1995).

We monitored all nests for 6 days following treatments to document rejection responses (egg ejection or nest desertion versus egg acceptance, yes/no bivariate category). Thus, our study was designed to examine the role of prior experience and memory in antiparasite defences within a single breeding attempt rather than across breeding attempts and between different years (Lotem *et al.* 1995). Hosts' rejection decisions were similar across years ($\chi^2_2 = 1.12$, $p = 0.572$) and data were combined from all breeding seasons, assuming each nest to be an independent data point for our contingency analyses using Fisher's exact tests.

3. RESULTS

Host great reed warblers suffered from a relatively high risk of multiple common cuckoo parasitism in our population. Among 441 clutches, 186 (42.2%) were singly parasitized and 76 were multiply parasitized (17.2%). Accordingly, 29.0% of parasitized nests contained two, rarely three or four, cuckoo eggs. Cuckoo eggs in multiply parasitized clutches ($n = 35$) were more likely to be laid on different days (91.4%) than on the same day (8.6%).

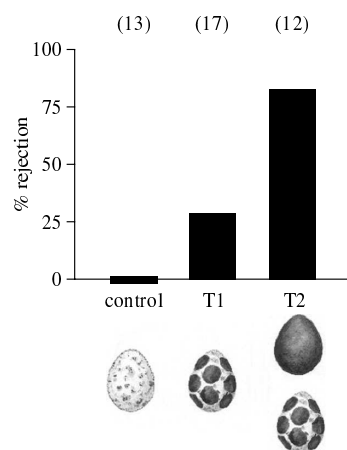


Figure 2. Experience-dependent proportions of accepted and rejected experimentally modified own eggs of great reed warbler hosts of common cuckoos in Central Hungary. Rejection rates refer to percentage of the spotted egg rejected (T1 and T2); in the control treatment no own egg was rejected.

Hosts with control nests did not reject any of their own eggs (0/13, 0%; figure 2), thus showed no recognition errors. Hosts with T1 nests that included a spotted egg, had a moderately higher rejection rate of experimentally spotted eggs (5/17, 29.4%, $p = 0.0525$; figure 2). Again, no unmanipulated host eggs were rejected or disappeared, showing evidence for neither rejection errors nor rejection costs (*sensu* Stokke *et al.* 2002).

Hosts with T2 nests rejected the dark brown eggs at a high rate (12/14, 85.7%; figure 2) compared to both controls ($p < 0.0001$) and T1 spotted eggs ($p = 0.0032$). In those T2 nests, where the original dark brown eggs had been ejected, the subsequent spotted eggs were also rejected in most nests (10/12, 83.3%; figure 2). Each experimentally spotted egg was introduced at similarly late stages of the laying stage in both T1 and T2 nests but the difference between rejection rates of single spotted eggs in great reed warbler nests was highly significant between the two treatments ($p = 0.0078$). Hosts rejected all dark brown eggs (12/12, T2 nests) and all but one of the spotted eggs (14/15, T1 and T2 nests combined) by the method of ejection while one clutch with a spotted egg was deserted.

4. DISCUSSION

Optimal acceptance threshold theory (Reeve 1989) has predicted successfully patterns of social discrimination decisions of several taxa, including eusocial insects and social mammals in the context of kin favouritism (Liebert & Starks 2004). Implicitly, acceptance threshold theory also explains increased host rejection behaviour when predictability of parasitism is greater (i.e. when adult parasites are present versus absent near hosts nests within reproductive bouts (Davies & Brooke 1988; Moksnes & Røskaft 1989; Bártol *et al.* 2002), across the breeding season

(Alvarez 1996) and between years (Brooke *et al.* 1998)).

Experimental data are in support of the acceptance threshold model in the great reed warbler that is a host species of the common cuckoo with high rates of multiple parasitism in central Hungary and intermediate levels of responses to naturally laid cuckoo eggs (Moskát & Honza 2002). This host showed higher rejection rates of artificial eggs in response to multiple parasitism than to single parasitism (Honza & Moskát 2005) and it demonstrated plasticity in the responses to the same phenotypic traits involved in egg rejection decisions (this study). Specifically, we found that within the same laying cycle, hosts' experience with presumed brood parasitism (as predicted by the appearance and rejection of a non-mimicking egg) results in the rejection of simulated parasite eggs with an otherwise accepted phenotype. A potential consequence of discrimination decisions based on flexible acceptance thresholds is that inaccurate mimicry of host eggs by parasites may be evolutionarily stable even when variability in local parasite egg phenotypes is reduced (Johnstone 2002). This scenario is likely to be relevant to common cuckoos and many of their hosts, and may explain the close but not perfect mimicry of host eggs by cuckoo eggs (Takasu 2003), because female cuckoos of the same *gentes*, laying similar eggs, are more likely to search nearby habitats and encounter nests of the same host species (Honza *et al.* 2002).

At the time when rejection decisions were made by great reed warbler hosts in treatments T1 and T2, the nest contents were identical in each nest (1 spotted and 3–4 unmanipulated own eggs). These results are consistent with the concept of a template-based recognition system for host–parasite egg discrimination in great reed warblers (Hauber & Sherman 2001), because the brown egg, as the indicator risk of parasitism, did not need to be present at the time when the rejection decision of the spotted egg was made (figure 2). The implication for the cognitive plasticity of host egg-recognition templates is that experience and memory play critical roles in the antiparasite-discrimination decisions of great reed warblers and, perhaps, generally in other host–parasite systems.

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IV.

Diszkriminációs képesség és mechanizmusok

Discrimination ability and mechanisms

4.

Discordancy or template-based recognition? Dissecting the cognitive basis of the rejection of foreign eggs in hosts of avian brood parasites.

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Discordancy or template-based recognition? Dissecting the cognitive basis of the rejection of foreign eggs in hosts of avian brood parasites

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SUMMARY

Many avian hosts have evolved antiparasite defence mechanisms, including egg rejection, to reduce the costs of brood parasitism. The two main alternative cognitive mechanisms of egg discrimination are thought to be based on the perceived discordancy of eggs in a clutch or the use of recognition templates by hosts. Our experiments reveal that the great reed warbler (*Acrocephalus arundinaceus*), a host of the common cuckoo (*Cuculus canorus*), relies on both mechanisms. In support of the discordancy mechanism, hosts rejected their own eggs (13%) and manipulated ('parasitic') eggs (27%) above control levels in experiments when manipulated eggs were in the majority but when clutches also included a minority of own eggs. Hosts that had the chance to observe the manipulated eggs daily just after laying did not show stronger rejection of manipulated eggs than when the eggs were manipulated at clutch completion. When clutches contained only manipulated eggs, in 33% of the nests hosts showed rejection, also supporting a mechanism of template-based egg discrimination. Rejection using a recognition template might be more advantageous because discordancy-based egg discrimination is increasingly error prone with higher rates of multiple parasitism.

Key words: brood parasitism, egg discrimination, learning, discordancy, recognition template.

INTRODUCTION

Avian brood parasites cause hosts to provide parental care for genetically unrelated young together with or instead of their own offspring (Kilner et al., 2004; Krüger, 2007). Many hosts have evolved strategies to reduce the costs of parasitism (Davies and Brooke, 1988; Takasu et al., 1998; Schulze-Hagen et al., 2009). One of the most prevalent adaptations against brood parasitism is egg rejection, showing individual (Avilés et al., 2009), sex-related (Palomino et al., 1998; Soler et al., 2002; Honza et al., 2007b; Pozgayová et al., 2009), population (Lindholm, 1999; Stokke et al., 2007a) and species-specific variability (Brooke and Davies, 1988; Davies and Brooke, 1989; Moksnes et al., 1991; Røskaft et al., 2002; Servedio and Hauber, 2006). Eggshell characteristics (e.g. coloration and maculation) play a critical role in the recognition of parasitic eggs by hosts of several brood parasite species, including the common cuckoo *Cuculus canorus* (e.g. Nakamura et al., 1998; Stokke et al., 1999; Cherry et al., 2007a; Honza et al., 2007a; Polaciková et al., 2007; Moskát et al., 2008a; Moskát et al., 2008c), the little and Himalayan cuckoos *Cuculus poliocephalus* and *Cuculus saturatus* (Higuchi, 1989), the great spotted cuckoo *Clamator glandarius* (e.g. Soler et al., 2000) and the brown-headed cowbird *Molothrus ater* (e.g. Rothstein, 1982; Underwood and Sealy, 2006). Cognitive mechanisms which correspond to context-dependent egg discrimination (Rothstein, 1975; Welbergen et al., 2001; Lahti and Lahti, 2002; Hauber et al., 2006; Moskát and Hauber, 2007), including the avian-specific sensory perception of egg coloration (Avilés, 2008; Cassey et al., 2008a; Cassey et al.,

2008b; Safran and Vitousek, 2008; Langmore et al., 2009), seem to be key factors in explaining behavioural variability of rejection decisions in host–brood parasite co-evolution. Nonetheless, the extent to which variability in the host's flexibility of species-recognition systems impacts on adaptive responses to parasitism (Sherman et al., 1997; McLean and Maloney, 1998; Stokke et al., 2005; Hauber et al., 2006; Moskát and Hauber, 2007) remains to be fully understood.

Egg discrimination is a prominent antiparasite defence mechanism, implying the successful recognition of the foreign eggs followed by the behavioural decision of the hosts to reject them (Davies and Brooke, 1989; Moksnes et al., 1991; Hauber and Sherman, 2001; Moskát and Hauber, 2007), even in conspecific parasitism (e.g. Jamieson et al., 2000). The two most prevalent mechanisms for egg recognition (Rothstein, 1974; Lahti and Lahti, 2002; Moskát et al., 2009) are (i) discordancy and (ii) recognition from template (Table 1). The latter mechanism requires knowledge of the appearance of the host's own eggs and has been termed 'true recognition' (Hauber and Sherman, 2001) because it requires neural encoding of the recognition template of own eggs. Rejection from template does not require the presence of host and parasite eggs at the same time (Moskát and Hauber, 2007).

Discordancy is the simplest mechanism for egg recognition, and is based on the differences between egg phenotypes within the same clutch (Rothstein, 1974). This mechanism predicts that hosts typically eject the egg types that are most dissimilar (and thus,

Table 1. Examples of egg recognition methods used by hosts in interspecific brood parasitism, as revealed by previous studies

Host	Brood parasite	Reference
Discordancy ¹		
<i>Phylloscopus humei</i>	<i>Cuculus poliocephalus</i>	Marchetti, 2000
<i>Sylvia borin</i>	<i>Cuculus canorus</i>	Rensch, 1925
Recognition template		
<i>Dumetella carolinensis</i>	<i>Molothrus ater</i>	Rothstein, 1974; Rothstein, 1975; Strausberger and Rothstein, 2009
<i>Icterus galbula</i>	<i>Molothrus ater</i>	Rothstein, 1978; Strausberger and Rothstein, 2009
<i>Toxostoma rufum</i>	<i>Molothrus ater</i>	Strausberger and Rothstein, 2009
<i>Quiscalus mexicanus</i>	<i>Molothrus ater</i>	Peer and Sealy, 2001
<i>Luscinia svecica</i>	<i>Cuculus canorus</i>	Amundsen et al., 2002
<i>Fringilla coelebs</i> and <i>F. montifringilla</i>	<i>Cuculus canorus</i>	Moksnes, 1992; Vikan et al., 2009
Discordancy and/or template		
<i>Dendroica petechia</i>	<i>Molothrus ater</i>	Sealy, 1995
<i>Turdus migratorius</i>	<i>Molothrus ater</i>	Rothstein, 1982
<i>Acrocephalus orientalis</i> ²	<i>Cuculus canorus</i>	Lotem et al., 1995
<i>Acrocephalus arundinaceus</i>	<i>Cuculus canorus</i>	Cherry et al., 2007a ³ ; Moskát et al., 2009 ³ ; Hauber et al., 2006 ⁴ ; Moskát and Hauber, 2007 ⁴

¹Discordancy has never clearly occurred; some template recognition was expected (cf. Rothstein, 1974).

²Previously considered as *Acrocephalus arundinaceus orientalis*, a subspecies of the great reed warbler.

³Discordancy.

⁴Recognition template.

necessarily, in the minority) in clutches, irrespective of whether these are their own eggs or parasitic eggs.

Another mechanism is recognition from a template. In this scenario, birds compare the characteristics of the eggs with a recognition template, which could be inherited and/or learned (Rothstein, 1974; Moksnes, 1992; Hauber and Sherman, 2001; Hauber et al., 2006; Moskát and Hauber, 2007; Petrie et al., 2009). Young birds might imprint on their own eggs during their first breeding attempts (Rothstein, 1975; Rodríguez-Gironés and Lotem, 1999; Stokke et al., 2007b) by examining the totality of their clutch (Hauber et al., 2004; Hoover et al., 2006), but first-time breeders might also have some knowledge of their eggs even at their first breeding if the template is inherited or if they imprint immediately on the phenotype of their own first-laid egg (Victoria, 1972; Lotem et al., 1995; Amundsen et al., 2002). In these last cases, additional learning throughout the first breeding attempt or later clutches serves as template updating, an adaptive cognitive mechanism (Hauber and Sherman, 2001) to accommodate the individual's changing extended phenotype (i.e. variability of egg appearance throughout the laying cycle). The aim of our study was to test in a parallel set of experiments whether egg discrimination in the great reed warbler *Acrocephalus arundinaceus* L. occurs by the discordancy or the template-based recognition mechanism.

The great reed warbler is a commonly parasitized cuckoo host in Hungary (41–68% parasitism rate) (Moskát et al., 2008b), with parasitism rate mainly dependent on the availability of trees in the close vicinity of the nests, serving as vantage points for the cuckoo (Moskát and Honza, 2000). This host rejects ca. 34% of naturally laid cuckoo eggs by egg ejection (12%), nest desertion (20%) or egg burial (2%) at Apaj, central Hungary (Moskát and Honza, 2002). However, hosts' rejection frequency does not depend on population-specific parasitism rate across the Hungarian Plain, as a consequence of low site fidelity of this host species (Moskát et al., 2008b). In previous experimental studies template-based egg recognition was revealed by Hauber and colleagues (Hauber et al., 2006), including its variant 'the phenotype distribution', in which all host eggs form a compound template of acceptable phenotype variability and hosts adjust rejection thresholds (Reeve, 1989) based on external contexts (Moskát and Hauber, 2007). However, the possibility of egg

discrimination by discordancy was also suggested in this species by Cherry and colleagues (Cherry et al., 2007a) and by Moskát and colleagues (Moskát et al., 2009), while Moskát and Hauber (Moskát and Hauber, 2007) provided experimental evidence for clutch imprinting through a template-updating mechanism. Because the behavioural evidence for hosts' egg-rejection decisions does not always allow distinction between predictions and outcomes of the discordancy *versus* the template recognition mechanisms (Moskát et al., 2008c; Antonov et al., 2009), contemporary experiments are required that specifically aim to contrast the predictions of these alternative cognitive models using the same experimental paradigm.

