Ekologie a chování drozdů (*Turdus* spp.) v původních a introdukovaných populacích

Disertační práce

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ABSTRAKT

V této disertační práci jsem se zaměřil na výzkum dvou modelových druhů, kosa černého (*Turdus merula*) a drozda zpěvného (*T. philomelos*). Zkoumal jsem původní evropské populace ze severní polokoule a populace, které byly před přibližně 140 lety introdukované na Nový Zéland na jižní polokouli. Větší část výzkumu je věnovaná hnízdnímu parazitismu, jmenovitě protiparazitickému chování a změnám v tomto chování mezi původními a introdukovanými populacemi. V další části využívám introdukovaných populací ke studiu změn v hnízdních charakteristikách.

Oba druhy se dokážou velmi účinně bránit proti mezidruhovému parazitismu především díky vysoce vyvinutým schopnostem rozlišit a vyhodit cizí vejce. Zjistili jsme, že tato schopnost je u každého jedince vysoce konzistentní a proti parazitaci obvykle reaguje opakovaně vyhozením nebo přijetím cizího vejce. Přes existující protiparazitické schopnosti je prokázaný hnízdní parazitismus u obou druhů výjimečný, a proto jsme v další práci hledali příčiny stojící za absencí parazitismu a za evolucí protiparazitických schopností. Ve srovnávací průzkumné části jsme porovnali life-history znaky deseti nejběžnějších hostitelů kukačky obecné (*Cuculus canorus*) se šesti evropskými drozdy rodu *Turdus* spp., abychom nejdříve určili kandidátní znaky, které by mohly být příčinou absence parazitismu kukačkou (např. srovnání velikosti těla, hnízda, vajec, mláděcí potravy drozdů a hostitelů). Poté jsme experimentální části ukázali, že tyto příčiny jsou druhově specifické a navíc jsou výsledkem působení interakce několika faktorů, které se uplatňují v mláděcí periodě (např. velikost a/nebo design hnízda, neschopnost kukačky konkurovat hostitelským mláďatům). V další experimentální práci jsme na populacích žijících v sympatrii a alopatrii s kukačkou ukázali, že schopnost odmítat cizí vejce u obou druhů je výsledkem evolučního tlaku ze strany vnitrodruhového parazitismu. To také podporují námi zjištěné případy vnitrodruhového parazitismu ve studovaných populacích.

Evropské a novozélandské populace modelových druhů vykazují několik rozdílů v hnízdních parametrech, přičemž nejvíce zkoumanou změnou je menší velikost snůšek u populací na jižní polokouli. Přesto jsou příčiny takového rozdílu nejasné a stále chybí

komplexní experimentální studie, které berou v úvahu více potenciálních faktorů. V naší experimentální studii se jako nejvýznamnější faktor jevila sezonalita, zatímco hnízdní predace a hustoty nedokázaly pokles snůšky vysvětlit. Dále jsme zjistili, že pokles velikosti snůšky introdukovaných populací se již stabilizoval a netrval déle než 100 generací. K lepšímu pochopení příčin by bylo vhodné vzít v úvahu i míru přežívání dospělců, která může úzce souviset s mírou sezonality. Trade-off velikosti snůšky a vajec predikovaný klasickou life-history teorií jsme prokázali pouze u kosa. Jiným významným faktorem ovlivňujícím snůšku i míru predace je urbanizace, ale naše studie provedená na několika prostorových replikátech a dvou druzích její vliv nepotvrdila.

ABSTRACT

Ecology and behaviour of thrushes (*Turdus* spp.) in native and introduced populations

The aim of this project was to explore behaviour and selected life-history traits in two model species, the blackbird (*Turdus merula*) and the song thrush (*T. philomelos*). I studied native European populations in the northern hemisphere and introduced populations to New Zealand in the southern hemisphere. A major part of my research was devoted to avian brood parasitism, examining antiparasitic strategies and differences in such strategies between native and introduced populations in particular. Further, I studied causes of changes in breeding characteristics between these populations.

Both species have evolved effective antiparasitic strategies against interspecific parasitism. They have developed especially good abilities to recognize and eject an alien egg. We found that rejection behaviour was higly repeatable at the individual level and that host birds responded highly consistently by ejection or acceptance. While antiparasitic strategies are shown to exist, the evidence of interspecific parasitism is very scarce. Thus, we posed the questions 'why is parasitism rare in these species' and then 'how did their rejection abilities evolve'. We compared life-history traits of ten most common cuckoo (*Cuculus canorus*) hosts in Europe with six European *Turdus* thrushes. We subsequently identified candidate traits that could be responsible for the absence of cuckoo parasitism in thrushes (e.g. comparisons of body size, nest size, egg size, chick diet). Our results showed experimentally that multiple potential factors and their interactions, which are species-specific and restricted to nestling period, are responsible for absence of parasitism (e.g. nest size and/or design, poor cuckoo chick competition abilities with host nestlings). In another experimental study we explored populations occuring in allopatry and sympatry with cuckoo to show that intraspecific parasitism is responsible for the evolution of rejection abilities in both the

blackbird and the song thrush. This result further support few documented cases of intraspecific parasitism in our study populations.

The breeding parameters of the New Zealand populations of our study species differed from those of their European conspecifics. The most documented pattern is that clutch size is smaller in the southern hemisphere than in the northern one. However, potential causes are still debated and complex experimental studies considering more factors are lacking. We showed in our experimental study that seasonality partly explained variaton in clutch size, whereas predation rates and breeding densities are insufficient to explain this pattern. We also showed that reduction of clutch size had stabilized in introduced populations and took place in less than 100 generations . Future studies should also include the analysis of adult survival rates which can be closely related to seasonality. Avian life-history theory predicts a trade-off between clutch size and egg size which we demonstrated only in the blackbird. Urbanisation is another significant factor influencing clutch size and predation rates but we did not confirm such effects in either of the two study species.

OBSAH

1. SEZNAM PŘILOŽENÝCH PŘÍSPĚVKŮ

Příspěvek I. **Samaš P.**, Hauber M.E., Cassey P. & Grim T. (2011): High repeatability of foreign egg rejection by brood parasite hosts. Ethology 117(7): 606–619. (doi: 10.1111/j.1439-0310.2011.01917.x)

Příspěvek II. Grim T., **Samaš P.**, Moskát C., Kleven O., Honza M., Moksnes A., Røskaft E. & Stokke B.G. 2011: Constraints on host choice: why do parasitic birds rarely exploit some common potential hosts? Journal of Animal Ecology 80(3): 508–518. (doi: 10.1111/j.1365-2656.2010.01798.x)

Příspěvek III. Igic B., Cassey P., **Samaš P.**, Grim T. & Hauber M.E. 2009: Cigarette butts form a perceptually cryptic component of song thrush (*Turdus philomelos*) nests. Notornis 56(3): 134–138.

Příspěvek IV. **Samaš P.**, Grim T., Hauber M.E., Cassey P. & Evans K.L. (rukopis): Ecological predictors of reduced avian reproductive investment in the southern hemisphere.

Příspěvek V. **Samaš P.**, Cassey P., Hauber M.E. & Grim T. (rukopis): Host responses to interspecific brood parasitism: a by-product of adaptations to conspecific parasitism?

Příspěvek VI. Hauber M.E., **Samaš P.**, Anderson M.G., Grim T. & Cassey P. (rukopis): Life-history theory predicts alternative egg rejection strategies of introduced *Turdus* thrushes in New Zealand.

2. ÚVOD A CÍLE PRÁCE

Ptáci se dokážou efektivně adaptovat na měnící se prostředí, díky čemuž úspěšně kolonizovali nejrůznější biotopy na Zemi. Osídlování nových míst jim umožňuje existence různorodých životních, například hnízdních, strategií. Právě fenomény spojené s hnízdním chováním fascinovaly mnoho přírodovědců, z nichž někteří jim zasvětili podstatnou část své výzkumné kariéry (např. Lack 1968, Møller 1994, Davies 2000). Hnízdění se také stalo nejčastěji zkoumaným životním obdobím ptáků, protože představuje ústřední etapu jejich životního cyklu a je zároveň logisticky snáze zkoumatelné než ostatní fáze (např. migrace apod.). Hnízdní chování a řada life-history znaků podléhá často výrazným změnám u populací, které kolonizují nové prostředí a biologům tak pomáhají sledovat příčiny vnitro- i mezidruhové variability life-history znaků (Blackburn a kol. 2009). Významným zprostředkovatelem mnoha ptačích kolonizací se stal sám člověk (Wilson 2004, Blackburn a kol. 2009).

2.1. Introdukce

Při objevování a osídlování nových míst sebou lidé introdukovali řadu ptačích druhů (Blackburn a kol. 2009). Tyto historické události, jako úmyslná nebo neúmyslná zavlečení, se díky tomu často stávají významnými nechtěnými biologickými experimenty, někdy globálních měřítek (Diamond 1986). Pomáhají nám poodhalit existenci, funkci a efektivitu strategií, uplatňujících se při zvládání nástrah nového prostředí (Blackburn a kol. 2009). Důležitou výhodou bývají také přesné záznamy o období introdukcí, počtu zavlečených jedinců a někdy i další podrobnější informace, které při zkoumání autochtonních populací prakticky vždy chybí. Biologům se tak díky umělým introdukcím naskytuje příležitost testovat řadu jinak velmi těžko ověřitelných hypotéz.

Například přerušení genového toku mezi zdrojovými a introdukovanými populacemi a vystavení populace novým životním podmínkám je neocenitelným pomocníkem pro řešení otázek hnízdního parazitismu a evoluce reprodukčního chování. Introdukované populace jsou totiž často alopatrické s původními hnízdními parazity (Lahti 2005). Při absenci parazitismu byly doloženy změny v chování takových populací (Briskie a kol. 1992, Hale a Briskie 2007). Také life-history znaky jako například velikost těla, vajec, snůšky podléhají změnám v čase a prostoru (Stearns 1992). Obecně známým příkladem pozorovaným u řady pěvců je pozitivní vztah velikosti snůšky a zeměpisné šířky, přičemž velikost snůšky v tropech i na jižní polokouli má tendenci být menší než na polokouli severní (Moreau 1944, Lack 1948, Ashmole 1963, Cody 1966, Martin a kol. 2000, Cardillo 2002). Podobně velikost snůšky negativně závisí na nadmořské výšce (Bears a kol. 2009). Především příčiny závislosti na zeměpisné šířce zůstávají stále předmětem debat, kvůli velké převaze srovnávacích studií, které nám narozdíl od experimentální studie mohou kauzální vztah mezi jevy pouze naznačit (Aldrich 1995). Právě zavlečené populace nám můžou významně pomoci při hledání odpovědí (Evans a kol. 2005).

Dobře známým příkladem introdukce je zavlečení evropských pěvců ze severní polokoule do Austrálie a na Nový Zéland na jižní polokouli. Neocenitelnou výhodou jsou přesně známé období introdukčních událostí včetně velikostí takových populací a se známým historickým ekologickým kontextem (Briskie a Mackintosh 2004). Thomson (1922) takto zaznamenal téměř pro každý druh počet jedinců vypuštěných v konkrétním roce a na konkrétním místě Nového Zélandu, někdy dokonce i s dalšími poznámkami, například jejich kondicí. Důležitá je znalost původu zdrojových populací, která je ve zmíněné knize také často uvedena. Například v případě pěvců se v naprosté většině jedná o jih Velké Británie.

Výše uvedené introdukční události lze vnímat jako neplánované biologické experimenty (Diamond and Robinson 2010). Díky nim můžeme studovat i hnízdní chování, pro jehož výzkum se využívá několik odlišných základních metodologických přístupů nebo i jejich kombinace. Ve srovnávací a korelativní studii jednoduše srovnáváme a korelujeme dva nebo více jevů s cílem nalézt společný trend v datech. Tyto studie nemanipulují s podmínkami, ale pouze využívají dostupných dat ke srovnání. Přestože oba přístupy nám mohou odhalit důležité rozdíly a souvislosti, nelze z nich činit přímé závěry o příčinách pozorovaného jevu. Takový závěr nám umožňuje učinit pouze experimentální studie, která na základě hypotézy predikuje očekávaný výsledek a potom provede manipulativní experiment k jejímu otestování. V této disertační práci jsem se pokusil využít experimentálních podmínek, které nám lidé vytvořili změnami prostředí a zavlečením konkrétních druhů na Nový Zéland (Evans a kol. 2005).

2.2. Modelové druhy

Studoval jsem dva druhy ptáků, kosa černého (*Turdus merula*) a drozda zpěvného (*T. philomelos*), kteří se přirozeně vyskytují na severní polokouli a jejich introdukované populace na jižní polokouli jsou dostatečně početné pro nasbírání reprezentativních vzorků. To mi umožnilo zabývat se různorodými otázkami z oblasti hnízdního parazitismu a proměnlivosti vybraných life-history znaků. Pro mou práci jsou nejdůležitější následující charakteristiky obou modelových druhů:

1) Hnízdí v sympatrii (severní polokoule) i alopatrii (severní i jižní polokoule) s mezidruhovými hnízdními parazity (Higgins 2006, **Příspěvek V**).
2) Jsou u nich zdokumentovány případy vnitro- (Perrins 1998, Grim a Honza 2001, Moskát a kol. 2003, K. Weidinger osobní sdělení, **Příspěvek V**) i mezidruhového parazitismu (Møller 1976, Moksnes a Røskaft 1995).
3) Jsou schopni rozeznat a vyhodit cizí vejce (Polačiková a Grim 2010, **Příspěvek V**).
4) Introdukované populace na jižní polokouli jsou zcela izolovány od zdrojových populací na severní polokouli (Higgins 2006).
5) U zavlečených populací na Nový Zéland jsou známy přesné roky a počty introdukovaných jedinců (Thomson 1922).
6) Vzhledem k vysokým počtům introdukovaných jedinců tyto populace netrpí efektem zakladatele (Briskie a Mackintosh 2004, Congdon a Briskie 2010).
7) Oba druhy si staví dobře nalezitelná a snadno dostupná hnízda a mají relativně vysoké hnízdní hustoty, což terénní výzkum logisticky usnadňuje.

Cíle práce

V této práci jsem se zaměřil na výzkum rodu *Turdus*, především pak jejich dvou zástupců, kosa černého a drozda zpěvného, které jsem zkoumal ve specifické fázi životního období, kterým bylo hnízdění. V první části jsem studoval behaviorální strategie proti hnízdnímu parazitismu, jejich změny a příčiny absence mezidruhového parazitismu. Ve druhé části jsem se zaměřil na změny life-history znaků, přičemž u obou témat jsem využil domácích (Evropa) i introdukovaných populací (Nový Zéland).

Konkrétně jsem studoval 1) konzistenci chování jednotlivce proti hnízdnímu parazitismu, 2) zda jsou zástupci rodu *Turdus* vhodnými či nevhodnými hostiteli kukačky obecné (*Cuculus canorus*), 3) jaký selekční tlak vedl ke vzniku protiparazitických schopností a konkrétních odmítacích strategií (vyhození parazitického vejce nebo opuštění parazitované snůšky), 4) které faktory ovlivňují velikost snůšky a vajec, 5) jaká je funkce hnízdního materiálu u drozda zpěvného, přičemž práce slouží jako příklad využití teoretického modelování vizuálních schopností ptáků v terénním výzkumu.

3. HNÍZDNÍ PARAZITISMUS – OBRANA HOSTITELE

Kladení vajec do cizích hnízd je jedním z nejpozoruhodnějších chování v ptačím světě. Jedná se o alternativní reprodukční strategii, kdy parazitická samice klade svá vejce do cizího hnízda a dále se nepodílí na rodičovské péči (Davies 2000). Druhy výhradně parazitující jiné jedince jsou *obligátní* parazité (např. kukačka obecná), zatímco k příležitostnému parazitismu se uchylují tzv. *fakultativní* parazité, kteří mohou klást i vlastní snůšky, o které se dále starají (Lyon a Eadie 1991). Hnízdní parazitismus můžeme také rozlišovat jako *vnitrodruhový*, kdy parazit klade svá vejce do hnízd vlastního druhu a *mezidruhový*, kdy samice všechna vejce klade jiným druhům. Úspěšně parazitovaný hostitel pak může utrpět škodu tím, že a) parazit sežere nebo rozbije jedno nebo více hostitelských vajec (kukačka obecná, kukačka chocholatá

Clamator glandarius), b) opustí vlastní snůšku, c) se zvýší náklady na inkubaci (Siikamäki 1995), d) se sníží líhnivost vajec (Lyon 2003), e) se zvýší konkurence mezi mláďaty (Riehl 2010) a f) investuje energii do výchovy nepříbuzného jedince (Riehl 2010). V extrémních případech parazitické mládě po vylíhnutí hostitelská mláďata zabije ostrým zobákem (medozvěstka malá *Indicator minor*) nebo je vytlačí z hnízda (kukačka obecná) a rodič dále vychovává pouze parazitické mládě (Davies 2000). Není proto divu, že mezi parazitem a hostitelem eskaluje koevoluční závod ve zbrojení, který vede ke vzniku adaptací na straně parazita a protiadaptací na straně hostitele (Davies 2000).

Z protiparazitických adaptací na straně hostitele je rozpoznání a vyhození parazitického vejce jednou z nejběžnějších a nejúčinnějších strategií hostitelů, přičemž se zároveň jedná o nejčastěji zkoumanou reakci hostitele (Davies 2000, Grim 2007). Všechny empirické studie reakcí hostitelů na cizí vejce stojí, ať už explicitně nebo implicitně, na teoretických modelech, které předpokládají, že hostitel své chování za konkrétních podmínek nemění. Například základním vstupním parametrem modelů je, že jedinec za konstantních podmínek výhradně vyhazuje (rejecter) nebo akceptuje (acceptor) parazitické vejce (Lotem 1993, Yamauchi 1993, 1995, Davies a kol. 1996, Takasu 1998, Ruxton a Broom 2002, Lawes a Marthews 2003, Servedio a Lande 2003, Hauber a kol. 2006, Servedio a Hauber 2006, Moskát a Hauber 2007). Tento zásadní předpoklad však zdaleka není dostatečně experimentálně podložený (**Příspěvek I**) a jeho narušení by mohlo mít výrazný vliv na predikce, které vycházejí ze současných teoretických modelů (Ruxton, osobní sdělení).

V naší práci (**Příspěvek I**) jsme se zaměřili na testování předpokladu, že vyhazovací chování konkrétního jedince je konzistentní. Opakovaně jsme parazitovali stejnou samici v jednom hnízdění a sledovali její reakci. Výsledky ukazují vysokou opakovatelnost v chování jedinců proti parazitickému vejci. 95 % kosů černých a 86 % drozdů zpěvných se zachovalo v obou pokusech stejným způsobem a opakovaně parazitické vejce vyhodili nebo jej konzistentně akceptovali. Přesto část jedinců změnila své chování z akceptace na vyhození nebo naopak, a proto je potřeba hlouběji prozkoumat nakolik taková změna může ovlivnit výstupy teoretických modelů.

Podobně jsme nyní testovali opakovatelnost vyhazovacího chování uvnitř a mezi hnízděními v jedné hnízdní sezóně a mezi hnízděními mezi různými hnízdními sezónami. Ptáme se, jestli konzistence v chování zůstává stejná i v různých časových rovinách. Naše předběžné výsledky potvrzují vysokou opakovatelnost chování i mezi hnízděními, jak uvnitř tak mezi sezónami (Samaš a kol. v přípravě).

Nicméně dosud zůstávalo záhadou, jak se u těchto běžných druhů naší fauny vyvinula vysoká schopnost rozlišit a vyhodit cizí vejce (Polačiková a Grim 2010). Ke vzniku takových schopností je zapotřebí dostatečně silného selekčního tlaku buď ze strany vnitrodruhového (Lyon 2003, Riehl 2010) a/nebo mezidruhového parazitismu (Davies 2000). U obou druhů jsou však dokumentované případy obou typů hnízdního parazitismu velmi ojedinělé. Například Lack (1963) našel mezi 22656 kosími hnízdy pouze tři hnízda parazitována kukačkou obecnou. Podobně Moksnes a Røskaft (1995) identifikovali pouze 11, respektive 21 kukaččích vajec u kosa a drozda mezi 11870 snůšek. Také dosavadní záznamy vnitrodruhového parazitismu jsou vzácné (Perrins 1998, Grim a Honza 2001, Moskát a kol. 2003, K. Weidinger osobní sdělení, **Příspěvek V**). Přesto je třeba zdůraznit, že identifikace vnitrodruhového parazitismu je v mnoha ohledech těžší a často uniká pozornosti badatelů i při pravidelných a častých kontrolách hnízd (Rohwer a Freeman 1989, Latif a kol. 2006).

V **Příspěvku II** jsme ukázali, že evropští „drozdi“ jsou pro kukačku nevhodní hostitelé a evoluční tlak na jejich obranné mechanismy proti mezidruhového parazitismu (kukačkou obecnou) byl a je naprosto zanedbatelný (**Příspěvek V**). „Drozdi“, tedy zástupci rodu *Turdus*, běžně hnízdí v sympatrii s kukačkou obecnou a staví velká, lehce nalezitelná hnízda. Proč tedy tento běžně dostupný zdroj není kukačkou využíván k parazitaci? Dosavadní studie nebraly v úvahu více faktorů a jejich interakce a testovaly pouze izolované hypotézy, např. zda za absenci parazitismu může vyhazování vajec (Davies a Brooke 1989, Honza a kol. 2004) nebo agresivita rodičů (Røskaft a kol. 2002) nebo růst mláďat (Grim 2006). Tyto studie nebyly v principu schopny uspokojivě odpovědět na výše zmíněnou komplexní otázku, protože testovaly pouze izolované hypotézy a neuvažovaly alternativní vysvětlení nebo interakci více faktorů. V naší studii (**Příspěvek II**) jsme spojili srovnávací a experimentální přístup. Ve srovnávací části jsme vybrali kandidátní znaky, které by mohly být odpovědné za absenci parazitismu, např. velikost těla, vajec, typ mláděcí potravy. Tyto znaky jsme pak podrobili dalším tentokrát experimentálním testům. Naše výsledky ukázaly, že příčinou absence parazitismu u „drozdů“ není časté a rychlé odmítání parazitických vajec ani agresivita rodičů k dospělé kukačce, ale interakce faktorů jako velikost, design hnízda a neschopnost kukaččích mláďat soupeřit s hostitelskými mláďaty. Velikost hnízda například komplikuje kukaččímu mláděti vytlačování hostitelských vajec, která pak kukačku v konkurenčním boji přerostou. U každého druhu hrají zjištěné znaky různě významnou úlohu a zásadním závěrem naší studie je, že studované druhy jsou nevhodní hostitelé kukačky.

Protože jsme v **Příspěvku II** ukázali, že kos černý, drozd zpěvný, drozd kvíčala (*Turdus pilaris*) a drozd cvrčala (*T. iliacus*) neprošli koevolučním soubojem s parazitickou kukačkou, zaměřili jsme se v **Příspěvku V** na otázku, jak se konkrétně u kosa a drozda zpěvného vyvinula velmi dobrá (Polačiková a Grim 2010) a konzistentní (**Příspěvek I**) schopnost vyhazovat cizí vejce. Kos černý i drozd zpěvný jsou schopni rozpoznat a vyhodit i vysoce mimetická konspecifická (vlastního druhu) vejce (**Příspěvěk V**). Evoluce takových vysoce vyvinutých schopností může být selektovaná mezidruhovým parazitismem (např. Davies a Brooke 1989, Procházka a Honza 2004) anebo vnitrodruhovým parazitismem (Lyon 2003, Lyon a Eadie 2004, 2008, Riehl 2010) a v úspěšných případech může vést až k „vítězství“ nad parazitem (Honza a kol. 2004). V naší práci jsme využili skutečnosti, že oba druhy se vyskytují jak v sympatrii (Česká republika), tak alopatrii (Česká republika, Nový Zéland) s hnízdním parazitem kukačkou obecnou. Hypotéza mezidruhového parazitismu předpokládá, že u alopatrických populací budou protiparazitické reakce ve srovnání se sympatrickými populacemi slabší, zatímco alternativní hypotéza vnitrodruhového parazitismu očekává, že se reakce lišit nebudou, popřípadě mohou být dokonce výraznější v alopatrii (jsou zde vyšší hnízdní hustoty, a tudíž i pravděpodobnost vnitrodruhového parazitismu). Do kosích a drozdích hnízd jsme vkládali nemimetický model, mimetický model nebo skutečné konspecifické vejce a sledovali, zda introdukované vejce jedinec vyhodí nebo akceptuje. To jsme provedli jak u alopatrických, tak sympatrických populací. Výsledné frekvence odmítaní se skutečně statisticky významně nelišily, u alopatrických populací byla dokonce tendence k silnějším reakcím. Experimentální výsledky tak podporují spíše hypotézu vnitrodruhového parazitismu. Tuto hypotézu dále podporují výsledky **Příspěvku II**, kde ukazujeme, že kos černý i drozd zpěvný jsou nevhodnými hostiteli pro kukačku a koevoluční souboj parazit-hostitel mezi nimi neprobíhal. Navíc se nám v naší studii podařilo identifikovat více případů vnitrodruhového parazitismu u obou druhů a žádný případ mezidruhového parazitismu (**Příspěvek V**).

Rozlišovací schopnosti ptáků jsou předmětem intenzivního výzkumu, a proto stále více rozumíme i fyziologickým procesům spojenými s diskriminačními procesy (Bennett a Théry 2007). Například pomocí pokročilé spektrofotometrie spolu s modelováním diskriminačních vizuálních schopností dokážeme alespoň částečně předpovídat, zda a nakolik je jedinec schopen cizí objekt/vejce v hnízdě rozlišit (Cassey a kol. 2008). Pomocí tohoto modelování se na drozdu zpěvném ukázalo, že čípky oka senzitivní na ultrafialové a krátkovlnné části světelného spektra hrají nejdůležitější roli při rozpoznávání cizího vejce (Cassey a kol. 2008). Taková modelování mohou být využita i k testování jiných, např. signalizačních, hypotéz (**Příspěvek III**). V **Příspěvku III** jsme konkrétně testovali, zda lidské výrobky (zde cigaretové nedopalky) zabudované do hliněné hnízdní kotlinky drozda plní nějakou signalizační roli nebo jsou jen strukturním doplňkem. Spektrofotometrická data ukazují, že pro drozdí oko nedopalky splývají s vnitřkem hnízdní kotlinky a zároveň jsou výrazně odlišné od jeho vajec. Výsledky tak naznačují, že nedopalky slouží spíše jen jako příležitostně využívaná strukturní komponenta hnízda.

Hostitel, který cizí vejce v hnízdě rozliší, se proti němu může bránit kromě vyhození také opuštěním snůšky (Davies 2000). Při studiu hostitelských reakcí je zcela nezbytné rozlišovat, zda vyhození vejce a/nebo opuštění snůšky je skutečně adaptací hostitele, která vznikla koevolucí s hnízdním parazitem (Moskát a kol. 2011). Teoretické modely předpokládají, že hostitel zvolí strategii protiparazitické reakce (vyhození versus opuštění) také podle velikosti snůšky (Davies a kol. 1996, Reeve 1989, Servedio a Lande 2003, Takasu a kol. 1993). Podle life-history teorie by druhy s větší snůškou měly cizí vejce vyhazovat, druhy se snůškou menší pak spíše opouštět

(Servedio a Hauber 2006). Zatímco srovnávací data odpovídají predikci modelů napříč několika liniemi hnízdních parazitů (Hauber 2003, Servedio a Hauber 2006), v **Příspěvku VI** jsme poprvé testovali tento předpoklad experimentálně. Kosi, mající menší snůšku, skutečně opouštěli výrazně více parazitovaných snůšek, zatímco drozdi s většími snůškami převážně vyhazovali. Výsledky tak podporují předpoklady teoretických modelů.

Opuštění snůšky po parazitaci může být zapříčiněno zcela jinými faktory, například přítomností člověka u hnízda, jeho manipulací s obsahem hnízda (Rutila a kol. 2006), počasím apod. Přesto velká většina dosavadních prací nerozlišovala mezi reakcemi „vyhození cizího vejce“ a „opuštění snůšky“, ale jejich spojením vytvořila jednu proměnnou „odmítnutí“ vejce. Pro ověření, zda opuštění je skutečně reakcí, je nezbytné souběžně s experimenty zaznamenávat osudy i kontrolních (neparazitovaných) hnízd, které nám po statistickém vyhodnocení umožní rozlišit skutečnou příčinu opouštění snůšek (**Příspěvek V**). Pokud data na kontrolní hnízda jsou nedostatečná nebo chybí, je důležité analyzovat výsledky odmítání cizích vajec jak s proměnnou opuštění (odmítnutím je pak vyhození i opuštění), tak bez ní (pouze vyhození), a ve studii uvést, zda se výsledky kvalitativně liší (**Příspěvek II**).

4. EVOLUCE LIFE-HISTORY ZNAKŮ – VELIKOST SNŮŠKY

Ptáci flexibilně reagují na měnící se podmínky a podle toho investují do reprodukce optimální množství energie (Stearns 1992). Jedním z dobře pozorovatelných znaků, který odráží reprodukční úsilí, je velikost snůšky (Lindén a Møller 1989). V současnosti je to také asi nejvíce prozkoumaný life-history znak u ptáků (Jetz a kol. 2008). Nicméně, přes značné výzkumné úsilí je stále obtížné rozlišit konkrétní efekt široké škály biotických a abiotických faktorů a jejich interakcí, kterými investice do snůšky může být ovlivněna (Ricklefs 2000). Patří mezi ně například potravní nabídka, množství a druhová skladba predátorů, vnitro- a mezidruhová konkurence, délka života nebo klimatické podmínky (Stearns 1992).

Mezi řadou faktorů, které mohou hrát důležitou roli, je pozoruhodná pravidelnost, s jakou klesá velikost snůšky se snižující se zeměpisnou šířkou, a dále i menší velikosti snůšek na jižní polokouli než v podobných zeměpisných šířkách na polokouli severní (např. Martin a kol. 2000, Jetz a kol. 2008). Tento fenomén byl popisován již v prvních publikacích Davida Lacka nebo Reginalda Moreaua v polovině minulého století a lze jej pozorovat jak na mezidruhové, tak vnitrodruhové úrovni (Stutchbury a Morton 2001). Od té doby se nejvíce diskutovaly dvě příčiny pozorované pozitivní korelace snůšky se zeměpisnou šířkou.

1) „Ashmoleova hypotéza“ (Ashmole 1963) tvrdí, že snůšku zásadně ovlivňuje sezonalita. V sezónnějším prostředí je vyšší úmrtnost jedinců během zimních měsíců. Dochází k redukci velikosti populace a v hnízdní sezóně pak vychází větší množství potravy na každého jedince, který pak může investovat více do své snůšky.
2) „Hypotéza hnízdní predace“ tvrdí, že vyšší predace negativně ovlivňuje velikost snůšky (Skutch 1949, Martin a kol. 2006, Cassey a kol. 2009). Menší snůška může zkrátit dobu kladení, dobu inkubace (Hepp a kol. 2005), a také zmenší krmící úsilí (a tím pádem častost příletů a odletů z hnízda) a s ní pravděpodobnost detekce predátorem (Skutch 1949, Martin a kol. 2000).

Uvádí se i další potenciální vlivy, které mohou výrazně ovlivnit investici do snůšky. Například vyšší hnízdní hustoty populace mohou negativně ovlivnit velikost snůšky snížením množství dostupné potravy na jedince a/nebo zvýšenou pravděpodobností predace hnízda (např. Chamberlain a kol. 1995, Keyser a kol. 1998, Lloyd 2006, viz ale Padyšáková a kol. 2010). Podle klasické life-history teorie je důležitým faktorem, který určuje investici do snůšky, míra přežívání dospělců – vysoká sezonalita v podmínkách mírného pásma je příčinou vyšší úmrtnosti dospělců, kteří se proto snaží investovat více úsilí do současného hnízdění (Martin 2004).

Popisované trendy nacházíme v globálním měřítku. Provedení experimentální studie v takovém měřítku je prakticky nemožné (Diamond a Robinson 2010), proto nepřekvapuje velká převaha srovnávacích a korelativních studií (např. Lack 1947, Martin a kol. 2006, Jetz a kol. 2008). Komparativní biologové využívají ohromného množství záznamů v hnízdních kartách z různých zemí. Například Jetz a kol. (2008) provedli srovnávací typ studie na 5 290 druzích ptáků a ukázali, že v globálním měřítku se větší snůšky relativně více soustřeďují na severní polokouli. Ve srovnání s tropy jsou pak průměrné temperátní snůšky přibližně dvakrát vyšší. Navzdory překážkám s provedením experimentální studie, historické události spojené s introdukcemi konkrétních druhů do nových lokalit nám poskytly půdu pro přirozený experiment, aniž bychom museli sami manipulovat podmínky, což je v takovém měřítku logisticky těžko proveditelné a navíc eticky nepřijatelné (Diamond 1986, Diamond a Robinson 2010).

V našem výzkumu (**Příspěvek IV**) jsme využili introdukce kosa černého a drozda zpěvného z Evropy na Nový Zéland, abychom experimentálně testovali tři základní hypotézy vysvětlující rozdíly ve velikosti snůšek – jmenovitě Ashmoleeovu hypotézu a hypotézy hnízdní predace a hnízdní hustoty. Dohromady jsme získali data na 2215 kosích vajec a 988 snůšek a 1696 drozdích vajec a 686 snůšek z 8 lokalit. Snůšky byly na jižní polokouli nižší o ~1 vejce u kosa a ~0.6 vejce u drozda. Podařilo se nám prokázat, že pokles ve velikosti snůšky u introdukovaných populací netrval déle než maximálně 100 generací. V souhlasu s Ashmoleeovou hypotézou, změny ve velikosti snůšky v průběhu hnízdní sezóny byly výraznější u evropských populací, kde je také vyšší sezonalita než na Novém Zélandu. Naopak míra predace byla nižší na jižní polokouli, kde jsme zaznamenali nižší velikosti snůšky, což je v protikladu k hypotéze hnízdní predace (Skutch 1949).

Hustoty hnízdění byly podle predikce skutečně vyšší na Novém Zélandu (u drozda byl však rozdíl statisticky nevýznamný), kde byly i nejnižší velikosti snůšek. Přestože tento trend byl prokázán i u dalších devíti pěvců zavlečených z Evropy na Nový Zéland (Macleod a kol. 2009, Weller 2009), ukázali jsme, že introdukované druhy s nejvyššími hnízdními hustotami mají nejmenší pokles ve velikosti snůšky (**Příspěvek IV**). Proto ani hnízdní hustoty nedokáží vysvětlit pokles snůšek introdukovaných populací pěvců na jižní polokouli. V dalším výzkumu bude nezbytné také rozlišit, zda i vyšší míra přežívání dospělců není významným faktorem zapříčiňujícím menší investice do snůšek na jižní polokouli. Vyšší přežívání dospělců je totiž spojeno s mírnějším klimatem, což předpovídá i Ashmoleova hypotéza. Zjistili jsme tedy, že Ashmoleova hypotéza v naší práci nejlépe vysvětluje rozdíly ve velikosti snůšek mezi severní a jižní polokoulí.