In this study we set out to investigate alternative cognitive mechanisms of experimentally induced egg discrimination in this host species, contrasting the potential roles of discordancy and the template recognition mechanism (Table 2), as previous studies suggested that great reed warblers use either the discordancy or the template recognition mechanism (see Table 1). To resolve this uncertainty, here we hypothesized (1) that discordancy plays some role, but not an exclusive one, in egg discrimination by great reed warblers. As discordancy means the rejection of the egg type in the minority (*sensu* Rothstein, 1974), it might be maladaptive in a population with heavy multiple cuckoo parasitism, including our Hungarian study site (Moskát et al., 2009). Therefore learning may also be important in egg recognition in great reed warblers (e.g. Rothstein, 1974; Rodríguez-Gironés and Lotem, 1999; Hauber et al., 2006; Stokke et al., 2007b). We hypothesize (2) that hosts' opportunity to inspect and learn the appearance of eggs in a nest helps egg recognition. Accordingly, we predict that hosts that had the opportunity to observe manipulated eggs during the laying process would show fewer rejections of the manipulated egg(s), when the hosts' own eggs are in the minority within the clutch, relative to those hosts with own eggs in the minority without the opportunity to inspect manipulated eggs during laying. Finally, a third factor for self-phenotype recognition is the presence and varying numbers of own eggs during parasitism where female parasites remove hosts' own eggs (Moskát and Hauber, 2007). Accordingly, we hypothesize (3) that the disappearance of hosts' own eggs is a cue for actual and impending parasitism and increases hosts' egg discrimination, because cuckoos predictably remove host

Table 2. Predicted variation in hosts' egg rejection responses according to different hypothetical cognitive mechanisms used for egg discrimination of moderately mimetic parasitic eggs: discordancy (Rothstein, 1974) and recognition template (cf. Hauber et al., 2006)

Treatment	Egg type		Predicted responses of hosts (rejection rates)	
	Minority	Majority	Discordancy	Recognition template
Spotted egg	Spotted	Own	Moderate	Moderate
Negatively spotted egg	Negatively spotted	Own	High	High
All spotted eggs	None	Spotted	None	Moderate*
'Without learning'	Own	Spotted	None	Moderate
'With learning' [†]	Own	Spotted	Moderate	None [‡]

The basic egg type used in the experiments ('spotted egg') had 20 large brown spots painted on the natural eggshell of the host's own egg. (Note that throughout this table we assume that hosts use only one of the recognition mechanisms.) The predicted reactions against hosts' own eggs are shown in parentheses.

*Relatively good mimicry allows hosts to reject the parasitic egg phenotype at a low frequency, but the lack of the own phenotype is expected to increase rejection rates.

[†]The treatment 'with learning' means the false imprinting on the parasitic eggs' phenotype. In the other treatments hosts had the chance to learn their own eggs' phenotypes.

[‡]This may depend on the mechanism by which hosts acquire the recognition template.

eggs prior to or during parasitism (see above). Hosts might use this cue to shift their acceptance thresholds to be more discriminating (Reeve, 1989; Hauber et al., 2006).

In addition, we studied the importance of eggshell pattern in self-phenotype recognition. A recent study by Moskát and colleagues (Moskát et al., 2008c) revealed that experimentally increased spot density had little effect on hosts' egg discrimination up to 75% cover of the eggshell surface, but when no piece of the original was seen, rejection rate abruptly increased to 100%. For this reason we tested how different components of the eggshell pattern (i.e. background colour *versus* maculation) contribute to the recognition (*versus* non-rejection) of the own egg phenotype. Accordingly, we hypothesize (4) that all hosts' own eggs whose background colour is left visible are available for the recognition of own-egg phenotypes and thus are rejected less often than parasitic eggs.

MATERIALS AND METHODS

Study site and species

The study was conducted in the surroundings of the village of Apaj (47°07'N; 19°06'E) in central Hungary, ca. 40–60 km south of Budapest. The study was performed in 2008, from mid-May until mid-June. We did not band individuals for identification in the study site, but pseudoreplication chance is low (Moskát et al., 2009). In this study area great reed warblers are particularly heavily parasitized by cuckoos. For details of the study site and basic methods, see Moskát and Hauber (Moskát and Hauber, 2007). The experiments performed in this study complied with Hungarian laws on the care and use of animals.

We systematically searched for great reed warbler nests in the 2–4 m wide channel-side reedbeds (*Phragmites australis*) once or twice a week. We used nests which were not parasitized by the cuckoo for experiments, and we deleted those nests from the data set which were parasitized during the control period. In Apaj, great reed warblers lay in the early morning, but cuckoos can lay throughout the whole day, with evening laying times more prevalent (C.M., unpublished observations), as observed in other hosts (Wyllie, 1981; Davies and Brooke, 1988; Honza et al., 2002). Cuckoos typically remove one randomly selected host egg from parasitized clutches [0 egg: 8%, typically when cuckoos lay into empty nests; 1 egg: 86%; 2 eggs: 6%; calculation based on Moskát and Honza (Moskát and Honza, 2002)]. For treatments, we manipulated one or more of the hosts' own eggs in a nest, painting dark brown spots (see below) onto the eggshell. We used painted

real eggs, following Honza and Moskát (Honza and Moskát, 2008). The eggs of great reed warblers are suitable for such types of painting experiments because a great reed warbler egg is as large as a cuckoo egg (Török et al., 2004). The mimicry of natural cuckoo eggs is typically good in our study area, both as perceived by humans (Moskát and Honza, 2002) and as scored by spectrophotometry (Cherry et al., 2007a; Cherry et al., 2007b), and hosts accept ca. two-thirds of real cuckoo eggs (Moskát and Honza, 2002). For this reason we chose an egg phenotype for experimental parasitism (except for the 'negatively spotted egg' treatment; see below) that is rejected at a similarly moderate frequency. We compared our experimental results on rejection rates with hosts' responses to cuckoo eggs from our observations of natural parasitism as a reference.

Experimental egg types

Spotted egg

On the day when the fourth host egg was laid (modal clutch size: 5; great reed warblers start incubation just after the penultimate egg is laid) (Moskát et al., 2008b), one egg, chosen randomly, was manipulated by painting 20 large dark brown spots of approximately 3–4 mm diameter on it. Artificial spots covered 40–60% of the whole egg surface (Fig. 1B). For all experiments we used dark brown waterproof fibre pens (Faber-Castel OHP-Plus permanent, size code: 1525; colour code: 78; thickness: M-size). In this category, besides the experiments carried out in 2008 ($N=9$), we also used data from previous years ($N=10$), when 20 large spots of 4–5 mm diameter were marked on the eggshell with the same type of pen (Moskát et al., 2008c), because host reactions did not differ between these sets of manipulations (2/10 and 4/9 rejections, Fisher's exact test, $P=0.350$). The colour of these artificial spots was found to be very similar to the dominant type of natural spots of host eggs in our population regarding avian-visible spectra, when assessed by spectrophotometer (300–700 nm) (Moskát et al., 2008c).

Negatively spotted egg

On the fourth day of laying, instead of painting spots on the fourth great reed warbler egg, it was painted 'negatively': large spots of approximately 3–4 mm diameter were left in the original background colour of the eggshell, but inter-spot areas were painted with the dark brown pen. We used 20 negative spots for this treatment, which covered 40–60% of the whole egg surface (Fig. 1C).

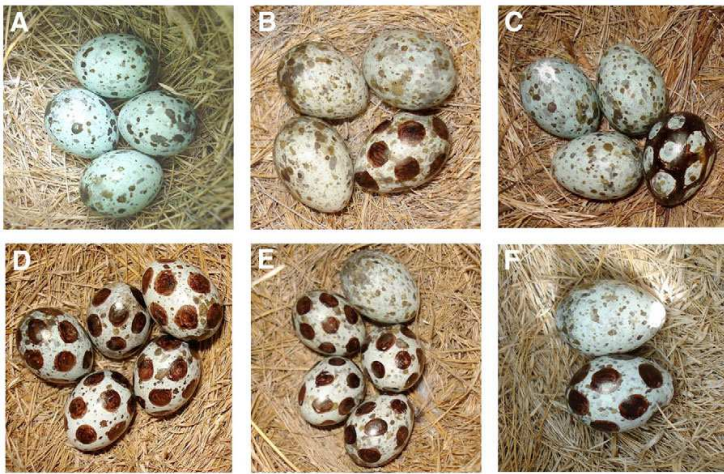


Fig. 1. (A) A non-parasitized great reed warbler clutch ('control'). (B) A great reed warbler clutch with one egg manipulated by adding large brown spots ('spotted egg'). (C) A great reed warbler clutch containing one egg with a painted dark brown background leaving original colour spots ('negatively spotted egg'). (D) All eggs in the clutch manipulated by large brown spots ('all spotted eggs'). (E) All except one egg manipulated by painting large brown spots in the discordancy experiments ('without learning'; see Materials and methods for details). (F) A variant of the 'without learning' case ('with learning') where host had the chance of observing the egg type manipulated by the researcher in which the new egg is painted each day in the early morning. The picture shows one brown spotted egg from the previous day and a new natural egg, which is waiting to be manipulated. (Photo credit: István Zsoldos.)

All spotted eggs

On the fourth day of laying, all four host eggs were painted with 20 large brown spots as for the 'spotted egg' treatment, above (Fig. 1D).

All spotted eggs except one, 'without learning'

Three randomly chosen host eggs were painted with 20 large brown spots as for the 'spotted egg' treatment, above (Fig. 1E).

All spotted eggs except one, 'with learning'

We manipulated newly laid host eggs by painting them with 20 large brown spots (see above; Fig. 1F) each day in the early morning to give hosts the chance to observe the manipulated egg as their own egg. However, we did not manipulate the fourth egg, and typically removed it between 05:30 and 06:30 h local time. In three cases the females laid later, so we checked these nests every 20–30 min until the new egg was laid (not later than 08:00 h). After 3 h we returned this non-manipulated fourth egg to the nest. In this category hosts had the chance to imprint the manipulated phenotype as their own, while in all of the other categories hosts could observe their own egg phenotype following egg laying.

Control

No egg was manipulated, but nest contents were monitored as for the treatments described above (Fig. 1A). We increased the sample size of both controls and the 'spotted egg' treatment using previous years' data sets [$N=13$ in 2008, and $N=12$ from Moskát et al. (Moskát et al., 2008c); with no egg rejected in any subset of the controls, Fisher's exact test, $P=1.000$].

If the host laid one more egg after manipulation at the four-egg state, we left the new (fifth) egg unmanipulated in treatments 'spotted egg' and 'negatively spotted egg', or manipulated by addition of 20 spots for treatments 'all spotted eggs', 'without learning' and 'with learning'. We started experiments with a clutch size of four, for the reason that birds started to incubate clutches just after the fourth egg was laid. At 48 nests (69%) females continued laying to 5 eggs (45 nests) or 6 eggs (3 nests). However, hosts' responses did not differ depending on maximum clutch size (Fisher's exact tests, two-tailed: spotted eggs: $P=0.617$; negatively spotted eggs: $P=1.000$; all spotted eggs: $P=1.000$; all spotted eggs except one

(combined): $P=0.303$). As we did not detect a significant difference when clutches where host females stopped laying just after the fourth eggs were compared with clutches where females continued laying in all of the categories, the number of untreated eggs in clutches did not influence hosts' decisions in response to parasitism.

We monitored nests for 6 days after the fourth day of laying to reveal hosts' reactions, and scored responses as: no response (acceptance) or rejection (egg ejection or nest desertion), following Moksnes and colleagues (Moksnes et al., 1991). This period of monitoring was justified by our previous studies on this host species, where latency of rejection was 1–5 days for each type of parasitic egg: for real cuckoo eggs in natural parasitism (Moskát and Hauber, 2007), and plastic model cuckoo eggs and painted great reed warbler eggs in experimental parasitism (e.g. Honza and Moskát, 2008).

Statistical analyses

We used binary logistic regression analyses for evaluation of the effects of our experiments on the dependent variable (acceptance versus rejection). We treated each experiment as an independent data point, even though hosts were not colour banded [see Moskát et al. for justification (Moskát et al., 2009)], and also because only a single observation or experiment was recorded from each territory, with breeding philopatry also generally low in this population (Moskát et al., 2008b), thereby limiting the possibility of pseudoreplication. For the sake of avoiding the use of two nests of a pair in the season, we did not use replacement clutches in the territory where the first nest failed. We also avoided the use of late nests in the season, which to our knowledge were either replacement clutches or second broods (Moskát et al., 2008b). We included experimental treatment as an independent fixed factor. We also introduced clutch size and egg-laying date in our analyses. This is justified by clutch size being a trait related to the age of the host parent in the great reed warbler, with young females often having smaller clutches than older ones (Bensch, 1996). We entered laying date (laying of the first egg) as a covariate as younger breeding great reed warbler females often lay in the middle of the breeding season, as revealed in the oriental reed warbler (*Acrocephalus orientalis*) in Japan (Lotem et al., 1992) (previously considered as a subspecies of the great reed warbler) (Leisler et al., 1997; Helbig and Seibold, 1999). However, seasonal effects on rejection had not been shown previously at our site (e.g. Moskát and Hauber, 2007;

Moskát et al., 2008a). We also included year as a covariate. Treatment, clutch size and year were used as categorical variables in the analysis, and the option ‘backward conditional’ was chosen for the selection of covariates.

All statistical analyses were carried out using the program SPSS version 9 (SPSS Inc., Chicago, IL, USA).

RESULTS

Altogether 71 experiments were conducted with known outcomes (i.e. not depredated, parasitized naturally by common cuckoos, or destroyed by storms until response and/or 6 days post-experiment) (Table 3). Rejection rates of the manipulated eggs in the different treatments varied between 25% and 85% (Fig. 2). In the binary logistic regression models, year was not significantly related to rejection rates ($P=0.397$) and was removed at step 2. Laying date ($P=0.377$) was also removed from the model at step 3. In the final model, treatment showed a significant relationship with host responses to parasitism (acceptance/rejection) (Wald=10.09, d.f.=4, $P=0.039$).

Specifically, among the treatments, only the ‘negatively spotted egg’ was significant in the model (Wald=8.27, d.f.=1, $P=0.004$). The rejection rate of manipulated eggs in this treatment was the highest, as 11/13 of the negatively spotted eggs were rejected by the hosts. Although clutch size was also retained in the model, this covariate was not statistically significant (Wald=6.72, d.f.=3, $P=0.081$). The discriminatory ability of the model was 73%, indicating a good predictive power. The Hosmer and Lemeshow goodness-of-fit test ($\chi^2=2.36$, d.f.=8, $P=0.968$) corresponds to the conclusion that there is an adequate fit of the data to the model. In the model the spotted egg treatment was used as the reference category, where hosts showed moderate rejection rate of the manipulated eggs (6/19 nests). In contrast, the rejection rate was greater in the negatively spotted egg treatment (11/13), which was highly significant (see above). Rejection rates of manipulated eggs in any other treatment (Fig. 2) did not differ from the reference category (rejections in 3/9, 4/16 and 4/14 nests in the ‘all spotted eggs’, ‘without learning’ and ‘with learning’ treatments, respectively, which did not enter as significant in the model, with corresponding P -values of 0.451, 0.733 and 0.295).

Our experiments started on the day when the fourth eggs were laid in the clutches. However, in the ‘with learning’ experiment hosts were confronted with the manipulated (spotted) egg type every day before this stage during the laying period, i.e. clutches with one,

two or three eggs. This meant that hosts had the chance to reject any of the spotted eggs in this pre-experimental period. We found that hosts rejected at least one spotted egg from 33% of the nests in this period (6/18 *versus* 4/14 rejections in the experimental period). When we compared all rejections of manipulated eggs (10/18) in the ‘with learning’ treatments with rejections in the ‘without learning’ treatment (4/16), the difference was not significant (Fisher’s exact test, two-tailed, $P=0.092$).

We documented no ejection costs or rejection errors (i.e. rejection of own eggs) (*sensu* Stokke et al., 2002) in most of the treatments, except in the ‘without learning’ and ‘with learning’ treatment. In the ‘without learning’ treatment we found one nest out of 16 where the host’s own egg was ejected with no ejection of any spotted egg (rejection error). In the ‘with learning’ treatment we also found rejection error in one nest out of 14, and in two nests one or two spotted eggs were successfully ejected together with a host egg (rejection cost). We recorded the loss of 8 spotted eggs during the laying process from 6 out of 18 nests (one egg in 5 nests and 3 eggs in one nest). The number of nests where rejection cost or rejection error occurred did not differ between the ‘without learning’ and ‘with learning’ treatments (Fisher’s exact test, two-tailed, $P=0.604$).