Klasická life-history teorie dále předpokládá negativní vztah mezi velikostí snůšky a vajec (Roff 1992, Martin a kol. 2006). Přestože se snůšky mezi hemisférami liší, celková investice do snůšky by mohla být vyrovnanější, pokud by evropští kosi a drozdi měli při větších snůškách menší vejce než jejich novozélandští protějšky. Naše výsledky ukazují negativní vztah velikosti snůšky a vajec u kosa, nicméně na Novém Zélandu byl tento vztah výraznější, což naznačuje, že investice do snůšky je menší na jižní polokouli. U drozda jsme žádný významný vztah mezi velikostí snůšky a vajec nepozorovali. Podobně jiné studie ukazují negativní, pozitivní nebo žádné vztahy mezi velikostí snůšky a vajec u řady různých druhů bez nějakých pravidelností u různých taxonů (např. Bernardo 1996, Hořák a kol. 2008).

Jiným novodobým lidmi vytvořeným fenoménem, který ovlivňuje hnízdní parametry pěvců, je urbanizace. Městské prostředí představuje pro kolonizující druhy často velkou překážku díky změnám v klimatu, fenologii, dostupnosti potřebných zdrojů, ekologických charakteristikách a podobně (Grimm a kol. 2008). Přesto se zde setkáme s druhy, které dokážou v městském prostředí prosperovat a jejich hustoty jsou zde vyšší než v přirozeném prostředí (Blair 1996). Mezi úspěšné kolonizátory měst se řadí také kos černý a drozd zpěvný. Srovnáním 46 studií bylo zjištěno, že kos (6 studií), drozd (4 studie) a většina z 17 dalších sledovaných druhů měla vyšší snůšky u neměstských populací (Chamberlain a kol. 2009). Nicméně, pouze jediná studie na kosu černém tento rozdíl ověřila statisticky. V naší práci (**Příspěvek IV**) jsme však žádný statisticky významný rozdíl při srovnání 4 neměstských se 2 městskými populacemi nepozorovali, ať už u kosa nebo drozda. Podobně jsme nezaznamenali rozdíl ve velikosti vajec mezi rurálnímí a urbánními ani u jednoho studovaného druhu. Ukazujeme tak, že efekt urbanizace na hnízdní parametry nemusí být natolik významný a pravidelný, jak se uvádí v současné literatuře. Dále jsme nenašli rozdíl v míře predace mezi neměstskými a městskými populacemi, přestože hustoty predátorů obou studovaných druhů jsou vyšší právě ve městech (Evans a kol. 2009a). Podobně ani Chamberlain a kol. (2009) ve své srovnávací práci rozdíly v míře predace nenašli.

5. ZÁVĚR

V této disertační práci jsem se zaměřil především na experimentální oblast výzkumu (**Příspěvky I, IV, V, VI**), přesto je využit i srovnávací přístup (**Příspěvek II**). Zatímco předešlé studie se zaměřovaly na protiparazitické chování konkrétního ontogenetického stádia, my jsme testovali obranné linie ve všech stádiích ontogeneze hostitele (agresivita proti parazitovi, odmítání vajec, odmítání mláďat) na více druzích a lokalitách (**Příspěvek II**). Dále byl kladen větší důraz na metodologickou konzistenci sběru dat mezi studovanými lokalitami (**Příspěvky I, IV, V**). **Příspěvek I** podrobněji také probírá metodická doporučení pro studium opakovatelnosti chování.

V této práci ukazuji, že 1) chování kosa i drozda proti hnízdnímu parazitismu je vysoce konzistentní, 2) oba druhy jsou nevhodní hostitelé kukačky, 3) v souladu s tím se ukazuje, že vysoce vyvinuté rozlišovací schopnosti cizích vajec jsou spíše výsledkem dlouhodobého působení vnitrodruhového a nikoli mezidruhového parazitismu, 4) velikost snůšky může ovlivnit způsob odmítání cizího vejce, 5) menší snůšky introdukovaných populací pěvců ze severní na jižní polokouli jsou částečně vysvětlitelné sezonalitou, zatímco hnízdní hustoty a častost predace s nimi nekorelují, 6) urbanizace neovlivňuje významně investici do snůšky, 7) modelování vizuálních schopností ptáků lze aplikovat na testování různorodých „signalizačních" hypotéz.

Další práce (Samaš P., Hauber M.E., Cassey P. a Grim T. v přípravě) ukazuje, že konzistence v reakci konkrétního jedince na hnízdní parazitismus zůstává vysoká nejen uvnitř jednoho hnízdění, ale také napříč hnízděními uvnitř hnízdní sezóny i mezi roky. Výsledky změn velikosti snůšek dále naznačují, že ne všechny druhy pěvců introdukovaných z Evropy na jižní polokouli (zde Nový Zéland), mají nižší snůšky, jak ukazujeme na příkladu zvonka zeleného (*Carduelis chloris*) (Samaš P., Polačiková L., Hauber M.E., Cassey P. a Grim T. v přípravě). U tohoto druhu také dokumentujeme schopnost rozlišit a vyhodit cizí vejce, což dosud nebylo prokázáno u evropských populací. V další studii (Baylis S.M., Hauber M.E., Samaš P. a Grim T. v přípravě) jsme experimentálně testovali vliv dvou trofických úrovní predátorů („meso-“ a „top-predátoři“) na lokalitách Nového Zélandu, kde byli „top-predátoři“ vyhubeni anebo naopak introdukovaní. Podle tzv. „mesopredator release“ hypotézy se mají populace kosa a drozda a „mesopredátorů“ (např. myš domácí *Mus musculus*) navzájem významně negativně ovlivňovat na místech, kde byli odstraněni tzv. „top-predátoři“ (např. kočka *Felis catus*, krysy rodu *Rattus* spp.). Naše předběžné výsledky však tuto hypotézu nepotvrzují.

V naší poslední práci (Samaš P. a Grim T.) se zabýváme vlivem urbanizace na disperzní chování kosa černého. Vliv urbanizace na chování ptáků je často výrazný a městské populace např. kosa černého se od svých rurálních protějšků již dokázaly během několika desítek generací odlišit morfologicky i geneticky (např. Partecke a kol. 2005, Partecke a Gwinner 2007, Evans a kol. 2009b, Evans a kol. 2009c). My ukazujeme, že městské populace jsou na rozdíl od neměstských extrémně sedentární a u svých teritorií se zdržují i v mimohnízdním období. Podle předpokladu byla disperze u dospělců nižší než u mláďat. Vysoká sedentárnost je jedním z klíčových faktorů, které pomáhají vysvětlit existující rozdíly mezi urbánními a rurálními populacemi, neboť věrnost místu omezuje genový tok, který jinak „rozmívá“ místní adaptace (Futuyma 1998).

V budoucím výzkumu bych se chtěl zaměřit především na studium vlivu urbanizace na populace pěvců a evoluci nových adaptací na městské prostředí, které se u jejich rurálních nebo lesních protějšků neprojevují. Dále by jsem chtěl zkoumat klíčové faktory, kterými mládě kukačky manipuluje krmící úsilí hostitelských rodičů. Pro oba projekty už jsem nasbíral předběžná data. Pro lepší pochopení evoluce snůšky pak bude nezbytné analyzovat i vliv přežívání dospělců, na které může mít právě sezonalita zásadní vliv. Dále by bylo vhodné získat data pěvců na přežívání dospělců, hnízd, hnízdní hustoty, délku hnízdní sezóny a vybrané klimatické charakteristiky z jižní části Velké Británie, kde byla chycena většina jedinců později introdukovaných na Nový Zéland a porovnat je se stejnými typy dat na jižní polokouli. Při studiu opakovatelnosti by jsem chtěl analyzovat i konzistenci dalších parametrů u jedince, jmenovitě investici do snůšky mezi hnízděními, přežívání a růst mláďat konkrétního rodiče/ů, preference prostředí a jeho konkrétních charakteristik, to vše mezi hnízděními. Dále mě zajímá opakovatelnost chování, stresových reakcí a tělesné kondice při opakovaném odchytu. Pro některé výše jmenované projekty již získávám se svými kolegy data.

6. LITERATURA

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Příspěvek I.

Samaš P., Hauber M.E., Cassey P. & Grim T. (2011): High repeatability of foreign egg rejection by brood parasite hosts. Ethology 117(7): 606–619. (doi: 10.1111/j.1439-0310.2011.01917.x)

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Repeatability of Foreign Egg Rejection: Testing the Assumptions of Co-Evolutionary Theory

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Abstract

Most theoretical models of coevolution between brood parasites, whether interspecific or conspecific, and their hosts explicitly assume consistent individual behaviour in host egg-rejection responses. Accordingly, hosts cast as acceptors always accept, whereas ejectors always reject parasitic eggs when exposed to stable ecological conditions. To date, only few studies have attempted to test this critical assumption of individual repeatability in egg-rejection responses of hosts. Here, we studied the repeatability of egg rejection in blackbirds (*Turdus merula*) and song thrush (*T. philomelos*), species in which females are reported to reject simulated, non-mimetic foreign eggs at intermediate frequencies at the population level. However, intermediate rates of acceptance and rejection can be consistent with either or both intra- and interindividual variability in rejection behaviours. Our experiments revealed generally high individual consistency in these hosts' responses to experimentally introduced non-mimetic and mimetic model foreign eggs. Individuals also responded faster on average to second than to first trials within the same breeding attempts, but the difference was statistically significant only in blackbirds. These results are consistent with the critical assumption of co-evolutionary models, that statistically egg rejection is mostly individually repeatable, but also reveal that some individuals in both species change their responses even within the short time-window of one breeding attempt. The data suggest that individuals reject foreign eggs faster when perceived parasitism risk is greater because of repeated introductions of experimental parasitic eggs. We provide methodological recommendations to facilitate experimental and meta-analytical studies of individual egg rejection repeatability and discuss how to reduce technical constraints arising from disparate treatments and variable sample sizes for future studies.

Introduction

Avian brood parasitism selects for the evolution of host defences; the rejection of foreign eggs is the most often documented type of defence (Davies 2000). Evolutionary theories of brood parasite–host arms-races explicitly assume that parasitic egg-laying strategies are 'strictly heritable' (Ruxton et al. 2001) in either interspecific (Gibbs et al. 2000) or conspecific parasites (May et al. 1991; Nee & May 1993; Takasu 2004; Shaw & Hauber 2009). Further, several models of interspecific and conspecific parasitism also assume that individual hosts are either rejecters (that always reject) or acceptors (that always accept) in response to parasitism (Yamauchi 1993, 1995; Takasu 1998; Ruxton & Broom 2002; Servedio &

Lande 2003; Servedio & Hauber 2006). Other models permit that rejecter hosts modulate behavioural responses based on experience (Lotem 1993; Lawes & Marthews 2003) and the cost-benefit trade-offs of rejection decisions (Moskát & Hauber 2007); or modulate the perceptual acceptance threshold among own and foreign eggs based on parasitism risks (Davies et al. 1996; Hauber et al. 2006). However, all these models incorporate that selection to reject parasitism produces host individuals which are consistent in their responses to parasitic eggs within the same ecological and perceptual context, otherwise host responses would represent random variation and not the outcome of selection.

Accordingly, all these evolutionary models of brood parasite–host coevolution assume that when a particular host individual rejected a particular foreign egg, it will reject *the same type of model* again when parasitised for the second time. Importantly, such consistency, and the resulting statistical measure of individual repeatability, can be interpreted as a ceiling value of heritability (Bell et al. 2009; Nakagawa & Schielzeth 2010). Thus, estimating repeatability provides a preliminary approach, and also a valuable set of behavioural details, about potential maximum heritability of the response trait (Bell et al. 2009). Violation of the critical assumption of the models that host's egg-rejection response is individually consistent could potentially have a dramatic effect on model predictions, because of the inherent non-linearities in the parasite–host systems dynamics (G. D. Ruxton, pers. comm.) and would call for the need for new theory and for the reassessment of conclusions drawn from data based on previous, theory-driven experiments.

A thorough literature search revealed to us that few studies collected data on individual repeatability of egg rejection and all those studies tested only hosts of interspecific brood parasites. For example, experimentally induced individual repeatability of egg rejection was 'very high' within the same breeding attempt in several species (Honza et al. 2007b; Peer & Rothstein 2010) but very low in common grackles (*Quiscalus quiscula*; Peer & Rothstein 2010). Repeatability between breeding attempts of individual hosts within one breeding season was found to be high in one study (Lotem et al. 1995) but low in another one (Alvarez 1996), when considering different host species of the common cuckoo (*Cuculus canorus*). Finally, repeatability of egg rejection was very low within the same individuals between breeding seasons (Soler et al. 2000; see also Palomino et al. 1998). Additional studies by Hauber et al. (2006) and Vikan et al. (2009) performed repeated experiments, but they used different models in subsequent trials on the same individual hosts, thus, those data could not be used to estimate repeatability.

Some previous studies also included several methodological limitations to allow for explicit testing of individual repeatability. For example, Peer & Rothstein (2010) repeated their experiments only at the nests of rejecters but not at the nests of acceptors, so that repeatability of acceptance/rejection responses could not be estimated. Further, Palomino et al. (1998) pooled host responses across various temporal periods (from within one breeding attempt to between year time scales). However, repeatability depends on the length of time between two consecutive behavioural measurements, irrespective of the outcome (see, e.g. Bell et al. 2009; Weidinger & Kočvara 2010). Egg ejection likely requires sophisticated cognitive abilities that are highly likely affected by memory and experience (e.g. Lotem 1993; Moskát & Hauber 2007; Moskát et al. 2010). Thus, there is good reason to expect that repeatability of egg ejection will be dependent on time-window between successive trials (e.g. naive acceptor will accept in both trials within one breeding attempt, but will reject parasitism in the next breeding attempt; see Lotem et al. 1995). Indeed, Palomino et al. (1998) discuss this potential bias.

Some other previous work also focused on overwhelmingly rejecter (Honza et al. 2007b) or acceptor (Hoover et al. 2006) species. Here, we argue that repeated experiments on *intermediate* rejecters (Røskaft et al. 2002; see also Discussion) provide the most relevant system for the study of consistency and, by proxy, heritability of individual hosts' rejection responses to parasite eggs. This is because strong rejecter species (that always reject, e.g. blackcaps *Sylvia atricapilla*; Honza et al. 2007b) or pure acceptor species (that never reject, e.g. dunnocks *Prunella modularis*; Davies & Brooke 1989) show little or no individual variation in their responses. Such species with invariable behavioural responses are not suitable for measuring repeatability because it can be *a priori* assumed that repeatability (and heritability) would be low (Bell et al. 2009; Nakagawa & Schielzeth 2010).

Quantitatively, meaningful measurement of repeatability also requires some, ideally high, between individual variance (Bell et al. 2009). This is because, conceptually, observations and experimental data revealing very high inter-individual variability at the population level are compatible with mechanisms involving *either* high individual repeatability (i.e. inconsistent behaviour between individuals, consistent behaviour within individuals) or low repeatability (inconsistent behaviour both between and within individuals). Thus, brood parasite host populations showing intermediate egg rejection rates are best suited to test these alternative mechanisms and estimations of individual repeatability of egg-rejection behaviour in the context of brood parasitism (Bell et al. 2009).

Another important aspect of host responses is whether only females (Soler et al. 2002), or both females and males (Honza et al. 2007b), eject parasitic eggs. In the latter case, one member of the pair can pre-empt the other's rejection response by removing the parasitic egg before the other member of the pair has a chance to recognise and reject the egg. Under such conditions, it is impossible to measure repeatability unless nests are video-recorded, but even then all nests where successive parasite eggs were not ejected by the same individual would have to be discarded from analyses because ejections by different individuals cannot be used to estimate individual repeatability (Bell et al. 2009). Thus, we argue that those host species in which *only one sex* (typically the female) ejects parasite eggs provide logistically and heuristically better models to study repeatability.

For this study of the repeatability of host responses to experimental brood parasitism, we selected two appropriate species: the blackbird (*Turdus merula*) and the song thrush (*T. philomelos*). These two species are intermediate rejecters of both dissimilar and similar foreign eggs, with average egg-rejection rates close to 50% (e.g. Grim & Honza 2001). For example, published rejection rates of immaculate blue cuckoo-sized model eggs, the most frequently used non-mimetic model type in studies of brood parasitism in Europe (Davies 2000; Grim et al. 2011), are 48.0 ± 22.6% ($\bar{x}$ ± standard deviation, n = 7 studies) for the song thrush, and 57.6 ± 14.1% (n = 6 studies) for the blackbird (reviewed in Table 1 in Polačiková & Grim 2010). Furthermore, in both of these species, the female is typically the sex that incubates (Cramp 1988; our own unpubl. data). As in other species with female-only incubation (e.g. Amundsen et al. 2002), only females eject eggs in blackbirds (Soler et al. 2002; Weiszensteinová J., Samaš P., Hauber M., Cassey P., Grim T. unpubl. data).

In both thrush species, there are documented cases of brood parasitism both by common cuckoos (Moksnes & Røskaft 1995) and by conspecifics (Grim & Honza 2001; Moskát et al. 2003; Higgins 2006). However, a recent large-scale study (Grim et al. 2011) showed that thrushes (*Turdus* spp.) are unsuitable cuckoo hosts and could not have been involved in long-term coevolution with the cuckoo. Thus, these thrushes' fine-tuned egg-ejection abilities most likely evolved in the context of conspecific brood parasitism (which is known in both thrush species; own unpubl. data), which can also be highly costly to hosts (Lyon 2003; Shizuka & Lyon 2010) and is known to select for anti-parasite adaptations (López-de-Hierro & Moreno-Rueda 2010; Riehl 2010). Thus, the present study is the first to study egg-ejection repeatability in the context of conspecific brood parasitism. However, our results are methodologically and empirically relevant for studies of either interspecific or conspecific parasitism because both types of parasitism create fundamentally similar selection pressures both theoretically (Yamauchi 1995) and empirically (Jackson 1998) and 'it is difficult to separate the effects of the two' (Davies 2000, p. 223).

Moskát et al. (2003) suggested that egg rejection in some potential cuckoo hosts can be explained as an extension of nest sanitation (i.e. removal of non-egg-like objects). Leaving aside theoretical objections (apparent nest sanitation can be simply a by-product of egg-rejection abilities, not *vice versa*), it is highly unlikely that egg rejection in thrushes represents a by-product of nest cleaning. This is because fine-tuned *specific* discrimination abilities (high rates of rejection of conspecific eggs, own unpubl. data) are not likely to be in principle a by-product of general *non-specific* nest cleaning behaviour (see discrimination threshold theory by Reeve 1989).

Repeatability of any trait can be estimated for various temporal scales (Weidinger & Kočvara 2010). For egg rejection, one could estimate repeatability (1) within one breeding attempt, (2) between different breeding attempts within one breeding season, and (3) between breeding seasons. The logical first research step for experimentally estimating repeatability is the short-term consistency in responses: if the short term repeatability were very low, it would follow that the long-term consistency would also be very low, in line with empirical data (Bell et al. 2009). In contrast, high short-term repeatability allows for either high or low long-term repeatability. Thus, we began to study short-term consistency in host responses within one breeding attempt (see also previous studies at the same time scale: Honza et al. 2007b; Peer & Rothstein 2010).

We predicted high repeatability of egg rejection within a single breeding attempt because (1) the shorter the time window between two trials, the higher the behavioural repeatability (Bell et al. 2009), and (2) most birds reject alien eggs at similar frequencies throughout the nesting period, i.e. they do not respond differently between laying and incubation stages (Davies & Brooke 1989; Moksnes et al. 1991; Peer & Rothstein 2010). The latter was reported for blackbirds and song thrush too (Davies & Brooke 1989; Polačiková & Grim 2010; Grim et al. 2011). We also predicted shorter latencies until rejection of the foreign eggs from the first to the second trial in line with both current theoretical models (Stokke et al. 2007) and empirical data (Honza et al. 2007b). Finally, we predicted that responses to mimetic model eggs (conspecific model, see Methods) should be less repeatable than responses to non-mimetic blue model eggs because the higher similarity between recognition cues imposes higher risk of discrimination errors (Reeve 1989).

In any study of brood parasitism, acceptor nests by definition have longer exposure to potential predation than rejecter nests (see Results). As predated nests are excluded from analyses, this methodological constraint might inflate the apparent rejection rates in any brood parasitism study and also egg rejection consistency estimates in studies of repeatability. Therefore, we also re-analysed our data by excluding nests where the host rejected the egg model but the nest was predated within the overall exposure period of acceptor nests (13 d, see Methods). This was to test whether different predation rates at acceptor vs. rejecter nests biased our results. We present both estimates because (1) to our knowledge, almost none of the previous studies made this correction (but see Vikan et al. 2010); thus, to make results comparable across studies we need to present also the uncorrected estimates (this allows inclusion of our data into meta-analyses), and (2) the comparison of corrected and non-corrected estimates may become valuable, especially if these estimates do not match; we propose that (re)analyses with this correction factor of past and ongoing or future studies of brood parasitism will follow this preferable approach.

Methods

We carried out the study in the city of Auckland, New Zealand (36°51′S, 174°46′E) in 2007–2009. Both the blackbird and song thrush are European species that were introduced to New Zealand in late 19th century (Thomson 1922). Both species reject foreign eggs from their nests at similar or even higher frequencies in New Zealand, as in their European populations (Hale & Briskie 2007; Polačiková & Grim 2010) as can be expected if egg rejection is not an evolved response to cuckoos but to conspecific brood parasitism (see Grim et al. 2011).

Model eggs

We tested both species with a plain light blue model egg, which is the most commonly used non-mimetic model in studies of common cuckoo parasitism across Europe (Davies 2000). We are aware of the problem that terms ‘mimetic’ vs. ‘non-mimetic’ are confusing and being used inconsistently across various studies (discussed in Grim 2005). This is because these terms dichotomize continuous variation in host–parasite egg similarity (Grim 2005, p. 76). Here, for the readers’ convenience, we use terms ‘mimetic’ (large models painted to resemble respective host eggs) vs. ‘non-mimetic’ (small models painted to resemble plain blue cuckoo eggs) as done in previous published studies of *Turdus* thrushes, but also present physical reflectance data on the colour similarity of egg models to our model host species’ eggs (Fig. 1a,b) and provide illustrations of the models, too (Fig. 2).

Our non-mimetic models were made from polysynthetic material and painted with acrylic paints to resemble eggs laid by the common cuckoo into the nests of the redstart (*Phoenicurus phoenicurus*; Moksnes & Røskaft 1995; Grim et al. 2009b). The size ($\bar{x} \pm SD = 22.7 \pm 0.54 \times 17.4 \pm 0.48$ mm, n = 10), mass (3.7 ± 0.45 g, n = 10) and the shape of these non-mimetic blue egg models were similar to real, common cuckoo eggs (size range: 20–26 × 15–19 mm, mass range: 2.9–3.8 g, Cramp 1985). Our preliminary experiments showed that blackbirds and song thrushes reject model blue eggs at intermediate frequencies in our New Zealand study populations (see Results).

Further, we tested both thrush species with mimetic model eggs (again made from polysynthetic material and painted with acrylic paints). The ‘conspecific model’ was painted to replicate appearance of the natural blackbird egg (i.e. greenish-blue background with dense red-brown spotting) and the natural song thrush egg (i.e. blue background with dark spots concentrated at the blunt egg pole), respectively. The size ($\bar{x} \pm$ standard deviation: $29.6 \pm 0.26 \times 21.5 \pm 0.28$ mm, n = 10), mass (7.8 ± 0.49 g, n = 10) and shape of blackbird conspecific models were similar to real blackbird egg (size range: 25–35 × 19–24 mm, estimated mass: 7.2 g, Cramp 1988). The size ($\bar{x} \pm$ standard deviation: $25.8 \pm 0.40 \times 21.0 \pm 0.25$ mm, n = 10), mass (6.4 ± 0.31 g, n = 10) and shape of song thrush conspecific models were similar to real song thrush egg (size range:

24–31 × 19–22 mm, estimated mass: 6.0 g, Cramp 1988). For representative reflectance spectra of natural and model eggs, see Fig. 1.

Preliminary experiments with single (i.e. non-repeated) introductions of the blue model were performed during the austral summer of 2007–2008. Repeated experiments with conspecific models were carried out in 2008, and repeated experiments with blue models in 2009.

Experimental procedures

Each experiment consisted of two consecutive trials. The nest was first parasitised during the laying stage or in the first 5 d of incubation (we visited nests each day; thus, clutch ages were not estimated but known exactly, assuming one egg laid per day). We did not remove the host's egg, as egg removal has no effect on rejection probability in these species (Davies & Brooke 1989). We decided *a priori* to employ the standard 6-d exposure period of Moksnes et al. (1991) to evaluate host responses. We aimed to check the nests daily until ejection or final acceptance, and despite logistically constraints (e.g. transportation and weather), we were able to check nests on 90% of subsequent days across the two species. Each nest was also checked several hours after the model egg was introduced on the same day. If the model disappeared during the day of the experiment, we assigned the nest latency to rejection of 0. The egg model was assumed to be accepted if it was still in an active nest on 6th day after the start of the experimental parasitism, then the model was removed. Although several previous studies included nest desertions as a specific response to parasite eggs, we did not do so because there were only 2 and 1 desertions (of blue models) in song thrushes and blackbirds, respectively. Thus, we assumed that desertion was not a response to parasitism by the specific egg models in our study population.

Two days after the outcome of the first trial (acceptance or rejection), we placed the same type of model into the nest. Again, we checked the nest daily until ejection or acceptance up to 6 d. Egg laying and incubation periods in both species last approximately 16 d (3–4 d of laying and 13 d of incubation; Higgins 2006) which provided enough time to test repeated acceptors (i.e. individuals that accepted both the first and second experimental egg; 6 + 1 + 6 d). We followed the same procedure in experiments with either blue (non-mimetic) models or with conspecific (mimetic) model eggs. Nests depredated before the first trial finished were excluded from analyses, whereas nests

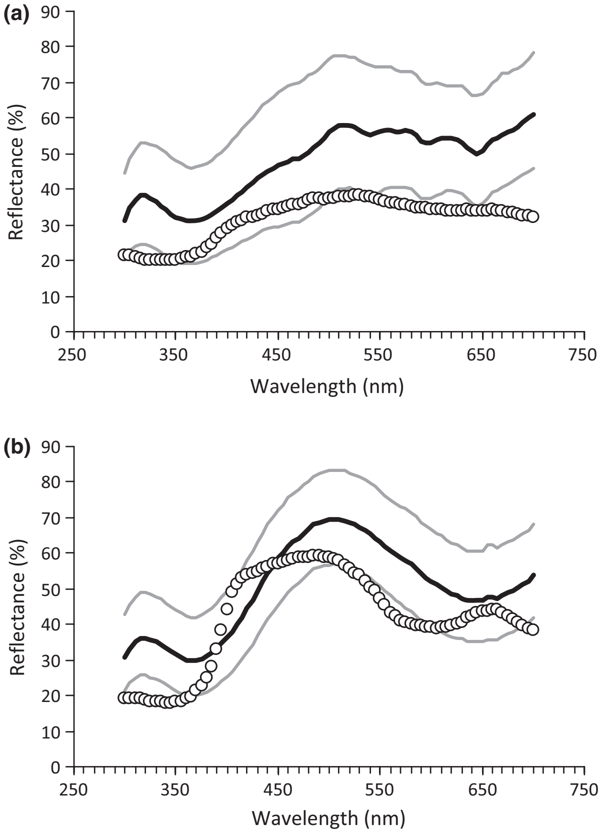

Fig. 1: Average reflectance spectra of the background coloration of natural eggs (black line) and artificial conspecific model eggs (○) for (a) blackbirds *Turdus merula* (n = 108) and (b) song thrushes *T. philomelos* (n = 156) in New Zealand. Spectra of natural eggs are shown with the range of natural variation (5th and 95th percentile ranges, grey lines) and the averages of three measurements are shown for each model egg (n = 5 models per type, because all model eggs were painted identically and variation would represent only measurement errors). In Fig. 1b, the artificial conspecific model reflectance curve refers to the blue background colour of both the conspecific song thrush model and the smaller model resembling the egg laid by the common cuckoo *Cuculus canorus* into redstart *Phoenicurus phoenicurus* nests, as these two types of models differed only in size and the presence of maculation (song thrush-like dark spots) on the conspecific song thrush model. Artificial conspecific models in both blackbirds and song thrushes can be considered highly mimetic because of the extensive overlap between artificial model eggs' reflectance curves and the models' size compared with the range of natural host eggs. The discrepancy between artificial and natural eggs is within the range of differences that had been considered highly mimetic in previous studies (e.g. spectral curves of artificial and natural eggs in Fig. 1 in Honza et al. 2007a). In contrast, artificial redstart-cuckoo (immaculate blue) model eggs are considered non-mimetic, as there is no overlap in size of hosts' eggs and models (see Methods) and coloration differences are striking between natural blackbird eggs and redstart-cuckoo models. Although the latter are similar as for background colour to natural song thrush eggs, the redstart-cuckoo models lack maculation that is used as a discrimination cue in song thrush (Polačiková et al. 2010).

that were depredated only after the first trial successfully ended were used to estimate the population-wide rate of rejection of alien eggs.

Statistical analyses

We applied two approaches to estimate the repeatability of binary egg-rejection (accept or eject) responses. First, we estimated consistencies of individual responses with Spearman's correlation coefficients. This is a statistically relevant approach (Martin & Bateson 2008, p. 74–78) and the resulting r_s-values and p-values are identical as when calculated with Pearson's correlation or phi contingency coefficient (see Weidinger & Kočvara 2010). Yet, approaches based on simple correlations are disadvantageous because they cannot control for possible confounding factors.

Second, to test for the possible confounding effects of covariates, we also estimated the consistency of a binary response between two trials within the nest using Generalised Linear Mixed Models (GLMM) implemented in R 2.12.0 (R Development Core Team 2010: package lme4 v. 0.999375-37; Bates et al. 2008). We used Laplace approximation to estimate the parameters in our GLMM with binary response variable (Bolker et al. 2009). We built four separate models for each species (blackbird, song thrush) and for each egg model type (non-mimetic, mimetic) combination. The global models for blue model eggs included nest identity (random variable) and following explanatory variables: nest age (age of the nest at start of experiment in days, day 0 = start of incubation, negative values denote start of experiment in laying stage), first egg-laying date (including its quadratic term to test for non-linear seasonal trends) and final clutch size. We selected the most parsimonious model based on Akaike information criterion corrected for small sample (AIC$_c$) (Burnham & Anderson 2002). Statistical models for conspecific model eggs did not include covariates because of low sample sizes so as to avoid overparametrization (Burnham & Anderson 2002).

Intraclass correlation coefficients (i.e. repeatability) of egg ejection (binary response variable) were calculated as $r = VA/(VA + VE + \pi^2/3)$, where VA denotes between-nest variance component, VE = component due to overdispersion (Guo & Zhao 2000) and within-nest residual component $\pi^2/3$ represents the distribution-specific variance for the logit GLMM (Nakagawa & Schielzeth 2010), where $\pi = 3.14$. We estimated asymptotic 95% confidence intervals for the correlation coefficients calculated in our GLMM model, using the public calculator http://faculty.vassar.edu/lowry/rho.html. The calculation is based on the Fisher r-to-z transformation according to the formula $z = ½*\ln*(1 + r)/(1–r) = \text{arctanh}(r)$ (where ln is the natural logarithm function and arctanh is the inverse hyperbolic function).

Spearman's correlation coefficients with exact 95% confidence intervals (binary response) were calculated using StatXact 7 (Cytel Inc. 2005). Results are presented as $\bar{x} \pm$ SE.

Individual repeatability of latency to ejection (a continuous response variable) between trials was calculated as $r = VA/(VA + VP)$ (Lessells & Boag 1987), where VA denotes between-nest variance component, and VP = within-nest variance component. Confidence intervals for their intraclass correlation coefficients were calculated according to formula in McGraw & Wong (1996).

Results

Responses of blackbirds to non-mimetic blue egg models

The overall rejection rate of blue egg models by blackbirds in the focal study season (2009) was 71.4% (n = 56, data from nests that were tested only once plus results of first trials from repeatedly tested nests). This is an intermediate rejection rate by definition

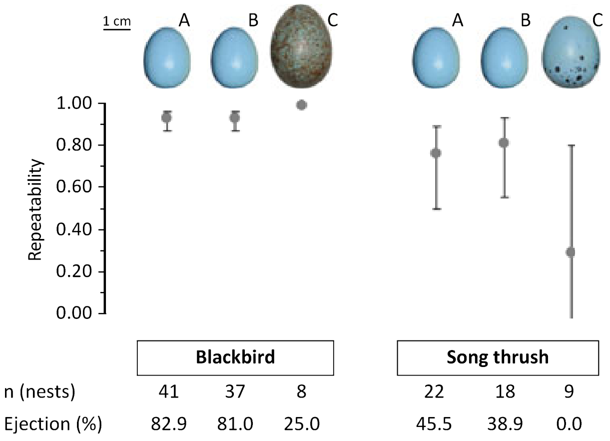

Fig. 2: Repeatability estimates from GLMM (see Methods) with 95% confidence intervals (CIs) of egg rejection in blackbirds and song thrushes. Results shown for (A) non-mimetic blue eggs with all nests included, (B) blue eggs with rejecter nests depredated within 13 d excluded (see Discussion for rationale behind this re-analysis) and (C) mimetic conspecific models. Note that CIs for mimetic conspecific models in the song thrush overlap zero; lower 95% CI (–0.46) is not shown in the graph. Sample sizes and ejection rates from 1st trials are shown below.