DISCUSSION

Regarding our first hypothesis, these results provide evidence against a general rule of thumb of discordancy in great reed warblers’ egg discrimination of foreign eggs. A few hosts appeared to apply a discordancy mechanism for egg rejection, even though it would be maladaptive for hosts, which use this mechanism in areas where there are high rates of multiple parasitism, as is typical in our population (see above), because hosts would end up rejecting their own eggs once these became the minority in the parasitized clutch. Nevertheless, our results suggest that discordancy is a constituent of the cognitive repertoire of some rejecter great reed warblers which can use it to reduce or escape the costs of cuckoo parasitism. Specifically, hosts in our experiments rejected a few of their own eggs above control levels when they were in the minority in both the ‘without learning’ and ‘with learning’ treatments (Table 3).

Our second hypothesis tested the importance of observational inspection of hosts’ eggs during egg laying in egg discrimination by the great reed warbler, by exposing hosts to false learning of the parasitic eggs (Strausberger and Rothstein, 2009). We found consistently similar results in the ‘without learning’ and ‘with learning’ variants of the ‘all spotted eggs except one’ treatment as

Table 3. Great reed warblers’ responses to manipulated and unmanipulated own eggs

Treatment	Egg in the minority			Eggs in the majority			N
	Egg type	Acceptance	Ejection+desertion	Egg type	Acceptance	Ejection+desertion	
Spotted egg	Spotted	13 (68%)	6+0	Natural	19 (100%)	0+0	19
Negatively spotted egg	Negatively spotted	2 (15%)	11+0	Natural	13 (100%)	0+0	13
All spotted eggs	None	–	–	Spotted	6 (67%)	3+0	9
‘Without learning’	Natural	14 (88%)	1+1	Spotted	12 (75%)	3+1	16
‘With learning’	Natural	12 (86%)	2+0	Spotted	10 (71%)	4+0	14
Control	None	–	–	Natural	25 (100%)	0+0	25

Data are number of nests (N).

‘Egg in the minority’, one egg, natural or manipulated, representing a different phenotype from the dominant egg type in the clutch (*sensu* Rothstein, 1974); ‘eggs in the majority’, the dominant egg type, natural or manipulated, in the clutch. (Frequency of acceptance is also shown as a percentage.)

*In each of 2 nests, 1 egg was ejected and 2 eggs in 1 nest were also rejected. In 2 out of these 3 cases hosts abandoned their nest after the successful ejection.

†In each of 2 nests, where the unmanipulated egg was accepted, all other (manipulated) eggs were ejected, i.e. 4 or 3 eggs. In 1 nest 1 spotted egg was ejected.

‡In each of 2 nests, 1 spotted egg was ejected. Two eggs in 1 nest and 3 eggs in 1 nest were also ejected.

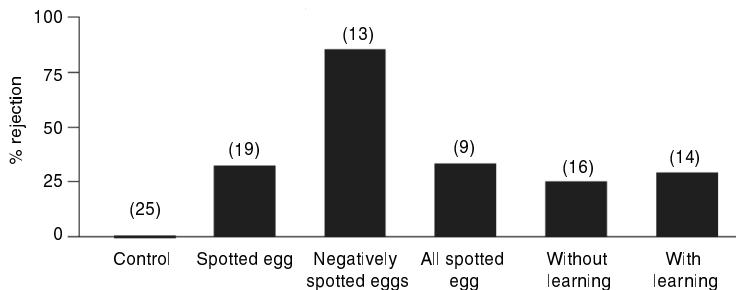


Fig. 2. Rate of rejection by great reed warbler hosts of manipulated own eggs in different treatments, within the 6 day control period following the laying of the fourth egg.

predicted (Table 2). Accordingly, these series of experiments did not support the scenario that this type of clutch inspection (*sensu* Hauber et al., 2004) contributes to rejection decisions in the great reed warbler.

Several types of learning may take place in hosts' behaviour, including learning their own eggs during their first breeding attempt (e.g. Rodríguez-Gironés and Lotem, 1999; Stokke et al., 2007b), learning of own eggs during egg laying (e.g. Rothstein, 1974; Moskát and Hauber, 2007), or learning of the parasite eggs (Hauber et al., 2006). Hosts may also inherit some kind of knowledge of their own egg template (Lotem et al., 1995; Hauber and Sherman, 2001). Prior experience-based recognition templates [e.g. during prior breeding attempts (Lotem et al., 1995) or during juvenile nest prospecting (Safran, 2004)] and inherited templates could both explain why 'learning' (inspecting hosts' manipulated eggs just after they were laid) did not increase tolerance of the manipulated (spotted) eggs, as we predicted. We explain it by the importance of a template recognition-based mechanism. Although learning of the own phenotype during egg laying may contribute to correction of the recognition template (Hauber and Sherman, 2001), this process cannot be prevalent in our great reed warbler populations, where cuckoo parasitism is high and cuckoos frequently parasitize host nests just before hosts have started egg laying (Moskát and Honza, 2002). Otherwise hosts may memorize the foreign cuckoo egg as their own, especially during their first breeding attempts, when they are confronted with eggs in their nests for the first time (Lotem et al., 1995; Hauber et al., 2004).

Our results nonetheless support the cognitive scenario that some great reed warblers possess a stored recognition template of acceptable egg phenotypes and reject parasite eggs based on their dissimilarity to the traits of the template. Further research is needed to evaluate whether learning during prospecting successful clutches or imprinting on own eggs by first year breeders really has consequences for egg discrimination for their later years. As an alternative possibility, it also remains to be studied whether the recognition template of the own eggs could be inherited. However, discriminating between these alternatives cannot be addressed by a simple age-specific comparison of rejection rates in the field between first time and experienced breeders, as proposed previously (Lotem et al., 1995). For example, at our Hungarian study site parasitism rates are so high that older breeders may be equally split between prior experience with parasitized *versus* unparasitized broods. Alternatively, even when first-time breeders reject parasitism (Mark and Stutchbury, 1994), inherited templates may not fully explain this own breeding experience-independent process, because many young birds may inspect eggs and nests during their hatch year, thereby potentially both evaluating the success of previous nesting attempts (Boulinier et al., 1996; Safran, 2004) and forming

recognition templates of conspecific eggs. Furthermore, gene-environment interactions are too complicated to enable us to categorize learned or inherited templates (Hauber and Sherman, 2001). Our findings are nonetheless conclusive, suggesting that both discordancy and template-based rejection to occur in great reed warblers, as independent mechanisms of anti-parasite adaptations.

The 'all spotted eggs' experiments revealed that many hosts (33%) were able to reject one or two parasitic eggs from a clutch, but others were not. In this way these results supported our third hypothesis, that the disappearance of hosts' own eggs facilitated foreign egg rejection. However, the manipulation of hosts' own eggs caused only a small shift in egg appearance (cf. the 32% rejection rate towards a manipulated egg in our 'spotted egg' treatment), so our results suggest that some of the hosts might have regarded the manipulated eggs as their own.

We also tested the importance of eggshell colour and maculation pattern in self-phenotype recognition. Maculation on the eggshell surface may enable individual recognition of an egg ('egg signature') (Davies, 2000) especially in species where intraspecific parasitism commonly occurs, with background colour also playing a part in egg signature (e.g. Lahti and Lahti, 2002). In our great reed warbler population background colour has more importance than spottedness in egg recognition (Moskát et al., 2008c). Spottedness proved to be important in intraclutch variation of eggs. An experimental study showed that increased variation of host eggs' spottedness, by painting on different numbers of dots, reduced hosts' efficiency in foreign egg rejection (Moskát et al., 2008a). Other studies revealed the importance of certain wavelength spectra, e.g. the effects of UV (+) and green (-) reflectance in the song thrush *Turdus philomelos* in foreign egg discrimination (Honza et al., 2007a; Cassey et al., 2008b) [for UV see also Honza and Polaciková (Honza and Polaciková, 2008) in the blackcap *Sylvia atricapilla*]. We used 2-tailed tests in our experiments because there is also a record of non-mimetic manipulation being attractive for hosts (Alvarez, 1999).

In contrast to the prediction of our fourth hypothesis, painting to produce 'negative spotting' showed that great reed warblers reacted differently to pattern types of egg markings, when the paint covered the same proportion of the eggshell surface. This is a surprising result because in a previous study we showed that increasing spot density had no effect on rejection rate by hosts, but when the whole eggshell was painted, rejection rate greatly increased (Moskát et al., 2008c). We explain the present result by differences in rejection behaviour with the higher contrast between the overall colour of experimental eggs and nearby non-manipulated own eggs (Fig. 1), supporting the scenario that rejection is based on contrast between different eggs within the clutch (Braa et al., 1992; Moknes, 1992; Procházka and Honza, 2003; Honza et al., 2004). However, both the discordancy and template recognition mechanisms predict

higher rejection rates for the negatively spotted egg type in contrast with the 'spotted egg' treatment (Table 2), so the 'negatively spotted egg' treatment, when it is evaluated alone, is not suitable for determining the exact egg discrimination mechanism.

Our study utilized several different but parallel experiments on great reed warblers' egg discrimination mechanisms to conclude that egg recognition cannot be explained by one single method. These results provide a methodologically uniform reconciliation of the many contrasting results and claims in the published literature on cuckoo hosts' egg-rejection mechanisms. Specifically, the hosts in our experiment clearly applied different cognitive mechanisms underlying recognition for the resulting behavioural patterns of egg discrimination, including the methods of discordancy and one kind of true recognition, i.e. the template-based mechanism. Rejection rates in different experiments implied a similar relative importance of rejection based on discordancy and template-based rejection. These results are thus consistent with those of Lotem and colleagues (Lotem et al., 1995), who suggested that egg discrimination in the oriental reed warblers cannot be explained only by the discordancy theory. Some birds behaved as if they were using a template.

On the one hand, foreign egg discrimination is clearly a complex process, showing variations in mechanism and proximate context-dependent factors influencing behavioural decisions between hosts. On the other hand, it seems to be a general rule that many bird species that discriminate foreign eggs use one or more variants of the true recognition process. Further studies are needed to clarify the relative importance of discordancy and true recognition in different host species. Great reed warblers' variable tolerance of multiple cuckoo eggs with different egg types (Honza and Moskát, 2005; Hauber et al., 2006; Moskát et al., 2009) suggests that the method of egg discrimination, or the efficiency of the method applied, may depend on the type of parasitism (e.g. single versus multiple, sequential parasitism, egg mimicry and the variation in the level of mimicry of parasitic eggs within the same clutch). Future research should also concentrate on the different functions of eggshell components in egg discrimination by hosts of brood parasites (e.g. shape, size, UV, spottedness, marking patterns). *Acrocephalus* warblers are good subjects for such studies, as they are parasitized by the cuckoo in many parts of Europe (Cramp, 1992), representing different stages of ongoing co-evolution (Stokke et al., 2008). Overall, our multi-treatment study revealed fine-tuned differences in great reed warblers' perceptual mechanisms. Our predictions for egg discrimination by hosts proved to be useful when different hypotheses were compared. However, we typically recorded lower rejections than expected, because great reed warblers' responses were shared between the discordancy and the recognition from template model. That is, about half of the great reed warblers, which rejected the parasitic eggs, applied the discordancy method for the recognition of the parasitic eggs, but rejections by others were based on templates.

Data on egg discrimination in hosts of brood parasites suggest that discordancy is the simplest method for egg discrimination (Rothstein, 1974; Marchetti, 2000), which could be used for recognition of brood parasite eggs when mimicry of the parasitic egg is poor. When cuckoos evolve better mimicry, template-based recognition probably gives a better chance of more accurate egg discrimination. Accordingly, two host species of the cuckoo which have been abandoned or nearly abandoned by the parasite, the brambling (*Fringilla montifringilla*) and chaffinch (*Fringilla coelebs*), both remain strong rejecters, including of closely mimetic eggs, through the use of a template-based discrimination (Vikan et

al., 2009). The presence of the cognitive repertoire of alternative egg recognition methods, as indicated in our study for great reed warblers, probably indicates an intermediate stage in the evolution of antiparasite defences, towards an eventual rejection method solely based on template recognition. We suggest that further studies are needed to test the novel prediction derived here and to describe in more detail the evolutionary stages that hosts may be using to rely on different egg-recognition methods, as a function of mimicry by parasitic eggs, host age and breeding experience.

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IV.

Diszkriminációs képesség és mechanizmusok

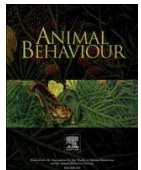
Discrimination ability and mechanisms

5.

Increased host tolerance of multiple cuckoo eggs leads to higher fledging success of the brood parasite.

Csaba Moskát, Márk E. Hauber, Jesús M. Aviles, Miklós Bán, Rita Hargitai, Marcel Honza

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Increased host tolerance of multiple cuckoo eggs leads to higher fledging success of the brood parasite

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In birds, multiple parasitism is the laying of two or more eggs by one or more parasitic females in a single host nest. Several cognitive mechanisms may explain how multiple parasitism could affect parasite egg discrimination by hosts. Rejection based on discordance predicts that multiple parasitism provides a perceptually more error-prone way for hosts to reject parasitism because more foreign eggs decrease the chance that any one egg is perceived as most dissimilar and recognized as foreign, unless parasite eggs are all similarly highly nonmimetic. In contrast, rejection based on clutch uniformity predicts that in multiple parasitism egg rejection is more error-proof if mimicry by parasite eggs is variable, because increased variation in egg appearance makes for easier egg rejection for hosts. Finally, true egg recognition, that is, rejection based on memory of the host's own eggs, predicts no differences in rejection rates from nests with single or multiple parasitism. We studied common cuckoos, *Cuculus canorus*, parasitizing a population of great reed warblers, *Acrocephalus arundinaceus*, in Hungary where multiple parasitism was frequent. Hosts rejected parasite eggs less often in nests with multiple parasitism than in nests with single parasitism. These observations were confirmed by experimental parasitism and support the rejection based on discordance hypothesis. As hosts were more likely to tolerate cuckoo eggs in nests with multiple parasitism, we found that multiple parasitism more than doubled cuckoos' reproductive output per host nest compared to single parasitism.

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Avian obligate brood parasites lay their eggs in nests of host species, and leave parental care of their offspring to the unrelated foster parents (Davies 2000). Caring for a parasite offspring is

a costly task for a host (Hauber 2006). For example, in evictor species the young brood parasite attempts to displace all nest content (Wyllie 1981; Honza et al. 2007a), including eggs and nestmates. Consequently, the brood parasite typically grows up alone, eliminating all host reproduction (Kilner 2005; Krüger 2007). Hosts can reduce the negative consequences of brood parasitism through antiparasite defence, involving aggression against the adult parasite (Davies & Brooke 1988; Røskaft et al. 2002a; Davies et al. 2003; Moskát 2005; Dyrce & Halupka 2006; Honza et al. 2006), egg discrimination (e.g. Davies & Brooke 1989; Moksnes et al. 1991) and/or chick discrimination (Payne et al. 2001; Langmore et al. 2003; Schuetz 2005; Grim 2006; Anderson & Hauber 2007). Consequently, the evolution of both brood parasitism and host responses reflects naturally and sexually selected morphological and life history trade-offs within and across parasite taxa and refined perceptual and cognitive mechanisms of host species' defences (Krüger & Davies 2002; Garamszegi & Avilés

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2005; Hauber & Kilner 2007; Krüger et al. 2007; Parejo & Avilés 2007).