Table 1: GLMM models of egg ejection of blue models as the binomial response variable, with NEST = nest identity as random variable and fixed factors of AGE = age of clutch at start of experiment, CLUTCH = final clutch size, SEASON = first egg-laying date and SEASON2 = squared first egg-laying date. Models are ranked from the best to the worst based on Akaike information criteria, corrected for small sample size (AIC_c) (Burnham & Anderson 2002). K = number of parameters in the model. $\Delta_i = AIC_{c(i)} - AIC_{c(min)}$. w_i = Akaike weights

Models	K	AIC_C	Δ_i	w_i
Blackbird (n = 41)				
NEST	2	56.31	0.00	0.29
NEST + CLUTCH	3	57.20	0.89	0.19
NEST + AGE	3	57.41	1.10	0.17
NEST + SEASON	3	58.05	1.73	0.12
NEST + AGE + CLUTCH	4	59.29	2.98	0.07
NEST + CLUTCH + SEASON	4	59.89	3.58	0.05
NEST + AGE + SEASON	4	59.96	3.65	0.05
NEST + SEASON + SEASON2	4	60.66	4.35	0.03
NEST + AGE + SEASON + CLUTCH	5	62.17	5.86	0.02
NEST + SEASON + SEASON2 + CLUTCH	5	62.34	6.03	0.01
NEST + AGE + SEASON + SEASON2	5	63.29	6.98	0.01
NEST + AGE + SEASON + SEASON2 + CLUTCH	6	895.63	839.32	0.00
Song Thrush (n = 22)				
NEST + SEASON	3	50.96	0.00	0.40
NEST + CLUTCH + SEASON	4	53.25	2.30	0.13
NEST + AGE + SEASON	4	53.27	2.31	0.12
NEST + SEASON + SEASON2	4	53.37	2.41	0.12
NEST + CLUTCH	3	54.26	3.30	0.08
NEST + AGE + SEASON + CLUTCH	5	55.68	4.73	0.04
NEST + AGE + SEASON + SEASON2	5	55.81	4.85	0.04
NEST + SEASON + SEASON2 + CLUTCH	5	55.81	4.85	0.04
NEST + AGE + CLUTCH	4	56.57	5.61	0.02
NEST	2	57.67	6.72	0.01
NEST + AGE + SEASON + SEASON2 + CLUTCH	6	58.37	7.42	0.01
NEST + AGE	3	59.94	8.98	0.00

(*sensu* Røskaft et al. 2002; see also Discussion). This rate was statistically similar to rejection rates in previous years at our study site (2007–2008, 54.2%, n = 24; $\chi^2 = 2.24$, df = 1, p = 0.14). All results below refer only to repeatedly tested nests (in 2009).

In the first trials, at 83% tested nests (n = 41), we documented ejection of the model egg. In the second trials, 78% of females ejected the egg in the same sample of nests. Only two females changed their response (both from ejection to acceptance), and the responses of all other females, both acceptors and rejecters, remained identical between the first and the second trial. Repeatability for the binary data (see Methods) showed very high consistency (Spearman's correlation: $r_s = 0.86$, exact 95% CI = 0.67–1.00, n = 41, p < 0.0001).

The most parsimonious GLMM included only random variable nest (Table 1; Fig. 2). However, three other models had $\Delta_i < 2$ (Burnham & Anderson 2002). These models included, additional to nest identity, also final clutch size, nest age, and laying date in the season (Table 1). As 95% confidence intervals for model-averaged parameter estimates of these variables included 0 in all cases, we did not consider these variables further (Burnham & Anderson 2002). The latency to ejection of the model in the second trial was significantly shorter than in the first trial (Fig. 2, paired *t*-test: $t_{32} = 2.43$, p = 0.02).

In the first trials, the probability of egg ejection increased with advancing nest stage (logistic regression: $\chi^2 = 6.88$, df = 1,39, p = 0.009, intercept = −1.75 ± 0.56, slope = 0.69 ± 0.36). Still, this pattern did not confound our results because individual responses remained virtually identical between the first and second trial (39 of 41 females did not change their rejection responses). In the second trials, the egg ejection probability did not covary with nest age (logistic regression: $\chi^2 = 0.41$, df = 1,39, p = 0.52).

Latency to ejection had a tendency to decrease with nest age in the first trials ($F_{1,30} = 4.11$, p = 0.052). In the second trials, latencies to ejection did not correlate with nest age ($F_{1,30} = 0.62$, p = 0.44). Individual repeatability of the latency in egg ejection between trials was $r = 0.46$ (CI = 0.17–0.71), when controlling for nest-age effect.

Responses of blackbirds to mimetic conspecific egg models

In the first trials (2008 breeding season), blackbirds ejected only two and accepted 14 conspecific models. Out of these 16 nests, we successfully completed the second trial at only eight nests (50%). Rejection response in the second trials was identical in all cases. The same two females ejected conspecific egg models, whereas the remaining six females accepted the model eggs, i.e. incubated the model egg successfully for at least 6 d. Non-parametric correlation with the binary data showed high consistency ($r_s = 1.00$, exact 95% CI = 1.00–1.00, n = 8, p = 0.03). GLMM-estimated repeatability for the binary data was quantitatively very similar (Fig. 2).

The latency to respond at the two rejecter nests in the first trial was 1 and 3 d respectively. In the second trial, both rejecters responded with 1 d latencies.

Responses of song thrush to non-mimetic blue egg models

The overall rejection rate by song thrush of blue egg models in the focal study season (2009) was 39.5%

(n = 48, data from nests that were tested only once plus results of first trials from repeatedly tested nests). This rate did not differ statistically from the rejection rate in 2007–2008 (21.7%, n = 23, χ^2 = 2.04, df = 1, p = 0.15). All results below refer only to repeatedly tested nests (in 2009).

In the first trials, 45.5% of females (n = 22) ejected the model egg. In the second trials, 50.0% of females ejected the egg in the same sample of nests. Three females changed their response to the repeat trials (one from ejection to acceptance and two from acceptance to ejection) and all other females, both acceptors (n = 10) and rejecters (n = 9), responded identically between the first and the second trial. Repeatability for the binary data showed high consistency (Spearman's correlation: $r_s = 0.73$, exact 95% CI = 0.45–1.00, n = 22, p = 0.002).

The most parsimonious model included, additional to nest identity, also a covariate of laying date (Table 1, Fig. 2). Early in the season more individual rejected our experimental blue eggs, whereas later in the season acceptance was more prevalent (effect size of laying date: intercept = 4.15 ± 1.95, slope = −0.09 ± 0.04; CIs for slope: −0.17 to −0.01). Latencies were similar in the second compared with the first trial (Fig. 3, Wilcoxon matched pairs test: $Z = -3.00$, n = 8, p = 0.25).

In the first trials, the probability of egg ejection did not covary with the nest age (logistic regression: χ^2 = 1.45, df = 1,20, p = 0.23). In contrast, in the second trials, the egg ejection probability decreased with nest age (logistic regression: χ^2 = 5.48, df = 1,20, p = 0.02, intercept = −4.60 ± 2.44, slope = −0.56 ± 0.28).

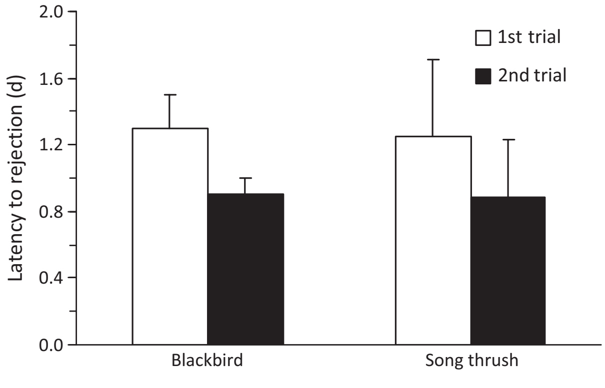

Fig. 3: Latency to rejection ($\bar{x}$ + SE) by blackbirds (n = 32 paired experiments) and song thrushes (n = 8 paired experiments) to experimental parasitism with non-mimetic blue model eggs. Data taken only from rejecters' nests where individuals rejected both in the first and second trial (i.e. nests where there was a non-consistent response to models eggs between the first and the second trial were excluded, see Methods for details).

Latency to ejection decreased with nest age in the first trials ($F_{1,6} = 7.99$, p = 0.03, latency to ejection = 1.24–0.16*nest age). In the second trials, latencies to ejection did not correlate with nest age ($F_{1,6} = 0.42$, p = 0.54). Individual repeatability of the latency in egg ejection between trials was r = 0.22 (CI = −0.53–0.77), when controlling for nest age effect.

Responses of song thrush to mimetic conspecific egg models

All song thrush in the focal study season (2008) accepted conspecific models in the first trials (n = 15). We successfully completed the second set of trials within the same breeding attempt for nine song thrushes. In all cases, song thrushes accepted models in both trials. Non-parametric correlation (Spearman's) on the binary data could not be calculated because of the absence of variation in the data. GLMM repeatability for the binary data (see Methods) was, as expected because of the absence of variation in the data, low and CIs included zero (Fig. 2).

Can different exposure of acceptor and rejecter nests bias repeatability estimates?

Importantly, despite the statistical similarity of the data across study years, some of the individual subjects' rejection rates of model eggs detected at repeatedly tested nests might still be inflated in comparison with the overall population rate of that season. This is because repeatedly tested acceptor nests had much longer exposure to potential predation (13 d) than singly tested acceptor nests (6 d) or repeatedly tested rejecters (mostly 3 d, see also latencies to rejection in Fig. 2) (see also Vikan et al. 2010). As predated nests were excluded from our analyses, this methodological constraint could have inflated the apparent rejection rates and possibly also repeatabilities for responses to blue model egg (all rejecter nests survived at least 13 d in conspecific egg treatments). Therefore, we repeated the aforesaid analyses but excluded all rejecter nests that did not survive at least 13 d from the introduction of the first egg model (i.e. from the start of the first trial).

In the first trials, blackbirds ejected the model egg at 81.0% nests (n = 37). In the second trials, 75.7% of females ejected the egg in the same sample of nests (Spearman's correlation: $r_s = 0.85$, exact 95% CI = 0.67–1.00, n = 37, p < 0.0001; GLMM: Fig. 2).

In the first trials, song thrush ejected the model egg at 38.9% nests (n = 18). In the second trials, 38.9% of females ejected the egg in the same sample of nests (Spearman's correlation: $r_s = 0.76$, exact 95% CI = 0.46–1.00, n = 18, p = 0.002; GLMM: Fig. 2). These corrected estimates are statistically identical (see CIs) to those not corrected for different periods of exposure and survival of acceptor or rejecter nests. Furthermore, the results of latencies to ejection remained statistically the same for the blackbirds (shorter for the second trials; paired t-test: $t_{27} = 2.46$, p = 0.02) and the song thrush (similar between first and second trials; Wilcoxon matched pairs test: $Z = 3.00$, n = 5, p = 0.35).

Discussion

Our experiments demonstrate very high levels of individual repeatability of blackbird and song thrush responses towards non-mimetic (both hosts) and mimetic (blackbirds) parasitic eggs. Specifically, 95% blackbirds and 86% song thrush responded identically to parasitic non-mimetic blue eggs (where we reached sufficient sample sizes) in two trials within the same breeding attempt. As documented in only one other species tested thus far (the blackcap, Honza et al. 2007b), in the blackbird, too, the latency to respond to model eggs was shorter between the first and second trials at the same nests.

Individual (in)consistency

Despite high individual repeatability of rejection of non-mimetic blue egg models, 5% blackbirds (n = 41) and 14% song thrush (n = 22) changed their behaviour from rejection to acceptance or *vice versa*. This is unlikely to be explained by altered host experiences (Rodríguez-Gironés & Lotem 1999), female age (Lotem et al. 1995), or light conditions at the nest (Langmore et al. 2005) because individuals experienced only few days of a short time-window between successive trials at the same nest with the same structure of vegetation cover.

Probability of egg rejection depends on a contrast of the parasite egg against host own eggs (e.g. in *Turdus* spp.: Cassey et al. 2008). Such variation of own egg colours, relative to the standardised paint of model eggs, is an inherent feature of any biological system; thus, it cannot be avoided in any experimental study of egg discrimination. However, this relative variation in contrast would also reflect natural variation in similarity between any parasite and any host eggs (see Fig. 3 in Antonov et al. 2010) and must be faced by both the parasites and the hosts, as well as the experimenters. Nevertheless, variation in similarity is not critical for our conclusions as far as host population is sampled randomly and, thus, reflects natural variation in the study system. Therefore, because our approach measured response behaviours within individuals, whose eggs themselves are less likely to vary within individuals than between individuals (Cassey et al. 2009), the resulting tests of repeatability remain valid. Additionally, this confounding effect was minimised in the present study because (1) host eggs remained identical between two within-breeding attempt trials, and (2) model non-mimetic eggs were painted simply (Fig. 2) and thus had highly standardised appearance. Hosts may commit more errors when cues to discriminate are too similar (Reeve 1989), but non-mimetic eggs we used (i.e. small plain blue cuckoo-egg-sized models) are perceivable for discrimination by our subject host species, both theoretically (Cassey et al. 2008) and empirically (Grim et al. 2011; this study). Still, Peer & Rothstein (2010) showed very low egg rejection repeatability in common grackles using a non-mimetic egg model in two trials within one breeding attempt (i.e. same design as our study).

Our findings have important implications for those theoretical models of parasite-host coevolution which assume that individuals are fixed in their responses once the foreign egg is perceived to reach an acceptance threshold (e.g. Takasu 1998; Servedio & Hauber 2006) or may change behaviour only when the parasitic egg mimicry is very high (Rodríguez-Gironés & Lotem 1999; Hauber et al. 2006). In turn, although the rates of inconsistency may not seem very large, our finding of 5% and 14% of host individuals changing behaviours between parasitism attempts could potentially have a very dramatic effect on model predictions, because of the inherent non-linearities in the system (G. D. Ruxton, pers. comm.). Our findings provide impetus for more modelling work to address rigorously the question how sensitive the various theoretical models of parasite–host co-evolution are with respect to their implicit and explicit assumptions of consistency and heritability. We provide novel empirical insights for future modelling efforts as the tested individuals in our study and those of Peer & Rothstein (2010) changed their behaviour even when (1) the parasite eggs were non-mimetic (i.e. parasite vs. own egg contrasts in size and colour were high across tested nests), (2) effects of altered experience were minimised, and (3) host clutch appearance remained identical.

We also detected some covariation with breeding stage on egg rejection probability in both blackbirds (only in first trials) and song thrush (only in second trials), despite previous work having showed that neither blackbirds nor song thrush differed in their egg-rejection responses between nesting stages (Davies & Brooke 1989 p. 212; Polačiková & Grim 2010 p. 114). Still, our analyses have shown that this had no statistical effect on estimates of repeatability: the model term nest age was statistically non-significant in all cases and was removed from all models (see Results).

Latency to ejection

Latency to ejection showed moderate repeatability in blackbirds but was low and not significantly different from zero in song thrush. That egg-ejector blackbirds responded faster during their second trials compared with the first ones may seem surprising. At the fitness level, late-laid and, thus, late-hatched interspecific parasites cannot eliminate host offspring (Moskát & Hauber 2007; Grim et al. 2009b), and late-hatched interspecific and conspecific parasites cannot successfully compete with host young (Hauber 2003; Hauber & Moskát 2008; Grim et al. 2009a; Moskát and Hauber 2010; Grim et al. 2011). However, even late parasitism may be costly because of increased costs of incubation and/or reduced hatching success (Siikamäki 1995).

At proximate level, the shifts in the latency of rejection in our results may reflect some combination of more extensive or accurate learning of a females' own eggs during incubation (Lotem et al. 1995; Stokke et al. 2007; Moskát et al. 2010) and/or the shifting of a discrimination threshold because of birds being alerted to a higher risk of parasitism by repeated exposure to foreign eggs, as suggested by both theoretical models (Reeve 1989; Rodríguez-Gironés & Lotem 1999) and experimental data (Davies et al. 1996; Hauber et al. 2006).

Importance on non-zero between-individual variation

We found that blue egg model rejection rates for the blackbird were at the upper limit, whereas those for song thrush were at the lower limit of standard definition of 'intermediate rejection' rates (i.e. 40–80%; Røskaft et al. 2002). Still, we stress that the definition of intermediate rejection should be fluid (E. Røskaft, pers. comm.) and what critically matters is that there is non-zero inter-individual variation in host behaviour (see Bell et al. 2009). This condition was fulfilled in the present study in responses to both non-mimetic models in the blackbirds and song thrush and to mimetic models in the blackbird, but not in the song thrush (although sample sizes were small for the last comparison, see Results).

In contrast, responses to mimetic models in the song thrush demonstrate how no variation in host behaviour affects repeatability estimation: the repeatability measures calculated by GLMM were low and statistically not significantly different from zero (Fig. 2). This inevitably follows from definition of repeatability: 'the proportion of phenotypic variation that can be attributed to between-subject (or between-group) variation' (Nakagawa & Schielzeth 2010, p. 935). Although host responses were seemingly highly consistent (all model eggs accepted in both first and second trials), there was no 'between-subject' variation and repeatability was bound to be statistically equivalent to zero, by definition.

Similarly to some of our data, Honza et al. (2007b) reported that all individuals whose responses were observed in both the first and second trials ejected parasitic eggs. These authors concluded that '[r]epeatability for host responses within the nest was very high' (p. 344). However, there was no 'between-subject' variation in host behaviour in that study. We recalculated repeatability for data from Honza et al. (2007b) and, as expected, repeatability was low and not significantly different from zero ($r = 0.28$; CI = −0.20–0.65; n = 19). This may have been caused by finding that 'one pair member may pre-empt the other's rejection response' (Honza et al. 2007b, p. 349). Accordingly, at some nests, one member of the pair ejected the first parasite model egg, whereas the second member of the pair ejected the second model egg. Although such data may be informative about some issues (e.g. 'do rejecters show assortative mating with rejecters?'), they cannot be used to estimate repeatability of individual behaviour (Bell et al. 2009). After excluding such data (five nests) from data set presented in Honza et al. (2007b), only individuals that ejected in both trials remained in the analysable data set, leading to zero repeatability estimate earlier.

Can differential predation risk at acceptor vs. rejecter nests inflate rejection rates?

Egg-rejection estimates in virtually all studies of brood parasitism may be inflated if there were uneven risks of predation at acceptor vs. rejecter nests (see also Vikan et al. 2010). For example, in many studies, by definition, an acceptor nest must survive at least 6 d to be included in analyses (Davies & Brooke 1989; Moksnes et al. 1991), whereas rejecter nests may be included in analyses after much shorter exposure to potential predation. For example, in the present study, most model eggs were rejected after 1 or 2 d (Fig. 1). Therefore, we reanalysed our data sets including only nests that survived the maximum exposure period of 13 d.

The reanalyses produced the same conclusions and the resulting new parameter estimates (repeatabilities) were statistically identical (based on 95% CIs) to uncorrected estimates. Therefore, biases resulting from differential survival of acceptor and rejecter nests were not detectable in this study. However, this does not exclude the possibility that in other data sets and studies such biases may in fact affect parameter estimates and conclusions. We recommend that future reanalyses and new studies of brood parasitism should explicitly test whether egg-rejection rates are not biased because of varying predation risks at acceptor vs. rejecter nests.

Methodological suggestions for future work

Future studies of individual repeatability of egg-rejection behaviours should focus on host species where (1) solely one sex incubates (and presumably the same sex rejects parasitic eggs), (2) egg-rejection rates are variable between individuals, and (3) egg rejection is by ejection (i.e. deserters cannot be used for estimations of within one breeding attempt repeatability). Also, (4) such studies should use models that are known to (or can be expected to) elicit intermediate rejection rates, and, (5) repeatability studies should use appropriate state of the art statistical tools that can control for possible confounding variables, i.e. GLMM (for details and examples, see Bates et al. 2008; Bolker et al. 2009; Weidinger & Kočvara 2010). Using highly mimetic model eggs leads to a potential outcome that all such models will be accepted, as we have shown with conspecific models in song thrush here, and under such circumstances, estimating repeatability may be unfeasible (Bell et al. 2009).

In any ecological study, authors should strive for representative sample sizes (Taborsky 2010). Although our samples for the mimetic conspecific models were small, we present even these data and the resulting analyses because not presenting collected data as part of a comprehensive experiment increases publication biases. Even small samples should be published because these could be later used in meta-analyses (weighted by sample size) (Johnson 2002; Nakagawa & Hauber 2011).

We used artificial model eggs whose colours can be perceived differently from natural eggs by the avian sensory system (Cassey et al. 2008). However, we did not specifically investigate whether and how individual birds perceived the eggs in our study. This is because we presented them with two identical stimuli which is the most important prerequisite for reliable estimates of repeatability (Martin & Bateson 2008).

Overall, our data imply that egg rejection decisions are highly consistent for individual female blackbirds and song thrush within the same breeding attempt. It remains to be seen in future studies whether, and how, consistent egg rejection decisions are across breeding attempts of a single individual and across generations of acceptor or rejecter females (Hauber et al. 2004; Hoover & Hauber 2007). The implications of these results are that a critical assumption of the evolvability of egg-rejection behaviours has been roughly met in both *Turdus* species. Also, contrary to assumptions of theoretical models, we confirmed that some individuals are flexible in egg-rejection behaviours even in the short time-window of one breeding attempt and may change their responses between subsequent exposures to brood parasitism. Thus, these extensive experimental data provide empirical impetus to assess rigorously how sensitive various theoretical models of parasite-host co-evolution are to their assumptions.

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Příspěvek II.

Grim T., Samaš P., Moskát C., Kleven O., Honza M., Moksnes A., Røskaft E. & Stokke B.G. 2011: Constraints on host choice: why do parasitic birds rarely exploit some common potential hosts? Journal of Animal Ecology 80(3): 508–518. (doi: 10.1111/j.1365-2656.2010.01798.x)

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Constraints on host choice: why do parasitic birds rarely exploit some common potential hosts?

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Summary

1. Why are some common and apparently suitable resources avoided by potential users? This interesting ecological and evolutionary conundrum is vividly illustrated by obligate brood parasites. Parasitic birds lay their eggs into nests of a wide range of host species, including many rare ones, but do not parasitize some commonly co-occurring potential hosts.

2. Attempts to explain the absence of parasitism in common potential hosts are limited and typically focused on single-factor explanations while ignoring other potential factors. We tested why thrushes *Turdus* spp. are extremely rarely parasitized by common cuckoos *Cuculus canorus* despite breeding commonly in sympatry and building the most conspicuous nests among forest-breeding passerines.

3. No single examined factor explained cuckoo avoidance of thrushes. Life-history traits of all six European thrush species and the 10 most frequently used cuckoo hosts in Europe were similar except body/egg size, nest design and nestling diet.

4. Experiments ($n = 1211$) in several populations across Europe showed that host defences at egg-laying and incubation stages did not account for the lack of cuckoo parasitism in thrushes. However, cross-fostering experiments disclosed that various factors during the nestling period prevent cuckoos from successfully parasitizing thrushes. Specifically, in some thrush species, the nest cup design forced cuckoo chicks to compete with host chicks with fatal consequences for the parasite. Other species were reluctant to care even for lone cuckoo chicks.

5. Importantly, in an apparently phylogenetically homogenous group of hosts, there were interspecific differences in factors responsible for the absence of cuckoo parasitism.

6. This study highlights the importance of considering multiple potential factors and their interactions for understanding absence of parasitism in potential hosts of parasitic birds. In the present study, comparative and experimental procedures are integrated, which represent a novel approach that should prove useful for the understanding of interspecific ecological relationships in general.

Key-words: antiparasite defence, co-evolution, host selection, interactive effects, parasite avoidance

Introduction

Resource use lies at the heart of ecological research. Common and easily accessible resources (prey for predators or hosts for parasites) should be, all other things being equal, used frequently (Jaenike 1990). Still, some apparently accessible and suitable resources remain unexploited. Absence of resource use can be explained by various factors, such as inconspicuousness or unpalatability (Ruxton, Sherratt & Speed 2004). Such avoidance of cryptic or aposematic prey is easily explained. In contrast, avoidance of common, conspicuous, easily accessible and nonaposematic resources presents a real ecological conundrum. Here, we combine comparative and experimental approaches to study a

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particularly striking example of nonuse of an apparently suitable resource.

Absence of brood parasitism in potential hosts was previously explained mostly by inaccessible nests and unsuitable diet for parasite chicks (Davies 2000). In general, little effort has been made to explain the absence (Peer & Bollinger 1997) or low rates of parasitism (Briskie, Sealy & Hobson 1990) in potential hosts of parasitic birds. Further, hypotheses on host avoidance have been tested almost entirely among North-American brown-headed cowbird *Molothrus ater* hosts (Briskie, Sealy & Hobson 1992; Sealy & Bazin 1995; Peer & Bollinger 1997; Mermoz & Fernandez 1999), whereas common cuckoo *Cuculus canorus* (hereafter: cuckoo) hosts have received marginal attention (Davies 2000; Honza *et al*. 2004). Importantly, most previous studies did not reach strong conclusions, as most of the potential factors usually remained untested. The few studies that claimed to explain the absence of cuckoo parasitism have focused on single-factor explanations, namely extremely high egg rejection rates (Procházka & Honza 2003; Honza *et al*. 2004; Lovászi & Moskát 2004; Stokke *et al*. 2004; but see Sealy & Bazin 1995; De Mársico & Reboreda 2008). Thus, previous studies remain inconclusive because they in principle cannot exclude the possibility that other factors (aggression against adult cuckoos, chick discrimination, etc.) contributed to the extinction of specific cuckoo races parasitizing these hosts. Previous studies also did not consider the possibility that some factors *per se* might not constrain brood parasitism, but that *interactions* between factors might be important (see also Weidinger 2002). For example, nest design alone or egg size alone might not constrain cuckoo eviction behaviour, but their combination may do so (i.e. cuckoos might be able to evict small eggs from large nests, or large eggs from small hosts nests, but not large eggs from large nests).

Common blackbirds *Turdus merula*; hereafter: blackbird, song thrushes *Turdus philomelos*, fieldfares *Turdus pilaris* and redwings *Turdus iliacus* breed at high densities in their respective habitats over most of Europe (BirdLife 2004). All four species build large and open conspicuous nests – in fact, thrush nests are by far the most conspicuous nests of any forest passerine. Thrushes are striking exceptions to the result reported by Soler, Møller & Soler (1999) where host population size was the best predictor of parasitism rate. For example, the blackbird is roughly as common as the four most frequently used cuckoo host species taken together (BirdLife 2004). However, the number of cuckoo eggs found in blackbird nests is almost 300 times lower than that among these common hosts (Moksnes & Røskaft 1995). Available data from other European thrushes (including the mistle thrush, *Turdus viscivorus*, and ring ouzel, *Turdus torquatus*) lead to similar conclusions – most hosts that are considered 'rare' are parasitized infrequently but still orders of magnitude more than thrushes (Moksnes & Røskaft 1995). As there are observations of thrushes rearing a young cuckoo up till fledging (Glue & Morgan 1972), the big puzzle is why thrushes are almost never parasitized by cuckoos despite their high abundance, very poor nest concealment and apparently appropriate body size.

An apparent absence of cuckoo parasitism in thrushes can reflect either real absence of parasitism and hence history of co-evolutionary interactions with the cuckoo (*primary unsuitability* because of host life-history) or well-developed host defences that evolved owing to either intra- or interspecific brood parasitism (*secondary unsuitability*; e.g. hosts prevent cuckoos from entering their nests or remove their eggs very fast). Primary unsuitability prevents any co-evolution between the parasite and potential hosts, whereas secondary unsuitability is caused by previous co-evolution between the two parties. Therefore, we first employed simple *exploratory* comparisons (i.e. without performing statistical tests, see Materials and methods) between all six extant European *Turdus* thrushes and the 10 most common cuckoo hosts in Europe (Table 1 in Moksnes & Røskaft 1995) to identify candidate traits that could explain absence of cuckoo parasitism in thrushes. We *a priori* rejected traits that are known to affect host selection by cuckoos (Soler *et al*. 1999), but are identical between common hosts and thrushes or even make thrushes more available for parasitism than regular hosts: (i) thrushes cannot escape cuckoo parasitism by their habitat selection because they breed in forest and woodland edges just like many regular cuckoo hosts; (ii) cuckoos do not parasitize hole nesters (with the single exception of the semi-hole nesting common redstarts *Phoenicurus phoenicurus*; hereafter: redstart; Rutila, Latja & Koskela 2002), but all thrushes are open-nesters just like most regular cuckoo hosts; and (iii) some passerines with well-hidden nests may, at least partly, avoid being parasitized, but there are no more conspicuous passerine nests in forests than those of thrushes. The rationale behind selecting the particular traits and logic behind our directional predictions (Table S1) is given in previous studies (references in Table S1).

After excluding some candidate traits, we tested experimentally whether cuckoos fail to parasitize *Turdus* species owing to thrushes being unsuitable at (i) egg-laying, (ii) incubation and/or (iii) nestling stages. We made three major types of experiments: (i) we presented stuffed dummies of the cuckoo and a common avian nest predator, the hooded crow *Corvus cornix*, at host nests to test host aggression and enemy recognition abilities, (ii) we introduced model eggs into potential host nests to test their egg discrimination abilities and (iii) by cross-fostering of host eggs and cuckoo nestlings between the nests of common hosts and thrushes, we attempted to disentangle the effects of nest architecture, egg size and presence of cohabiting host chicks on the performance of parasite nestlings.

Materials and methods

EXPLORATORY COMPARISONS OF LIFE-HISTORY TRAITS

First, we collated data on thrush and regular cuckoo host life-history traits from the literature (Perrins 1998; Soler *et al*. 1999). We compared possible deviations of traits between all European *Turdus* 'thrushes' ($n = 6$ species) and 'common hosts' ($n = 10$ most common hosts reported in Table 1 of Moksnes & Røskaft 1995).

There are both obvious similarities (e.g. large body size) and striking differences (e.g. aggression to intruders near the nest) between various thrush species (Fig. S1). Therefore, some thrushes are more similar to common hosts than other thrush species in our sample. This implies that a particular thrush species may, in principle, differ from other thrush species in the reasons why it is avoided by cuckoos (indeed, our results support this view). Pooling of data into 'thrushes' vs. 'common hosts' groups for statistical analyses of variance would mask such differences. Therefore, we simply plotted original species-specific data for thrushes vs. common hosts to investigate whether particular thrush species deviate from the common hosts in any life-history traits. The rationale for this comparison was not to test any hypothesis (note that we did not perform any formal statistical test in this part of the study), but was simply to identify traits for future experimental work. Clearly, if a *particular* life-history trait of a *particular* thrush species is within the range of that life-history trait in common cuckoo hosts, then it follows that the *particular* life-history trait is not responsible for the absence of cuckoo parasitism in that thrush species. This, of course, does not exclude the possibility that the same trait (with different quantitative value) precludes cuckoo parasitism in another thrush species.

From a statistical point of view, the present study does not suffer from pseudoreplication because we are interested in the specific difference between thrushes and regular hosts ('location differences', Hurlbert 1984). Our comparisons of life-history traits between thrushes and regular cuckoo hosts are heuristic. The comparisons (Table S1, Fig. S2) do *not* test a hypothesis; in contrast, their aim is to identify what factors are meaningful candidates for experimental tests.

GENERAL FIELD PROCEDURES

To increase the power of our tests, we included both our unpublished data and our own already published results (see below). From 1986 to 2009, we studied 1016 thrush nests in 12 localities in Northern and Central Europe (Appendix S1; see Moksnes *et al.* 1991; Grim & Honza 2001a; Moskát, Karcza & Csörgö 2003, for descriptions of those areas and field procedures). The host population was considered sympatric when the cuckoo was breeding in that particular area ($n = 6$). Other populations, mainly in urban areas, were considered allopatric ($n = 6$). Additionally, for eviction and cohabitation experiments with cuckoo chicks, we studied 185 nests of common hosts in the Czech Republic, Hungary and Finland (see Grim & Honza 2001b; Grim *et al.* 2009a; Moskát & Hauber 2010, for descriptions of those areas and field procedures).

Blackbird populations in towns show very high philopatry that has even led to striking and partly genetically determined differences in biology of urban vs. rural blackbirds (Partecke & Gwinner 2007). Because cuckoos generally avoid towns (Perrins 1998), urban populations can be regarded as allopatric (this is confirmed by the absence of any records of cuckoo parasitism in any passerines in our intensely studied urban populations). Data from Hungary show very high site fidelity in both town and countryside blackbird populations (96·0% of recoveries in the city for birds banded in Hungarian towns; T. Csörgö pers. comm.; 87·9% of recoveries in Hungarian countryside for birds banded there; Z. Karcza pers. comm.). Fieldfares also show high fidelity to their natal areas (84·9% recoveries within 6 km from original nest; Norman 1994). We predicted stronger anti-parasite defences in sympatry than in allopatry (Davies & Brooke 1989; Stokke *et al.* 2008).

We measured the nest cup inside diameter and inner depth with a ruler to the nearest millimetre. The index of nest cup steepness was calculated as the nest cup depth divided by the nest cup width (Grim *et al.* 2009a).

DUMMY EXPERIMENTS

Responses of thrushes to adult brood parasites were tested using stuffed dummies of the cuckoo and the hooded crow as a control. The reason for using hooded crow dummies was to determine whether the absence of aggression against a cuckoo dummy in preliminary experiments reflected an overall absence of aggression to any, even clearly dangerous, enemies near the nest. We used one or two (depending on availability) stuffed specimens for both the cuckoo and crow at each study site. Responses did not vary between the specimens; thus, we pooled the data (see also Grim 2005). The dummy was placed between 0·5 and 1·0 m from the focal nest, level with it and facing the nest rim. We observed the responses of nest owners for 10 min after the first parent appeared near the nest and became aware of the dummy. We scored responses on the following scale: (i) no reaction = host(s) observed the dummy but ignored it, (ii) distress calls = host(s) uttered distress/alarm calls, (iii) mobbing = host(s) performed dives or flights around the dummy and (iv) attacks = host(s) aggressively attacked the dummy with contact attacks (Moksnes *et al.* 1991). In cases of attacks, the dummy was immediately removed to avoid its destruction. As some responses were rare in some data sets, we pooled scores 1 and 2 as 'no aggression' and scores 3 and 4 as 'aggression' (Røskaft *et al.* 2002). If no birds arrived at the focal nest during a 30-min period after the dummy was placed near the nest, the response was scored as 'no reaction'. Excluding data from such experiments had no effect on the results from blackbirds (excluded $n = 5$), song thrushes ($n = 10$), fieldfares ($n = 1$) or redwings ($n = 4$).

Each nest was tested only once to avoid pseudoreplication, and only one kind of dummy (cuckoo or crow) was presented near each nest. Nest defence experiments were performed during egg-laying, incubation and young nestling stages when the adult cuckoo is a threat to hosts – female cuckoos prey upon host nestlings (Davies 2000), and accordingly, hosts do not differ in their responses to adult parasites across breeding stages (Grim 2005). At some nests, both egg and nest defence experiments were done. In such cases, an aggression experiment was performed after the egg discrimination experiment was finished.

EGG EXPERIMENTS

We tested the rejection abilities of thrushes with cuckoo-sized model eggs painted (i) immaculate blue to mimic eggs of the cuckoo gens parasitizing the redstart (Moksnes & Røskaft 1995) and (ii) brownish and densely spotted to resemble those of the meadow pipit *Anthus pratensis* (Moksnes *et al.* 1991). Redstart type models were similar to song thrush eggs (as for background colour) and clearly dissimilar to eggs of the other three host species. Pipit type models were dissimilar to eggs of all hosts. Models were made of plaster of Paris or hard plastic. Thrushes are grasp-ejecters; thus, the model material should not affect their egg rejection decisions (Honza, Kuiper & Cherry 2005).