Consequences of Multiple Parasitism

When the relative population density of brood parasites is high compared to that of hosts, some host nests may contain more than one parasite egg, so that they are multiply parasitized (Wyllie 1981; Moskát et al. 2006). Irrespective of whether multiple parasitism (or superparasitism) is due to the laying of eggs by the same or different females (McLaren et al. 2003; Ellison et al. 2006), it is typically thought to be costly to brood parasites because parasitic chicks compete more vigorously than do host chicks (Hauber 2003), leading to the reduction of per capita egg-to-fledging success of the parasite (Trine 2000; Hoover 2003). Multiple parasitism is especially costly for evictor brood parasites whose chicks displace all eggs and nestmates following hatching (Honza et al. 2007a) and it has been hypothesized to be implicated in the evolution of both egg removal by laying parasites and the similarity between host and specialist parasite eggs (Davies & Brooke 1988; Brooker et al. 1990).

Multiple parasitism is common in some hosts of the brown-headed cowbird, *Molothrus ater* (Trine 2000; Hoover 2003; McLaren et al. 2003), the shiny cowbird, *Molothrus bonariensis* (Lea & Kattan 1998; Tuero et al. 2007), the bronzed cowbird, *Molothrus aeneus* (Ellison et al. 2006), and also in the great spotted cuckoo, *Clamator glandarius* (Martínez et al. 1998; Soler & Soler 1999). However, it is a relatively rare phenomenon in hosts of the common cuckoo, *Cuculus canorus* (hereafter 'cuckoo'), an evictor brood parasite, which are typically parasitized at a rate below 20% (e.g. Schulze-Hagen 1992; Davies 2000; Rutila et al. 2002; Avilés et al. 2005; Antonov et al. 2006, 2007; Stokke et al. 2007a). Thus, most cuckoo chicks that hatch successfully (Øien et al. 1998) do not need to compete with or evict other parasite eggs and chicks from parasitized broods. In contrast, an unusually high level (ca. 64%) of cuckoo parasitism occurs in the great reed warbler, *Acrocephalus arundinaceus*, in the Hungarian Great Plain (Moskát & Honza 2002), where a high rate of multiple parasitism has also been reported: 35% of the parasitized clutches ($N = 123$) were multiply parasitized by two to four cuckoo eggs, and so 58% of all cuckoo eggs ($N = 187$) were found in nests with multiple cuckoo eggs (Moskát & Honza 2002). Egg collections in Hungarian museums (C. Moskát, unpublished data) and other reports on historical data suggest that such a high level of parasitism has existed for at least the last 80–100 years (e.g. Baker 1942; Molnár 1944; Moskát & Honza 2002).

Behavioural Responses of Hosts

Multiple parasitism may also have critical implications for the host's reproductive success and behavioural responses to parasitism. At the fitness level, more parasite eggs mean greater chances of elimination or reduction of the host's own brood and caring for more genetically unrelated young, thus reducing the foster parents' current (Trine 2000; Hauber 2002; Hoover 2003) and future reproductive outputs (Payne & Payne 1998; Hauber 2002; Hauber & Montenegro 2002).

The evidence suggests a negligible effect of multiple parasitism on recognition by hosts of nonevictor parasites. Indeed, experiments with *Molothrus* cowbird hosts have shown that single parasite eggs are as likely to be accepted or rejected as multiple foreign eggs (e.g. Rothstein 1975a; Davies 2000). Similar results were reported for magpie, *Pica pica*, hosts of great spotted cuckoos in Spain (Soler & Møller 1990). In contrast, although studies of multiple parasitism on hosts of evictor parasites are sparse (e.g. Brooker et al. 1990), these suggest that multiple parasitism may affect host recognition. For instance, Honza & Moskát (2005) experimentally parasitized great reed warbler clutches in Hungary

by two different, but both nonmimetic, model common cuckoo eggs, and found increased host rejection of the nonmimetic eggs relative to experimental single parasitism with a nonmimetic egg. However, in natural cases of cuckoo parasitism in the Hungarian great reed warbler population, the cuckoo eggs generally show a good visual match as judged by human eyes (Baker 1942; Southern 1954; Moskát & Honza 2002; Lovász & Moskát 2004; Fig. 1) and spectrophotometry (Cherry et al. 2007a). If mimicry is so good, hosts may treat foreign eggs as their own (Hauber et al. 2006; Moskát & Hauber 2007). Therefore, the research by Honza & Moskát (2005) cannot be regarded as a typical case of parasitism in that naturally parasitized great reed warbler population. In addition, the use of two nonmimetic eggs in that earlier experiment did not allow a test for contrasting predictions of alternative cognitive explanations for egg rejection. Here, we designed a new treatment to simulate parasitism more realistically to add experimental data to observations of natural parasitism and to address alternative cognitive scenarios involved in the hosts' responses to multiple parasitism.

Alternative Cognitive Mechanisms of Host Responses

The rejection by discordance hypothesis (Rothstein 1975b, 1982; Marchetti 2000; Servodio & Lande 2003) assumes that egg appearance is perceived relative to the appearance of other eggs in the clutch so as to generate a distribution of pairwise dissimilarities. In this cognitive scenario, therefore, it is parasitism per se, rather than the identity of individual parasite eggs that is perceived by the host (Hauber et al. 2004; Hoover et al. 2006). Accordingly, this cognitive scenario predicts that multiple parasite eggs of different levels of mimicry will lead to lower rejection rates than single parasite eggs because more foreign eggs would reduce the chance that any single egg is considered the most dissimilar. In contrast, when two parasite eggs are highly dissimilar in multiple parasitism, each foreign egg is expected to be recognized, so this method would result in increased rejection rates.

Alternatively, the hypothesis that rejection is based on limited intraclutch variation (Davies & Brooke 1989; Stokke et al. 1999; Moskát et al. 2008a; Schulze-Hagen et al. 2009) predicts that through evolutionary time, host species or populations of a host species that are more frequently parasitized by cuckoos would evolve eggs more homogeneous in appearance within a nest than populations with lower rates of parasitism (Øien et al. 1995; Soler & Møller 1996; Moskát et al. 2002; Avilés & Møller 2003). From a cognitive perspective, individual hosts would use clutch uniformity of their perceived own eggs to identify parasitism (Stokke et al. 1999; Moskát et al. 2008a). Thus, provided that foreign eggs are nonmimetic (i.e. they cannot be considered among the host's own set of eggs), multiple parasitism in a host clutch will lead to increased recognition of the parasite eggs. If a nest contains two or more parasite eggs that differ in the extent of their mimicry relative to the host's eggs, this should also increase the perceived variation of the entire clutch within the nest and lead to increased rejection of parasitism.

Finally, the true egg recognition (recognition from memory) hypothesis predicts that birds compare the characteristics of eggs in a clutch to a memory template of their own or otherwise acceptable egg phenotype, which could be inherited and/or learned (Rothstein 1974; Moksnes 1992; Hauber & Sherman 2001; Hauber et al. 2006; Moskát & Hauber 2007). If the match of a parasite egg to the internal template is close, hosts would accept this egg as their own (Reeve 1989; Davies et al. 1996; Hauber et al. 2006; Moskát & Hauber 2007; Stokke et al. 2007b). Thus, this scenario predicts the same frequency of rejection for foreign eggs for single and multiple

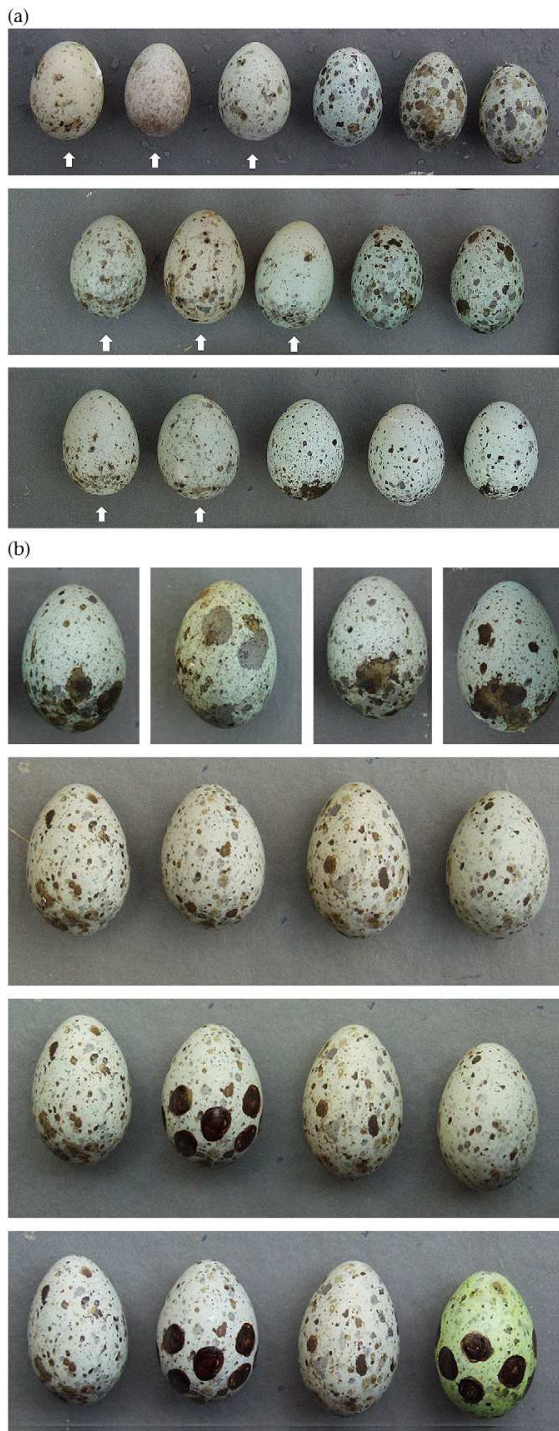


Figure 1. (a) Photos of great reed warbler clutches parasitized with multiple common cuckoo eggs. Parasite eggs are indicated by arrows. (b) Row 1: highly maculated great reed warbler eggs from central Hungary (rare type). Rows 2–4 provide an example of our experimental manipulation on typical host eggs. Row 2: the clutch before experimental manipulation; row 3: clutch with one 12-spot mimetic egg; row 4: clutch with one 12-spot mimetic and one yellow/12-spot nonmimetic egg.

parasitism by parasite eggs that have the same degree of similarity to host eggs.

Importantly, these alternative cognitive mechanisms also make contrasting predictions about the method of parasite egg rejection (i.e. egg ejection or nest desertion). Specifically, the rejection by clutch uniformity and the true recognition hypotheses do not predict differences in the method of rejection between nests with single and multiple parasitism, but the rejection based on discordance hypothesis predicts more nest desertions than ejections for multiple parasitism because it is parasitism per se and not the identity of the foreign egg that is recognized (Servadio & Hauber 2006).

Our aim in this study was to contrast egg rejection behaviours of great reed warbler hosts towards real cuckoo eggs in nests with single or multiple cuckoo parasitism in the context of predictions of these alternative cognitive mechanisms of foreign egg recognition. We did not address instances of repeated or sequential parasitism of the same clutch in this study (i.e. parasitism of a host clutch with a previously ejected cuckoo egg, which represents novel cognitive challenges for cuckoo hosts: Hauber et al. 2006; Honza et al. 2007b) and we did not have the genetic tools to identify whether one or more female cuckoos were responsible for multiple parasitism. We compared these observations on natural parasitism with the outcomes of a consistent methodology of experimental single or multiple parasitism, where we artificially parasitized a set of non-parasitized host clutches by painting one or two of the hosts' own eggs.

Impact of Host Responses on Cuckoo Fitness

If the rejection rate of cuckoo eggs by hosts differed between nests with single versus multiple parasitism, we would also find an effect on cuckoos' reproductive success from clutches with single versus multiple parasitism. We therefore assessed whether more evictor parasite eggs represented a cost for individual cuckoo young because hatchling cuckoos may eliminate other parasite eggs and nestmates. Alternatively, more than one parasite egg in the same host nests may decrease host rejection rates of foreign eggs and, thus, increase the probability that at least one parasite egg hatches successfully in a parasitized clutch. To achieve these goals, we contrasted the proportions of parasitized clutches yielding a fledgling cuckoo between nests containing single versus multiple cuckoo eggs.

METHODS

Study Area and Species

We conducted the study in the surroundings of Apaj and Kiskunlacháza-Bankháza, central Hungary (47°07'N, 19°06'E), ca. 40–50 km south of Budapest (Moskát & Honza 2002). Great reed warblers breed in 2–4 m wide strips of reed along both sides of small channels. We collected observational data by monitoring great reed warbler nests and measuring behavioural responses to single and multiple parasitism by cuckoos across three years 1998–2000. Nests were found at the building stage, or at the start of the laying stage, and monitored daily during the egg-laying stage (day 0 = day of first egg laid), and for at least 6 consecutive days or until rejection if parasitism took place until rejection, or until successful fledging if rejection did not take place. Newly laid eggs were assigned to host or parasite species based on size and maculation and marked with a fibre pen for individual identification. Laying female cuckoos typically remove one egg from the clutch (86%), either a host egg or an already present cuckoo egg, but rarely two eggs (6%), or no egg (8%) at all (Moskát & Honza 2002).

Although spectrophotometry studies revealed a generally close match across all wavelengths between cuckoo and great reed warbler eggs in this population (Cherry et al. 2007a, b), a wide range of host–parasite egg mismatch was revealed by human scoring (28% perfect, 23% good, 27% moderate, 16% poor and 6% bad as reported by Moskát & Honza 2002, following the categories proposed by Moksnes et al. 1993). Furthermore, great reed warblers reject ca. 34% of naturally laid cuckoo eggs, and are more likely to reject more poorly mimetic eggs, as judged by human observations (Moskát & Honza 2002) or measured by spectrophotometry (Cherry et al. 2007a). Common cuckoo eggs are typically larger than eggs of most of their hosts (Krüger & Davies 2002); however, in our study site egg volumes of this relatively large cuckoo host and its brood parasite were similar (Török et al. 2004; see also Honza et al. 2001 for data from the Czech Republic), justifying the use of painted conspecific eggs as experimental cuckoo eggs (see below).

Host Responses to Natural Parasitism

Host reactions towards each cuckoo egg were categorized as acceptance or rejection with the mode of rejection specified (i.e. ejection, burial of the parasite egg or the desertion of the parasitized nest; for more details see Moskát & Honza 2002). In nests with more than one cuckoo egg, hosts showed the same reactions towards each of the two or more cuckoo eggs in clutches with multiple parasitism, except in one case when one of three cuckoo eggs was ejected, but the other two were accepted. This clutch was omitted from the data set because of its unsuitability for an analysis where the response variable was binary, that is, acceptance or rejection of all parasitism per nest. We also identified cases of ejection costs (Stokke et al. 2002) when host eggs disappeared together with the cuckoo eggs from the nests, while ejection errors were recorded when host eggs disappeared but cuckoo eggs remained in the nest.

Our quantitative analyses included clutches that contained one to four cuckoo eggs at the same time. In the present study we did not use data on repeated or sequential parasitism, that is, when the laying cuckoo removed a cuckoo egg, or a female cuckoo laid another egg after a burial or ejection of a previously laid cuckoo egg by the host. This ensures that we evaluated host reaction to multiple parasitism, that is, when the host had the chance to inspect two or more parasite eggs at the same time. Also, only clutches that had at least three eggs (host and parasite egg numbers combined) were considered (the modal clutch size in this population is five eggs; Moskát et al. 2008b). Great reed warblers' responses to parasitism are affected by the absence of their own eggs in the nest at the early laying stage (Moskát & Hauber 2007), so we considered parasitized clutches that contained at least one or, generally, more host eggs. However, we included two cases of egg burial in cases of single parasitism, when burial was incomplete and the upper half of the parasite eggs were visible in the clutch. The conclusions did not change when we omitted these two nests from the analyses.