We introduced the parasitic egg to the host nest during the egg-laying or incubation stages. In some of the experiments, one host egg was removed, but in the majority of the cases, no host egg was removed (removal of one egg has no effects on thrush responses; Davies & Brooke 1989). We made an effort to monitor experimental nests daily during a standard 6-day-period following experimental parasitism (Davies & Brooke 1989; Moksnes *et al.* 1991). Nests depredated before the 6-day-period finished were excluded from analyses. We scored three kinds of responses: acceptance, ejection and desertion. Desertion can largely be considered as a rejection response in thrushes because (i) parasitized nests are deserted more frequently than unparasitized nests (Grim & Honza 2001a) and (ii) hosts tested with nonmimetic eggs desert more often than those parasitized with mimetic models (Davies & Brooke 1989). Exclusion of deserted nests from analyses did not change our results and inferences.

CHICK CROSS-FOSTERING EXPERIMENTS

None of the thrush nests monitored in this study were naturally parasitized by the cuckoo. Therefore, we cross-fostered cuckoo hatchlings from *Acrocephalus* warblers to thrush nests to test their eviction and survival abilities. We did not introduce cuckoo eggs as that would result in an unnecessary waste because of egg rejection by hosts (see Results). This was also the reason we did not test whether there was decreased incubation efficiency of relatively smaller cuckoo eggs in the presence of larger thrush eggs. Although hatchability decreases with increasing clutch volume (Lerkelund *et al.* 1993; Tuero, Fiorini & Reboreda 2007), cuckoo females remove at least one host egg before laying their own (Davies 2000). This would result in decreased clutch volume and thus *improved* incubation efficiency. However, a cuckoo egg has a volume 1·4–2·0 times smaller than *Turdus* eggs which might decrease hatching success of the parasite (but see Tuero *et al.* 2007). Still, successful and increasing rates of cuckoo parasitism in azure-winged magpies *Cyanopica cyana* (Davies 2000) suggest that size discrepancy in parasite vs. host eggs does not constrain cuckoos from parasitizing hosts as large as thrushes – azure-winged magpie eggs (26·0 × 21·2 mm; Hosono 1983) are sized between redwing and blackbird eggs. However, we note that some model cuckoo eggs in our experimental nests were not very visible in blackbird nests, and blackbirds probably could not properly rotate and heat such eggs, which were positioned in the bottom of nests. This factor may contribute to lower benefits for cuckoos from parasitizing thrushes, but it does not totally prevent successful parasitism (see records of successfully hatched and fledged cuckoos from blackbird nests; Glue & Morgan 1972).

We weighed cuckoo chicks to the nearest 0·1 g. Sample sizes in these experiments are relatively small because of logistic constraints (low availability of cuckoo chicks, high predation) and ethical and conservation reasons (recently cuckoo populations show declining trends across Europe; BirdLife 2004). Cuckoo nestlings showed very low survival in thrush nests. Hence, the results were clear-cut and did not justify increasing samples at the cost of animal suffering (see Taborsky 2010).

To disentangle the effects of nest sizes and egg sizes on the eviction success, we tested for eviction of large (thrush) and small (Eurasian reed warbler *Acrocephalus scirpaceus*; hereafter: reed warbler) eggs from both large (thrush) and small (reed warbler) nests. In these experimental nests, cuckoo chicks faced eviction of two to five thrush or warbler eggs (depending on original host clutch size). To study the effects of cohabitation with host chicks, we transferred newly hatched cuckoo chicks into host nests with three same-aged host chicks (each cuckoo into a different host nest). We matched chicks for age (days) because average incubation periods are similar and their ranges overlap considerably among cuckoos (mean = 12·4, range = 11–14; Hudec & Šťastný 2005), and both blackbirds (mean = 12·8, range = 11–17) and song thrushes (mean = 12·6, range = 10–16; Hudec 1983) in Central Europe where we performed the experiments.

We scored chick fate as 'predated', 'survived' or 'deserted'. Predated chicks either disappeared from nests (that showed typical signs of being disturbed by a predator) or only the remains of fresh corpses were found in the nest. Deserted chicks were found dead in the nest cups without any injuries, but parents were present near the nest – showing that chick death was not caused by death of host parents instead suggesting that hosts decided not to feed the chick further but remained in the territory (see also Langmore, Hunt & Kilner 2003). Death because of unsuitable diet was excluded because cuckoo chicks were shown to be able to digest and survive on diet that is typically fed by thrushes to their chicks (predominantly earthworms and molluscs: Grim 2006a; rarely fruits: Martín-Gálvez *et al.* 2005).

As a control for possible effects of the cross-fostering itself, we transferred cuckoo chicks among (i) reed warbler nests, (ii) great reed warbler *Acrocephalus arundinaceus* nests and (iii) between reed and great reed warbler nests. No nestlings suffered from low growth or survival because of cross-fostering (Kleven *et al.* 1999; Grim & Honza 2001b).

STATISTICAL ANALYSES

In the exploratory part of this study, we did not analyse data statistically (see above). In the experimental part of the study, we were primarily interested in the effects of sympatry and allopatry and type of experimental dummy and egg on host behaviour. We also included factors that were shown to affect host discrimination behaviour in some host species in previous studies (as recommended by Grafen & Hails 2002). We analysed our experimental data using either generalized linear mixed models (aggression to dummies, egg rejection) or general linear mixed models (GLM) (latency to egg rejection in days).

The full models contained the following explanatory variables: (i) random effects of 'year' (nominal) and 'locality' (nominal), (ii) fixed effects of 'geography' (nominal: sympatry vs. allopatry with the cuckoo), 'dummy' type (nominal: cuckoo vs. crow) or 'egg model' type (nominal: blue vs. spotted) and the interaction of geography with dummy or model type, respectively and (iii) covariates of final 'clutch size' (continuous), 'laying date' of the first egg in the clutch (continuous) and 'nest stage' (nominal: egg laying, 1–3 days of incubation, 4–9 days of incubation, 10 days of incubation to hatching, nestlings – the last two categories only for dummy experiments). 'Year' was entered as a random (i.e. not fixed) effect because we had no specific year-based temporal predictions.

The response variables were host reaction either to experimental eggs (nominal: accepted vs. rejected) or to dummies (nominal: aggression vs. no aggression) and latencies to egg rejection (continuous: in days). We followed backward elimination of nonsignificant terms, starting with the interaction term, then covariates and we kept two major fixed effects of interest (geography, dummy/egg type) in the models until the last step regardless of their significance (Grafen & Hails 2002). Additionally, in another series of analyses, we removed all covariates and confirmed that the interaction was nonsignificant even when covariates were not present in the models.

The final clutch size was included as a surrogate measure of host quality. In 'egg' models, we did not test for a possible effect of number of host eggs at the time of addition of the experimental egg because this variable inevitably positively covaried with nest stage (which was already included in the models; see the issue of multicollinearity: Graham 2003). The 'laying date' in the breeding season was centred for each host species separately by mean within each year to exclude a possible confounding effect of between-year variation in our seasonal sampling effort. Test statistics and *P*-values reported in Results for nonsignificant terms are from backward elimination procedure just before the particular term (being the least significant) was removed from the model.

We analysed data separately for each of the study host species. Nominal responses to dummies and eggs were analysed using GLIMMIX macro of SAS (generalized linear mixed model with binomial error and logit link; Bolker *et al.* 2009). Continual response of latency to egg rejection was analysed using GLM.

In all our models, the random effects of year and locality were very small (likelihood ratio tests; Bolker *et al.* 2009), i.e. there was no significant spatio-temporal variation in the data. When removed, the resulting simpler models with the same structure of fixed effects had a dramatically better fit (much lower AIC_c) and very similar parameter estimates. Hence, we decided to present results of the models without random effects (Bolker *et al.* 2009).

In all, we successfully finished 543 dummy experiments (including data from Grim & Honza 2001a; Røskaft *et al.* 2002), 421 egg rejection experiments (including data from Moksnes *et al.* 1991; Grim & Honza 2001a) and 247 experiments and observations at nests with chicks (including some data from Grim 2006a; Grim *et al.* 2009a,b). The vast majority of data are new: 80·1% of dummy, 74·8% of egg and 83·8% of chick data points have not been published before. Importantly, all data on host responses to eggs and dummies from six Czech and Hungarian populations are new, and none of 193 nests where eviction success was studied (the most important part of the present study) were included in any previously published work. Sample sizes differ among analyses because some data points were missing (e.g. the final clutch size was unknown for nests where experimental egg was ejected during laying stage, and the nest was depredated before clutch completion). We fitted all models in SAS 9.2 (SAS Institute 2008). All statistical tests are two-tailed (Lombardi & Hurlbert 2009), and values are given as mean ± SE.

Results

DO LIFE-HISTORY TRAITS OF THRUSHES AND REGULAR HOSTS DIFFER?

Regression of number of cuckoo eggs in European collections (Moksnes & Røskaft 1995) against minimum breeding population estimates (in millions pairs) of all six European *Turdus* thrushes explained 78% of variation ($F_{1,4}$ = 14·3, P = 0·019; slope = 0·48 ± 0·13). This pattern suggests that cuckoos parasitize thrushes only by mistake (random sampling effect).

Thrushes did not deviate from regular cuckoo hosts in the majority of the investigated general life-history characteristics (Fig. S1a). Thrushes are well available to cuckoos in both space and time, but some of them show lower breeding success. Still, this factor cannot explain avoidance of thrushes by cuckoos: the most common cuckoo host, the reed warbler, experiences a breeding success (42·7%) similar to some thrush species.

Values of specific parasitism-related traits of thrushes were either within the range of those traits in common hosts or deviated in the direction opposite to the hypothesis that thrushes are abandoned cuckoo hosts (intra-clutch variation; Fig. S1b). Thrushes tended to show lower aggression towards adult cuckoos and rejected alien eggs more slowly than common hosts (Fig. S1b).

Overall, there are some striking differences between thrushes and common hosts (Fig. S1a):

1. Average body size of thrushes is five times larger than that of common hosts. The smallest thrush – the redwing – is more than twice the size of the largest commonly used European host (the great reed warbler). However, body size *per se* has no direct causal negative influence on cuckoo parasitism and, thus, cannot obviously explain avoidance of thrushes by cuckoos (see Materials and methods for a discussion of the azure-winged magpie as a cuckoo host).
2. Because of a general positive correlation between body size and egg size, thrushes lay large eggs that consequently already produce large nestlings at hatching.
3. Thrushes feed their nestlings with less insects and spiders (which are almost exclusively brought to nestlings by common fosterers) and more earthworms (which are almost absent in the diet of current common hosts).
4. A review of cuckoo chicks reported in previous studies showed that cuckoos may fail to evict host clutch or brood from nests of almost any host (T. Grim & P.

Table 1. Responses (mean ± SE) of the four thrushes towards dummies and model eggs in sympatry and allopatry with cuckoos. Effect sizes for aggression and rejection rates are back-transformed values from the final models. For statistics and sample sizes (degrees of freedom), see Table S2. For details on analyses, see Materials and methods

Host response	Factor	Blackbird	Song thrush	Redwing	Fieldfare
Dummy (% aggression)	Cuckoo	49·2 ± 6·4	14·3 ± 4·4	8·2 ± 3·9	0·0 ± 0·0
	Crow	33·3 ± 4·7	20·8 ± 5·6	78·4 ± 4·8	83·2 ± 3·5
Dummy (% aggression)	Sympatry	38·9 ± 6·0	15·1 ± 3·9	–	–
	Allopatry	42·7 ± 5·3	23·3 ± 7·7	33·5 ± 7·4	72·9 ± 3·9
Egg model (% rejection)	Blue	65·7 ± 4·8	54·2 ± 10·2	44·4 ± 11·7	26·3 ± 10·1
	Spotted	50·0 ± 8·8	82·0 ± 5·4	30·5 ± 4·1	14·0 ± 4·9
Egg model (% rejection)	Sympatry	61·8 ± 6·6	78·6 ± 7·3	19·1 ± 8·6	9·4 ± 5·1
	Allopatry	61·8 ± 5·6	61·4 ± 8·6	34·4 ± 4·2	24·3 ± 7·1
Latency to rejection (days; mean ± SE)	Blue	2·0 ± 0·2	3·0 ± 0·5	2·7 ± 0·5	5·9 ± 0·7
	Spotted	2·5 ± 0·4	2·3 ± 0·3	3·0 ± 0·3	3·9 ± 0·6
Latency to rejection (days; mean ± SE)	Sympatry	1·7 ± 0·3	2·7 ± 0·3	1·8 ± 0·9	4·1 ± 1·4
	Allopatry	2·5 ± 0·2	2·1 ± 0·4	3·0 ± 0·3	5·0 ± 0·5

Procházka, unpublished data). However, unsuccessful evictions happened much more frequently in thrushes than in common hosts (Fig. S1).

Taken together, these differences suggest that the major obstacle for cuckoos is present during the nestling period. The large size of host eggs could decrease the effectiveness of eviction behaviour leading to detrimental competition with host chicks. Alternatively, the diet of thrushes with high proportions of earthworms and lower proportions of insects could pose digestion problems for cuckoo chicks independently of presence of host chicks. We tested these hypotheses experimentally.

EXPERIMENTS – DUMMIES

Out of 543 dummy experiments, 60% were done during laying or early incubation (nest stage had no significant effect on host responses, Table S2). Aggression towards cuckoo dummies was generally weak, with fieldfares ignoring the cuckoo dummy completely (Table 1). Sympatry/allopatry had no effect on thrush responses to either crows or cuckoos (Table 1; no such data were available for fieldfares and redwings).

Fieldfares and redwings attacked the crow much more frequently than the cuckoo, whereas the responses of song thrushes and blackbirds were similar towards the two kinds of dummies (Table 1). Most importantly, aggression towards cuckoos was generally lower in thrushes than in common hosts (Fig. S1). Therefore, the intensity of nest defence cannot be responsible for the absence of cuckoo parasitism in thrushes.

EXPERIMENTS – EGGS

Out of 421 egg experiments, 84% were done during laying or early incubation (nest stage had no significant effect on host responses, Table S2). Song thrushes rejected significantly more meadow pipit type models than more mimetic redstart type eggs, while other thrushes tended to reject more the latter type (Table 1). Contrary to our predictions, rejection rates were not higher in sympatry with cuckoos (Table 1).

Effects of egg type and sympatry/allopatry on latency to rejection were generally small and nonsignificant (Tables 1 and S2). Importantly, latencies were almost always longer than 2 days thus excluding the possibility that natural cuckoo parasitism goes undetected because of extremely fast egg ejection by hosts.

EXPERIMENTS – CHICKS: EVICTION SUCCESS

Egg eviction experiments (n = 193) showed that cuckoos evicted all host eggs in natural nests of reed and great reed warblers (Table 2). They also succeeded in evicting all large song thrush eggs from reed warbler nests and even larger blackbird eggs from old used blackbird nests attached at the top of active great reed warbler nests (all tested host pairs were willing to accept this nest change; we did not test eviction success in active blackbird nests because cuckoo chicks survived poorly in such nests, see below). This indicates that egg size itself does not constrain eviction behaviour.

In contrast, cuckoo chicks were unable to evict not only large song thrush eggs, but also small reed warbler eggs from song thrush nests (Table 2). Cuckoo chicks successfully evicted some host eggs only from one nest that was relatively shallow (4·5 cm deep) and slightly tilted; the cuckoo chick accomplished the eviction of the whole host clutch only when 5 day old. Video-recordings revealed that chicks tried to evict experimental eggs, but the cuckoo's legs slipped on the hard and smooth lining of nest cups and effectively prevented the chicks from pushing host eggs higher than half-way to the nest rim. But it was nest size (not the smooth lining) that impeded eviction success because cuckoo chicks were also unable to evict any eggs/chicks from similar sized fieldfare nest cups that do not have smooth structure (like song thrush nests) but are lined with grasses. Unsuccessful evictions were not caused by large host egg size *per se* as cuckoo chicks (i) were also unable to evict small reed warbler eggs from the same song thrush nests, and (ii) they evicted song thrush eggs from reed warbler nests in all cases (Table 2).

Table 2. Eviction success of cuckoo chicks, i.e. the percentage of *nests* where the cuckoo chick succeeded in complete elimination of host eggs by eviction. Only at one nest (of the fieldfare) did the cuckoo chick partly succeed, evicting two out of three eggs

Nest	Treatment	Sample size (nests)	What was evicted?	Eviction success (%)	Locality
Reed warbler	Natural	99	Reed warbler eggs	100	Czech Republic
Reed warbler	Experimental	7	Song thrush eggs	100	Czech Republic
Great reed warbler	Natural	18	Great reed warbler eggs	100	Czech Republic
Great reed warbler	Natural	36	Great reed warbler eggs	100	Hungary
Blackbird	Experimental	10	Blackbird eggs	80	Hungary
Song thrush	Experimental	3	Song thrush chicks	33	Czech Republic
Song thrush[a]	Experimental	10	Song thrush eggs	10	Czech Republic
Song thrush	Experimental	4	Reed warbler eggs	0	Czech Republic
Fieldfare	Experimental	3	Fieldfare eggs	0	Norway
Fieldfare	Experimental	3	Fieldfare chicks	0	Norway

[a] Includes six natural active song thrush nests and four old thrush nests attached at the top of active reed warbler nests (nest owners always accepted the change immediately as evidenced by video-recordings).

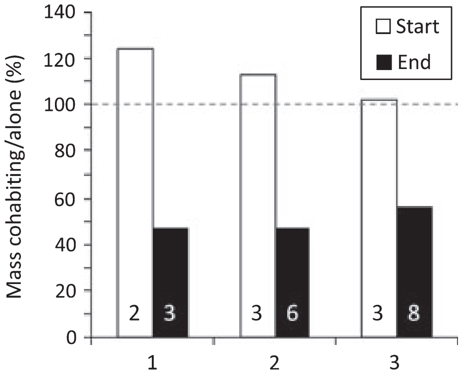

Fig. 1. Relative mass growth differences between cuckoo chicks in song thrush nests either cohabiting with host chicks or raised alone (in the latter treatment, we removed host eggs to eliminate possible confounding effects of costs of eviction; see Grim *et al.* 2009a). Shown are data for three successful (i.e. not depredated) experiments. Each cohabiting cuckoo chick was matched for age (at the start of experiment) and mass with one cuckoo chick raised alone (see Methods in Grim *et al.* 2009a). At the start of the experiment (i.e. when cuckoo chicks were cross-fostered from reed warbler nests, where they hatched, into song thrush nests; *open bars*), the cohabiting chicks had a slightly larger mass than lone chicks (as evidenced by mass ratio of 'cohabiting'/'alone' > 1). At the end of the experiment (i.e. when cohabiting cuckoo chicks died; *full bars*), the cohabiting cuckoo chicks had dramatically lower masses (by *c.* 50%) than their matched lone chicks (the last mass values for cohabiting chicks were measured when chicks were still alive and host parents were present at the nests as evidenced by video-recordings). Inset numbers show chick ages within each matched pair. The horizontal line shows the expectation of observed mass ratios under the null hypothesis of no costs of cohabitation with host chicks.

Cuckoo chicks evicted host song thrush chicks at only one nest (Table 2), but the cuckoo accomplished the task when 7 days old – much later than under normal conditions and just before the eviction instinct naturally ceases (Grim *et al.* 2009a). Thus, it probably was the result of nestling competition and not eviction *per se* (see Moskát & Hauber 2010). Also, this apparently successful evictor suffered from poor growth (chick no. 3 in Fig. 1) and soon after evicting the last (3rd) host chick it died.

Observed differences in eviction success across hosts make sense in the context of nest cup architecture. Mean nest depths (cm) decreased in the order fieldfare (6·86 ± 0·13, n = 36), song thrush (6·70 ± 0·11, n = 61), blackbird (6·32 ± 0·07, n = 144) and redwing (5·65 ± 0·09, n = 39). The differences were significant except between the fieldfare and song thrush (R^2 = 0·16, $F_{3,279}$ = 15·76, P < 0·0001; Tukey–Kramer HSD: P < 0·05). The index of nest cup steepness decreased in a similar order: song thrush (0·73 ± 0·01), fieldfare (0·66 ± 0·01), redwing (0·65 ± 0·01) and blackbird (0·63 ± 0·01). Song thrush nests cups were significantly steeper than those of the three other species which in turn did not differ from each other (R^2 = 0·15, $F_{3,279}$ = 16·55, P < 0·0001; Tukey–Kramer HSD: P < 0·005).

In line with the significant differences in nest sizes and shapes among thrushes, cuckoo chicks were significantly less successful in evicting at least one host egg from deep and steep song thrush and fieldfare nests (2 out of 13 chicks) in comparison to shallower and less steep blackbirds nests (eight out of eight chicks; χ^2_1 = 17·90, P < 0·0001). Overall, thrushes showed deeper (but not steeper) nest cups than common hosts (Fig. S1).

EXPERIMENTS – CHICKS: GROWTH AND SURVIVAL

Cuckoo chicks raised alone in the nests of song thrushes survived successfully to fledging (Table 3) and also grew at the highest rates observed among cuckoo hosts (Grim 2006a). This rejects diet composition as a possible explanation for absence of cuckoo parasitism in song thrushes. In striking contrast, cuckoo chicks that cohabited nests with song thrush nestlings fared extremely poorly – all decreased their growth rates to 50% in comparison to lone chicks (Fig. 1), and all died within a week after being cross-fostered to song thrush nests (Table 3).

Table 3. Survival of cuckoo chicks under natural and experimental conditions. We either removed host eggs (alone) or added cuckoo chicks into nests with freshly hatched host chicks (cohabiting). 'Survival age' is the number of days (mean and range) the cuckoo chicks survived until either fledging or death caused by parental desertion. In the *blackbird* nests in the Czech Republic, cuckoo chicks were either deserted (1) or predated (2), but the latter chicks grew very poorly and similarly to deserted chicks (thus, the predated chicks would most likely die anyway even in the absence of predation; see Grim 2006a). In the *song thrush* nests, lone cuckoos either survived to fledging (1) or were predated (2), but the latter chicks grew until predation similarly to fledged chicks (thus, the predated chicks did not suffer from host discrimination; see Grim 2006a)

Host	Treatment	Sample size (nests)	Survival age (days)	Survival rate (%)	Locality
Blackbird	Alone	4	1·75 (1–2)	0	Hungary
Blackbird (1)	Alone	2	4·0 (2–6)	0	Czech Republic[a]
Blackbird (2)	Alone	4	6·5 (3–13)	0	Czech Republic[a]
Song thrush	Cohabiting	3	5·7 (3–8)	0	Czech Republic
Song thrush (1)	Alone	3	18·3 (18–19)	100	Czech Republic[a]
Song thrush (2)	Alone	3	7·0 (2–11)	100	Czech Republic[a]
Fieldfare	Cohabiting	3	5·7 (1–12)	0	Norway
Redstart	Alone	16	19·3 (17–22)	100	Finland[b]
Redstart	Cohabiting	9	22·8 (11–26)	44	Finland[b]

[a] Data from Grim 2006a.
[b] Data from Grim *et al.* (2009a,b).

We found some interspecific variability in host responses to parasitic chicks. Both fieldfares and song thrushes did not show outright rejection of cuckoo chicks: fosterers fed both parasitic and own chicks, but cuckoos grew poorly in both song thrush (Fig. 1) and fieldfare (Fig. S2) nests and none survived (Table 3). In contrast, three out of four blackbird fosterers in Hungary did not feed the lone cuckoo chick at all and abandoned the nest, so cuckoos died. In another case, we found the cuckoo chick under the nest, but the female blackbird continued to brood her eggs just before hatching. Chicks survived only for 1 or 2 days (one and three chicks, respectively). Also in the Czech Republic, cuckoo chicks failed in nests of blackbirds even in the absence of the hosts own chicks: cuckoos grew very poorly, and out of six transferred chicks, none survived until fledging (also depredated nestlings grew very poorly before predation and similarly to nonpredated chicks; Grim 2006a). In contrast, lone blackbird chicks always ($n = 7$) survived well in blackbird nests until fledging (predated nests were excluded from analyses). These chicks included three cases where only one egg per clutch hatched (these chicks spent 11–13 days alone in their nests and successfully fledged) and another four cases when all chicks but one disappeared (fledged or died) extremely early (age: 9 days posthatch). The last chicks from the latter nests spent another 3–5 days alone in their nests. Thus, cuckoo chicks survival (0%, $n = 7$, excluding predated chicks) was significantly lower than that of lone own host blackbird chicks (100%, $n = 7$; Fisher exact test: $P = 0{\cdot}0006$).

Overall, cuckoo chicks in thrush nests suffered high mortality. These data come from song thrushes (Czech Republic), fieldfares (Norway) and two geographically distinct populations of blackbirds (Czech Republic, Hungary). We did not collect more data because of ethical considerations; moreover, despite modest sample sizes (overall $n = 22$), the results were clear-cut (see Taborsky 2010).

Discussion

The present study aimed to solve a long-standing ecological and evolutionary conundrum 'Why are thrushes, extremely common birds constructing highly conspicuous nests, avoided by the cuckoo?' In general, hosts can evade parasitism by cuckoos at three breeding stages – egg-laying, incubation and nestling stages (Davies 2000). Both comparative and experimental evidence concurred that the apparent absence of cuckoo parasitism in thrushes was not caused by host defences during the first two stages or by unsuitable host life-history traits. The negligible rates of observed cuckoo parasitism in thrushes were not explained by host aggression (excluding parasites from approaching host nests). Rapid ejection of foreign eggs was also excluded as an explanation, because experimentally parasitized thrushes did not reject eggs faster than current cuckoo hosts. Moreover, thrushes did not remove all foreign eggs, even highly nonmimetic ones, from their nests. In contrast, we demonstrated that a specialized 'thrush' cuckoo host race cannot establish itself because of problems experienced by the parasite at the chick stage. In some thrushes (song thrush, fieldfare), nest design hindered eviction success. Thus, cuckoo chicks were forced to compete with host hatchlings and suffered from decreased growth and high mortality. In other thrushes (blackbirds), hosts were unwilling to care even for lone cuckoo chicks.

IMPORTANCE OF THE CHICK STAGE

The great majority of studies on brood parasitism focused on host–parasite interactions at egg stages – aggression to adult parasites and host responses to eggs (see Grim 2007). Inevitably, host–parasite interactions at the chick stage remained neglected (reviewed in Grim 2006b). The present study highlights the importance of studying the chick stage because cuckoos seem to be impeded from colonizing thrushes as hosts exactly at the chick stage, but not at laying or incubation stages.

In contrast to some traditional explanations, neither large host body (Moksnes *et al.* 1991) nor egg (Kleven *et al.* 1999) sizes seemed to prevent cuckoo parasitism in thrushes. This is because cuckoo chick eviction attempts succeeded in nests of large hosts (blackbirds), but failed in nests of smaller hosts (song thrushes, fieldfares). Furthermore, and counterintuitively, host hatchling size is also not an important factor because (i) it is nest design that constrains eviction success, and (ii) cuckoo chicks are poor competitors irrespective of the size of fosterer chicks (Grim *et al.* 2009a). This raises a possibility that nest cup design in some thrushes evolved as an adaptation to prevent nest-mates eviction by cuckoo chicks. Although the special plaster-like lining of song thrush nests may contribute to hinder eviction success it is not necessary: cuckoo chicks were also unable to evict host eggs/young from fieldfare nests that are lined with grasses. Therefore, the nest size *per se* is sufficient to prevent cuckoo chicks from evicting nest contents. In fact, birds in 16 families/subfamilies (close to 5% of the world's birds) use mud in building their nests, and most of them are not victimized by evicting parasites (Rowley 1971). Special plaster-like lining in the song thrush may enhance nestling survival by rendering conditions inside the nest unsuitable for *insect* ectoparasites (Reichholf 2003).

Both song thrushes and fieldfares were willing to feed cuckoo chicks while blackbirds seemed to feed cuckoo chicks only at low rates (Czech Republic) or refused to feed them completely (Hungary). Additionally, Glue & Morgan (1972) reported 'nest abandonment' as a reason for cuckoo failure in blackbird nests in Britain. Lowered feeding rates of parasite chicks were documented in several hosts of other brood parasites (Lichtenstein 2001; Payne, Woods & Payne 2001). Importantly, blackbirds were willing to care for lone own chicks, sometimes for extensive periods from hatching till fledging. Thus, death of lone cuckoo chicks in blackbird nests seems not to be explained as a host strategy to desert any single-chick broods (Langmore *et al.* 2003). The evolutionary origin of low blackbird willingness to care for cuckoo chicks remains an exciting subject for future work (see also Grim 2006b).

A traditional explanation for absence of regular cuckoo parasitism in thrushes was 'unsuitable diet' (Moksnes *et al.* 1991). Cuckoo nestlings are never fed earthworms or food items of similar size by their most common hosts, reed warblers (Grim & Honza 2001a) and other regular hosts (Fig. S1). Interestingly, although this comparative evidence suggests that diet is an important factor explaining absence of cuckoo parasitism in thrushes, the experimental and observational data reject this hypothesis. Cuckoo chicks in song thrush nests were fed mainly with large earthworms and still achieved higher growth rates than in reed warbler nests (Grim 2006a). Cuckoo chicks may digest considerable amounts of plant diet (grapes; Martín-Gálvez *et al.* 2005), and Mikulica (1993) even observed cuckoo chicks fed with 'voles and bird nestlings in the nests of the red-backed shrike'. Still, more experimental studies are needed to test whether some particular host species or even populations specialize on diet that is indigestible for parasite chicks.

All comparative and experimental data combined strongly suggest that thrushes were not involved in long-term co-evolution with the cuckoo. This conclusion raises the question of what is the origin of egg discrimination in thrushes. That question is beyond the scope and aims of this study. In general, egg rejection could be because of conspecific parasitism, interspecific parasitism (or both) and/or nest sanitation (Honza *et al.* 2005). This provides an interesting subject for future studies. Whatever the evolutionary cause of egg rejection in thrushes, our conclusion remains the same – low or intermediate egg rejection rates and low rates of aggression to adult cuckoos cannot explain virtual absence of cuckoo parasitism in thrushes.

THRUSHES: SUITABLE OR UNSUITABLE CUCKOO HOSTS?

So far, there has been an inconsistency in classifying thrushes into 'suitable' (Davies & Brooke 1989) or 'unsuitable' (Moksnes *et al.* 1991) categories of hosts. Our data support the latter view. Nevertheless, there are anecdotal observations of cuckoo nestlings successfully fledging from nests of various *Turdus* species (Glue & Morgan 1972). Still, nonzero reproductive success of 'thrush' cuckoos cannot lead to the conclusion that thrushes are suitable hosts. The important variable is the difference between cuckoo reproductive success in the nest of a thrush and other available sympatric hosts. The extremely low breeding success of parasitic chicks in thrush nests indicates that these species are parasitized only by mistake or as a last resort when nests of main hosts are unavailable.

'Suitable hosts' and 'unsuitable hosts' are discrete categories. However, (un)suitability of hosts is a continuous variable: factors influencing the probability of successful recruitment from a host nest are continuous (host egg and nest size, food quality and quantity, etc.). This makes strict categorization of potential hosts difficult. However, selection by cuckoo females should lead to a preference for the most suitable hosts (Kleven *et al.* 1999), thus lowering parasitism rates in hosts of low or intermediate quality (De Mársico & Reboreda 2008). This could be the major evolutionary force behind the fact that some potentially suitable, but lower quality hosts are avoided by brood parasites (see also Jaenike 1990).

Røskaft *et al.* (2002) showed that a more detailed classification of host species (to five categories) can better explain the pattern of parasitism than the traditional suitable–unsuitable dichotomy. Interestingly, a category 'large nest and eggs' (thrushes) shows almost identical level of aggression against dummy cuckoos as the category 'seed eaters' and 'hole nesters' – birds that clearly cannot serve as cuckoo hosts. Thus, thrushes probably experienced similar selection pressures from cuckoos as the two latter categories.

In general, different thrush species prevented cuckoo parasitism at different stages. For example, song thrushes showed relatively low aggression and low rejection of alien eggs, but nest architecture (size) forced cuckoos to share the parental care with host young (which was fatal for young parasites). In contrast, blackbirds were more aggressive, rejected more alien eggs, but their nests were too shallow to prevent the cuckoo from evicting host progeny. Thus, in the case of successful eviction of their eggs/young by the cuckoo chicks, egg acceptors may adopt another line of defence: low willingness to care for lone alien chicks (alternatively, low willingness to feed the parasite chick may result from selection forces unrelated to brood parasitism, see Grim 2006b, but with the same consequences for the parasite; thus, evolutionary origin of such host behaviours does not have any bearing on the conclusions of the present study).

Fieldfare and blackbird clutches hatch asynchronously (Perrins 1998). These hosts start to incubate before clutch completion, thus parasitic eggs not laid very early in the host laying period could have low chances of hatching before host eggs. Molnár (1939) reported cases where the cuckoo chick hatched a few hours *after* the first great reed warbler nestlings; these cuckoo chicks were unable to evict their nestmates. Host nestlings started to grow faster than the cuckoo chicks in these nests, and all cuckoo chicks in these mixed broods died within 5 days posthatch. Recent studies showed that even competition with small passerine nestlings can have deleterious effects on cuckoo growth and survival (Soler 2002; Martín-Gálvez *et al.* 2005; Hauber & Moskát 2008; Grim *et al.* 2009b). Therefore, hatching asynchrony could decrease suitability of potential hosts irrespective of their body size.

These considerations suggest that comparing average traits of whole groups of species (common vs. rare/avoided hosts) and application of comparative methods (Soler *et al.* 1999) may mask real *species-specific* (vs. group-specific) differences in factors that affect the success of cuckoo parasitism.

Of course, it is always possible that cuckoos frequently parasitized thrushes a long time ago and that the anti-parasite responses we detected in thrushes represent evolutionary relics of adaptations against cuckoos that are no longer adaptive. Unfortunately, such a scenario is impossible to test. Moreover, it cannot explain *current* absence of parasitism. This is because relatively (in comparison to some current cuckoo hosts) weak anti-parasite defences would not prevent cuckoos from starting to parasitize thrushes again (assuming that thrushes were formerly used and later abandoned hosts).

Conclusions

Absence of a specialized 'thrush' cuckoo host race(s) cannot be explained by strong anti-parasite host defences at laying and incubation stages or by unusual host life-history traits. In contrast, it seems to be best explained by host–parasite interactions at the nestling stage. Importantly, although thrushes present superficially similar taxa, factors preventing successful cuckoo parasitism were species-specific. In song thrushes and fieldfares, nest size, rather than large egg size (as hypothesized previously; Kleven *et al.* 1999), turned out to be the primary impediment to egg eviction, forcing parasites to share parental care with host progeny. Owing to the low competitive ability of cuckoo chicks, parasites experienced an early death. In contrast, neither nest design nor competitive ability constrained cuckoo chicks' survival in blackbird nests – cuckoos successfully evicted host eggs but were soon deserted suggesting blackbirds possess traits that might serve as anti-parasite defences at the chick stage. In addition to these major factors, some others may additively decrease *Turdus* suitability as hosts, e.g. lower breeding success in some thrushes and/or larger eggs that incur larger energetic costs of eviction (Grim *et al.* 2009a). These results coupled with previous findings (e.g. there are no specialized cuckoo gentes parasitizing particular *Turdus* spp.) indicate that thrushes are poor quality hosts, were not regularly parasitized by cuckoos in the past and have not evolved specific adaptations against interspecific brood parasitism.

The aim of the present study was to answer a long-standing puzzle: 'Why do we hardly find any cuckoo eggs or chicks in nests of one of the most common and by far the most conspicuous forest passerines?' Still, our study highlights an important lesson for the study of host selection and resource use in general. To explain the rarity of parasitism in particular hosts, it was insufficient to consider single host traits in isolation and ignore other candidate traits as was done by virtually all previous studies. Furthermore, a comprehensive approach that tested host responses to all parasite developmental stages (eggs, nestlings, adults) proved to be more fruitful than the traditional single-stage (typically egg) approach. We predict that future studies of this fascinating phenomenon will reveal effects of various factors that additively and/or interactively (cf. Weidinger 2002) decrease overall reproductive success of parasites in the nests of particular hosts to levels that do not allow for long-term survival of parasite populations specialized on such hosts. Finally, future ecological studies of resource use might benefit from combining comparative and experimental approaches as exemplified by the present study.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Location of the study sites.

Fig. S1. Life-history traits of thrushes and common hosts.

Fig. S2. Cuckoo chick growth in the nest of the fieldfare.

Table S1. Predictions for differences in life-history traits between common hosts and thrushes.

Table S2. Statistical results of analyses of host anti-parasite responses.

Supplementary on-line material

Constraints on host choice: why do parasitic birds rarely exploit some common potential hosts?

Tomáš Grim, Peter Samaš, Csaba Moskát, Oddmund Kleven, Marcel Honza, Arne Moksnes, Eivin Røskaft and Bård G. Stokke

Journal of Animal Ecology, doi: 10.1111/j.1365-2656.2010.01798.x

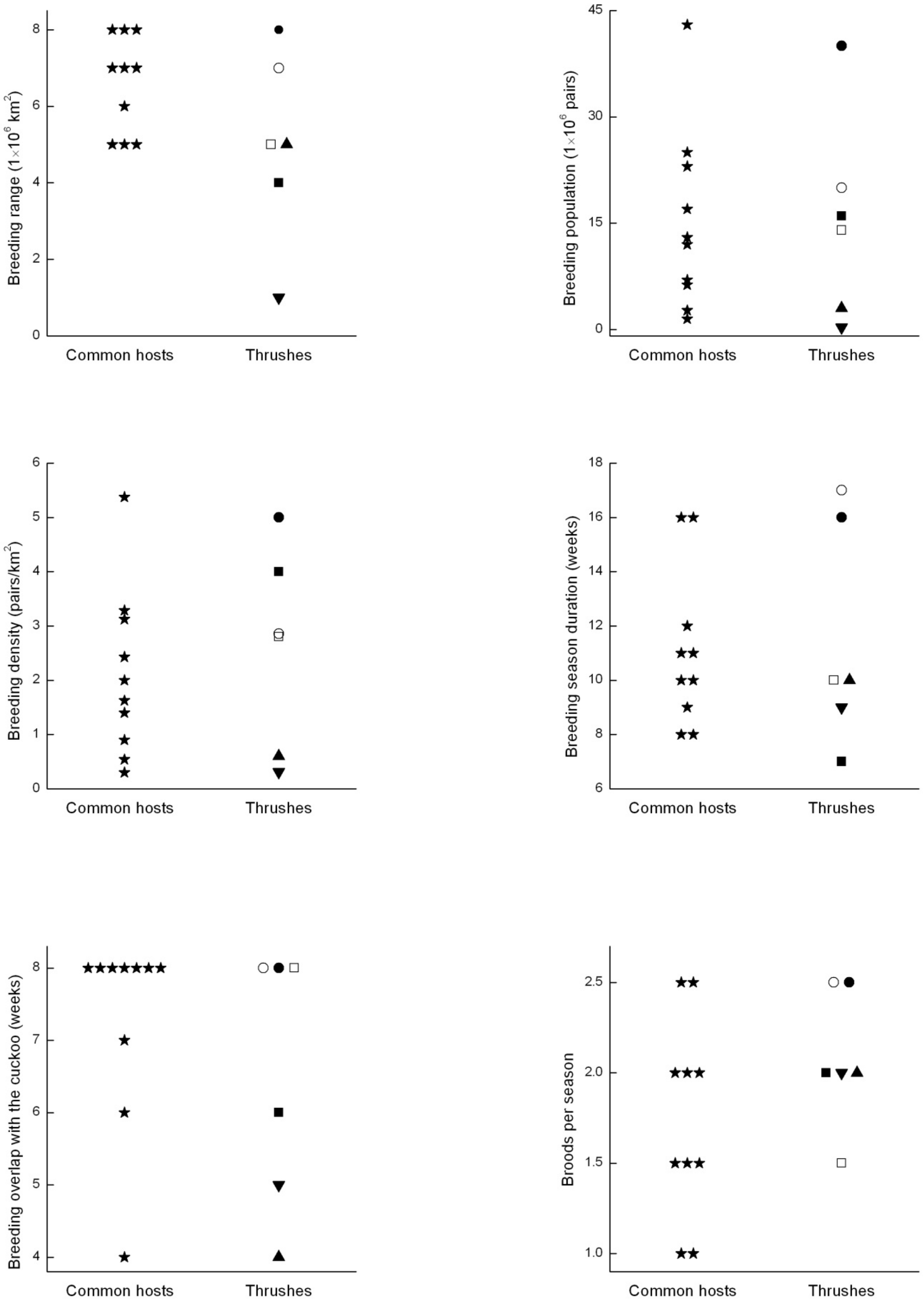
Appendix S1 Location of studied thrush populations living in sympatry or allopatry with cuckoos.

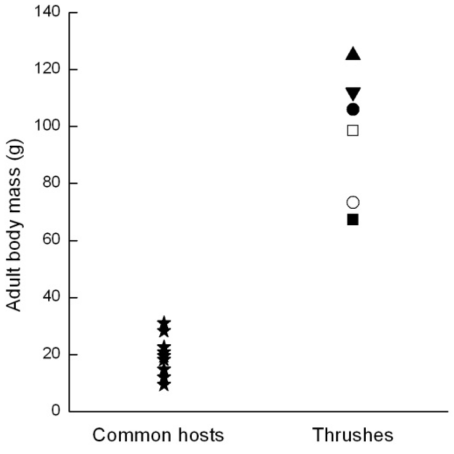
Study area	Country	Status	Latitude N	Longitude E
Buda hills	Hungary	sympatry	47° 01´	19° 00´
Budapest	Hungary	allopatry	47° 30´	19° 05´
Lužice	Czech Republic	sympatry	48° 51´	17° 04´
Brno	Czech Republic	allopatry	49° 12´	16° 38´
V. Knínice	Czech Republic	sympatry	49° 14´	16° 25´
Grygov	Czech Republic	sympatry	49° 32´	17° 19´
Olomouc	Czech Republic	allopatry	49° 35´	17° 15´
Rørkær	Denmark	sympatry	55° 25´	09° 14´
Lund	Sweden	allopatry	55° 42´	13° 10´
Oslo	Norway	allopatry	59° 55´	10° 45´
Tydal	Norway	sympatry	63° 04´	11° 34´
Stjørdal	Norway	allopatry	63° 27´	10° 57´

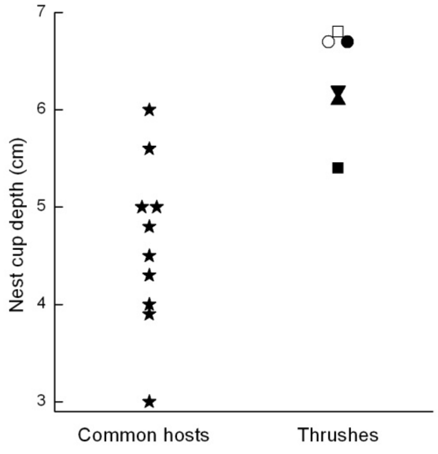
Figure S1. Variation in life-history traits between thrushes and common hosts: (a) general life-history traits, (b) specific parasitism-related traits (see Table S1 for explanation). Common hosts (★) include 10 most common hosts reported by Moksnes & Røskaft (1995). Thrushes include all 6 European members of genus *Turdus*: blackbird (●), song thrush (○), redwing (■), fieldfare (◘), mistle thrush (▲), ring ouzel (▼). We extracted data from literature (Perrins 1998; Soler, Møller & Soler 1999; N. B. Davies, pers. comm.; M. Martín-Vivaldi, pers. comm.). In some cases information on the particular trait was not available (e.g., latency to egg rejection in the dunnock *Prunella modularis* which is a pure acceptor of alien eggs).

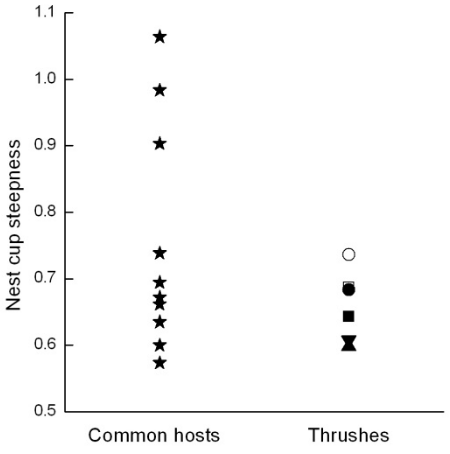
Figure S2. Cuckoo chick growth in the nest of the fieldfare. The cuckoo hatched one day before the host chick but was quickly overgrown by the host nestling. The latter fledged at a typical age of 13 days post-hatch whereas the cuckoo suffered from poor growth and died at the age of 16 days at extremely low mass.

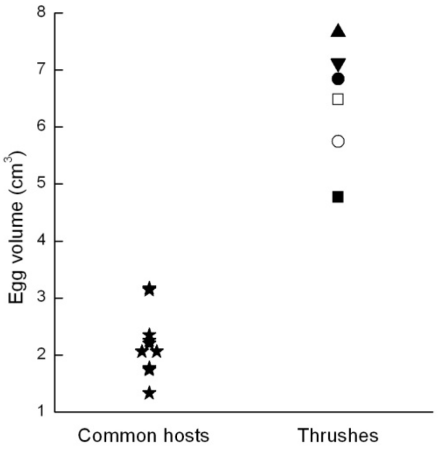
Figure S1a

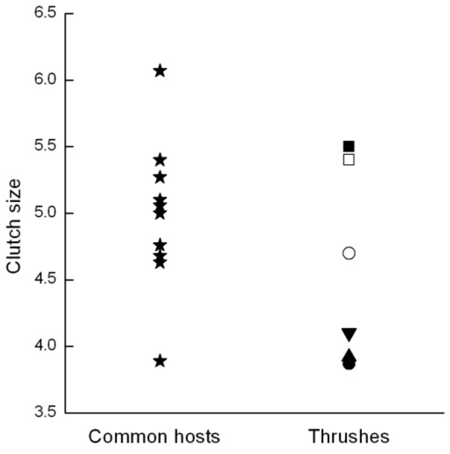

Breeding range (1×10^6 km^2)
0
2
4
6
8
Common hosts
Thrushes
Breeding population (1×10^6 pairs)
0
15
30
45
Common hosts
Thrushes
Breeding density (pairs/km^2)
0
1
2
3
4
5
6
Common hosts
Thrushes
Breeding season duration (weeks)
6
8
10
12
14
16
18
Common hosts
Thrushes
Breeding overlap with the cuckoo (weeks)
4
5
6
7
8
Common hosts
Thrushes
Broods per season
1.0
1.5
2.0
2.5
Common hosts
Thrushes

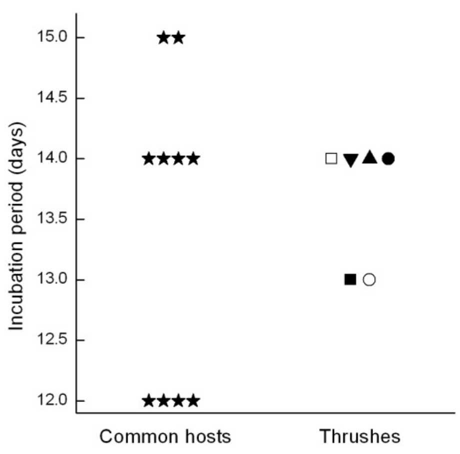

Adult body mass (g)
0
20
40
60
80
100
120
140
Common hosts
Thrushes

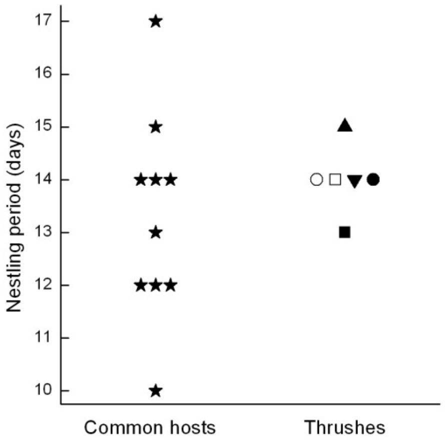

Nest cup depth (cm)
3
4
5
6
7
Common hosts
Thrushes

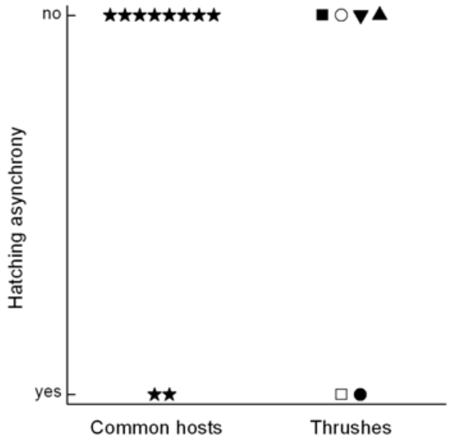

Nest cup steepness
0.5
0.6
0.7
0.8
0.9
1.0
1.1
Common hosts
Thrushes

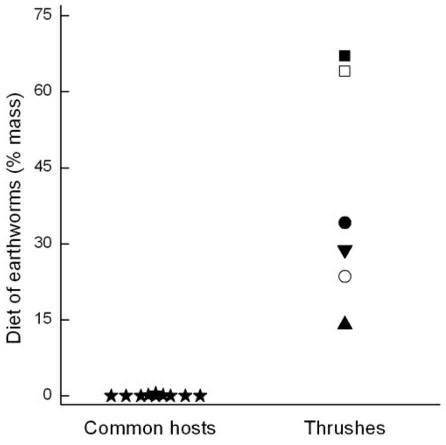

Egg volume (cm^3)
1
2
3
4
5
6
7
8
Common hosts
Thrushes

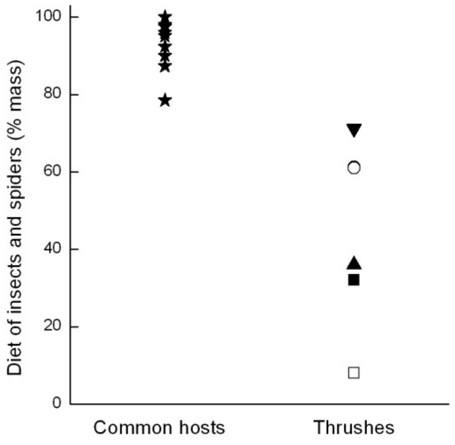

Clutch size
3.5
4.0
4.5
5.0
5.5
6.0
6.5
Common hosts
Thrushes


Incubation period (days)
12.0
12.5
13.0
13.5
14.0
14.5
15.0
Common hosts
Thrushes


Nestling period (days)
17
16
15
14
13
12
11
10
Common hosts
Thrushes


Hatching asynchrony
no
yes
Common hosts
Thrushes


Diet of earthworms (% mass)
75
60
45
30
15
0
Common hosts
Thrushes


Diet of insects and spiders (% mass)
100
80
60
40
20
0
Common hosts
Thrushes

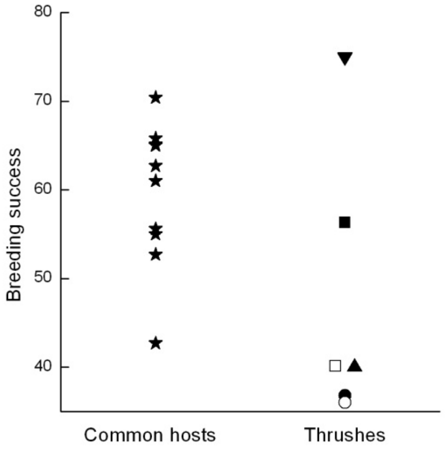
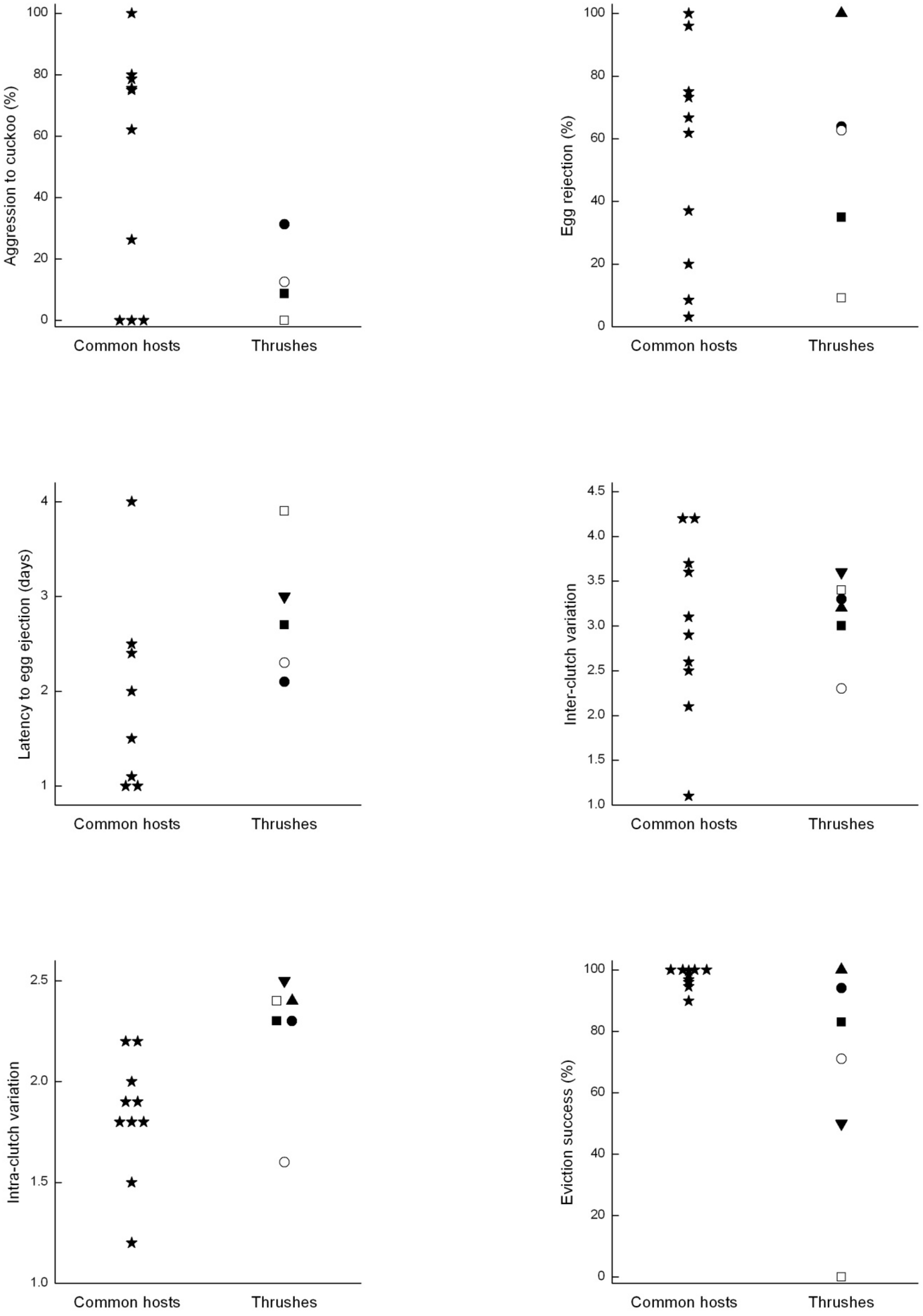

Breeding success
80
70
60
50
40
Common hosts
Thrushes

Figure S1b


Aggression to cuckoo (%)
0
20
40
60
80
100
Common hosts
Thrushes
Egg rejection (%)
0
20
40
60
80
100
Common hosts
Thrushes
Latency to egg ejection (days)
1
2
3
4
Common hosts
Thrushes
Inter-clutch variation
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Common hosts
Thrushes
Intra-clutch variation
1.0
1.5
2.0
2.5
Common hosts
Thrushes
Eviction success (%)
0
20
40
60
80
100
Common hosts
Thrushes

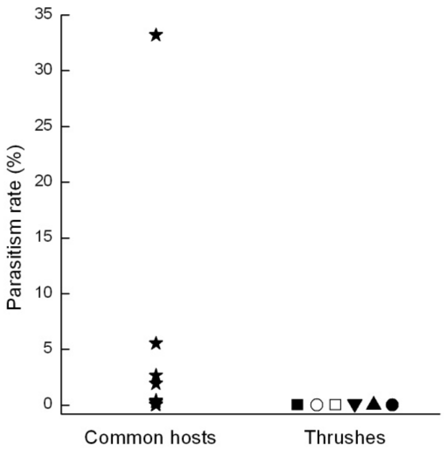

35
30
25
20
15
10
5
0
Parasitism rate (%)
Common hosts
Thrushes

Figure S2

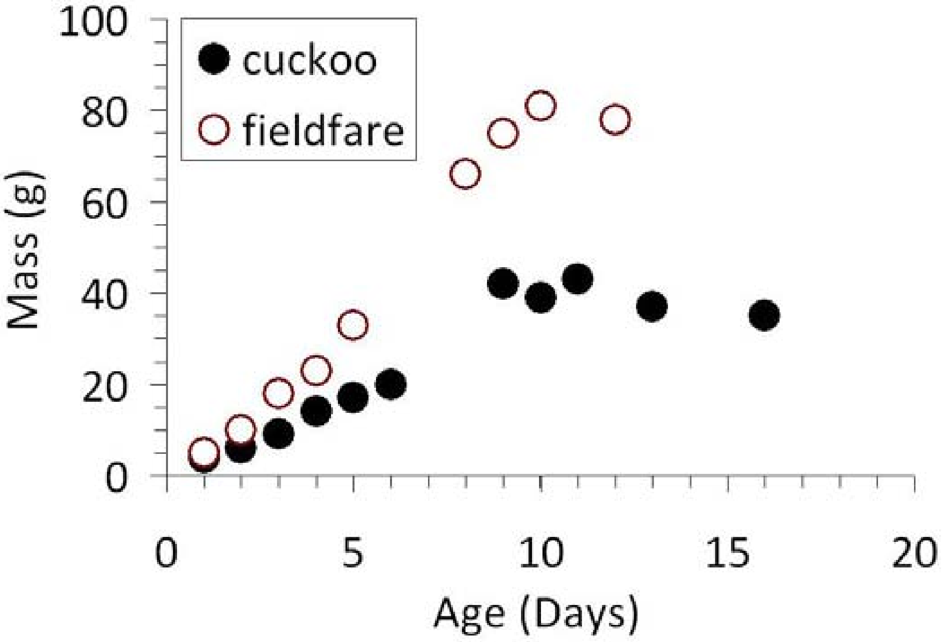

100
80
60
40
20
0
Mass (g)
0
5
10
15
20
Age (Days)
cuckoo
fieldfare

Table S1 Predictions for differences in candidate life-history characteristics between thrushes and regular cuckoo hosts. "Predicted differences" under the hypothesis that the particular factor explains the absence of cuckoo parasitism in thrushes. "–" = predicted lower value of the trait in thrushes than in common hosts, "+" = the opposite. "**General life-history traits**" may cause primary unsuitability of hosts for exploitation by parasites (such traits prevent host-parasite co-evolution). "**Specific parasitism-related traits**" may cause secondary unsuitability and represent host defences previously evolved due to brood parasitism (such traits do not enable parasites to colonize such hosts again). For rationales behind each hypothesis see sources in "References" column.

Trait	Predicted difference	References
General life-history traits		
Breeding range	–	Soler, Møller & Soler (1999)
Breeding population	–	Soler, Møller & Soler (1999)
Breeding density	–	Soler, Martín-Vivaldi & Møller (2009)
Duration of the breeding season	–	Soler, Møller & Soler (1999)
Overlap of breeding with the cuckoo	–	Honza, Kuiper & Cherry (2005)
Broods per season	–	Soler, Møller & Soler (1999)
Adult body mass	+	Soler, Møller & Soler (1999)
Nest cup depth	+	Grim *et al*. (2009a)
Nest cup steepness (depth/diameter ratio)	+	Grim *et al*. (2009a)
Egg volume	+	Moksnes *et al*. (1991)
Clutch size	+	Soler, Møller & Soler (1999)
Incubation period duration	–	Soler, Møller & Soler (1999)
Nestling period duration	–	Soler, Møller & Soler (1999)
Hatching asynchrony (presence)	+	Soler (2002)
Diet – insects + spiders	–	Grim & Honza (2001)
Diet – earthworms	+	Grim (2006)
Breeding success	–	Avilés et al. (2006)
Specific parasitism-related traits		
Aggression to cuckoo	+	Røskaft *et al*. (2002)
Egg rejection	+	Honza *et al*. (2004)
Latency to egg ejection	–	Honza *et al*. (2004)
Responses in sympatry vs. allopatry	S > A	Stokke *et al*. (2008)
Inter-clutch variation	+	Øien, Moksnes & Røskaft (1995)
Intra-clutch variation	–	Øien, Moksnes & Røskaft (1995)
Eviction success	–	Grim *et al*. (2009a,b)

Table S2 Responses by thrushes to simulated brood parasitism. Host responses include aggression to stuffed dummies of the common cuckoo and the hooded crow, rejection of parasitic model eggs (blue and spotted) and latencies to egg rejection in sympatry and allopatry with the cuckoo. For effect sizes see Table 1. For statistical and experimental procedure details see Methods. *d.d.f.* = denominator degrees of freedom. Nominator degrees of freedom = 1 in all cases except "nest stage" that had 4 *d.f.* in aggression analyses and 2 *d.f.* in egg rejection and latency analyses).

Predictor	Blackbird			Song thrush			Redwing			Fieldfare		
	d.d.f.	*F*	*P*	*d.d.f.*	*F*	*P*	*d.d.f.*	*F*	*P*	*d.d.f.*	*F*	*P*
Aggression												
Geography	160	0.22	0.64	114	1.04	0.31	120	2.09	0.15	138	0.00	1.00
Dummy	161	3.97	0.05	113	0.52	0.47	121	39.07	<0.0001	139	0.00	0.97
G*D	136	0.70	0.41	79	0.92	0.34	.	.	.	.	.	.
Clutch	137	0.11	0.74	94	2.09	0.15	105	2.47	0.12	118	0.12	0.73
Nest stage	156	1.43	0.23	90	2.20	0.08	102	0.79	0.50	115	0.05	0.98
Laying date	148	0.12	0.73	80	0.00	0.98	101	0.09	0.77	137	0.95	0.33
Egg rejection												
Geography	128	0.01	0.93	71	2.18	0.14	144	1.87	0.17	67	2.50	0.12
Egg model	129	2.47	0.12	72	6.00	0.02	143	0.93	0.34	66	0.08	0.77
G*E	121	3.15	0.08	67	0.00	0.95	.	.	.	.	.	.
Clutch	123	0.50	0.48	69	0.15	0.70	137	0.01	0.94	62	0.56	0.46
Nest stage	127	2.47	0.12	70	0.18	0.67	141	0.35	0.55	65	1.85	0.18
Laying date	122	0.27	0.61	68	0.04	0.85	.	.	.	64	0.56	0.46
Latency to egg rejection												
Geography	91	6.43	0.01	54	1.49	0.23	52	1.78	0.19	13	0.37	0.55
Egg model	90	1.41	0.24	53	1.41	0.24	51	0.46	0.50	14	4.64	0.049
G*E	84	0.72	0.40	49	1.36	0.25	.	.	.	.	.	.
Clutch	86	0.99	0.32	51	0.05	0.83	45	0.04	0.84	14	6.91	0.02
Nest stage	85	0.58	0.45	50	0.01	0.94	50	0.34	0.56	12	0.16	0.70
Laying date	89	0.18	0.68	52	1.97	0.17	49	0.19	0.67	14	11.84	0.004

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Příspěvek III.

Igic B., Cassey P., Samaš P., Grim T. & Hauber M.E. 2009: Cigarette butts form a perceptually cryptic component of song thrush (*Turdus philomelos*) nests. Notornis 56(3): 134–138.

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Cigarette butts form a perceptually cryptic component of song thrush (*Turdus philomelos)* nests

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Abstract Bird nests often contain objects produced and manipulated by other animals, including human rubbish. The function, if any, of these items remains unclear, and it is unknown whether they might serve a signalling role to increase the conspicuousness of the nest lining or contribute to its crypsis. We located several nests of the introduced song thrush (*Turdus philomelos*) in New Zealand containing discarded cigarette butts. These items were embedded into the dried mud-matrix of the nest and appeared visually inconspicuous to the human observer. However, human and avian visual sensitivities are dramatically different. We used full-spectrum reflectance spectrophotometry, combined with perceptual modelling of the avian visual system to assess the contrast between mud lining, garbage, and the colours of thrush eggs. Our analyses confirmed that, when perceived by birds, cigarette butts were similar in appearance to the nest lining and showed sharp contrast with the eggs. We suggest that cigarette butts form an opportunistic structural component of the song thrush nest. It remains to be determined whether human-made objects in song thrush nests serve anti-predator or an olfactory signalling function. This study illustrates the application of avian perceptual modelling to test signalling based hypotheses for the extended phenotype of birds, including nest architecture.

Igic, B.; Cassey, P.; Samas, P.; Grim, T.; Hauber, M.E. 2009. Cigarette butts form a perceptually cryptic component of song thrush (*Turdus philomelos)* nests. *Notornis 56(3):* 134-138.

Key words avian vision, nest architecture, perceptual modelling, spectrophotometry, waste

INTRODUCTION

Birds are well known to be proficient nest builders and the most important function of nests is to provide protection for developing offspring (Collias 1964). The need for protection and thermoregulation is thought to be a critical factor driving selection on nest location, and the shape, composition, and size of nests (Hansell 2000). Selection should thus favour nest building behaviour that provides optimal breeding conditions in particular habitats (Mertens 1977).

The use of specific nesting materials can increase concealment or crypsis (Solis & de Lope 1995), but in some cases they may also serve as anti-predatory material to keep away predators (e.g. snake sloughs and carnivore scat; Strecker 1926; Schuetz 2005). Other functions include protection against ectoparasite infestations through the incorporation of odorous leaves and moss (Clark & Mason 1988; Banbura *et al.* 1995; Lambrechts & Santos 2000), and the regulation of nest microclimate through the use of fine grasses, feathers, and mammalian fur (Bartholomew *et al.* 1976; Blem & Bleam 1994; Winkler 1993; Lombardo *et al.* 1995). Finally, nest building and the resulting size of the nest can also

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be associated with courtship behaviour and serve as a sexually selected trait; in turn, signalling to conspecifics, including mates, about the quality and quantity of individual reproductive investment into the pair-bond and the breeding attempt is predicted to be beneficial in species with biparental care (Soler *et al.* 1998; Brouwer & Komdeur 2004).

These alternative, though not mutually exclusive hypotheses for the adaptive use of nest materials may be classed into either non-signalling or signalling functions. Non-signalling roles include the structural and physical functions of the materials, while the sensory appearance (e.g., odour, colour, texture) of the nest materials may contribute to a signalling function (Brouwer & Komdeur 2004). Birds normally incorporate a variety of plant and animal materials into the nest structures (Kessel 1957; Collias 1964; Briel & Moyle 1976; van Ripper 1977; Collias 1986; Nadkarni & Matelson 1989), and in some situations, birds also use human rubbish to build nests. It is unknown whether human-made objects function either as a structural element or play a signalling role.

A specific prediction of any conspecific hypothesis invoking signalling to mates about the content and the structure of the nest is that sensory cues of the particular nesting material be perceivable and discriminable by the intended receiver. We tested whether cigarette butts form a conspecifically conspicuous element of material in the nests of introduced songbird, the biparental song thrush (*Turdus philomelos*) in New Zealand. We modelled the perceptual ability of thrushes to discriminate cigarette butts incorporated into the nest's inner lining from the colouration of the eggs and the rest of the interior nest lining. Such a perceptual approach is a first step in testing the assumptions of whether human objects play a signalling role in song thrush nests. Human-based estimates of conspicuousness, crypsis, and mimicry may be poorly applicable to foreign objects in bird nests because the avian visual system differs from visual systems of humans and other mammals in relation to the number of photoreceptors, the types of photoreceptors and sensitivity of photoreceptors (Hunt *et al*. 2009).

METHODS

Field observations

We located song thrush nests in the Auckland Botanic Gardens (37° 0′ 46.8″ S, 174° 54′ 27″ E) and Meola Reef Reserve (36° 51′ 3.3″ S, 174° 42′ 38.15″ E), New Zealand during the 2007–2008 austral breeding season. Both are urban sites. We inspected the contents of active song thrush nests and recorded the proportion of nests that had cigarette butts incorporated into the lining of the nest interior.

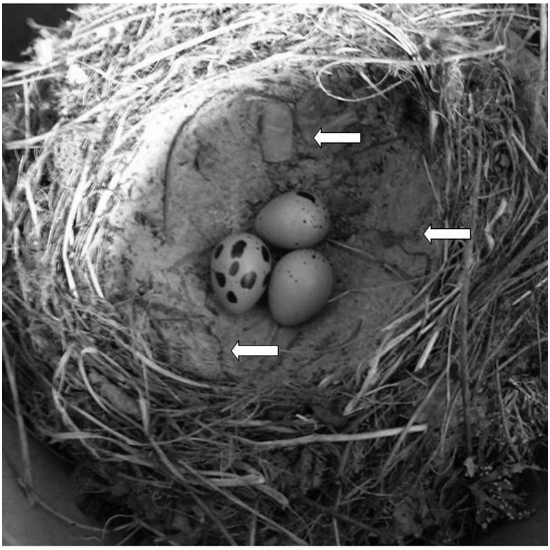

Fig. 1. Song thrush nest and eggs (1 manipulated and 2 control) from which nest background and cigarette reflectance measurements were taken. White arrows indicate the positions of the three cigarette butts incorporated into interior nest matrix. Photo credit: Mark Hauber.

Song thrush nests are very suitable for rapid visual inspection, especially in comparison with the congeneric European blackbird (*T. merula*), because the interior is lined with a relatively uniform layer of mud and plant fibres, making the presence of foreign material in the lining obvious (Fig. 1).