Cuckoos lay an egg every second day (Wyllie 1981) and in parasitized clutches multiple cuckoo eggs are more likely to be laid on different days (91% of cases; Hauber et al. 2006). Although we did not analyse the parentage of the cuckoo eggs by molecular techniques (Marchetti et al. 1998), our photos of multiple parasitism showed that the same nests were typically parasitized with eggs that clearly differed in colour and pattern (for examples see Fig. 1a), suggesting that they were parasitized by different cuckoo females (cf Moksnes et al. 2008).

In our study area the first cuckoo eggs were laid in a nest at a mean stage \pm SD of 0.9 ± 1.5 days (range -2 to 4 , $N = 20$; day

$0 = 1$ st egg laid). Additional cuckoo eggs were also laid after day 9. In an exceptional case, the first cuckoo egg was laid in the nest 2 days before the host female started her egg laying, and a second cuckoo egg was laid in the incubation stage, 2 days after the hosts completed the clutch.

Experimental Manipulations

Egg discrimination by great reed warblers in response to single versus multiple cuckoo parasitism was also tested through experimentation in 2006 with different egg types. Experimental eggs were produced by the manipulation of the hosts' own eggs; thus clutch size did not change, which simulated egg removal and replacement by the laying cuckoo.

Single experimental parasitism

Mimetic egg. For single parasitism we used two egg types. First, 12 dark-brown spots 4–5 mm in diameter were painted on the eggshell surface with a brown fibre pen (Faber-Castell OHP permanent, colour code 78, size 1525, Faber-Castell Inc., Stein, Germany; Fig. 1b, rows 3 and 4, second egg on the left). The colour of this pen was very similar to the dominant spot colour of hosts as revealed by spectrophotometry, and the size of these spots was within the range of natural spots (range < 1 – 6.1 mm; Moskát et al. 2008c). Typically the clutch needed a close inspection for humans to realize that the manipulated egg differed from the host's own eggs, so we called this egg type 'mimetic'. A previous experimental study revealed only 8% rejection rate for this egg type in single parasitism (one case out of 12 eggs and nests; Moskát et al. 2008c), and in the present study great reed warblers also tended to accept these eggs in clutches when no other eggs were manipulated (20% rejection of $N = 10$ eggs and nests) in similar frequencies (Fisher's exact test, two-tailed: $P = 0.571$). The disappearance of non-manipulated great reed warblers' own eggs at unparasitized nests (8.7%, in 4/46 nests between 1998 and 2005, C. Moskát, unpublished data) did not differ significantly from the rejection rate of these artificially spotted eggs in our population (Fisher's exact test: $P = 0.673$).

Nonmimetic egg. The mimetic egg type was expected to be rejected at a relatively low rate by hosts, so we also designed a less mimetic egg type, to be distinguishable from the host's own eggs, causing hosts to reject them at a relatively high rate. We modified host eggshell colour by painting the natural eggshell surface with a highlighter pen, because a previous study revealed that such changes in eggshell surface colours increased hosts' rejection rates significantly (Moskát et al. 2008c; see also Honza et al. 2007c for another species). So for the second egg type we painted the background colour of a host egg with a yellow highlighter pen (Stabilo Boss art No. 70/24, Stabilo Inc., Haroldsberg, Germany). This pen made the eggs a light greenish-yellow (Fig. 1b). We called this egg type nonmimetic. Many kinds of green and yellow shades can be found on both host and parasite eggshells in our population; however, these are very rare forms ($< 1\%$ frequency, Moskát & Honza 2002; Moskát et al. 2008c). We also painted 12 brown spots as for the mimetic egg over the yellow highlighter marking (Fig. 1b, row 4, egg on the right), to ensure that the two egg types differed in only one parameter (i.e. background colour). Great reed warblers showed the same strong rejection responses (100% rejection) against this combined yellow/12-spot (nonmimetic) egg type as seen against other egg types used in previous experimental parasitism on which the overall background colour, not the maculation, of great reed warbler host eggs was manipulated (i.e. dark-brown-painted host eggs: 92% rejection rate; Hauber et al. 2006). We checked the content of

experimentally parasitized nests daily for 6 consecutive days to detect rejection.

Multiple experimental parasitism

At our study site, half of naturally laid cuckoo eggs showed good to perfect mimicry and the other half showed moderate to bad mimicry (Moskát & Honza 2002). For this reason, we induced multiple parasitism experimentally by introducing one of our mimetic eggs and one of our nonmimetic eggs on day 4 in the hosts' egg-laying period. As we manipulated two host eggs from each nest, the clutch size remained the same, as in natural cuckoo parasitism (see above). This approach was specifically aimed at testing the prediction of the decreased clutch uniformity hypothesis by introducing two different artificially manipulated egg types into nests to simulate multiple parasitism.

Control

We also used a control group of nonparasitized host clutches in the year of the experiment where we did not manipulate eggs, but only checked and marked them as in cases of experimental parasitism. No egg was ejected or buried and no nest was abandoned or showed recognition error ($N=8$), which is the same result obtained in previous years for control clutches (0/13 desertions, Hauber et al. 2006; 0/12 desertions, Moskát et al. 2008c), indicating no host sensitivity for nest checks. However, this is a relatively small sample size for monitoring recognition errors.

Cuckoo Reproductive Success

We monitored the fate of parasitized nests until the cuckoo chick fledged. We calculated hatching success of cuckoos as the proportion of nests with cuckoo chicks that hatched. We also calculated fledging success as the proportion of nests that produced a cuckoo fledgling, in relation to the number of parasitized clutches. Both hatching and fledging success were calculated for single and multiple parasitized nests separately, with depredated nests removed from the analyses.

Statistical Tests

Individual breeding pairs of hosts were not colour-banded in the population, but only a single observation or experiment was recorded from each territory and breeding philopatry is generally low in this population (Moskát et al. 2008b), thereby limiting the possibility of pseudoreplication. All statistical tests were two-tailed. Analyses were carried out using the programs Statistica 5.1 (Statsoft Inc., Tulsa, OK, U.S.A.) and Statview 5.0.1 (SAS Institute Inc., Cary, NC, U.S.A.), and binary logistic regressions were computed in SPSS version 9.0 (SPSS Inc. Chicago, IL, U.S.A.). For the binary logistic regression analyses, the host's response was the dependent variable (accept or reject), with the number of cuckoo eggs, host clutch size, laying date (Gregorian day) and year treated as covariates in the initial model. Stepwise variable selection was applied by choosing the option 'backward conditional' with standard criteria.

Ethical Note

Our observational data came from monitoring the nesting attempts of wild birds as part of a long-term project on the breeding biology of great reed warblers. No birds abandoned nests because of our visits. The experimental test of our hypotheses also required the manipulation of some great reed warbler eggs. No great reed warbler eggs were crushed during our treatments, and we detected no effect of the manipulation on egg hatchability in comparison with nonexperimental nests. Our study was licensed

by the Duna-Ipoly National Park, the Kiskunság National Park and the Hungarian Inspectorate for Environment, Nature and Water.

RESULTS

Host Responses to Natural Parasitism

Altogether, 45 cases of single parasitism and 34 cases of multiple parasitism with 78 cuckoo eggs (two, three or four cuckoo eggs in the frequency of 25, 8 and one, respectively) were detected in this study. Rejection rates of great reed warblers towards cuckoo eggs differed significantly between naturally parasitized clutches with single versus multiple parasitism (40% and 12% rejection rates in nests of single and multiple natural parasitism, respectively; Fisher's exact test: $P=0.004$; Fig. 2). Rejection rates did not differ whether nests were parasitized by two or three to four cuckoo eggs (12% and 11% rejection rates with corresponding sample sizes of $N=25$ and $N=9$, respectively; Fisher's exact test: $P=1.0$). A binary logistic regression (with the Wald statistic, W) revealed that laying date was not significantly related to rejection rates ($\beta = -0.036$, $W_1 = 1.5$, $P = 0.30$; laying date was removed at step 2), nor was the year of observation ($\beta = 0.65$, $W_1 = 2.4$, $P = 0.13$; year was removed at step 3). In the final model, the rejection rate of cuckoo eggs by great reed warbler hosts was negatively associated with the increasing number of cuckoo eggs per clutch ($\beta = -1.76$, $W_1 = 6.6$, $P = 0.010$) and positively with number of host eggs ($\beta = 0.84$, $W_1 = 7.8$, $P = 0.005$). The discriminatory ability of the logistic model was 77.6%, and the Hosmer–Lemeshow goodness-of-fit test was not statistically significant ($\chi^2_2 = 8.9$, $P = 0.26$), indicating good power and good calibration of the model, respectively. Nests deserted during the laying stage had no chance of being completed and, consequently, they had smaller clutches, which resulted in the negative relationship between clutch size and egg rejection (Spearman rank correlation: $r_s = -0.31$, $P = 0.006$). None the less, even without the clutch size term included, the relative contributions of each predictor variable to the overall model remained similar (model discriminative power: 72.4%; the number of cuckoo eggs per nest: $\beta = -1.7$, $W_1 = 6.8$, $P = 0.009$; laying date: $P = 0.72$; year: $P = 0.18$).

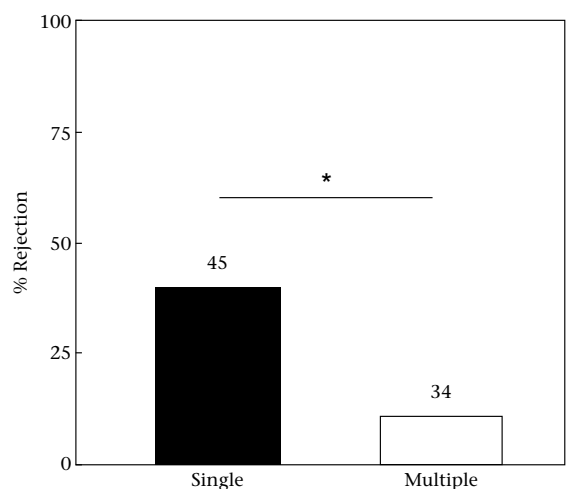


Figure 2. Rejection rate in response to real cuckoo eggs in nests naturally parasitized by one ('single') and two or three ('multiple') parasite eggs. In calculations a multiply parasitized nest was considered as one case. Numbers above bars indicate numbers of cases. * $P < 0.005$; Fisher's exact test.

The method of rejection differed consistently between cases of single and multiple parasitism: in clutches with single parasitism ($N = 18$ of 45 nests) ejection was the most frequent method of rejection (13/18, 73%) with the remaining cases being 11% of burial and 16% of nest desertion. In multiple parasitism, hosts rarely rejected cuckoo eggs (4/34 nests, 7/78 eggs). Egg ejection was observed in only one case (1/4 nests, 1/7 eggs) and all other rejections were by desertion (3/4 nests, 6/7 eggs). Thus, nest desertion was the more frequent method of rejection at nests with multiple parasitism (Fisher's exact test: $P = 0.046$), and ejection the more frequent response in nests with single parasitism (Fisher's exact test: $P = 0.005$).

Ejection costs were observed only in nests with single parasitism (4/13 nests, i.e. 31% of nests where the cuckoo egg was ejected). Typically one host egg was lost per nest, but in one case three host eggs disappeared. Ejection errors were observed in four nests where the cuckoo eggs were accepted, but only in cases of single parasitism (4/27 cases, i.e. 15% of nests where the parasite egg was accepted, with the loss of three eggs in one nest and one egg in each of the other three nests).

Experimental Parasitism

With respect to the nonmimetic egg, great reed warblers rejected all manipulated eggs in response to single parasitism (14 nonmimetic eggs in 14 nests). When hosts were exposed to experimental parasitism by the two different egg types (multiple parasitism with one mimetic and one nonmimetic experimental eggs), they rejected 60% of nonmimetic eggs (nine nonmimetic eggs in 15 nests; Fig. 3). The mimetic egg was also rejected in six cases from 15 nests of multiple experimental parasitism (40%) together with a nonmimetic egg. The rejection rate of the mimetic egg when it was introduced together with a nonmimetic egg did not differ significantly from that when the mimetic egg was introduced alone (two mimetic eggs rejected in 10 nests, 20%;

Fisher's exact test: $P = 0.68$). In contrast to natural parasitism, only ejection was observed as the method for rejection of the parasite eggs in both single and multiple experimental brood parasitism.

Hosts with multiple experimental parasitism showed a significantly lower rate of rejection compared to single parasitism with respect to the nonmimetic egg only (binary logistic regression: $\beta = -2.6$, $W_1 = 4.7$, $P = 0.032$; model discriminative power was 84%). Neither laying date ($P = 0.37$) nor clutch size ($P = 0.34$) had an effect on rejection, and they were removed from the binary logistic model by the stepwise procedure ($\beta = 0.16$, $W_1 = 0.79$, $P = 0.37$ and $\beta = 0.91$, $W_1 = 0.91$, $P = 0.34$ for laying date and clutch size, respectively). The lack of laying date and clutch size effects suggests similarities in the putative age and quality of host parents between our experimental treatments (Lotem et al. 1992).

Cuckoo Reproductive Success

In a counterintuitive pattern of this evictor parasite's breeding success, having more than one cuckoo egg per host clutch increased the chance that a cuckoo egg would be accepted and eventually hatch. This meant that a greater proportion of multiply parasitized nests contained a cuckoo hatchling than did singly parasitized nests (19/64 and 15/28 in single and multiple parasitism, respectively; Fig. 4, $\chi^2_1 = 4.2$, $P = 0.041$).

In each case only a single cuckoo chick survived and there was no difference in the hatching-to-fledging survival rate of cuckoo chicks between clutches with originally single versus multiple parasitism: 93% of hatched cuckoo chicks fledged successfully from nests with single parasitism ($N = 14$ hatchlings), and 75% from multiply parasitized nests ($N = 20$ hatchlings, with four nests containing two cuckoo hatchlings; $\chi^2_1 = 0.17$, $P = 0.68$). We also found similar productivities of those nests where at least one cuckoo egg hatched (single parasitism: 93%, $N = 14$ nests; multiple parasitism: 94%, $N = 15$ nests; $\chi^2_1 < 0.01$, $P = 0.99$). In contrast, the proportion of host broods with the cuckoo chick surviving to fledging (ca. 18–21 days; C. Moskát, unpublished data) in nests with multiple parasitism proved to be greater than that in nests with single parasitism (13/59 and 15/30 in single and multiple parasitism, respectively; $\chi^2_1 = 7.2$, $P = 0.007$; Fig. 5).

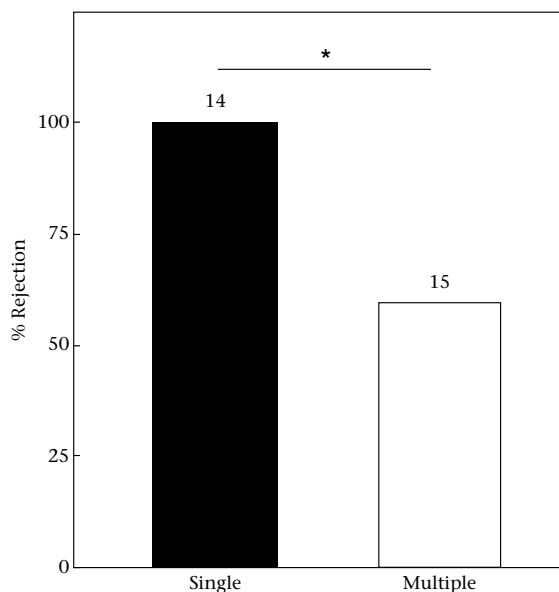


Figure 3. Rejection rates by great reed warblers against nonmimetic eggs when nests were experimentally parasitized with one nonmimetic egg ('single'), or with one mimetic and one nonmimetic egg each ('multiple'). Numbers above bars indicate numbers of cases. * $P < 0.005$; Fisher's exact test.