An additional song thrush nest was located in the Rose Garden on the grounds of the University of Auckland, City Campus, Auckland, New Zealand (36° 51′ 2″ S, 174° 46′ 4″ E) in Nov 2007. The nest was the subject of an egg painting experiment for a separate study, resulting in an egg painted with a Sharpie™ felt tip black pen. Following completion of the study, the nest was collected for spectrophotomeric analysis in the lab. The nest cup interior contained 3 pieces of cigarette butts incorporated into the interior nest lining (Fig. 1). The nest was stored in a dark place at room temperature until analysis.

Reflectance spectra measurements

Reflectance measurements of eggs, the mud lining, and cigarette butts were taken using an Ocean Optics USB2000 Miniature Fiber Optic Spectrometer, illuminated by a DT mini-lamp and OOIBase32TM operating 136 software (Ocean Optics, Inc., Dunedin, FL, USA). We used a probe with a custom built cap lined with black velvet so that it was maintained at a 90° angle and omitted all outside light sources. Three measurements were taken at randomly chosen locations on the nest background.

Table 1. Mean values for ΔS (or the just noticeable differences: JND for chromatic contrasts) ± standard error.

JND	Nest	Egg
Cigarette	1.29 ± 0.29	1.96 ± 0.17
Sharpie Pen	4.72 ± 0.51	1.99 ± 0.11
Nest	–	3.19 ± 0.23

Table 2. Mean values for ΔfQ (or the just noticeable differences: JND for achromatic contrasts) ± standard error.

JND	Nest	Egg
Cigarette	1.18 ± 0.55	2.59 ± 0.45
Sharpie Pen	3.17 ± 1.31	0.81 ± 0.19
Nest	–	3.77 ± 0.67

A single measurement (*ca.* 2 mm diameter) was taken on a random location along the length of each cigarette for a total of 3 cigarette measurements, and 3 measurements were taken on random equatorial locations on each of the 3 eggs found in the nest. Measurements were taken at 0.48 nm intervals in the avian-visible, ultra-violet (UV) inclusive range of 300–700 nm. White and dark standard reflection calibration measurements were taken between each set of 3 reflectance measurements using the Ocean Optics WS-1 diffuse reflectance standard and a custom-built black box, respectively.

Perceptual modelling

To model the avian visual perception of the cigarettes and estimate the ability of a song thrush to discriminate a cigarette from the nest background, we employed the approach developed by Vorobyev & Osorio (1998) for tetrachromatic vision of UV sensitive birds (300–700 nm) by using Avicol v2 software (Gomez 2007; Doutrelant *et al.* 2008). Due to the lack of published data on the song thrush cone sensitivity, sensitivity data used in the analysis were those for a close relative, the European blackbird (Cassey *et al.* 2008). Sensitivity data were extracted from published information in Hart *et al.* (2000) using VistaMetrix v1.3 (SkillCrest, LLC., available from http://www.skillcrest.com/) within the visual range between 330–700 nm. As the Avicol software requires a full set of sensitivity data between 300–700 nm, photoreceptor absorbance between 300–330 nm was set as zero. The song thrush is an open-cup nesting species, and so to simulate natural nest illumination, open nest irradiance spectra were extracted from the published information in Avilés *et al.* (2008) in the 300–700 nm range.

The Weber fraction for the long-wave sensitive cone (LWS) used in the model was set at 0.1 (Vorobyev *et al.* 1998) and the signal to noise ratio used in the calculations was independent of the signal and was based on neural noise only ($e_i = \omega / \sqrt{\eta_i}$; Vorobyev & Osorio 1998). A ratio of UVS 1: SWS 2: MWS 3: LWS 3 for the density of the 4 avian photoreceptor classes was assumed (see Stuart-Fox *et al.* 2008; Vorobyev & Osorio 1998). We assumed the achromatic contrast was based on the sum of the spectra of MWS and LWS cones, because the sensitivities of these photoreceptors are similar to that of rod and principle double cone cells (Hart *et al.* 1998; 2000).

The Avicol software extracts the bird's ability to distinguish, for instance, a cigarette butt from the nest background as the perceptual distance between the two spectra (ΔS) or the 'just noticeable difference' (JND). A JND greater than 1.0 indicates that a bird is able to distinguish the colour of the cigarette from the colour of the nest interior (Osorio & Vorobyev 1996). The software also estimates the ability of a bird to distinguish the brightness of a cigarette butt from that of the nest background as a JND for the achromatic contrast (ΔfQ).

Achromatic mechanisms become increasingly important for discrimination in dimmer light conditions while chromatic mechanisms are more important in brighter environments (Vorobyev & Osorio 1998). Because the song thrush nest is an open cup and is more similar to a bright light environment, we would predict that the ability of birds to distinguish cigarette butts from the nest background would be mostly influenced by chromatic mechanisms. The average JNDs for pair-wise comparisons were therefore calculated by pairing egg colour spectra randomly for each cigarette, black-pen painted egg surface and nest background, to remove statistical independence violations. This was then repeated for nest background spectra.

RESULTS

We found cigarette butts in 1 of 32 nests song thrush nests at the Auckland Botanic Gardens, and in 7 of 34 nests at Meola Reef Reserve (Fisher's exact test: P = 0.05). In total, 12% of song thrush nests had cigarette butts visible in the nest lining.

The cigarette and nest interior both appear to have a similar spectral curvature, as compared to the song thrush egg and the marker pen, indicating that the chromatic components of each object are similar (Fig. 2). In comparison, the cigarette butt has a greater total reflectance indicating that it is brighter than the nest interior background.

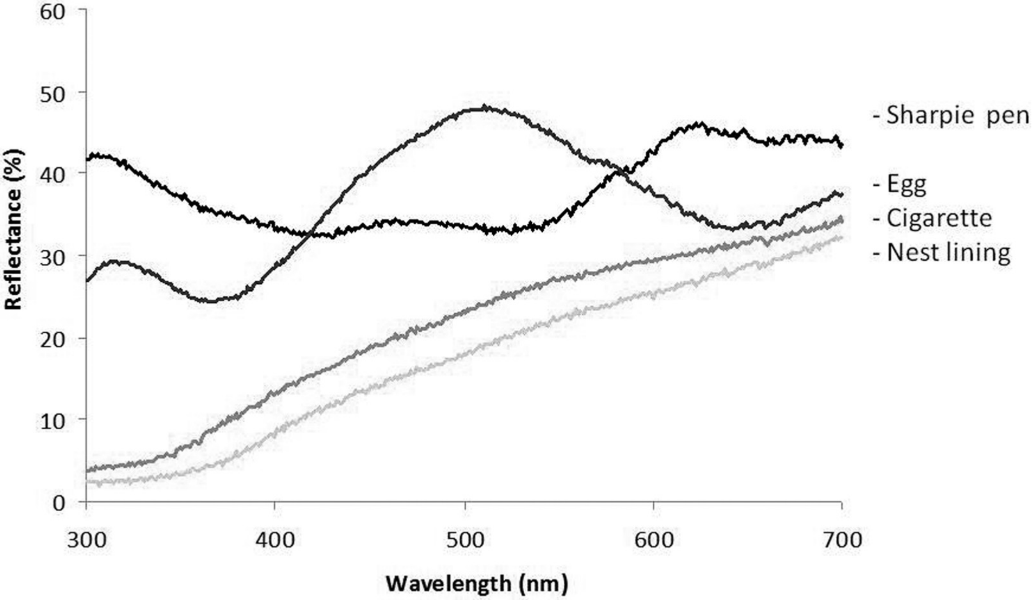

Fig. 2. Representative reflectance spectra of the colours of the cigarette butts, nest background, sharpie marker pen and song thrush egg background. Shown are the means from 3 reflectance measurements for cigarette butt, nest background and sharpie pen, and 9 reflectance measurements for song thrush eggs.

The chromatic components of cigarette butt and nest interior were found to be perceived as similar by the *Turdus* visual system (Table 1). The cigarette butt was found to be less conspicuous against the nest interior than the song thrush eggs for both the chromatic and achromatic components of colour (Tables 1, 2). The mean and standard errors suggest that cigarette butts can be perceived by birds as consistently different from egg surfaces but not from the nest lining.

DISCUSSION

We found no evidence that cigarette butts are visually conspicuous against the interior nest lining of a song thrush nest included in this study. Clearly, more work is needed to increase sample sizes and the types of human-made objects that are included in song thrush nest linings. Nonetheless, we suggest that it is unlikely that thrushes selected cigarette butts to incorporate into their nests to function as a signal to mates or other conspecifics (c.f. Brouwer & Komdeur 2004). Rather, it appears that thrushes are simply using discarded cigarette butts as a convenient construction material.

Although the number of nests we found with cigarette butts was small, we detected a greater proportion of nests with cigarette butts in the Meola Reef Reserve than at the Auckland Botanic Gardens, despite the greater public density in Gardens during daytime hours (*pers. obs.*). However, institutional policy of the Auckland Botanic Gardens prohibits smoking on the grounds, while the Meola Reef Reserve does not, and also the latter has an additional source of particulate garbage from washed up items along the nearby ocean shore. Thus, the difference in availability of cigarette butts may play a role in the rates at which such items are included into nests across different sites.

In our approach we have modelled colour differences as perceived by a blackbird visual system but inferred our results in relation to song thrush perceptual ability. We consider this appropriate because of the close phylogenetic relationship and similar behaviours of blackbirds and song thrushes in New Zealand. As with most Northern hemisphere oscines, both of these *Turdus* species are UV sensitive (Ödeen & Håstad 2003), and the known photoreceptor sensitivities of blackbirds are also very similar to those of an even more distantly related passerine species, the blue tit (*Cyanistes caeruleus*; Hart *et al.* 2000).

It is likely that cigarette butts are more conspicuous in nests composed of different plant or other naturally occurring materials and it is unknown how frequently other species in New Zealand incorporate cigarette butts into their nest lining. Further investigation could possibly include assessment of possible roles (reviewed in Møller 1984; Winkler 1993; Lombardo *et al.* 1995) of human-made objects, include cigarette butts, in regulating nest microclimate, olfactory signalling and deterring odour-driven predators. Overall, whether some anthropogenic waste is beneficial or harmful also remains to be evaluated in future work quantifying the success of clutches and broods in nests that incorporate human garbage.

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Příspěvek IV.

Samaš P., Grim T., Hauber M.E., Cassey P. & Evans K.L. (rukopis): Ecological predictors of reduced avian reproductive investment in the southern hemisphere.

Ecological predictors of reduced avian reproductive investment in the southern hemisphere

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Abstract Life-history traits exhibit strong spatial patterns, and despite intensive research the forces driving these patterns remain poorly understood. One of the most frequently documented patterns is for avian clutch sizes to be smaller at southern latitudes than equivalent northern ones. We exploited a natural experiment provided by the introduction of European passerines to New Zealand to test prominent potential drivers of geographic patterns in avian reproductive parameters, the seasonality, nest predation, and breeding density hypotheses. We used the blackbird and song thrush as case studies as introduced populations do not experience founder effects. Using a consistent methodology our results conflict with a recent meta-analysis that urbanisation reduces avian clutch size. Both species laid smaller clutches in the southern hemisphere. Clutch size reductions had stabilised when introduced populations had been established for one hundred generations. We found evidence for a trade-off between offspring number (i.e. clutch size) and investment in individual off-spring (i.e. egg size) only in blackbird. The smaller southern clutches were associated with lower nest predation rates, contradicting Skutch's nest predation hypothesis, and were not consistently associated with higher breeding densities. Moreover, across all eleven European passerines introduced to New Zealand that those with the greatest reductions in clutch size in the southern hemisphere exhibited the smallest increases in population density. Evidence for Ashmole's seasonality hypothesis was also scant. We suggest that future studies should also test for spatial variation in the trade-off between reproductive investment and if adult survival promote smaller clutches in the southern hemisphere.

Key-words Biogeographic rule, Clutch size, Egg size, Nest predation, Reproductive investment

Introduction

Life history traits exhibit strong geographic patterns (Cardillo 2002, Gaston et al. 2008). Amongst the most consistent of which is the tendency for avian clutch size (both intra- and inter-specifically) to co-vary positively with latitude, and to be smaller in the southern hemisphere than at equivalent northern latitudes. A diversity of mechanisms that may drive these spatial patterns have been identified, but despite decades of research their relative contributions remain unclear (Moreau 1944, Lack 1947, 1948, Ashmole 1963, Cody 1966, Martin et al. 2000*a*, Evans et al. 2005, 2009*a*, Jetz et al. 2008).

One of the strongest candidates for a general driver of spatial variation is Ashmole's hypothesis (Ashmole 1963). This states that in seasonal environments mortality during periods of low food supply, typically winter, reduces population size below that which the environment can support the following breeding season. Reduced competition thus increases the per capita food supply during the breeding season enabling clutch size to be increased. The rapid increase in spring food availability may further promote larger clutches in seasonal environments (Stutchbury and Morton 2001). Therefore, individuals experiencing higher seasonality should have larger clutch sizes than those in areas of lower seasonality. Whilst such patterns have been found in many studies, both intra- and inter-specifically, seasonality rarely provides a complete explanation of spatial variation in clutch size (Martin 2004, Jetz et al. 2008), including smaller southern clutches (Evans et al. 2005). Ricklefs (1980) view of Ashmole's hypothesis is that resource availability during the non-breeding season plays a greater role in determining clutch size than breeding season resource availability. Temporal variation in the latter appears, however, to control seasonal clutch size patterns within species. Single brooded species tend to exhibit a linear decline in clutch size, whilst multi-brooded species exhibit an initial increase in clutch size with a mid-season peak followed by a decline towards the end of the breeding season (Lack 1954, Klomp 1970, Perrins 1970, Crick et al. 1993).

The nest predation hypothesis is also widely considered to be a strong driver of spatial patterns in clutch size; it states that an increased risk of nest predation reduces clutch size (Skutch 1949, Snow 1962, 1978, Slagsvold 1984, Eggers 2006, Martin et al. 2006, Cassey et al. 2009, but see Ricklefs 1977). A number of mechanisms may contribute to this pattern (Roff 1992). Smaller clutches will be promoted in areas with high predation risk as the duration of the nesting period, and thus the probability of predation, are reduced by decreasing both the egg-laying period (because birds are physiologically constrained to lay no more than one egg per day) and incubation period (Engstrand and Bryant 2002, Hepp et al. 2005). Smaller clutches also reduce the probability of nest predation at the chick stage because parents can reduce provisioning rates and thus the probability of visually orientated predators detecting the nest (Skutch 1949, Martin et al. 2000b). Alternatively, if provisioning rates are not reduced, food availability per nestling is higher than that in larger broods increasing chick development rates and reducing the age of fledging and thus the period during which nestlings are vulnerable to nest predators (Martin and Briskie 2009). Finally, when predation risk is high parents may lay smaller clutches to reduce investment in each nesting attempt because this increases the resources available for re-nesting within the same season, and increases the probability of adults surviving to another breeding season due to a trade-off between reproductive effort and survival (Martin 1995).

It is routinely recognised that avian life history traits, including clutch size, typically respond to population size in a density dependent manner (Perrins 1965, Ricklefs 1980, but see Both 1998). This density dependence partly arises through interactions with other mechanisms that influence clutch size, for example high population densities typically increase the risk of nest predation (Krebs 1970, Chamberlain et al. 1995, Keyser et al. 1998, Schmidt and Whelan 1999, Lloyd 2006). It is thus useful to take population density into account in analyses of spatial patterns in clutch size, but few studies do so. This is perhaps because such studies frequently rely upon the haphazard collection of data on nest contents by volunteers, often across multiple years, and thus associated data on population densities are rarely available.

The majority of large scale studies assessing geographic patterns in clutch size are comparative (Moreau 1944, Cardillo 2002, Jetz et al. 2008) and are consequently somewhat limited in their ability to assign underlying causality. This situation can be improved by exploiting natural experiments in which species are introduced to novel locations where environmental conditions differ from those in the ancestral range. For example, many bird species were introduced from the United Kingdom to New Zealand, which has a less seasonal climate than that experienced by the source population (Evans et al. 2005, Table 1). The translocation history of these species is well documented (Thomson 1922), providing researchers with an opportunity to estimate the flexibility of avian breeding biology traits under a known historical ecological context (Briskie and Mackintosh 2004). Previous work has exploited these experiments and documented smaller clutch sizes in the southern hemisphere, relative to ancestral populations at equivalent northern latitudes, but the mechanisms driving these patterns are still uncertain (Niethammer 1970, MacMillan 1985, Evans et al. 2005). These reduced clutch sizes also appear to be associated with smaller eggs (Cassey et al. 2005, Congdon and Briskie 2010), which contrasts with predictions drawn from avian life history theory that these variables should be negatively correlated (reviewed in Roff 1992, Martin et al. 2006).

Here we advance these previous analyses of clutch size variation between native European bird populations and conspecific introduced populations in New Zealand by assessing both clutch size and egg size in the focal populations and taking nest predation rates and breeding population densities into account. Instead of relying upon nest record cards we use data from intensive fieldwork conducted by the same researchers in both regions. Whilst nest record cards are certainly valuable (Hušek et al. 2010) our approach increases the methodological consistency, reducing the potential influence of confounding variables and enabling greater precision in estimating egg-laying dates (Weidinger 2001). We use the blackbird *Turdus merula* and song thrush *T. philomelos* as case studies because these species occupy similar habitats in the native and non-native ranges, where their reproductive biology has not been influenced by founder effects (Briskie and Mackintosh 2004, Congdon and Briskie 2010). This is presumably because relatively large numbers of individuals were released with approximately 800 blackbirds and 400 song thrushes being introduced between 1862 and 1879 (Thomson 1922).

Our primary objective is to exploit the human-mediated introduction of several species of birds from the northern to the southern hemisphere, i.e., a natural experiment (*sensu* Diamond 1986), to assess whether spatial patterns in clutch size are driven by Ashmole's seasonality hypothesis, Skutch's nest predation hypothesis or density dependence. We also test three additional hypotheses: (i) the marked environmental differences between urban and rural environments (Evans et al. 2009*b*, Grimm et al. 2008) results in conspecific urban and rural populations exhibiting divergence in reproductive parameters and nest predation rates (Chamberlain et al. 2009, Evans 2010); (ii) reductions in clutch size are associated with increased egg size as predicted by avian life-history theory (Martin et al. 2006); (iii) that changes in clutch size in response to novel southern hemisphere conditions are still in progress.

Material and methods

Study sites

Our European study populations are situated in the Czech Republic (hereafter CZ) at similar latitudes as the United Kingdom (hereafter UK) source populations (~50°N). Although our northern hemisphere data were not collected in the general area of source populations (most likely rural southern England; Thomson 1922) this does not present a problem because we are interested in *between*-hemisphere differences (i.e. differences independent of local variation *within* hemispheres). Moreover, rural blackbirds from the UK and the CZ form a genetically panmictic population (Evans et al. 2009*c*), and the major aspect of climate that is relevant from the point of view of the present study (i.e. seasonality) is more similar between the UK and the CZ than NZ (Table 1). Data were collected from four rural (agricultural landscape with hedges and small forest patches) and two urban sites in CZ, and two urban sites in New Zealand (hereafter NZ; Table 2). Urban areas were defined as those with a human population density greater than 900 km^{-2} (Table 2). Data were collected in CZ in 1992 and each breeding season from 2000 to 2010, and in NZ in 2007, 2008, and 2009.

Field procedures

Nest searching was conducted in both regions throughout the breeding season, i.e. from the last week in March to the end of July in CZ, and from the start of September to the end of January in NZ. Effort was spread uniformly across these periods, although in order to calculate breeding population densities very intensive searches were conducted at one urban CZ site (Olomouc, size 33.7 ha) in 2005 and 2006, and two urban NZ sites in Auckland of 14.1 ha and 36 ha in 2008. To standardise habitat types further we selected sites with similar vegetation structure, i.e. urban parks with solitary trees and shrubs. These intensive searches were conducted for two weeks in the middle of the breeding season (CZ: second half of April, Strachoňová 2008; NZ: the last week of September and first week of October, Bull 1946). We defined breeding density as the maximum number of simultaneously active nests per unit area.

Nest contents were checked daily during laying, every one to three days during incubation and every one to four days during the nestling phase. Egg length and width were measured using digital callipers to the nearest 0.01 mm. Egg size was calculated using Hoyt's (1979) equation, i.e. egg volume (cm^3) = 0.51 x length x $(width)^2$. We collected data on 2215 eggs in 988 blackbird clutches, and 1696 eggs in 686 song thrush clutches. Sample sizes differ between analyses because for some nests we recorded final clutch size but were not able to measure the eggs due to early predation or poor accessibility.

Daily nest predation rates were calculated for Olomouc and Bystřice in CZ and Auckland and Hamilton in NZ. These were calculated following Mayfield (1975) with standard errors calculated according to Johnson (1979). These calculations require data on the duration of the egg laying, incubation and nestling periods which were estimated using published data (Weidinger 2007, Strachoňová 2008) supplemented with our own observations (Table 3). The nest predation rates for Olomouc and Bystřice have previously been reported by Strachoňová (2007, 2008) who helped us with field research in CZ and also NZ. To test whether reductions in the clutch size of

NZ populations have occurred during recent decades we used nest record card data collected by the Ornithological Society of New Zealand (OSNZ) in 1950–1998.

Statistical analyses

First egg dates (FEG) were transformed prior to analysis so that they were directly comparable between regions and to take variation between years in the timing of breeding into account. This was achieved in two steps. First, FEG dates were centred around the mean so that for each species, region and year the mean FEG date equalled zero; second, a constant was added so that the earliest FEG date then had a value of zero ensuring that all dates were positive before square terms were calculated.

We first tested the hypotheses that conspecific urban (N = 2 sites) and rural (N = 4 sites) populations differed in their clutch size and egg size. These analyses were conducted only using data from CZ, as only urban sites were sampled in NZ. We constructed linear mixed models and used backward elimination of non-significant terms to construct a minimum adequate model (Grafen and Hails 2002). Clutch size and egg size data exhibited normal distributions and were thus modelled assuming a continuous distribution and an identity link (analogous ordinal logistic models generated qualitatively identical results which are thus not presented). Our predictor variables were habitat type (urban vs. rural; a fixed binary factor), continuous first egg date (FEG) and its square term (FEG^2); year and site were included as nominal random effects to take potential confounding effects of spatio-temporal correlation into account. We then repeated these analyses for egg-size in which clutch size was added as a continuous variables and clutch identity was included as an additional random effect. We also tested the hypothesis that urbanisation influences nest predation rates by comparing these between the urban Olomouc site and the rural Bystřice site in each of three years. This was achieved using the *t*-test for unequal variances (Welsch's test; Ruxton 2006).

We then assessed the effects of region on clutch size and its seasonal trends, data were pooled across urban and rural habitat types as we did not detect significant effects of habitat on clutch size (see results). As an initial assessment of the later we constructed models of clutch size separately for the NZ and CZ populations and used FEG and FEG^2 as continuous predictors and year and site as nominal random effects. We then constructed models that pooled data across regions and included region and its first order interaction terms as additional predictors.

The influence of region on egg size was then assessed in multiple regression models that used clutch size, FEG and FEG^2 as continuous predictors, region as a binary fixed factor, and clutch identity, year and site as random effects. First order interaction terms with region were also included as predictors.

Regional differences in daily nest predation rates were compared using the t-test for unequal variances (Welsch's test; Ruxton 2006). Analyses described above found no significant influence of habitat type on nest predation rates (see results) so data were pooled across habitat types. Variation in the breeding densities of CZ and NZ populations was assessed following Payton (2003) by testing for significant overlap in confidence 84% intervals, a lack of overlap in which corresponds to a significant differences at $\alpha = 0.05$. As other studies report significant effects of urbanisation on song thrush and blackbird densities (Tratalos et al. 2007, Evans et al. 2009*b*) our tests of regional effects on densities are only conducted within equivalent habitat types, i.e. urban areas.

Finally, we test whether blackbirds and song thrushes have continued to evolve smaller clutch sizes in NZ during recent decades by comparing the clutch sizes we found in 2008 with those reported from the same regions of NZ (Auckland and Hamilton) in the 1950s and 1960s using data from the OSNZ nest record scheme. We do so by comparing confidence intervals in the manner described above. In addition, we construct multiple regression models of the clutch size recorded in the OSNZ scheme for our study area between 1950 and 1998 (blackbird) and 1956 and 1986 (song thrush); the time periods differ between species due to the variation in the availability of nest record cards. These models include year and its square term, FEG, FEG^2, and altitude as continuous predictors together with area of New Zealand (Hamilton or Auckland) as a fixed binary variable.

All analyses were conducted using SAS vs 9.2. All t-test we used were t-tests for unequal variances (Welsch's test) as recommended by Ruxton (2006). Throughout this paper all mean values are given ± one standard error.

Results

Effects of urbanisation on clutch size, egg size and nest predation rates

Within CZ blackbird clutch sizes were very similar in rural (4.33 ± 0.07, *N* = 139) and urban (4.10 ± 0.03, *N* = 565) environments (Fig. 2a). Habitat type was not retained in multiple regression analyses ($F_{1,685}$ = 2.32, *P* = 0.13) that took first egg date, year and locality into account. Egg size did not differ between rural (6.66 ± 0.04 cm^3, *N* = 307) and urban areas in CZ (6.87 ± 0.02 cm^3, *N* = 1241; $F_{1,1532}$ = 2.66, *P* = 0.10).

Song thrush clutch sizes were also similar in rural (4.35 ± 0.05, *N* = 264) and urban environments in CZ (4.20 ± 0.08, *N* = 108; Fig. 2b); habitat type was not retained in multiple regression analyses ($F_{1,355}$ = 0.67, *P* = 0.41) that took season, year and locality into account. Egg size did not differ between rural (5.84 ± 0.02 cm^3, *N* = 615) and urban areas in CZ (5.79 ± 0.04 cm^3, *N* = 285; $F_{1,878}$ = 0.12, *P* = 0.73), but increased as the season progressed ($F_{1,878}$ = 7.09, *P* = 0.008; y = 5.67(± 0.10) + 0.004(± 0.002)*FEG).

Daily nest predation rates did not differ between rural blackbird populations (0.050 ± 0.006, *N* = 76) and urban ones (0.051 ± 0.006, *N* = 460; *t* = -0.12, df = 4, *P* = 0.91). Similarly, daily nest predation rates did not differ between rural (0.054 ± 0.013, *N* = 93) and urban song thrush populations (0.053 ± 0.013, *N* = 97; *t* = -0.04, df = 4, *P* = 0.97).

Effects of region on clutch size, its seasonal patterns and egg size

Blackbird clutch size in CZ peaked in the middle of the breeding season, when clutches contain an extra egg compared to clutch sizes at the start and the end of the breeding season (Table 4; Fig. 1a). There was no evidence for seasonal trends in blackbird clutch sizes in NZ (Table 4; Fig. 1b). Song thrush clutch sizes in CZ also exhibited a strong seasonal pattern, with clutch size declining linearly as the season advanced; maximum and minimum clutch sizes differed by 0.75 (Table 4; Fig. 1c). Song thrushes in NZ also exhibited a linear decline in clutch size, but there was evidence that the seasonal rate of decline was slower (although statistically not significantly) in NZ than in CZ with maximum and minimum clutch sizes differing by 0.5 (Table 4; Fig. 1d).

Mean blackbird clutch sizes on average contained an extra egg in CZ (4.14 ± 0.03, N = 704) than NZ (3.15 ± 0.04, N = 284; Fig. 2a). Region had a significant effect in multiple regression models ($F_{1,966} = 28.90$, $P < 0.0001$) that took the seasonal effects reported above into account and that also used year and site as predictors. Song thrush clutch size was larger in CZ (4.31 ± 0.04, N = 372) than NZ (3.73 ± 0.04, N = 314; Fig. 2b) by approximately half an egg. Region had a significant effect in multiple regression models ($F_{1,666} = 9.04$, $P = 0.002$) that took the significant seasonal effects reported above into account.

Mean blackbird egg sizes were similar in CZ (6.83 ± 0.02 cm^3, N = 1548) and NZ (6.92 ± 0.03 cm^3, N = 667). A multiple regression model that took first egg date, clutch size, clutch id, year and site into account retained the following predictors: egg volume = 7.49(± 0.30) - 0.19(± 0.08)*clutch size -0.66(± 0.08)*region + 0.18(± 0.09)*clutch size*region. The main effect of clutch size was significant ($F_{1,1507} = 4.84$, $P = 0.04$) and negative, region ($F_{1,1507} = 3.69$, $P = 0.06$) formed a significant interaction with clutch size ($F_{1,1507} = 4.17$, $P = 0.04$) indicating that the negative relationship between egg size and clutch size was steeper in NZ. Song thrush egg size was similar in CZ (5.82 ± 0.02 cm^3, N = 900) and NZ populations (5.67 ± 0.02 cm^3, N = 796). Multiple regression models of egg volume that took region, clutch size, first egg date, year and site into account retained neither region ($F_{1,1257} = 2.22$, $P = 0.14$) nor clutch size ($F_{1,1254} = 0.60$, $P = 0.44$).

Regional effects on daily nest predation rates and breeding densities

Daily nest predation rates were higher in CZ than in NZ. Blackbird mean daily predation rates were higher in CZ (0.051 ± 0.006, N = 536) than NZ (0.027 ± 0.007, N = 316; t =

2.60, df = 719, *P* = 0.009; Fig. 3). The same pattern occurred in song thrush populations (CZ 0.054 ± 0.010, *N* = 190; NZ 0.027 ± 0.007, *N* = 324; *t* = 2.21, df = 367, *P* = 0.03).

The mean urban blackbird density (Fig. 4) in CZ was 0.21 pairs/ha (84% confidence intervals (CIs): 0.09 - 0.33) and 0.90 pairs/ha in NZ (84% CIs: 0.55 - 1.25); the non-overlap of confidence intervals indicates that there was a significant difference at α = 0.05 (Payton et al. 2003). Song thrush densities (Fig. 4) also exhibited a strong trend towards lower densities in urban CZ (mean 0.14; 84% CIs: 0.00 - 0.34) than urban NZ (mean 1.38; 84% CIs: 0.00 - 4.04), but the overlap in confidence intervals indicates that differences were not statistically significant.

Temporal changes in clutch size within NZ populations

Mean blackbird clutch size in the Auckland and Hamilton areas during the 1950s and 1960s recorded in OSNZ nest record cards was 3.29 ± 0.09 (84% CIs 3.15-3.42; *N* = 70) compared with a current mean in this region of 3.15 ± 0.04 (84% CIs 3.11-3.20; *N* = 284). The overlapping confidence intervals indicate no significant difference at α = 0.05. Mean song thrush clutch size in the Auckland and Hamilton areas during the 1950s and 1960s recorded in OSNZ nest record cards was 3.69 ± 0.09 (84% CIs 3.56-3.81; *N* = 54) compared with a current mean in this region of 3.73 ± 0.04 (84% CIs 3.68-3.78; *N* = 314). The overlapping confidence intervals indicate no significant difference at α = 0.05. Multiple regression analyses that took FEG, FEG^2, altitude and area into account confirmed that there was no significant change in either blackbird or song thrush clutch size within the Auckland and Hamilton areas since the 1950s (blackbird: year *P* = 0.11; $year^2$ *P* = 0.55; song thrush: year *P* = 0.45; $year^2$ *P* = 0.47).

Discussion

Urban blackbird and song thrush populations did not exhibit significantly different clutch sizes from their rural conspecifics. This is somewhat surprising as a recent meta-analysis of 46 studies concluded that sub-urban and urban populations typically have smaller clutch sizes than rural ones, perhaps due to a limited availability of natural food during the breeding season (Chamberlain et al. 2009). Moreover, 22% of the studies included in the meta-analysis were conducted on our focal species, with all six previous blackbird and all four previous song thrush studies finding larger clutches in rural environments. However, very few previous studies tested for statistical significance and only one of those on our focal species did so (on blackbirds; Chamberlain et al. 2009), and we did find a tendency for slightly smaller urban clutches (blackbird: 0.27 of an egg; song thrush: 0.15 of an egg). We also found no significant differences in the egg size of rural and urban populations. The similarity of rural and urban egg sizes concurs with previous studies of blackbird (Vogrin 1997), starling *Sturnus vulgaris* (Vengerov 1992) and magpie *Pica pica* (Antonov and Atanasova 2003), but other studies report smaller eggs in urban song thrush, chaffinch *Fringilla coelebs* and magpie populations (Vengerov 1992), and larger eggs in urban kestrels *Falco tinnunculus* (Pikula et al. 1984). Our lack of evidence for differences in egg size between urban and rural environments is thus compatible with the apparent lack of consistency in the impacts of urbanisation on avian egg size. More importantly, we find that total reproductive investment per clutch was not influenced by urban development in either blackbirds or song thrushes, indicating that the impacts of urbanisation on avian reproductive investment can be less marked than the current literature suggests.

Predation rates of blackbird and song thrush nests did not differ significantly between urban and rural habitats. This is perhaps surprising given the higher densities of many nest predators in urban environments, including corvids and cats (Sims et al. 2008, Evans et al. 2009*b*), but concurs with previous studies that fail to find evidence for consistent patterns in nest predation rates between urban and rural environments (Chamberlain et al. 2009). Our finding that both clutch sizes and nest predation rates do not differ between urban and rural environments is compatible with Skutch's hypothesis (Skutch 1949) that spatial variation in clutch size is determined by variation in nest predation rates.

We found significantly smaller clutches in New Zealand blackbird and song thrush populations than those at equivalent latitudes in the northern hemisphere.

These reductions in clutch size appear to have occurred within one hundred generations of establishment of the introduced populations suggesting relatively rapid adjustment to novel environmental conditions, but this would be entirely expected if the reductions arose through phenotypic plasticity. There is little evidence that clutch size reductions in the southern hemisphere are associated with an increase in egg size and thus total reproductive investment per clutch is reduced in the southern hemisphere. Blackbirds exhibited the trade-off between clutch size and egg size predicted by avian life history theory (Martin et al. 2006). Interestingly, this trade-off is stronger in New Zealand than the Czech Republic indicating that reproductive investment is lower in the southern hemisphere even when taking clutch size into account. There was no evidence that larger song thrush clutches contained smaller eggs. The absence of such a trade-off has also been reported between populations in a large scale study of great tits *Parus major* (Horak et al. 1995). This is, however, considered to arise primarily from latitudinal gradients in female body size and from constraints preventing increased clutch size at very high latitudes which in combination results in populations with the largest clutch sizes also laying large eggs (Encabo et al. 2002). The mechanisms that break down the trade-off between clutch size and egg size in song thrush populations are unknown. They could involve constraints limiting clutch size which result in high quality individuals increasing egg size rather than clutch size; in addition, the increased investment in each offspring arising from larger eggs may increase offspring recruitment rates and thus parental fitness to a greater extent than increasing the number of offspring.