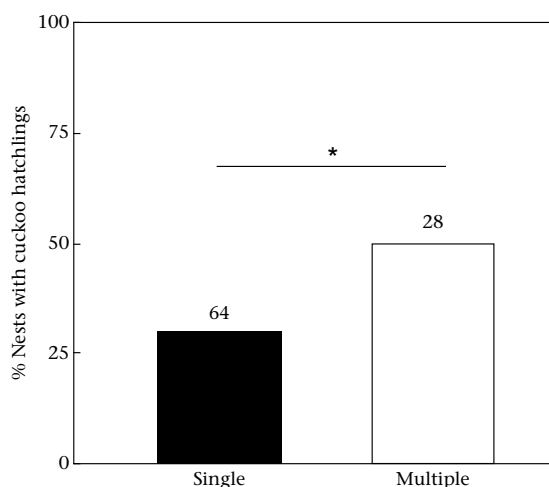


Figure 4. Percentage of host nests with cuckoo hatchlings from clutches with single or multiple parasitism. Numbers above bars indicate numbers of cases. * $P < 0.05$; chi-square test (see Results).

As cuckoo fledging rates were significantly greater in nests with multiple parasitism, we expected lower survival rates of cuckoo eggs in single parasitism. However, survival rates to fledging did not differ statistically in single and multiple parasitism (13 fledglings from 96 cuckoo eggs (0.135 fledgling/egg) in single parasitism, and 15 fledglings from 118 cuckoo eggs found in 50 nests (0.127 fledgling/egg) in multiple parasitism; $\chi^2_1 = 0.02$, $P = 0.88$). We explain this finding by the lower survival rate of cuckoo eggs on a per capita basis in nests with multiple than single parasitism (see above), which overcompensated for the effect of the hosts' lower rejection rate in nests with multiple parasitism.

DISCUSSION

Our results revealed a consistent difference, in both the observational cases and the experimental results, in great reed warblers' responses to variable numbers of cuckoo eggs, as we detected higher rejection rates to natural cuckoo eggs or experimental eggs in nests with single versus multiple parasitism. Thus, we reject the hypothesis of cuckoo egg rejection by clutch uniformity which predicts that more cues from more parasite egg appearances aid hosts to improve egg discrimination by decreasing egg similarity within the clutch and, thus, presumably by increasing behavioural rejection of foreign eggs. The significant differences in host responses between nests with varying numbers of cuckoo eggs also allow us to discount the true recognition scenario that predicts that rejection rates would be similar (and low for mimetic parasite eggs) in host nests with single or multiple parasitism.

In contrast, the hypothesis of rejection based on discordance was supported by both the observational and the experimental results. Specifically, this scenario predicts that more foreign eggs decrease the ability of the hosts to discriminate their own from foreign eggs based on pairwise differences between egg appearances (Marchetti 2000; Servadio & Lande 2003; Servadio & Hauber 2006) in parasitized clutches. The methods of the rejection of cuckoo eggs were also in support of the rejection by discordance hypothesis (as predicted by the model of Servadio & Hauber 2006), because cuckoo eggs were more likely to be rejected by ejection from nests with single parasitism while nests with multiple parasitism were more likely to be deserted. Increased rejection costs

and rejection errors of hosts' own eggs were associated with the increased rejection rates of single cuckoo eggs as they were documented only in nests with single parasitism.

A previous study revealed different results on great reed warblers' egg discrimination in multiple parasitism, yet they all seem to be the simple consequences of the mimicry of the experimental eggs: Honza & Moskát (2005) found that two types of experimental eggs, both rejected at a high rate (68–75%) in single parasitism, were rejected at about 96% when both were used for multiple experimental parasitism. In contrast to our present experiments, in previous experiments both of the parasite eggs fell outside the hosts' acceptance threshold for their own eggs (Hauber et al. 2006) and the two easily recognizable eggs appeared to facilitate the hosts' egg discrimination, in support of the discordance mechanism (see Introduction). Both the model cuckoo eggs used in the multiple parasitism experiments by Honza & Moskát (2005) and the natural cuckoo eggs in our study area (cf Moskát & Honza 2002) showed different levels of mimicry to host eggs (measured by the method of Moksnes & Røskaft 1995; Kruskal–Wallis test: $\chi^2_2 = 15.010$, $N = 158$, $P = 0.001$), which explains the differences in egg rejection rates in multiple parasitism by Honza & Moskát (2005) and the present study (96% versus 12%; Fisher's exact test: $P < 0.001$).

Apart from mimicry (Honza & Moskát 2005 versus this study), intraclutch variation in host eggs (Stokke et al. 1999; Cherry et al. 2007a; Moskát et al. 2008a), hosts' age (Lotem et al. 1992, 1995), nonrandom selection of hosts (Hauber 2001; Grim 2002; Hauber et al. 2004; Garamszegi & Avilés 2005; Parejo & Avilés 2007), previous experience with the parasite egg (Rodríguez-Gironés & Lotem 1999; Hauber et al. 2006; Honza et al. 2007b) and variable host genetic backgrounds (Martín-Gálvez et al. 2006) may also affect rates of hosts' egg rejection in response to multiple parasitism. In the great reed warbler, other important factors include the host's sighting of a cuckoo near the nest (Bártol et al. 2002), experience with its own eggs throughout the laying stage (Moskát & Hauber 2007), breeding stage (Moskát 2005) and time of day (Dyrce & Halupka 2007). Habitat structure and immigration of parasite-naïve individuals (Takasu et al. 1993; Røskaft et al. 2002b, 2006), through a metapopulation effect (Barabás et al. 2004; Hauber et al. 2004), might also affect antiparasite defence in our population. However, our results revealed no statistical relationship with laying date or interannual differences in rejection rate from nests with single versus multiple parasitism, and so increased tolerance to parasite eggs in multiple parasitism seems to be a fairly robust effect.

Surprisingly, fitness correlates of the parasite with multiple parasitism in our observational data set yielded an increased egg-to-fledging success of parasite eggs, owing to reduced rejection of parasite eggs in multiple parasitism. Yet, as with all observational data sets, consistent variation in rejection rates of cuckoo eggs by hosts in naturally parasitized nests can be explained by parameters not measured in the observational portion of our study. For example, differences in visit rates to host nests by prospecting and laying parasites have been implicated in shifting acceptance thresholds of cuckoo eggs by other host species (e.g. Brooke et al. 1998). However, we recorded this last confound in our experimental approach because we increased the variability of eggs in nests with simulated multiple parasitism solely by using two different egg types compared with one egg type in host nests with simulated single parasitism. This in turn resulted in the same directional change of decreasing host rejection rates as seen in the naturally parasitized nests. Nevertheless, experimental work is required to determine the factors influencing which evictor cuckoo chick survives in the nest with multiple parasitism, such as hatching asynchrony or sex-specific development of the parasite

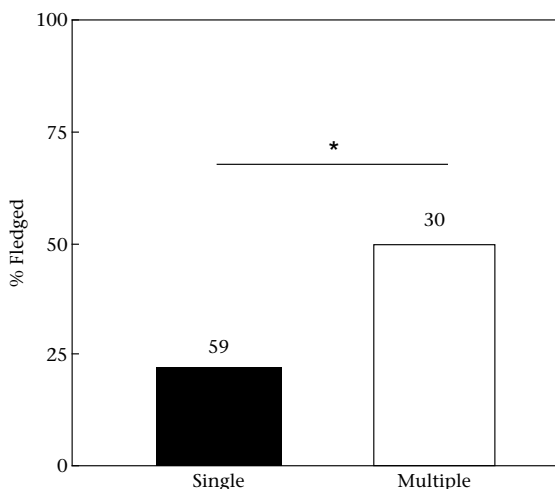


Figure 5. Percentage of nests with fledged cuckoo chicks from clutches with single or multiple parasitism. Numbers above bars indicate numbers of cases. * $P < 0.01$; chi-square test (see Results).

(Tonra et al. 2008). In our study, only one cuckoo chick survived per parasitized brood but in some hosts of the cuckoo, including the redstart, *Phoenicurus phoenicurus*, in Finland, multiple cuckoo chicks can survive per host brood, alongside the hosts' own chicks (Rutla et al. 2002; Grim et al. 2009), implying that perhaps genetic, host race differences might also mediate cuckoo success in the presence versus absence of multiple parasitism.

From the perspective of the brood parasites, counter to expectations for an evictor brood parasite whose chicks do not tolerate other host or parasite chicks in the nest (Hauber & Moskát 2008), our results surprisingly show that multiple parasitism seems not to be detrimental, because hosts' antiparasite defences against multiple parasite eggs in a clutch are weaker than against single parasite eggs. In the absence of genetic data on these eggs, we do not know for certain whether multiple cuckoo eggs were laid by the same females. None the less, our photos of nests with multiple cuckoo eggs showed consistent differences between parasite eggs, suggesting laying by different females (Moksnes et al. 2008), as seen in other avian brood parasite taxa (Martínez et al. 1998; McLaren et al. 2003; Ellison et al. 2006). The reduced rate of rejection of cuckoo eggs from multiply parasitized nests might also act against the evolution of the recognition and rejection of other parasite eggs by laying female cuckoos (Brooker et al. 1990; Brooker & Brooker 1998).

In conclusion, we have found that multiple natural parasitism may play a key factor in cuckoo egg discrimination of great reed warblers, causing a highly significant reduction of rejection rates. We demonstrated this shift in multiple experimental parasitism by using simultaneously one mimetic and one nonmimetic egg. As a consequence of hosts' limited egg rejection responses to multiple parasitism, cuckoos' reproductive success was higher in clutches with multiple parasitism. However, multiple parasitism is also costly for cuckoos: because of the multiple eggs invested in the same nest only one cuckoo chick can fledge (Wyllie 1981). An equilibrium between an individual female cuckoo's benefits of increased hatching and fledging success of her young and the costs of losing eggs and chicks to intraspecific competition by evictor parasite chicks may exist in populations exposed to a longstanding high parasitism pressure and a consistently high proportion of nests with multiple parasitism.

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IV.

Diszkriminációs képesség és mechanizmusok

Discrimination ability and mechanisms

6.

Viewing own eggs is not required for foreign egg rejection.

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ABSTRACT

Many hosts have evolved diverse cognitive mechanisms to recognize and reduce the cost of social parasitism. For example, great reed warblers (*Acrocephalus arundinaceus*) can accurately reject even mimetically colored brood parasitic common cuckoo (*Cuculus canorus*) eggs in a clutch of own eggs. Yet, these same hosts' antiparasite defenses are less effective when the clutch is parasitized by multiple cuckoo eggs, suggesting a role for discordancy or online self-referent phenotype matching between own and foreign eggs. We performed experiments to understand whether the presence of own eggs is required for the discrimination and rejection of foreign eggs in great reed warblers. We dyed hosts' own eggs with one of several artificial colours so that clutches contained (a) 1 dyed and 4 unmanipulated eggs, (b) 3 dyed and 2 unmanipulated and (c) 5 dyed eggs. Rejection rates of dyed eggs varied widely between the colours, and as predicted by both discordancy and online self-referencing, rejection rates were highest in treatment (a) with one dyed egg compared to treatments with the majority (b) or all (c) dyed eggs. However, relative rejection rates of dyed eggs were also consistent between the specific colours across the treatments, including (c) where no unmanipulated own eggs were available for viewing and irrespective of whether eggs in (c) were dyed all different colours or the same colours. We conclude that in the absence of discordancy or online self-referencing, hosts may rely on comparisons of foreign egg colours against an internal recognition template of acceptable (own) egg phenotypes.

INTRODUCTION

Obligate social parasites, including brood parasitic ants, fishes, amphibians, and birds, introduce their offspring into the clutch or brood of other species rely on

host species to provide costly parental provisioning for the parasitic young (Sato 1986; Davies et al. 1989; Grim 2006; Brown et al. 2009; Davies 2011; Kilner and Langmore 2011). By eliciting parental care for unrelated young, the opportunity and the cost of parasitism represent important co-evolutionary selection pressures (Rothstein 1990); parasites show morphological and behavioural adaptations to go undetected by hosts, and hosts show sensory and cognitive antiparasite adaptations to recognize and reject foreign young, respectively (Krüger 2007). From extensive studies of co-evolutionary arms races focused on the mimicry of host egg colours by brood parasitic birds and the increasingly complex behavioral responses of hosts to mimetic parasite eggs (Davies 2000), we know that some avian brood parasites only recently have begun to exploit host species which have not yet evolved antiparasite responses (Hauber et al. 2004), other hosts have won and eliminated their parasites altogether (Lovász and Moskát 2004), or parasitic mimicry has become so perfect that the hosts' sensory systems cannot discriminate foreign eggs or young from their own in the nest (Avilés 2008; Ranjard et al. 2010; Langmore et al. 2011). In most cases studied in both vertebrate and invertebrate systems, however, the arms race is ongoing, and some parasitic attempts succeed against the imperfect defense portfolios of hosts (e.g. Johnson and Herbers 2006, Takasu et al. 2009; Antonov et al. 2010, Martin et al. 2011).

Empirically, foreign egg discrimination is the most important antiparasite adaptation in avian hosts of brood parasites (Davies 2000, 2011), and historically has been studied most extensively using experimental approaches in the context of host egg mimicry by the common cuckoo *Cuculus canorus* (hereafter: cuckoo) (Payne 2000). cuckoo eggs typically resemble host eggs both in colour and pattern (Davies and Brooke 1988, 1989; Moksnes and Røskoft 1995), and sometimes also in size (Török et al. 2004), but not in shape (Bán et al. 2011) or thickness (Spottiswoode 2010; Igic et al. 2011a). The cognitive basis of how hosts discriminate between own and foreign eggs has been considered to include both self-referenced and socially learned cues of own and parasitic eggs', chicks', and adults' phenotypes (Lotem et al, 1995; Hauber and Sherman 2001; Moskát and

Hauber 2007; Stokke et al. 2007; Davies & Welbergen 2008, Sato et al. 2010). Typically, the discrimination of own vs. foreign eggs depends on perceivable differences between the visible appearances of own vs. foreign eggshells within the parasitized clutch (Cherry et al. 2007; Honza et al. 2007a; Avilés 2008; Cassey et al. 2008; Avilés et al. 2010; Stoddard and Stevens 2010, 2011; Spottiswoode and Stevens 2010, 2011; Vikan et al. 2011). However, some experiments already point to the possibility that viewing the maculation of the hosts' own eggs is not required to reject foreign eggs (Moskát et al. 2010) and learning of the overall appearance of the hosts' own eggs (i.e. both maculation and background colour) may also be involved in rejecting foreign eggs in the absence of own eggs in the clutch (Lotem et al. 1992; Moskát and Hauber 2007). Here for the first time we focus on the background coloration of host vs. foreign eggs to examine whether viewing own eggs is required to recognize and reject foreign eggs in the nest.

Regarding the cognitive basis underlying foreign egg recognition, a recent analysis by Moskát et al. (2010) demonstrated that a regular host of the cuckoo, the great reed warbler (*Acrocephalus arundinaceus*), relies on at least two different mechanisms of egg recognition. One of these is discordancy, where hosts reject those eggs whose phenotype is in the minority in the clutch (Rothstein 1974). Accordingly, in the great reed warbler, 10% of the hosts ejected their own eggs, when clutches contained four similar artificially maculated parasitic eggs and one own egg (Moskát et al. 2010). An alternative, but not mutually exclusive, cognitive process is online self referencing (hereafter: self referencing), whereby birds compare each egg against one of their known own eggs in the clutch, probably identified shortly after laying it (Hauber and Sherman 2001; Moskát and Hauber 2007). The experimental separation of the two decision mechanisms, especially when both processes contribute to rejection behaviours, requires specially designed experimental treatments of one or most of the eggs in the nest to assess these cognitive rules' relative contributions (Moskát et al. 2010).