Seasonal clutch size variation, especially in blackbirds, appears to be more limited in the southern hemisphere than the northern one, suggesting that birds are responding to the lower climatic seasonality in the southern hemisphere. Our results concur with previous studies reporting reduced clutch size and seasonality in New Zealand populations compared to British ones based on analyses of nest record cards (Evans et al. 2005). We find that the largest reduction in southern clutch size in blackbird (1.0 of an egg) than song thrush (0.6 of an egg); the blackbird also exhibited the strongest relaxation of seasonal clutch size trends. Whilst we were not able to locate nests in New Zealand at the very start of the breeding season we do not consider that this will bias our comparison of seasonal trends in the two focal species as both will have been affected similarly. Our data are thus compatible with Ashmole's hypothesis that reduced seasonality will promote smaller clutches in the southern hemisphere. This contrasts with analyses of nest record cards which found little evidence for Ashmole's hypothesis as the reduced seasonality in the southern hemisphere could not explain the magnitude of reductions in clutch size (Evans et al. 2005).

One of the most prominent alternative hypotheses for smaller clutches in the southern hemisphere is the nest predation rate hypothesis (Skutch 1949), but in contrast to its predictions we find that nest predation rates are lowest in the southern hemisphere where clutches are the smallest. This result is robust because predation rates were consistently lower in NZ across years, localities and study species. Also, whilst increased human visitation rates to blackbird nests can reduce the risk of nest predation (Ibáñez-Álamo and Soler 2010) our visitation rates were constant across study populations.

Blackbird population densities were significantly higher in our NZ study sites than comparable ones in the northern hemisphere, and we found a similar (albeit non significant) trend in song thrush population densities. These higher southern hemisphere densities may be driving reduced clutch sizes through density dependence effects. Other studies have also found higher song thrush and blackbird population densities in NZ than Europe, together with higher densities of each of the additional nine passerine species that have been introduced from Europe to NZ (Macleod et al. 2009, Weller 2009). However, the species with the largest increase in population density in NZ have the smallest decrease in clutch size (Fig. 5) and thus the later cannot be explained by density dependence.

Spatial patterns in clutch size, such as smaller clutches in the southern hemisphere, are one of the most frequently documented trends in avian life history traits, but two of the commonly proposed drivers (nest predation and density dependence) appear incapable of driving the pattern. We find some support for

Ashmole's seasonality hypothesis that smaller clutches arise in the southern hemisphere as reduced seasonality results in breeding population densities being closer to the environment's carrying capacity. Reduced seasonality is, however, associated with milder winters that could promote greater longevity in southern populations and thus reduced clutch sizes due to the trade-off between reproductive investment and longevity. Further tests are required to distinguish this adult survival hypothesis from Ashmole's hypothesis.

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Table 1. Differences in climatic variables across our study sites in Olomouc region in CZ (Tinklová 2007), Auckland region in NZ (NIWA 2010) and southern UK (UK Met Office 2010) in years 1971–2000.

Climate variables	CZ	UK	NZ
Mean annual temperatures (°C)	9.0	10.0	15.1
Annual range of mean monthly temperatures (°C)	22.3	13.5	9.0
Rainfall per year (mm)	529	767	1240
Range in monthly rainfall (mm)	92.1	119.4	71.0

Table 2**.** Location and characteristics of study sites. All rural sites comprised deciduous forest.

Locality	Region	Latitude	Longitude	Altitude (m)	Habitat	Human population density (km^{-2})
Bystřice	CZ	49°35' N	17°17' E	218	Rural	0
Grygov	CZ	49°31' N	17°18' E	205	Rural	0
Kníničky	CZ	49°14' N	16°30' E	250	Rural	0
Lužice	CZ	47°40' N	16°48' E	170	Rural	0
Olomouc	CZ	49°35' N	17°15' E	220	Urban	990
Brno	CZ	49°11' N	16°35' E	240	Urban	1760
Auckland	NZ	36°51' S	174°46' E	20	Urban	1228
Hamilton	NZ	37°46' S	175°16' E	35	Urban	1459

Table 3. Duration of the egg laying, incubation and nestling periods assumed when calculating daily nest predation rates using Mayfield's (1975) method. Data are obtained from Weidinger (2007), Strachoňová (2008) and personal observations.

	Czech Republic		New Zealand	
	Blackbird	Song thrush	Blackbird	Song thrush
Egg laying	5	5	3	4
Incubation	11	11	12	12
Nestling	8	8	8	8

Table 4. The relationship between clutch size and first egg date (FEG) in the Czech Republic (CZ) and New Zealand (NZ) in multiple regression analyses that also took year and site into account.

Species	Region	Model (± 1 S.E. of parameter estimates)	FEG (*F* ratio, *P* value)	FEG^2 (*F* ratio, *P* value)
Blackbird	CZ	Y = 3.24 (± 0.24) + 0.03 (± 0.01)* FEG - 0.0002 (± 0.00005)*FEG^2	$F_{1,685}$ = 24.79; *P* < 0.0001	$F_{1,685}$ = 27.18; *P* < 0.0001
	NZ	N/A	$F_{1,278}$ = 0.14; *P* = 0.71	$F_{1,277}$ = 0.52; *P* = 0.47
Song thrush	CZ	y = 4.67(± 0.18) - 0.007(± 0.003)*FEG	$F_{1,355}$ = 7.93; *P* = 0.005	$F_{1,354}$ = 2.41; *P* = 0.12
	NZ	y = 3.98(± 0.08) – 0.005(± 0.001)* FEG	$F_{1,308}$ = 13.36; *P* = 0.0003	$F_{1,307}$ = 3.75; *P* = 0.054

FIG. 1. Seasonal variation in clutch size of blackbirds in Czech Republic (a) and New Zealand (b) and song thrushes in Czech Republic (c) and New Zealand (d). Outlying data-points representing two clutches (most likely parasitized by conspecifics) with 8 eggs and one with 7 eggs are not displayed in graphs (a, c). We centred "first egg date" values by mean for each species/year/region combination separately, so that day zero corresponded to the average date of laying across species, years and regions. This ensured that seasonal patterns in clutch size were directly comparable between species, years and regions.

FIG. 2. Differences (least square means + S.E.) in blackbird (a) and song thrush (b) clutch sizes across rural and urban localities. Sample sizes (numbers of clutches) are given within bars. Data were collected in the Czech Republic (CZ, black bars) and New Zealand (NZ, white bars). Bars not connected by the same letter are significantly different ($P < 0.05$).

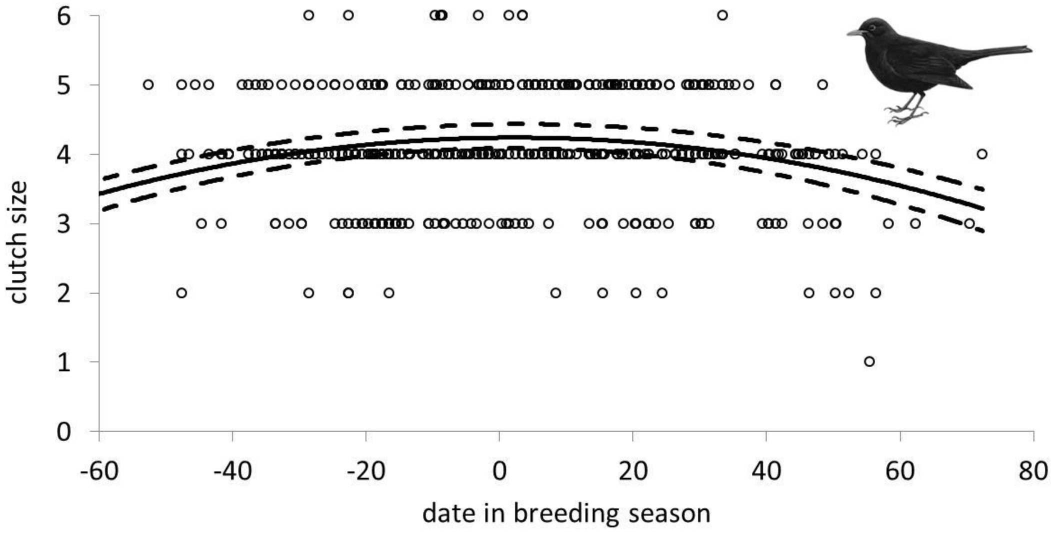
FIG. 3. Daily predation rates (estimate + S.E.) of blackbird and song thrush nests across different localities and years in NZ (white bars) and CZ (black bars; from Strachoňová 2007). Sample sizes are given within bars.

FIG. 4. Breeding density estimates for blackbirds and song thrush across years and spatial replicates in CZ (black bars) and NZ (white bars). Olomouc represents urban study site in CZ and Bystřice rural study site in CZ. Auckland 1 = Botanic Gardens, Auckland 2 = Western Springs represent urban NZ sites. See Methods for details.

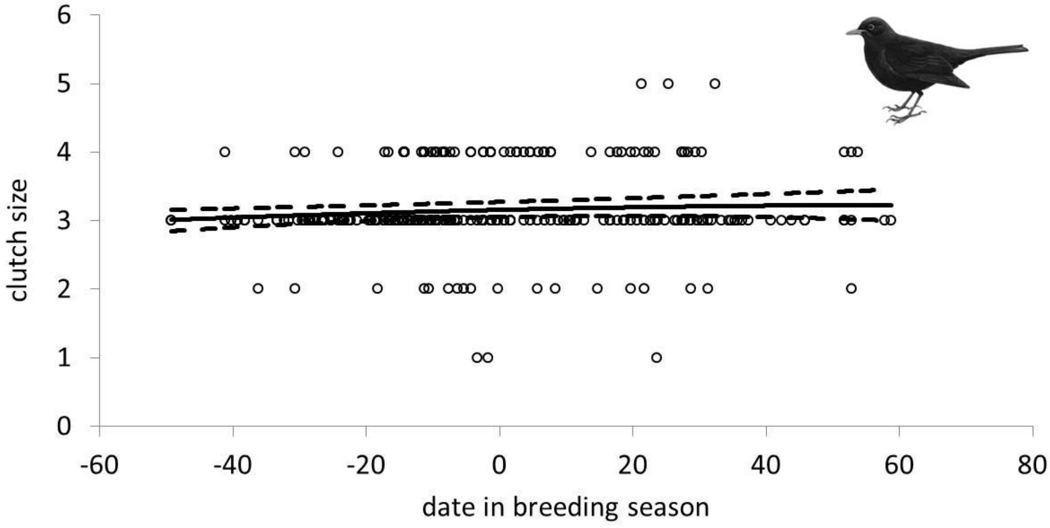
FIG. 5. The propotional reduction in clutch size of 11 passerines introduced from the UK to NZ is smallest in species with the greatest increase in population density in NZ relative to the UK $r^2 = 0.53$, $P = 0.01$). The relationship remains significant when taking into account phylogeny and species specific indices of the magnitude of reduced seasonality (which are not significant). Density data are from MacLeod et al. (2009), clutch size data and seasonality indices are from Evans et al. (2005).

FIG. 1

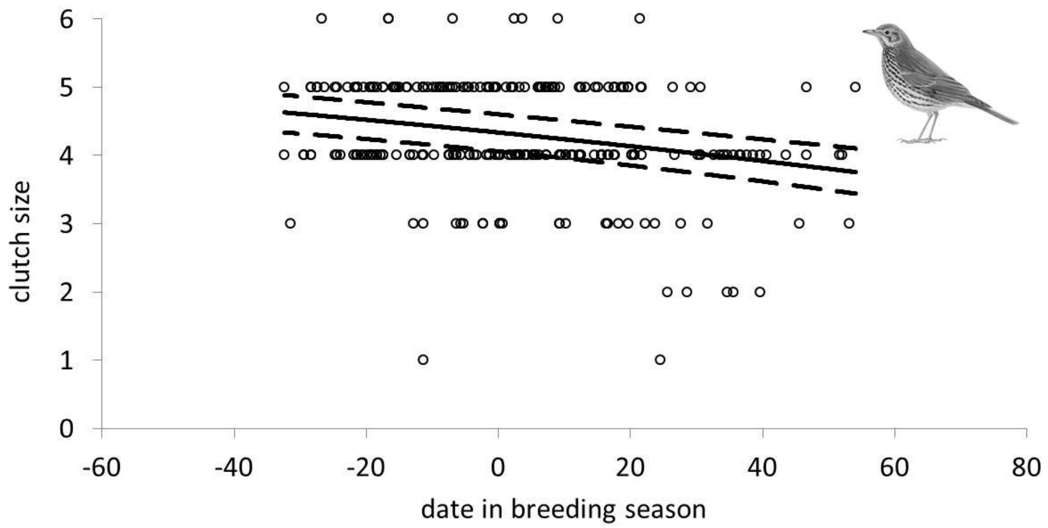
(a) Blackbird CZ



(b) Blackbird NZ



(c) Song Thrush CZ



(d) Song Thrush NZ

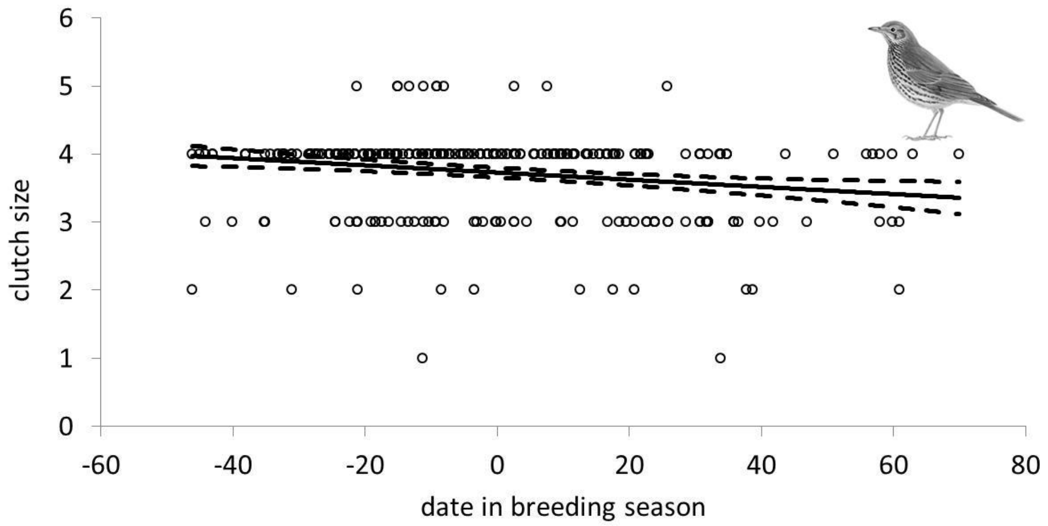


FIG. 2

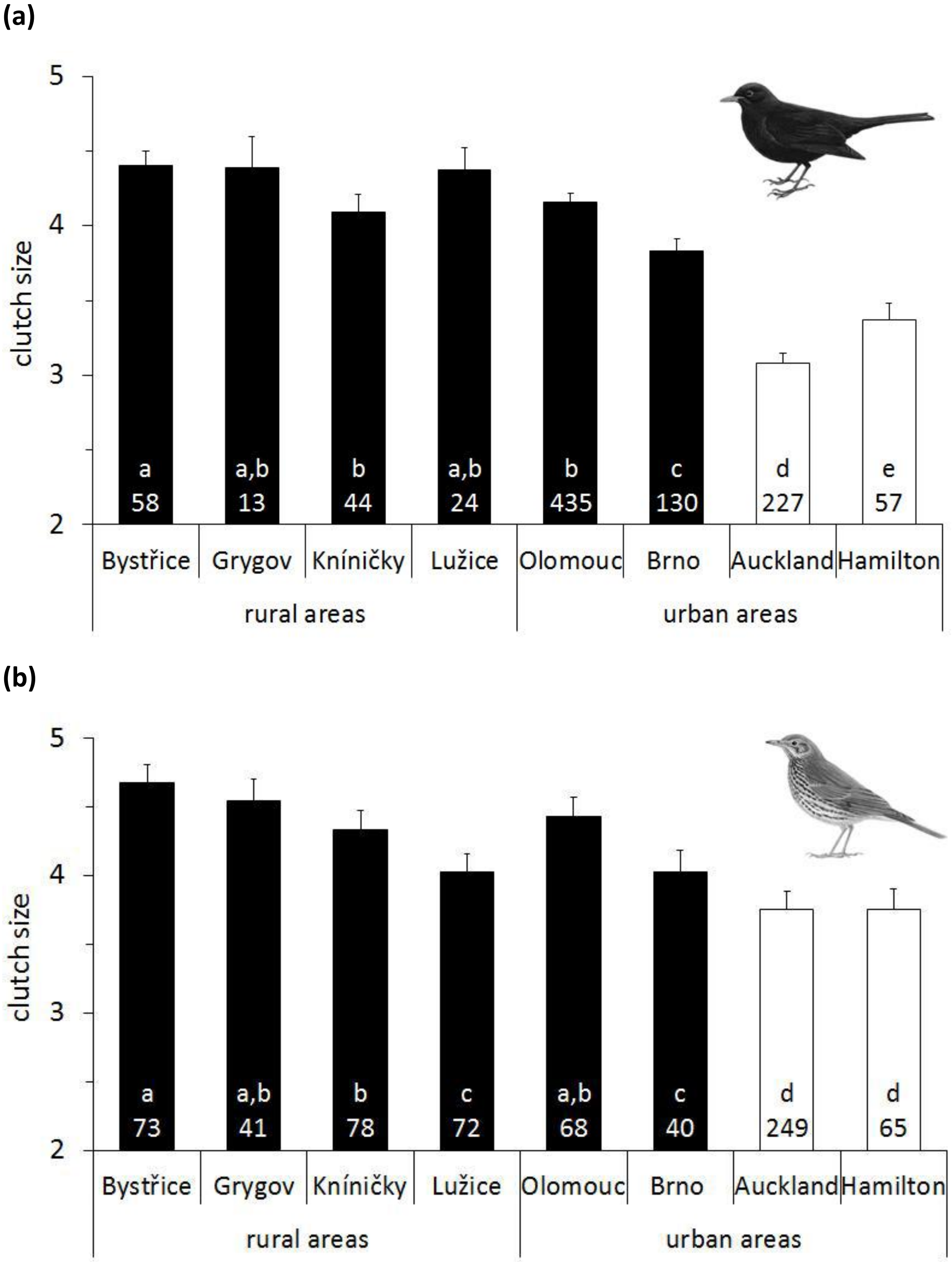

(a)
5
4
3
2
clutch size
a
58
a,b
13
b
44
a,b
24
b
435
c
130
d
227
e
57
Bystřice
Grygov
Kníničky
Lužice
Olomouc
Brno
Auckland
Hamilton
rural areas
urban areas
(b)
5
4
3
2
clutch size
a
73
a,b
41
b
78
c
72
a,b
68
c
40
d
249
d
65
Bystřice
Grygov
Kníničky
Lužice
Olomouc
Brno
Auckland
Hamilton
rural areas
urban areas

FIG. 3

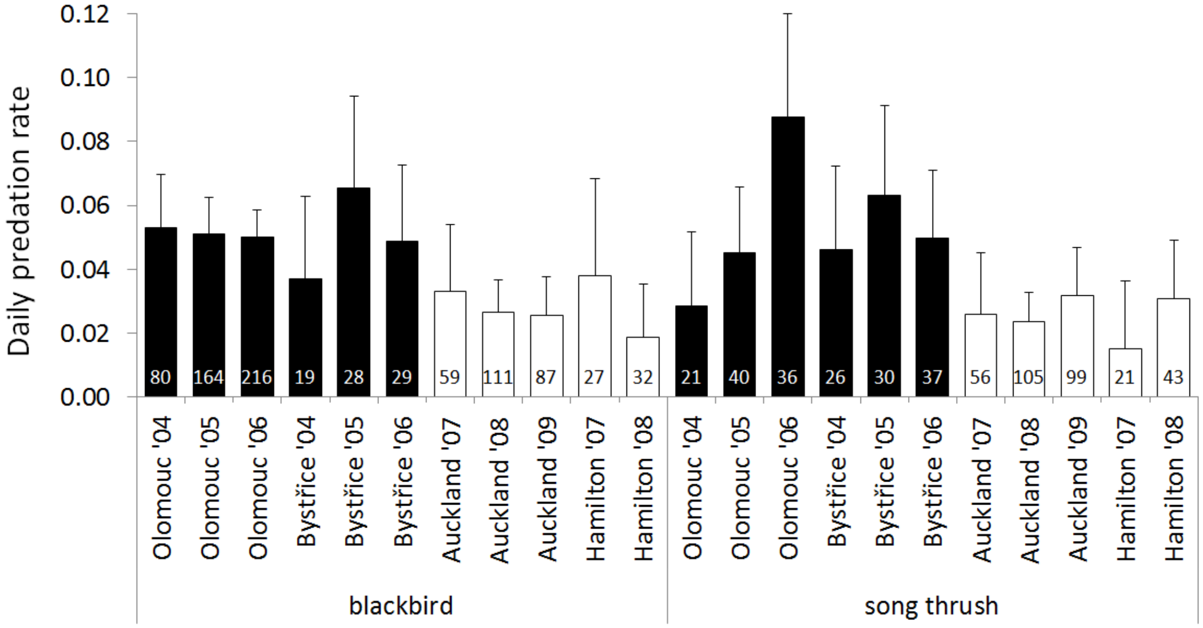

Daily predation rate
0.12
0.10
0.08
0.06
0.04
0.02
0.00
80
164
216
19
28
29
59
111
87
27
32
21
40
36
26
30
37
56
105
99
21
43
Olomouc '04
Olomouc '05
Olomouc '06
Bystřice '04
Bystřice '05
Bystřice '06
Auckland '07
Auckland '08
Auckland '09
Hamilton '07
Hamilton '08
Olomouc '04
Olomouc '05
Olomouc '06
Bystřice '04
Bystřice '05
Bystřice '06
Auckland '07
Auckland '08
Auckland '09
Hamilton '07
Hamilton '08
blackbird
song thrush

FIG. 4

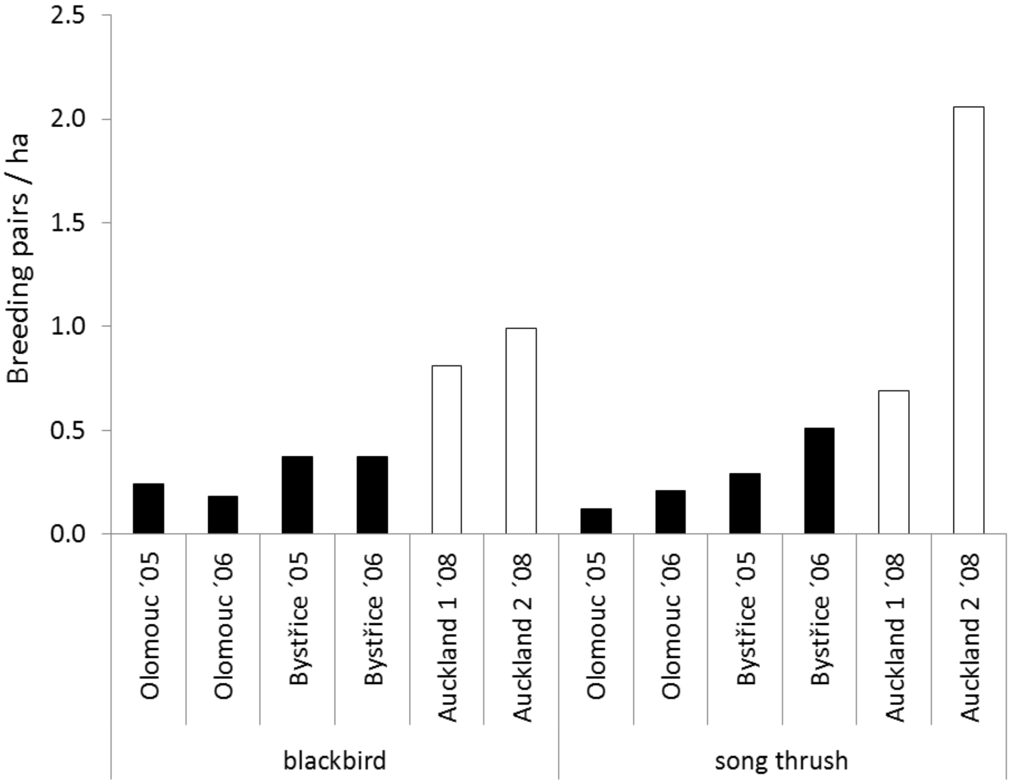
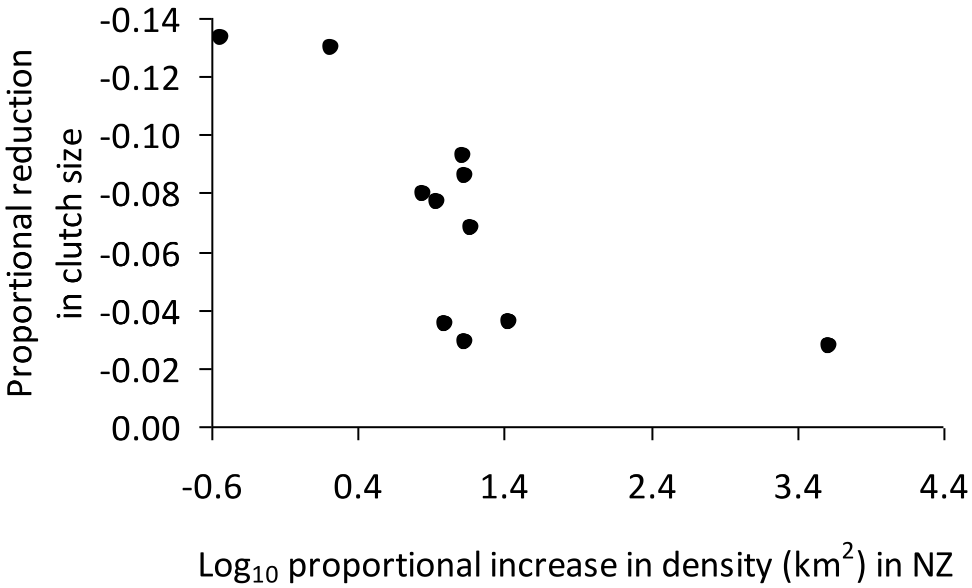

Breeding pairs / ha
2.5
2.0
1.5
1.0
0.5
0.0
Olomouc ´05
Olomouc ´06
Bystřice ´05
Bystřice ´06
Auckland 1 ´08
Auckland 2 ´08
Olomouc ´05
Olomouc ´06
Bystřice ´05
Bystřice ´06
Auckland 1 ´08
Auckland 2 ´08
blackbird
song thrush

FIG. 5


Proportional reduction in clutch size
-0.14
-0.12
-0.10
-0.08
-0.06
-0.04
-0.02
0.00
-0.6
0.4
1.4
2.4
3.4
4.4
Log$_{10}$ proportional increase in density (km^2) in NZ

Příspěvek V.

Samaš P., Cassey P., Hauber M.E. & Grim T. (rukopis): Host responses to interspecific brood parasitism: a by-product of adaptations to conspecific parasitism?

HOST RESPONSES TO INTERSPECIFIC BROOD PARASITISM: A BY-PRODUCT OF ADAPTATIONS TO CONSPECIFIC PARASITISM?

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Abstract

Why have abilities to recognize eggs evolve in birds? Typically, foreign egg discrimination is interpreted as evidence for interspecific coevolution with brood parasites. An alternative explanation not tested in most previous studies is that conspecific brood parasitism also selects for egg discrimination abilities. Importantly, at early stages of interspecific parasite-host coevolution – when there is low similarity between host and parasite eggs – interspecific parasitism does not select for fine-tuned discrimination abilities that are needed to respond to conspecific parasitism. This is supported by theory (the conspecific acceptance threshold model) and empirical data. The alternative, conspecific parasitism hypothesis predicts the evolution of fine-scaled discrimination by hosts. We studied responses to experimental interspecific and conspecific parasitism (n=957) in two model hosts (*Turdus* thrushes) using same methods in areas of sympatry (Europe) and allopatry (Europe and New Zealand) with their potential interspecific brood parasite, the cuckoo *Cuculus canorus*. Host responses were consistent with the conspecific parasitism hypothesis and in line with recent evidence that thrushes are unsuitable cuckoo hosts. Thus, the host rejection of non-mimetic eggs does not provide a clear evidence for interspecific parasite-host coevolution. The rejection of model or real cuckoo eggs may instead be a by-product of adaptations against conspecific parasitism.

KEY WORDS: co-evolution, discrimination, egg rejection, intraspecific brood parasitism, recognition errors.

Introduction

Why do birds recognize and reject foreign eggs? This question fascinated researchers for centuries (Davies 2000). During the last several decades dozens of studies performed thousands of experiments whereby artificial models were introduced into host nests (Grim 2007). Hosts rejecting model eggs of the common cuckoo, *Cuculus canorus* (hereafter: cuckoo), brown-headed cowbirds, *Molothrus ater*, and other brood parasitic birds (Rothstein 1975; Peer and Sealy 2004; Langmore et al. 2005), were typically interpreted as strong evidence for interspecific host-parasite coevolution.

The majority of egg discrimination studies tested host responses to experimentally introduced artificial model eggs of various types, including some studies with model or real conspecific eggs (Davies and Brooke 1989; Braa et al. 1992; Moskát et al. 2003; Honza et al. 2004; Stokke et al. 2004; Hale and Briskie 2007; Honza et al. 2007). The use of conspecific eggs is critical in testing evolutionary functions of egg rejection because discrimination can be selected not only by **interspecific parasitism (IP)** but also by **conspecific (intraspecific) parasitism (CP)** (Lyon 2003; Lyon and Eadie 2004, 2008; Riehl 2010). Although IP may be extremely costly to hosts (Davies 2000), CP is associated with non-negligible costs due to increased costs of incubation (Gibbons 1986; Siikamäki 1995), reduced hatching success of larger clutches (Hauber 2003; Lyon 2003), increased chick competition and parental investment into unrelated individuals (Riehl 2010). Overall, CP is costly to both host offspring and parents because parasitized nests have lower fledging success (Brown 1984; Evans 1988), and increased current reproductive effort decreases survival and future fecundity of parents (Dijkstra et al. 1990; Nager et al. 2001). Within particular host species CP can be even more costly than IP, depending on the frequency of CP relative to IP per clutch (Jackson 1998).

The many costs of CP are sufficient to drive evolution of host defences (Emlen and Wrege 1986; Lyon 2003; Riehl 2010) even if CP occurs at low frequencies (López-de-Hierro and Moreno-Rueda 2010). This is best evidenced by existence of egg rejection abilities in hosts that are not impacted by interspecific parasites. Rejection of conspecific eggs was found in many non-passerines that are not parasitized by heterospecifics, e.g., in gulls (Ležalová-Piálková and Honza 2009), terns (Saino and

Fasola 1993), murres (Gaston et al. 1993), coots (Lyon 2003), moorhens (McRae 1995) and other rails (Sorenson 1995).

Rejection of conspecific eggs was also documented in various passerines (Latif et al. 2006). Such ability was explained by either selection pressures from parasites laying *highly mimetic* eggs (Braa et al. 1992; Moksnes 1992; Welbergen et al. 2001; Stokke et al. 2004), or by combined effects of IP and CP (Victoria 1972; Lahti and Lahti 2002; Lahti 2005). Jackson (1998) argued that in northern masked weaver, *Ploceus taeniopterus*, species known to reject conspecific eggs, parasitism by *Chrysococcyx* cuckoos was unimportant selection force (due to extreme rarity of such IP) whereas it was CP which selected for this hosts ability to recognize conspecific eggs. Even passerines that are unambiguously unsuitable cuckoo hosts are able to reject alien eggs, e.g., cavity nesting starlings, *Sturnus vulgaris* (Davies and Brooke 1989). Most importantly, recent studies of the house sparrow, *Passer domesticus*, showed that sparrows, despite being clearly unsuitable hosts with no coevolutionary experience with the cuckoo (Moksnes and Røskaft 1995), are able to recognize and reject conspecific eggs at high rates (Kendra et al. 1988; Moreno-Rueda and Soler 2001; López-de-Hierro and Ryan 2008; López-de-Hierro and Moreno-Rueda 2010). This empirically rejects theoretical arguments that CP is not sufficiently costly to select for host defences.

“Cuckoo and conspecific parasitism could both be important agents of selection on … egg rejection behavior” (Lahti 2005, p. 18057). Therefore studies using only model or real heterospecific eggs – and not conspecific eggs – cannot lead to an unambiguous conclusion that songbirds evolved specific adaptations to reject IP. Indeed, some previous studies considered an alternative hypothesis that rejection of eggs of obligate interspecific parasites can be an “epiphenomenon of conspecific brood parasitism” in both cowbird (Briskie et al. 1992) and cuckoo hosts (Grendstad et al. 1999; Stokke et al. 2004; Lahti 2005). However, those studies did not support this scenario. Therefore, we tested this ’by-product‘ hypothesis in two passerines where roles of IP and CP for the evolution of their egg rejection abilities were discussed for decades but without reaching consensus.

The song thrush, *Turdus philomelos*, and the blackbird, *T. merula*, are good candidates to test whether IP, CP or both selected for anti-parasite adaptations: (1) Both species commonly breed in areas of either sympatry or allopatry with the cuckoo which leads to clear predictions on between-population variation of host antiparasite defences (Stokke et al. 2008; Table 1). (2) Previous experiments showed that these thrushes reject model eggs painted to look like heterospecific eggs (Davies and Brooke 1989; Moksnes et al. 1990; Grim and Honza 2001; Moskát et al. 2003; Hale and Briskie 2007; Polačiková and Grim 2010; Polačiková et al. 2010). (3) Visual modelling predicted that thrushes should be able to recognize and reject alien conspecific eggs (Cassey et al. 2008a,b). (4) Field observations documented only handful of cases of cuckoo parasitism in thrushes (Møller 1976; Polačiková et al. 2010). (5) Museum surveys indicate that there is not a cuckoo gens specialising on song thrush or blackbirds (Moksnes and Røskaft 1995). This suggests that the *Turdus-Cuculus* interaction, if any, would be in the early stages of coevolution (Grendstad et al. 1999). (6) There are observations of CP in song thrush and blackbirds (Perrins 1998; Grim and Honza 2001; Moskát et al. 2003; K. Weidinger pers. comm.; this study). The low number of such documented cases may stem from the context that CP is much harder to detect than IP (Latif et al. 2006). The contrast between abilities of thrushes to reject alien eggs and the rarity of both CP and IP in the same species led to long-term speculations in the literature about the evolutionary origin of host anti-parasite adaptations (Davies and Brooke 1989; Moskát et al. 2003; Polačiková and Grim 2010).

The relative importance of CP or IP as a current selective factor for *Turdus* thrush egg rejection behaviour is still in need of a critical evaluation. But if both IP and CP converge to produce the same behavioural antiparasitic adaptation in hosts, i.e., egg discrimination and rejection, how can we differentiate between the two alternative functional explanations? Several types of concurrent experiments with consistent methodologies but with alternative predictions are required to test the two hypotheses (Table 1). Here we list critical predictions of the IP hypothesis (predictions of CP represent the opposite).