Finally, in all cases of egg recognition, an internal, memory or perceptual threshold based rule could also guide discrimination decisions between

acceptance and rejection, independent of the presence and the diversity of the eggs that can be inspected and viewed in the nest at the time of the rejection behaviour (Lotem 1993; Hauber et al. 2006; Moskát and Hauber 2007). For example, relative difference in the perceived colours of the foreign eggs from a coloration deemed acceptable by the host's sensory filters (Cassey et al. 2008; Stoddard & Stevens 2011) may be compared to the attributes of a learned recognition template to guide behavioural thresholds between acceptance and rejection of own vs. foreign eggs (Reeve 1989; Hauber and Sherman 2001; Hauber et al. 2006; Ranjard et al. 2010; Igic et al. 2011b). In other words, hosts might have a cognitive algorithm of which consistent sensory responses to spectral properties of eggshell colour should lead to acceptance or rejection (Honza et al. 2007b; Cassey et al. 2008).

Irrespective of the exact type and timing of the acquisition for this recognition template, theory predicts that using multiple, non-exclusive cognitive processes of discrimination to generate a critical behavioural response may be especially beneficial in reducing recognition errors (i.e. mistakenly rejecting own eggs or accepting foreign eggs, through cognitive redundancy; e.g., Hauber et al. 2000). Integrating alternative decision rules to detect foreign eggs might be especially important in those host populations where egg mimicry is good, parasitism rates are high, and clutches are exposed often of multiple parasitism (Moskát et al. 2009). In these cases, hosts' own eggs may frequently be in the minority or, rarely, altogether absent in host nests during the laying or the incubation period (e.g., Rothstein 1974; Trine 2000; Hoover 2003; Gloag et al. 2011; Krüger 2011). As such, hosts faced with high rates of multiple parasitism would benefit from being able to recognize foreign eggs even in the absence of viewing their own eggs in the clutch. cuckoos parasitize great reed warblers in Hungary at unusually high rates, with more than 50% of nests parasitized in habitats where trees are available as vantage points for cuckoos (Røskft et al. 2002; Moskát et al. 2008b). A consequence of heavy cuckoo parasitism is the high frequency of multiple parasitism (Moskát et al. 2006). Previous work in Hungary

also revealed that the presence of more cuckoo eggs, and therefore fewer host eggs in a clutch are predictive of reduced rates of foreign egg rejection, including natural and experimental clutches with multiple parasitism (Moskát and Hauber 2007; Moskát et al. 2008a; Moskát et al. 2009). Critically, however, in all these prior studies, natural or experimentally parasitized clutches included one or more host eggs, making it possible for the *in situ* simultaneous, online comparison of viewing and inspecting own and foreign eggs in the nests, and, thus, allowing for both discordancy and self-referencing based recognition mechanisms (*sensu*: Hauber and Sherman 2001).

Here, we experimentally investigated the role that the presence of own eggs might play in facilitating egg discrimination in great reed warblers. Specifically, we manipulated the relative applicability of both discordancy- and self referencing-based cognitive rules, to assess their possible contributions to egg rejection decisions. We hypothesized that hosts' egg rejection ability would be reduced in multiple parasitism in contrast with single parasitism, including treatments where all eggs in the clutch were treated to appear foreign. Accordingly, we predicted that hosts would reject fewer eggs in multiple than in single parasitism, because in single parasitism both discordancy and self referencing can promote the discrimination against the parasitic egg, which is in the minority of the clutch. We also specifically tested an alternative hypothesis, namely that the presence of own eggs in a mixed clutch of own and parasitic eggs increased hosts' egg recognition rates through self referencing only. For this hypothesis we predicted that great reed warblers would reject more foreign eggs in multiple parasitism, when hosts eggs are still present (albeit in the minority), relative to clutches where all of the hosts' own eggs are experimentally replaced with foreign eggs. However, the two mechanisms would identify different eggs for rejection in clutches where the own eggs are in the minority (i.e.: self-referencing: foreign eggs; discordancy: own eggs), whereas in a clutch with all host eggs manipulated, a third cognitive mechanism, a recognition template based rejection decision (Moskát and Hauber 2007) must be operating. The use of the

varying numbers of dyed foreign egg types thus allows us to test this third recognition template mechanism, which predicts a consistent pattern of relative rejection rates of the same coloured eggs across each of our treatment types. To evaluate these hypotheses, we used a range of artificial colours to dye hosts' own eggs to determine whether colour-dependent egg rejection rates in great reed warblers are caused by the presence and proportion of own eggs in the clutch.

METHODS

The study was conducted in the surroundings of Apaj (47°07'N; 19°06'E), ca. 40-60 km south of Budapest, Hungary, in 2009 and 2010, from mid-May until mid-June. great reed warblers breed in 2-4 m wide reed-belts along both sides of irrigation channels. We monitored sections of the channels every week to find nests during the nest-building or egg-laying stages. On the day of laying the 5th egg in the clutch (the modal clutch size in this host population: Moskát and Hauber 2007), one or more host eggs in a clutch were experimentally manipulated using highlighter pens (type Stabilo Boss). We used blue, green, yellow, orange and red highlighter pens (No. 70/31, 70/33, 70/24, 70/54, and 70/40, respectively) for dyeing the hosts' eggs; representative reflectance spectra were taken by Ocean Optics USB 2000 spectrometer of the differently dyed eggs and are illustrated in Fig. 1. This dyeing approach had been demonstrated suitable for studying experimental parasitism in great reed warblers, because even though cuckoo eggs are thicker and stronger than host eggs (Hargitai et al. 2010; Igic et al. 2011a), this host is able to reject cuckoo eggs by puncture ejection (Honza and Moskát 2008) and parasite and host eggs are comparable in size (Honza et al. 2001; Török et al. 2004; Antonov et al. 2006). Also, highlighter-dyed eggs were found to be ejected consistently by this host species in several previous studies (Moskát et al. 2009, 2010; Avilés et al. 2009). These colours represent the main colour types within the colour spectrum of within the 400-700 nm interval falling inside the range of

avian vision (Fig. 1) and both prior work and the current study (see below) yielded egg rejection rates by hosts that encompassed their range of natural rates of cuckoo egg rejections (~33%). All unmanipulated eggs were handled and inspected to the same extent as dyed eggs, thereby representing a control for the experimental treatment within clutches. Prior experiments using colour dyes matching host egg colours have resulted in no significant increases in egg rejection rates compared to unmanipulated eggs (Hauber et al. 2006) thereby implying that simply applying dye to own eggs does not elicit rejection behaviour in this host species. Unmanipulated eggs in the control nests with no dying treatment were also never ejected or abandoned in this study ($n = 27$ nests).

One of the following four treatments was applied to nests in our study (Fig. 2):

(a) Treatment '1-egg': We manipulated one host egg per clutch (single parasitism, where the foreign egg is in minority), using one of five colour types at a time, with one of the five highlighter pen colours at a clutch, so the original maculation pattern also remained visible. Experiments were started in the second half of the laying stage (3-5 eggs already in the nest).

(b) Treatment '3-eggs': We manipulated three eggs (multiple parasitism, where the foreign eggs are in majority) in a clutch with different colours, using the blue, yellow and orange pens, which colours were recorded to evoke low, intermediate and high values in rejection frequency found in our single parasitism treatments (our unpublished results). All experiments were started when clutches were completed with five eggs.

(c1) Treatment '5-eggs with multiple colours': All eggs in five-egg clutches (multiple parasitism without unmanipulated eggs) were dyed by the different colours of treatment (a) simultaneously in each clutch. These colours identical with the previous experimental set plus contains two dyed eggs (green and red). Experiments were started when clutches were completed with five eggs.

(c2) Treatment '5-eggs with one colour': All eggs in five-egg clutches (multiple parasitism without unmanipulated eggs) were dyed by one of the three colours

which were also used in treatment (*b*).

Experimental nests were monitored until 6 days after the treatment on a daily basis to characterize host behavioral responses. If the manipulated eggs remained in the clutch at the end of the check period (6 days), the result was considered as acceptance (following: Moksnes et al. 1991; Hauber et al. 2006; Moskát et al. 2010). To determine if a clutch was deserted, we looked for indications of cold eggs and no rotation of the eggs between subsequent daily visits. Specifically, to look for egg rotation by females, we positioned the eggs with their sharp poles toward the centre of the nest. If this configuration was not altered within one day and the eggs remained cold, we categorised the result of the experiment as desertion. If hosts removed one or more eggs from clutch, the result was classified as ejection.

We did not colour band individual hosts in this study but studied only those nests that were sufficiently distant and synchronous within years to be deemed to belong to different pairs, so it is highly unlikely that we investigated two or more nests of the same pair of hosts. Pseudoreplication is also unlikely between years because this host species shows a low level of breeding philopatry in our study population (Moskát et al. 2008b). Accordingly, we considered each nest as the unit of statistical analysis.

Only nests which were not naturally parasitized by cuckoos were used for analyses to avoid the effect of external cues of parasitism (Svenningsen and Hølen 2010), such as sight of the cuckoo (Davies and Brooke 1988) and the differential confounds of sequential (Hauber et al. 2006) versus simultaneous multiple parasitism (Moskát et al. 2009). We applied linear models for the analyses of host rejections toward the parasitic eggs in the different treatments. For testing hosts' reactions toward the differently coloured eggs in single parasitism (treatment *a*), we applied nominal logistic regression, including host responses to parasitism (eject/accept) as a binary response variable, with laying date as a co-variate and treatments as factors. From rejections either by ejection or desertion, only ejections were considered. The sole case of desertion was

excluded from analysis.

We compared the behavioural outcomes of our first three treatments using a generalized linear mixed-effects model (Bates and Maechler 2009) with logit link function and binomial error distribution. Here the response variable was the ejection event of single eggs (binary factor), with laying date as co-variate, and type of treatment as fixed factor. Clutch ID was entered as random effect. As above, host responses included ejections and acceptances, as desertion was so rare as only one additional desertion was detected in treatment *c1*, again in response to the green colour. With this model, we could also estimate the interaction between individual colours and treatments on the ejection rates of an egg which is informative regarding whether the individual colours have their own differential effects against the number(s) of painted eggs. In this model we only considered the rejection rates of blue, yellow and orange dyed eggs which were present in all treatment types. The model has been fitted using the Laplace approximation criterion (Bates and Maechler 2009). We used non-parametric rank tests to determine the relative rejection rates of differently dyed eggs across the four treatments to look for a statistical interaction effect between the number of eggs manipulated (*a*: 1, *b*: 3, or *c1 and c2*: 5) and the number of colours used for manipulations per nest (*a*: 1, *b*: 3, *c1*: 5, or *c2*: 1). We used two-tailed tests and set $\alpha = 0.05$. For the analyses we used the R statistical environment (R Development Core Team 2006) and Statview 5.0.1 (SAS, Cary, NC).

RESULTS

Altogether, 134 experiments were conducted with suitably known outcomes (i.e. not depredated, parasitized naturally by common cuckoos, or destroyed by storms until the response could be recorded at 6 days following a manipulation). As we did not use nest desertions for calculations of rejection (one case of desertion was detected in treatment *a* and one case in treatment *c* (see above), our data set

included 69 cases for single parasitism (treatment *a*; blue: 14, green: 14, yellow 12, red 16 and orange: 13), 16 cases for multiple parasitism with three manipulated eggs per clutch (treatment *b*), 16 cases for multiple parasitism with five differently manipulated eggs per clutch (treatment *c1*) and 33 cases in the three colour categories for the multiple parasitism with five identically dyed eggs (treatment *c2*). The manipulations of egg coloration induced a broad range of reactions from hosts, rejection rates against these egg types varied in a wide range (7-77%) in treatment (*a*) (Fig. 3). It is interesting to note that the egg types that are ejected at the highest rates are those that appear to be less mimetic to the background colour of hosts' own eggs. Red and orange eggs were very different from host eggs both in term of peak reflectances and spectral shape characteristics (Fig. 1), and therefore, appeared highly non-mimetic to human observers. Laying date had no significant effect on the ejection ($B = -0.237$, S.E. = 0.14, $z = -1.693$, $P = 0.09$; Table 1), whereas the colour of the dyed egg significantly affected the hosts' responses (blue: $B = -4.864$, S.E. = 1.917, $z = -2.536$, $P = 0.011$; yellow: $B = 3.801$, S.E. = 1.457, $z = 2.608$, $P = 0.009$; orange: $B = 7.053$, S.E. = 1.763, $z = 4.0$, $P < 0.001$; Table 1). Specifically, ejection rates increased in the following order: blue < (green) < (red) < yellow < orange across all treatments *a* and *c* (Fig. 3).

To investigate the effect of the numbers of eggs painted on ejection rate across the three treatments, we compared ejection rates of the eggs dyed blue, red or orange, as these three colours were present in each treatment and hence allowed the calculation of interaction between egg colour and number of eggs painted. Ejection rate was significantly lower for eggs in clutches containing three colored eggs (treatment *b*) than for eggs in clutches with one (*a*) painted egg ($B = -7.571$, S.E. = 3.151, $z = -2.403$, $P = 0.016$; Table 1). Hosts' reactions in the three treatments of multiple parasitism (treatments *b*, *c1* and *c2*) proved to be statistically similar when ejections per nest were considered on a binary basis (yes/no) ($B = 1.264$, S.E. = 1.069, $z = 1.183$, $P = 0.237$). The interaction between egg colour and the number of eggs painted was not significant ($P > 0.39$), therefore we

removed the interaction term from the final model. This lack of significant interaction showed that the relative order of ejection rates of those egg colours that were used in all three treatments (restricted to comparisons of blue, yellow, and orange dyed eggs) was consistent among treatments. Accordingly, the relative rank of ejection rates of those three egg colours that were used across all types of treatment (i.e., restricted to comparisons of blue, yellow, and orange dyed eggs: *a*, *b*, *c1*, and *c2*) was significant across the three treatments, with relative rejection rates of blue < yellow < orange egg colours (Friedman ANOVA: $\chi^2 = 8.00$, $P = 0.018$). This consistency in relative egg colour rejection rates across our treatments also implies that whether 5 different colours (*c1*) or 1 of 5 colours (*c2*) were applied to all eggs in the nest, did not significantly impact the colour-based egg rejection decisions of these cuckoo hosts.

DISCUSSION

We demonstrated that there are consistent patterns of egg rejection in response to experimentally introduced avian brood parasitism in the presence or absence of hosts' own eggs so that no own eggs are needed to recognize foreign eggs. Across our treatments, we obtained the strongest rejection responses against the experimentally dyed eggs when host nests contained one (treatment *a*) over three or five experimentally dyed, 'parasitic' eggs (treatments *b*, *c1* and *c2*, respectively). Even though these manipulations included different combinations of colors and numbers of dyed eggs, we detected no interaction between the numbers of eggs and numbers of colours introduced in a nest to generate foreign egg phenotypes, so the results conform to the prediction of the hypothesis that hosts' egg rejection responses are reduced in multiple parasitism (Moskát et al. 2009). Critically, in the treatment when all the eggs were dyed, hosts could no longer use online comparison of own vs. foreign eggs, through either self referencing own eggs or discordancy between own and foreign eggs; Still hosts

continued to consistently reject differently foreign eggs based on their coloration in the same relative probability as they did in nests with some or the majority of their own eggs visible. We suggest that in this context, host used an internal template recognition rule, through a sensory filter and acceptance threshold based on egg coloration, to decide whether to eject or not an egg (Hauber et al. 2006; Moskát and Hauber 2007).

Hosts did not reject all dyed or foreign eggs in treatment *b* when both self-referencing and filter-based recognition mechanisms were available to identify foreign eggs, and in the case of orange eggs, they did so statistically less often than when no own eggs there were visible in the nest (treatment *c1*). One explanation is that in the case of three parasitic eggs and two own eggs (Treatment *b*), along with the recognition template-based discrimination mechanism, a special discordancy-based mechanism acts: discordancy means that hosts reject the egg phenotype in the minority in a clutch. When there is only one parasitic egg in a 5-egg clutch, this is a clear case (treatment *a*). Similarly, when all the eggs are parasitic except one, which is an own egg, some of the great reed warblers ejected their own eggs (Moskát et al. 2010). In our current experiments (treatment *b*), the parasitic eggs are still in the majority of the clutch (3 eggs; 60%), but the frequency of own eggs appeared to be also relevant (2 out of 5 eggs; 40%), which may reduce the accuracy of the discordancy effect relative to own eggs (c.f. Moskát et al. 2009). This results in lower overall rejection rates in multiple parasitism, which also explains our previous results, when we revealed a higher tolerance against variably mimetic cuckoo or manipulated eggs in multiple than in single cuckoo parasitism (Moskát et al. 2009).

In contrast to our study host species, in the brambling (*Fringilla montifringilla*) and chaffinch (*F. coelebs*) a separate study revealed similar rejection rates in single and multiple parasitism with two parasitic eggs (Vikan et al. 2009). These two finch species can be regarded as abandoned hosts by the cuckoo, and hence the winners of the evolutionary arms race with the parasite (Vikan et al. 2009). For these reasons it is likely that bramblings and chaffinches

detect parasitic eggs by recognition template only, and do not rely on discordancy-based discrimination, as do great reed warblers (Moskát et al. 2010). In support of the role of these alternative evolutionary histories, theoretical models confirm that hosts which tolerate more cuckoo eggs in multiple than in single parasitism, may have longer co-evolutionary interactions through more stable host-brood parasite population dynamics (Takasu and Moskát 2011).

Extensive previous work on the sensory basis of host-parasite egg rejection, conducted using subjects within the same species, consistently supported the pattern that increasing mimicry leads to reduced rejection (Hauber et al. 2006; Rutila et al. 2006; Cassey et al. 2008; Stoddard and Stevens 2011). However, in most of these prior studies only a single foreign egg was introduced into experimental nests, thereby allowing sensory mechanisms of concurrent contrast of own versus foreign eggs and/or internal filters assessing the sensory inputs from the foreign egg alone. We aimed here to disentangle these alternative or interactive factors through a comparable series of experimental tests of the sensory mechanisms of rejecter hosts by including nest manipulations where the background coloration of most or all of the clutch has been altered. Moskát et al. (2010) also conducted such a set of experiments by altering the maculation patterns of host eggs, where all eggs in great reed warblers' nests were manipulated and found that egg rejection rates increased above background/control levels. Still, even in that study, some nests had all host eggs manipulated in the same way, so that the relative roles of sensory filter-based thresholds of acceptance versus rejection could not be tested. Similarly, in a separate set of experiment Moskát et al. (2008a) also manipulated all host eggs by painting variable numbers of spots on hosts' own eggs during the laying stage and found for the first time clear experimental evidence for a reduced rejection rate of foreign eggs with increasing intraclutch variability in maculation. Once again, that experiment did not test the relative acceptance threshold of foreign eggs which themselves may vary in their appearance.

In the present study, we set out to design an experimental paradigm which

included an all-clutch manipulation where each of the hosts' five eggs was dyed with different or same colours. The results of the absolute rejection rates showed consistent decreases from single-egg to the multiple-egg treatments. In contrast, the relative rejection rates of the specific colours remained consistent between the single-egg and the five-egg experiments in that blue eggs were accepted the most and orange eggs were accepted the least often. The conclusion is that these hosts possess a consistent relative colour-based sensory acceptance threshold to detect foreign egg colours, but whether those foreign eggs elicit responses, and are behaviourally rejected, may be modified by context, including the proportion of hosts' own eggs present in the nest. An additional critical interpretation of these data is that hosts recognize more eggs than they actually reject (Moskát and Hauber 2007). This conclusion also has implications for the cognitive decision rules involved in mediating behavioural responses through sensation and perception. Specifically, the new results reveal that the lack of behavioural responses to foreign eggs cannot be considered as necessarily being due to limited cognitive complexity of host birds (Antonov et al. 2009; Moskát and Hauber 2007).

Overall, great reed warblers' rejection rates of dyed eggs depend on the experimental eggs per clutch, from the highest rates in treatment *a* to lower rates in treatments *b* and *c1 and c2*. In turn, the relative proportions of differently dyed eggs rejected in each treatment showed a consistent pattern across all three treatments, with blue eggs relatively the most likely to be accepted and orange eggs the most likely to be rejected. Given that both the presence/absence and also the relative numbers of great reed warblers' own eggs over dyed eggs varied globally across these experiments, these results are most consistent with a recognition template based discrimination mechanism operating in these hosts in response to sensory inputs from particular colours of foreign eggs. Because we manipulated host eggs at clutch completion, our results do not reveal whether hosts need to view their own eggs during the current or a past laying attempt to acquire their internal recognition template. Future experiments should address

egg rejection behaviour in young hosts which had not had the chance to inspect their own eggs even once (Victoria 1972).

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Legend to Table and Figures

Table 1: Estimates of ejection rates' logarithm for the different colours and treatments. Generalized linear mixed models were fit by the Laplace approximation, with reference category: ejection of single blue eggs.

Figure 1: Photographic exemplars of a natural great reed warbler clutch parasitized by one cuckoo egg on the right side of the nest, an experimental nest with five differently dyed host eggs) and the mean reflectances, relative to the white standard, of the background of natural great reed warbler eggs (N), natural cuckoo eggs (C) and experimental eggs dyed with one of five different colours (blue - B, green - G, yellow - Y, red - R, or orange - O).

Figure 2: The experimental schematics of three treatment (Tr.) types for testing egg ejection in single parasitism (a) and multiple parasitism, either when two natural coloured eggs (white) of the host are present (b) or when none are present in the clutch (c1: 5 different colours, c2: 5 same colours). Five colours were applied for treatments (a) and (c1): blue, green, yellow, red, and orange, and three colours were used for treatments (b) and (c2): blue, yellow, and orange.

Figure 3: The proportion of nests where at least one painted egg was ejected by hosts in response to experimental parasitism with dyed own eggs. Note that number of colours was five in Treatments *a* and *c1*, while the number of colours were three in Treatment *b* and *c2*, and did not include green and red, and only one colour was used to dye host eggs per nest in Treatments *a* and *c2*.

Table 1

Ejections	<i>B</i>	S.E.	<i>z</i>	P
Intercept	-4.86	1.92	-2.54	0.01
Laying date	-0.24	0.14	-1.69	0.09
Yellow	3.80	1.46	2.61	0.01
Orange	7.05	1.76	4.00	<0.01
Treatment (b) Painted 3 eggs	-7.57	3.15	-2.40	0.02
Treatment (c1) Painted 5 eggs	-4.91	2.54	-1.93	0.05
Treatment (c2) Painted 5 eggs	-3.26	2.31	-1.41	0.16

Fig. 1

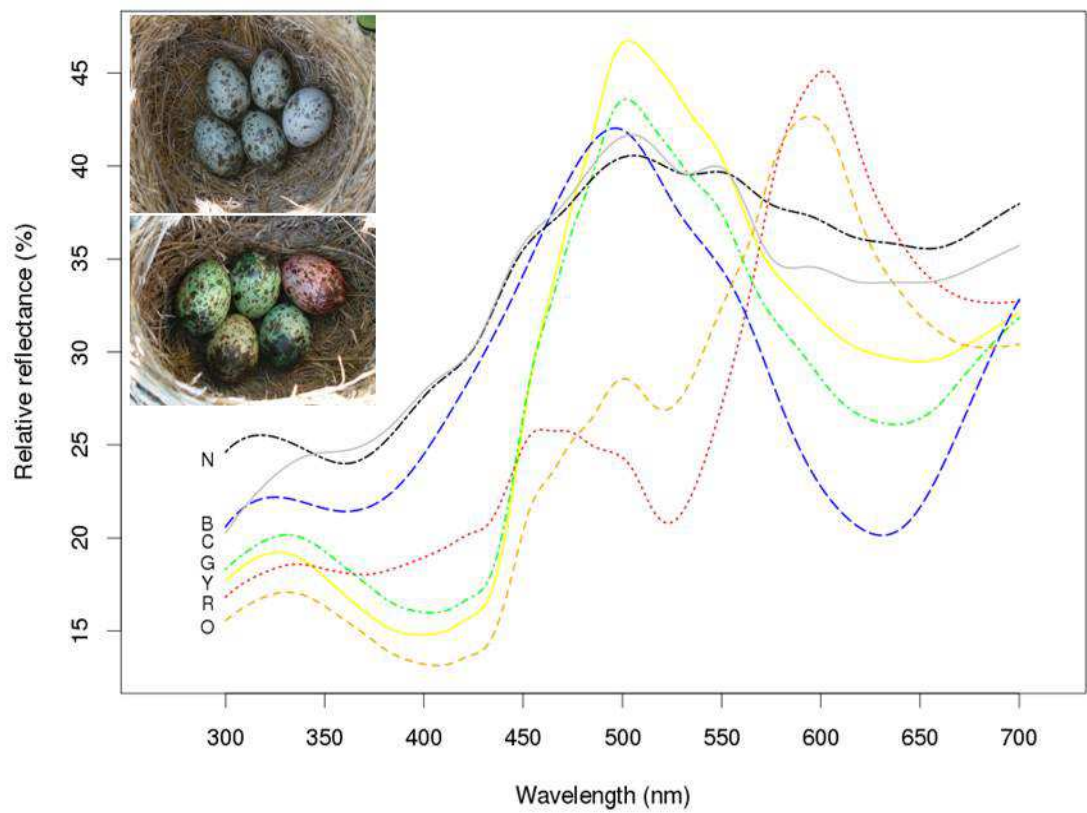


Fig. 2

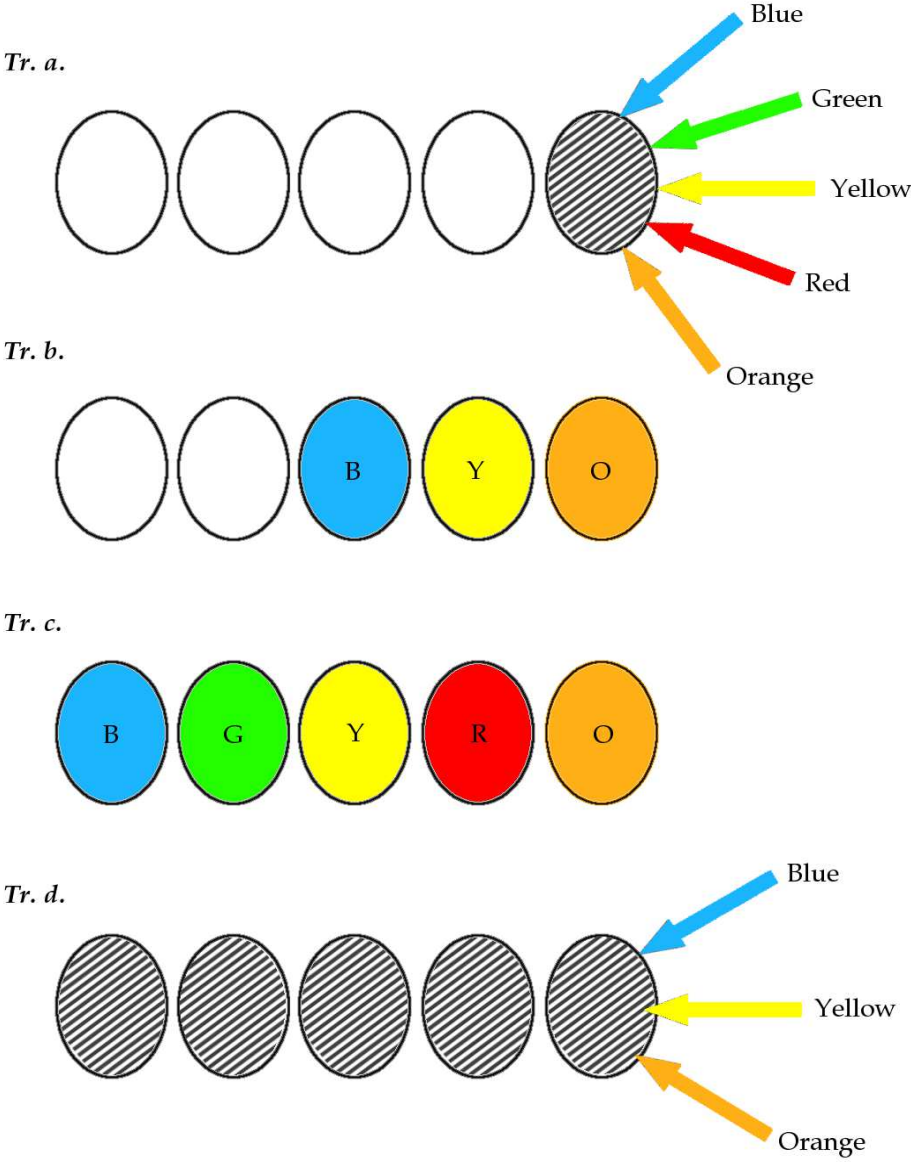
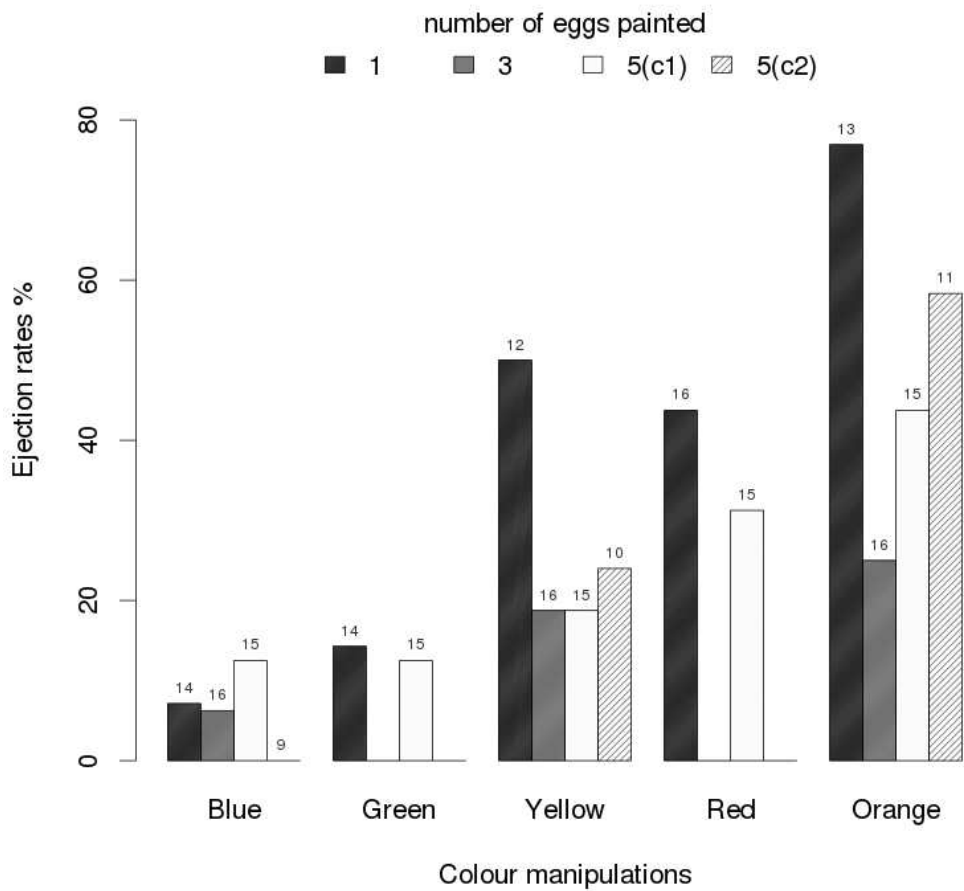


Fig. 3



Irodalom jegyzék - References

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