(1) Hosts are unable to discriminate against conspecific parasite eggs. Clearly, "evolution of a more fine-tuned [egg] recognition ability is unnecessary in the absence of intraspecific brood parasitism“ (Stokke et al. 2004, p. 459; Lahti 2006, p. 165). This view is supported both by theory and empirical data. Under the conspecific acceptance threshold theory (Reeve 1989; Sherman et al. 1997; Fig. 1) the optimal discrimination threshold is set by a trade-off between *acceptance errors* (accepting a parasite egg) and *rejection errors* (ejecting own egg). In early stages of interspecific parasite-host coevolution there is typically low similarity between host and parasite eggs (Davies 2000). At this point, the optimal discrimination threshold should be *permissive* (sensu Reeve 1989), i.e. far away from a distribution of host's own egg phenotypes so as to avoid rejection errors (Fig. 1a). In other words, a host individual showing too strong discrimination ability would commit too many rejection errors and would face fitness losses (Servedio and Lande 2003). Only after an advanced process of coevolution whereby the parasite evolves more mimetic eggs would the optimal discrimination threshold become more *restrictive* (sensu Reeve 1989), i.e. closer to the distribution of host's own egg phenotypes (Fig. 1b; Sherman et al. 1997). Thus, without prolonged arms-race between the parasite and the host IP could not select for fine-tuned discrimination abilities that are needed to cope with CP (Stokke et al. 2004; Lahti 2006). This prediction is supported by findings that hosts parasitized with highly mimetic cuckoo eggs show better egg discrimination abilities than hosts parasitized with poorly mimetic cuckoo eggs (Brooke and Davies 1988). Typical cuckoo hosts reject dissimilar eggs but accept conspecific eggs (e.g., Bártol et al. 2002). Known suitable cuckoo hosts that do reject conspecific eggs are often currently avoided by cuckoos but there is ample evidence for IP in historical records (Moksnes and Røskaft 1995) and, without exception, these species are/were parasitized by highly mimetic cuckoo eggs (Braa et al. 1992; Lotem et al. 1995; Moksnes and Røskaft 1995; Lovászi and Moskát 2004; Stokke et al. 2004). In contrast, cuckoo eggs are extremely dissimilar to *Turdus* eggs: the volume of cuckoo eggs (3.13±0.36cm^3, Moksnes and Røskaft 1995) is only 46% of blackbird egg volume (6.85±0.63cm^3, n=450, own data) and no cuckoo eggs resemble thrush eggs as for colour and patterning (Moksnes and Røskaft 1995).

Clearly, parasitism by cuckoo eggs alone could not provide sufficient selection pressure on thrushes to evolve abilities to discriminate *conspecific* eggs.

(2) Host populations allopatric with the cuckoo respond to experimental parasitism not at all or weakly at best (if there was no difference between allopatry and sympatry, then there should be no ejection costs/errors, see prediction 3). Importantly, 'allopatry' is defined by a severe reduction of overlap of individuals or their gametes, not by geographic distance (Futuyma 1998, p. 482). We studied populations in:

(1) 'micro-scale allopatry' (hereafter **micro-allopatry**) where (a) urban blackbirds and song thrush are highly philopatric (Cepák et al. 2008), (b) cuckoos avoid larger towns and cities (Perrins 1998), and (c) blackbirds were known to be the forest specialist until 150 years ago (Evans et al. 2009). Despite this short time in allopatry, urban populations of the blackbird already diverged in many behavioural and physiological traits, and these variation are partly genetic (Partecke and Gwinner 2007). To our knowledge, none of many studies of urban *Turdus* species across many countries with thousands of nests observed recorded a single case of cuckoo parasitism (Perrins 1998). The major drawback of prior studies between urban and forest *Turdus* populations regarding cuckoo parasitism and egg rejection (Table 1 in Polačiková and Grim 2010) was that the length of presumed sympatry/allopatry was unknown in all cases and no information on gene flow was available (cf. Soler et al. 1999). Here, we studied not only micro-scale allopatric populations but also included macro-scale allopatric populations with known length of allopatry and levels of gene flow. To our knowledge, only one study system with known length of allopatry with interspecific parasites was studied with consistent methods across different populations so far (Lahti 2005, 2006).

(2) 'macro-scale allopatry' (hereafter **macro-allopatry**). Both song thrush and blackbirds were introduced to New Zealand (hereafter NZ) in late 19th century (Thomson 1922) but (a) the European cuckoo was not introduced there, (b) neither song thrush nor blackbirds act as regular hosts of native cuckoos in NZ (Hale and Briskie 2007), (c) gene flow between NZ and Europe does not exist (both species are extremely

sedentary; Cepák et al. 2008), (d) thrushes do not serve as cuckoo hosts in Asia (Polačiková et al. 2010 and references therein) and there is no gene flow between Asia and NZ (Higgins 2006), and (e) NZ populations bred for ~140 generations in isolation from cuckoos and already show micro-evolutionary changes in their breeding biology from their European source populations (clutch size: Evans et al. 2005; egg morphology: Cassey et al. 2005). Importantly, long-term genetic isolation (Lahti 2005) is not necessary for decreased host anti-parasite responses in allopatry because hosts adjust their phenotypically flexible behaviour (Welbergen and Davies 2009) to current parasitism risk across years (Brooke et al. 1998), within season (Brooke et al. 1998, Lindholm 2000), and according to immediate threat of parasitism, e.g., based on whether they can see cuckoos near their nests (Bártol et al. 2002; Davies and Welbergen 2009). Nonetheless, studying allopatric NZ populations is valuable because we can be absolutely sure that our NZ populations did not experience any "environmental cues" (Lahti 2006), including visual (Davies and Welbergen 2009) or acoustic signals of the parasite presence (Lindholm 2000) for more than a century. Further, both species were introduced to NZ in very large numbers: each with 16 and 14 introductions with 800+ and 400+ individuals of blackbirds and song thrush, respectively (Thomson 1922). Therefore, a potential lack of egg rejection behaviour could not be explained by a bottleneck (Lahti 2005). Indeed, recent evidence showed an absence of bottleneck effects in the breeding phenology of both thrushes in NZ (Briskie and Mackintosh 2004).

(3) If hosts rejected parasite eggs in allopatry with the cuckoo, then they should not show ejection costs and/or errors. Theoretical models of parasite-host coevolution predict and field observations provide evidence that the maintenance of egg discrimination abilities in the absence of continued selection pressure is possible only when there is no counter-selection from ejection costs/errors (Stokke et al. 2007). Otherwise, egg discrimination as an error-prone cognitive trait, in the absence of parasitism, would weaken and become extinct (Stokke et al. 2007; Antonov et al. 2010). Thrushes are currently not parasitized by cuckoos in Europe (Moksnes and Røskaft 1995), thus, there is no benefit of antiparasite defence for thrushes in the context of cuckoo parasitism. If we speculate that an ancient cuckoo gens with excellent egg mimicry selected for high levels of egg discrimination in thrushes and then went extinct, we would expect that (a) egg discrimination might persist if it were cost free (Rothstein 2001), (b) egg discrimination would be lost if there were costly recognition errors (Stokke et al. 2007), or (c) egg discrimination would be maintained if benefits associated with recognition of conspecific eggs outweighed the costs of recognition errors. Such benefits could only be accrued during responses to CP.

(4) Latency from foreign egg introduction to rejection is less in sympatry with the cuckoo. Hosts should respond to experimental parasitism faster in sympatry than in allopatry if cuckoos were the selective agent behind the evolution of egg discrimination because the presence of adult cuckoos is known to increase host responsiveness to alien eggs (reviewed in Davies 2000). In contrast, the CP hypothesis predicts no difference in latencies or even shorter latencies in allopatry, especially if allopatric populations show higher breeding densities and, consequently, higher risks of CP (Weaver and Brown 2004).

We stress that predictions (2) and (4) do *not* depend on the plasticity of host behaviour. Both genetic change (micro-evolution) and non-genetic conditional adjustment of individual behaviour (phenotypic plasticity) predict decreased responsiveness in allopatry than in sympatry under IP hypothesis (Davies and Welbergen 2009, Welbergen and Davies 2009).

A survey of previous experimental work on song thrush and blackbirds in both sympatry (Davies and Brooke 1989; Moksnes et al. 1990; Grim and Honza 2001; Honza et al. 2007; Moskát et al. 2003) and allopatry (Moskát et al. 2003; Hale and Briskie 2007) demonstrated consistently higher rejection rates of non-mimetic cuckoo-type eggs than conspecific-like model or real conspecific eggs. Generally, authors interpreted their results as a support for IP hypothesis. However, those studies are equally consistent with CP hypothesis, which also predicts a graded response of higher rejection rates to increasingly dissimilar foreign egg phenotypes relative to own eggs.

Published experimental methods, treatments, tools, and criteria often varied between the species, study sites, and areas with and without cuckoos in previous studies, preventing meaningful quantitative comparisons. Different authors used different model eggs with respect to material, size, and colour (Davies and Brooke 1989 vs. Hale and Briskie 2007), employed different criteria for assessing acceptance of alien eggs (6 days in Moksnes et al. 1990 vs. 4 days in Honza et al. 2007), typically studied a single host population (Grim and Honza 2001) and did not include experimental treatments with conspecific eggs between site types within the same study (Moskát et al. 2003). In our current work, to differentiate between IP and CP hypotheses we used (1) taxonomical replicates (two host species of *Turdus*), (2) geographical replicates (several different locations and study sites within allopatry and sympatry, respectively) and (3) treatment replicates (three types of experimental eggs) at (4) very large numbers of host nests. To address methodological constraints of previous studies, we used identical models manufactured by one person in both thrush species and all studied populations and employed consistent experimental approaches in all population types of the two subject species to generate quantitatively comparable results and strong tests of the alternative hypotheses.

Methods

Study sites and species

We collected data in the Czech Republic (CR) in Olomouc (49°35' N, 17°15' E), Brno (49°12' N, 16°38' E), Bystřička forest 2–3 km from Olomouc, a forest close to Grygov village 7–10 km from Olomouc, and forests adjacent to the village of Lužice (48°51' N, 17°04' E). In NZ we collected data in Auckland city (36°51' S, 174°46' E) and Hamilton town and its rural neighbourhood (37°46' S, 175°16' E) and Tawharanui Regional Park (36°22' S, 174°49' E). Bystřička, Grygov, and Lužice populations were “sympatric”, Olomouc, and Brno “micro-allopatric”, and Auckland, Hamilton, and Tawharanui “macro-allopatric”. Olomouc town (where we collected majority of micro-allopatric data) is completely surrounded by open agricultural fields and, therefore, is lacking suitable cuckoo perches (Antonov et al. 2010). Therefore, urban thrushes are well isolated from cuckoos by both urban environment *per se* and the surrounding landscape matrix.

We conducted the experiments from April to July 2000–2010 in CR and from September to November 2007–2009 in NZ. Because we could not work at all sites simultaneously, even in the same country, we included date in the breeding season as a potential confounding variable in our models.

Egg experiments

We parasitized nests by introducing an artificial egg model or a natural conspecific egg (Fig. 2). (1) “Blue model” (“redstart” type) is an immaculate pale blue and cuckoo sized egg, representing a cuckoo gens which parasitizes the common redstart (*Phoenicurus phoenicurus*; Moksnes and Røskaft 1995). (2) “Spotted model” (“meadow pipit” type) is a dark brown-grey egg, spotted with brown matching meadow pipit (*Anthus pratensis*) eggs (Moksnes et al. 1990). (3) “Real conspecific eggs” were natural blackbird and song thrush eggs collected from abandoned clutches. Conspecific eggs were either transferred to host nests immediately or, when no well synchronized host nests were available, were stored in a fridge before use for a max. 3 days (to avoid the risk of eggs being rejected by hosts due to egg decay). All host eggs and conspecific experimental eggs were individually marked by water-proof marker. Each conspecific egg was used only once.

Egg models were made from the polymer clay and painted by acrylic colours. Size (mean±S.E.: 22.9±0.1 x 17.3±0.2 mm, n=10), mass (3.5±0.2g, n=10) and shape of blue and spotted egg models were similar to real cuckoo eggs (size range: 20–26 x 15–19mm; weight range: 2.9–3.8g; Perrins 1998). Host reactions toward egg models and real parasite eggs are similar (Rothstein 1975; Davies and Brooke 1989; Moksnes et al. 1990). Although smaller hosts species suffer higher rejection costs when ejecting model than real natural eggs (Martín-Vivaldi et al. 2002), large cuckoo hosts (including thrushes) did not damage any own eggs (i.e. there were zero rejection costs) when faced with artificial hard models in the same study.

We checked the nest content daily or every second day for the standard 6-day period (Davies and Brooke 1989; Moksnes et al. 1990). We classified host response as “ejected” if an experimental egg was missing whereas host eggs remained in the nest and were incubated. Depredated nests were excluded from the analysis.

Nest desertion may be a response to parasitism or could result from other causes (human disturbance or inclement weather). Therefore we also checked randomly chosen control nests for the 6-day period (these nests were handled just like experimental nests but no eggs were added).

To minimise nests disturbance we did not remove any host eggs. Previous experiments showed that egg removal had no effect on the rejection behaviour of several cuckoo hosts, including both thrushes (Davies and Brooke 1989). However, our experimental approach of adding (model) eggs rather than switching them with host own eggs might confound our results because clutch sizes are smaller in NZ than in CR (Evans et al. 2004). Thus, the addition of a (model) egg would increase the clutch size and its visible area disproportionately more in NZ than in European nests. We included variable “clutch size” in our models to test for such possible confounding effects.

Statistical analyses

We analysed all data separately for the two study host species. We were primarily interested in the effects of sympatry and allopatry with the cuckoo and the type of experimental egg on host behaviour but we also controlled for factors that were shown to affect host discrimination behaviour in some host species in previous studies. Ejection responses data were analysed using generalized linear mixed models (binomial error and logit link) in SAS (version 9.2; SAS Institute 2008). Latency to egg rejection (in days) was analysed with general linear mixed models in JMP 8.0.1 (SAS Institute 2009).

Initially, we fitted full models with explanatory variables: geography (sympatry, micro-allopatry, macro-allopatry), egg type (blue model, spotted model, conspecific egg), the interaction of the two terms, breeding stage (age of nest at start of experiment; measured as number of days elapsed from start of incubation, negative values denoted the start of experiment in laying stage), first egg laying date (including its quadratic term to test for non-linear seasonal trends), final clutch size (at clutch completion), daytime (time of egg introduction in hours). Date was centred within each year for CZ and NZ separately to remove confounding effects of between-year variation of seasonal breeding and timing of experiments. Random factors included population identity and year (entered as a nominal variable, Grafen and Hails 2002) to control for potential spatio-temporal correlation in the data. In analyses of nest desertion we, instead of egg type, included “treatment” variable (control, blue, spotted, conspecific).

We selected final models by backward elimination of non-significant terms. First, we sequentially examined significance of covariates and kept the two main factors of interest (geography and egg type) and their interaction in the model regardless their significance (Grafen and Hails 2002). After only significant covariates remained in the models, we proceeded with the interaction and the main factors of interest (Grafen and Hails 2002). In all models, the random effects (population identity and year) were very small (likelihood ratio tests; Bolker et al. 2009), i.e., there was no significant temporal and population-specific variation in the data (presence/absence of random effects had no effect on our conclusions). The simpler models without the random effects but with the same structure of fixed effects had dramatically better fits (much lower AIC_c) and very similar parameter estimates. Hence, we decided to present results of the models without random effects (Bolker et al. 2009).

Overall, we obtained information on host responses under the 6-day criterion for 365 nests (38 controls, 181 blue, 88 spotted, 58 conspecific) of song thrush and for 592 nests (54 controls, 325 blue, 106 spotted, 107 conspecific) of blackbirds. Sample sizes differed between various analyses because some data points were missing (e.g. the final clutch size was unknown for nests where experimental egg was ejected during laying and the nest was predated before clutch completion). Additionally, we excluded deserted nests based on our analyses (see below), therefore, samples presented in Fig. 3 are smaller. Effect sizes for desertion and ejection rates are back-transformed values from the final models. Results are shown as mean±S.E.

Results

Song thrush

Nest desertion

Frequencies of desertions were similar between controls (20.0±6.8%, n=38) and nests parasitized with blue (15.7±3.0%, n=181), spotted (15.8±4.2%, n=88) and conspecific eggs (28.2±6.3%, n=58). These frequencies were statistically not different according to the model that controlled for other significant terms (quadratic date in breeding season) and the term of main interest (geography) (treatment effect: $F_{3,357}$=1.48, P=0.22). Therefore, we excluded deserted nests from further analyses.

Egg ejection

Egg type and breeding stage remained the only significant variables in the final model (Table 2). Spotted eggs were rejected at significantly higher rates than blue models (t_{256}=4.86, P<0.0001) or conspecific eggs (t_{256}=4.01, P<0.0001). In contrast, there was no difference between rejection rates of conspecific eggs or blue models (t_{256}=0.38, P=0.70) (Fig. 3a). The probability of ejection increased with advancing breeding stage (test for slope: t_{256}=3.18, P=0.002; egg ejection=–0.78+0.11*breeding stage(days)).

The latency to egg ejection (Fig. 4a) was explained by the egg type and the breeding stage (Table 2). Latency to ejection decreased with advancing breeding stage ($F_{1,116}$=11.54, P=0.0009; latency (days)=2.46–0.10*breeding stage (days)). When the effect of the breeding stage was controlled for, the latency to rejection for blue models (2.01±0.19 days, n=57) was similar to spotted models (1.84±0.19 days, n=54; t_{116}=–0.64, P=0.53). The latency for conspecific eggs (2.97±0.37 days, n=15) was significantly longer than that for blue models (t_{116}=2.30, P=0.02) and spotted models (t_{116}=2.71, P=0.008).

Blackbird

Nest desertion

Frequencies of desertions were similar between controls (12.3±4.3%, n=54) and nests parasitized with blue (7.3±1.6%, n=325), spotted (12.9±3.4%, n=106) or conspecific eggs (22.3±4.2%, n=107). The overall effect of treatment was significant ($F_{3,585}$=5.88, P=0.0006) in the model that controlled for other significant terms (linear date in breeding season) and the term of main interest (geography). Post-hoc examination of least square mean differences CIs showed that the only significant difference was between blue and conspecific treatments. However, desertion rates at each of the three experimental groups were statistically the same as those at control nests. Therefore, we excluded deserted nests from further analyses.

Egg ejection

After the elimination of non-significant terms, the geography vs. egg type interaction and breeding stage significantly explained variation in the blackbird egg ejection responses (Table 3). Overall, spotted eggs were ejected at significantly lower rates than blue models (t_{455}=4.42, P<0.0001) and at similar rates to conspecific eggs (t_{455}=1.51, P=0.13). Conspecific eggs were significantly more often accepted than blue models (t_{455}=5.50, P<0.0001) (Table 3; Fig. 3b). Egg type vs. geography interaction was significant because responses were significantly different between sympatric and allopatric areas only in some cases (Fig. 3b). The probability of ejection increased with the advancing breeding stage (test for slope: t_{455}=3.64, P=0.003; egg ejection=–0.33+0.11*breeding stage(days)).

For the latency to ejection (Fig. 4b), the geography vs. egg type interaction and breeding stage explained significant variation in the blackbird responses (Table 3). Latency of response for blue models (1.41±0.11, n=218) was significantly less than that for conspecific eggs (3.38±0.33, n=24; t_{268}=5.74, P<0.0001) and spotted models (2.29±0.24, n=38; t_{268}=3.33, P=0.001). Spotted models were also ejected faster than conspecific eggs (t_{268}=2.69, P=0.008). Egg type vs. geography interaction was significant because latency of responses varied significantly between sympatric and allopatric areas only in some specific comparisons (Fig. 3b). Latency to ejection shortened with advancing breeding stage ($F_{1,268}$=11.07, P=0.001; latency (days)=2.51–0.06*breeding stage (days)).

Conspecific parasitism in the study populations

We observed:

1) two cases of CP in the song thrush in NZ (Auckland). In one case, a new egg appeared 4 days after the laying was finished (original clutch of 3 eggs). In the

second case, 2 eggs appeared in the nest within one day (total clutch was 5 eggs).

2) two cases of CP in the blackbird in the CR (Olomouc, under 2 new eggs per day criterion: Latif et al. 2006).
3) two cases of probable CP in Olomouc were extreme clutches of 8 eggs in the blackbird (later only 3 nestlings hatched; median clutch size of song thrush and blackbirds in Europe is 4, Perrins 1998). In an other blackbird nest, 6 eggs appeared in the nest within 4 days, thus, this nest was multiply parasitized.
4) one case of a blackbird clutch (two eggs) laid into a fresh song thrush nest (Brno), a probable case of nest usurpation (we do not know whether the blackbird removed any already laid song thrush eggs). Another blackbird female laid three eggs into an old song thrush nest (fledged earlier in the same year) but the nest was predated before the end of incubation (Olomouc).

Rejection costs/errors

We detected both ejection costs (own eggs damaged during successful ejection of a foreign experimental egg) and ejection errors (ejection of own eggs both with and without parasite egg). The latter may also represent ejection costs when damaged eggs were removed by nest owners before we checked the nest content. We found such events in both study species in areas of both sympatry and allopatry.

In song thrush, frequency of costs/errors varied across populations and years from 1.4 to 7.7% (3 population-year-specific estimates with at least 10 nests per sample) with overall frequency of 3.7% (n=107 nests). In blackbirds, frequency of costs/errors varied across populations and years from 2.8 to 10.0% (6 population-year-specific estimates with at least 10 nests per sample) with overall frequency of 5.7% (n=246 nests). We also observed cases of possible ejection errors at control nests both in the song thrush (7.9%, n=38 nests) and blackbird (3.7%, n=54 nests).

Discussion

Contrary to predictions of interspecific parasitism (IP) hypothesis, our study showed that *Turdus* thrushes frequently rejected conspecific eggs, did not reject more frequently in sympatry than allopatry with the cuckoo, in some cases even showed highest egg rejection rates in the most isolated allopatric populations of NZ, and did not reject faster in sympatry than in allopatry with the cuckoo. Since egg rejection was consistently costly (ejection costs/errors) to thrushes across years and populations, we dismiss the explanation that egg discrimination persists in allopatry because it is cost-free. Two scenarios are compatible with our empirical data: (1) high levels of egg discrimination evolved in response to parasitism by mimetic cuckoo egg, but – after cuckoo gens parasitizing thrushes went extinct – has been maintained due to the benefits of conspecific egg rejection, or (2) egg discrimination evolved in response to conspecific parasitism (CP). Under both scenarios CP plays a major role in maintaining egg rejection by these two *Turdus* species.

Importantly, recent evidence (Grim 2006) showed that thrushes are currently unsuitable cuckoo hosts – cuckoo chicks always die in competition with host chicks (song thrush) or even when alone in host nests (blackbirds). This excludes a possibility of recent cuckoo-thrush coevolution and lends stronger support to the latter CP scenario. Absence of current parasitism by cuckoos is not a by-product of strong anti-parasite adaptation in thrushes (e.g., too fast ejection of alien eggs; Fig. 4). Therefore the effective costs of cuckoo parasitism in thrushes is currently nil whereas any rates of CP (e.g., this study) will result in non-zero costs of CP. Thus, in thrushes CP inevitably provides stronger selection pressure than IP.

Contrary to theoretical expectations (Davies 2000) both song thrush and blackbirds ejected (1) with higher probabilities, and (2) faster with *increasing* age of the nest. This suggests that even late parasitism might be costly to hosts, perhaps due to decreased incubation efficiency and female condition and survival (Siikamäki 1995). Also longer experience with the appearance of own eggs could improve abilities to reject alien eggs (Lotem et al. 1995).

Conspecific egg discrimination

Methodologically, although many egg rejection studies used a comparison of mimetic vs. non-mimetic eggs to evaluate the effect of host-parasite egg similarity on the rate of egg rejection, mimetic model eggs are not all the same with respect to their effect on host perception (Cassey et al. 2008a) and rejection behaviours (Honza et al. 2007) and should not be taken as experimental proxies for conspecific eggs. Therefore, we advocate the use of non-mimetic model and/or real eggs, mimetic model eggs, and real conspecific eggs as three experimental treatments to be applied to potential host species within the same population/study area.

Evolutionarily, two specific selection pressures that may lead to the evolution of conspecific egg discrimination include either CP (Jackson 1998) or IP by *highly mimetic* parasite eggs (Stokke et al. 2004). As all cuckoo eggs known from field studies or museum collections are highly dissimilar to thrush eggs we can exclude the latter as an explanation for current maintenance of conspecific egg discrimination in thrushes.

Our study documented surprisingly high rejection rates of conspecific eggs, especially in NZ: both song thrush and blackbirds ejected ~50% of conspecific eggs in allopatric populations. These high rates were achieved solely by the *ejection* of conspecific eggs, as deserted nests were excluded from our analyses. The rarity of conspecific egg rejection in other species was traditionally explained by (1) low costs to conspecifically parasitized hosts and (2) the high costs/errors when attempting to eject closely mimetic parasite eggs relative to host egg phenotypes (Davies 2000). Still, sophisticated egg discrimination evolved in species where effects of IP can be excluded (Stouffer et al. 1987; Moreno-Rueda and Soler 2001; López-de-Hierro and Ryan 2008) or played secondary role to the CP (Jackson 1998; see also discussions in Braa et al. 1992; Welbergen et al. 2001; Lyon and Eadie 2004; Stokke et al. 2004). Mechanistically, the very high conspecific egg ejection rates in our study species are in line with sensory-perceptual studies based on modelling of avian vision, enabling fine scale conspecific egg discrimination in both song thrush (Cassey et al. 2008a) and blackbirds (Cassey et al. 2008b).

Sympatry/allopatry with cuckoos

One major consistent pattern of variation in anti-parasitism traits among hosts of cuckoos is that hosts show stronger responses in sympatry than in allopatry (but see Rothstein 2001 for some studies of cowbirds). Hosts reduce the incidence of egg rejection when parasitism risks are low (Davies and Brooke 1989; Davies et al. 1996; Brooke et al. 1998; Lindholm and Thomas 2000; Briskie et al. 1992; Soler et al. 1999; Stokke et al. 2008), flexibly adjust aggression to adult parasites according to long-term (Briskie et al. 1992; Røskaft et al. 2002) or immediate parasitism risk (Welbergen and Davies 2009; Davies and Welbergen 2009) and show higher intraclutch variation in allopatry than sympatry with parasites (Moskát et al 2002; Aviles and Møller 2003).

None of previous studies had any long-term information on cuckoo absence/presence in any host population. Populations considered currently allopatric from brood parasites could have been parasitized until recently and/thus, are effectively sympatric at evolutionary time scales (Lovászi and Moskát 2004). In contrast, populations considered sympatric, because of current presence of parasites, could have become parasitized only recently and thus, are effectively allopatric at evolutionary time scales (Zúñiga and Redondo 1992). Although we, similarly to all previous studies, have no information on parasitism status of sympatric populations, we can be confident that at least macro-allopatric populations were not in contact with the cuckoo for a long time. Both thrushes are highly sedentary: 96% song thrush and 99% blackbirds are philopatric within 10 km distance from the place of first banding (Cepák et al. 2008). The introduced NZ populations are ~13,000 and 9,500 km, respectively, from the edge of their regular ranges (Perrins 1998, Higgins 2006). Thus, we can be confident that since being introduced to NZ in late 19th century they bred for ~140 generations in complete isolation from cuckoos. Also urban thrushes (micro-allopatry) are very highly philopatric (Cepák et al. 2008) and cuckoos avoid parks in large cities (Perrins 1998). Although there might be some gene flow between our sympatric and micro-allopatric populations (cf. Soler et al. 1999) such process could not lead to higher responses in micro-allopatric than sympatric populations (Futuyma 1998) that we observed in some treatments (Fig. 3a).

Rejection costs/errors

Our study presents evidence of costs in terms of ejection costs/errors. We also detected skips in the laying sequence which might indicate ejection errors or costs (Underwood and Sealy 2006). Thus, we can dismiss possibility that the egg discrimination persists in these *Turdus* species because it is cost free (Rothstein 2001).

Previous authors argued that ejection costs/errors are important only at non-parasitized nests (Stokke et al. 2004). This is because errors at successfully parasitized nests do not lead to differential fitness between acceptors and rejecters (the fitness is zero in both). However, this suggestion does not hold for CP because an acceptor's fitness suffers only from the negative effects of intra-brood competition by unrelated parasite(s) and the extra parental care provided for unrelated young, whereas the erring rejecter's fitness is decreased both by such competition and misdirected parental care, as well as by mistakenly killing own offspring before hatching. In effect, ejection costs/errors at parasitized nests can further provide selection pressure against the maintenance of egg discrimination abilities in the context of CP but not IP.

Discrimination of heterospecific eggs as a by-product of adaptations to CP and vice versa

Lyon and Eadie (2004) explained host coots' (*Fulica armillata*, *F. rufifrons*) responses to IP by the black-headed duck (*Heteronetta atricapilla*) parasitism as "an incidental by-product of within-species conflict" (p. 390). Parasitism by the duck was associated with no 'recoverable' costs, i.e. costs that might select for host defences to alleviate the effects of parasitism, and the degree of similarity between various experimental parasite eggs and hosts eggs had no effect on rejection rates. Thus, there were no significant costs of parasitism to hosts and no selection for egg mimicry in the parasite. These results were used to reject IP hypothesis for the evolution of egg rejection behaviours by coots and to support CP through the observation that there was some CP within coot populations and coots rejected up to 26% of both naturally laid and experimentally added conspecific eggs.

As discussed above, there is also the possibility that advanced coevolutionary arms-races between mimetic cuckoos and rejecter hosts result in such fine scale parasite mimicry of host eggs and discrimination, that conspecific eggs too will be rejected. For example, Honza et al. (2004) found that the blackcap, *Sylvia atricapilla*, ejected ~30% of experimentally added conspecific eggs. In this particular case blackcaps' behaviour might be explained as a by-product of adaptations to IP. This is because (1) there is good experimental evidence that the blackcap had a coevolutionary interaction with the cuckoo (Honza et al. 2004; Grim 2005, 2008), (2) it was historically parasitized by a mimetic cuckoo eggs by a specific cuckoo gent (Moksnes and Røskaft 1995). A long-term coevolutionary process is predicted to fine tune host discrimination abilities as a counter-defence under increasing mimicry in cuckoo eggs until the stage when conspecific egg discrimination is possible (Reeve 1989).

To sum up, rejection of heterospecific eggs may be a by-product of adaptations to CP (Lyon and Eadie 2004), and rejection of conspecific eggs may be a by-product of adaptations to IP (but only under condition of highly mimetic parasite eggs; Stokke et al. 2004).

Evidence for CP

Reports of CP are quickly increasing in a number of species (Lyon and Eadie 2008) and do so disproportionately more in altricial birds (Latif et al. 2006). CP was also detected in both song thrush (Grim and Honza 2001; K. Weidinger pers. comm.; this study), blackbirds (Moskát et al. 2003; K. Weidinger pers. comm.; this study), and in the closely related redwings (*Turdus iliacus*; Grendstad et al. 1999), fieldfares (*T. pilaris*; Ringsby et al. 1993) and mistle thrushes (*T. viscivorus*; Yom-Tov 1980). Thus, CP might be relatively widespread in the genus *Turdus*.

Importantly, daily nest checks and counting the appearance of new eggs, in the absence of marking already present eggs are not sufficient to detect all cases of CP (Latif et al. 2006) because parasites may exchange a host egg with their own (Lombardo et al. 1989), or some hosts may eject parasite eggs very fast (between daily nest visits by researchers; Latif et al. 2006). We also directly observed the ejection of non-mimetic experimental eggs both by blackbirds and song thrush within several hours, including even minutes (immediately when the host individual first arrived at the nest after we introduced the experimental egg; pers. obs.). Moreover, in this study hosts frequently ejected even conspecific eggs – which represent the upper limit of similarity between parasite and host eggs – within mere 24 hours from the act of experimental parasitism (7 by song thrush, 4 by blackbirds). Thus, our study inevitably underestimated real rates of CP in thrushes.

Typical blackbird clutch contains 4 eggs whereas supernumerary clutches may indicate parasitism (Perrins 1998). Such clutch size outliers (circumstantial evidence for CP, see Yom-Tov 1980) were also reported in blackbirds in the South hemisphere where they have been introduced (Higgins 2006, p. 1861; see Results). Further, in the song thrush there are records of eggs laid several days after the clutch completion both from Europe (Grim and Honza 2001) and the South hemisphere (Higgins 2006, p. 1887). Wysocki and Walasz (2004) observed nest usurpation and nest sharing in the blackbird females in Poland. To sum up, based on criteria of 'more than one egg per day', 'appearance of new eggs after completion of the clutch' and 'abnormally large clutches' (Yom-Tov 1980) there is good evidence for CP in various species of the genus *Turdus*. Additionally, there is also evidence of IP between thrush species, including the song thrush parasitizing blackbird's nest (Erard and Armani 1986) and fieldfare parasitizing redwing's nest (Grendstad et al. 1999). Although being much easier to detect, cases of cuckoo IP in thrushes are extremely rare (Møller 1976).

In addition, very high rates of conspecific egg rejection (up to 50%) by thrushes are strikingly at odds with IP hypothesis but support CP hypothesis because extremely low rates of cuckoo parasitism with highly *non*-mimetic eggs cannot select for fine-tuned discrimination of conspecific eggs. This is supported both by theory (Reeve 1989) and empirical data (Brooke and Davies 1988). Importantly, potential observations of rarity or absence of CP in a bird species do not themselves reject the CP hypothesis, just as current absence of cuckoo parasitism in a host cannot be used to reject the IP hypothesis (see currently unused hosts/populations that won arms-race with the cuckoo; Moskát et al. 2002; Honza et al. 2004). The observed responses to experimental parasitism may be the “ghost of parasites past” (Thompson 1994, p. 163). Also, both IP and CP might select for the egg discrimination simultaneously. In fact, Braa et al. (1992) invoked both IP and CP to explain rejection of conspecific parasite eggs by bramblings, *Fringilla montifringilla*, despite the finding that this species is parasitized by mimetic cuckoo eggs.

CP as an alternative female reproductive behaviour might be favoured in populations with non-breeding floater females or failed breeders, high breeding densities, easy locatability of nests, large clutch sizes and an extended egg-laying season (Kempenaers et al. 1995; see also Lahti 2005). Both thrush species meet these conditions as they suffer from very high predation rates, breed at very high densities in urban areas, their nests are by far the most conspicuous passerine nests, they lay large clutches for open-nesting passerines and their egg-laying season is the longest of any urbanized birds (Perrins 1998).

Conclusions

Our study, through the use of consistent intra- and intercontinental methodology demonstrates that the rejection of model non-mimetic eggs does not provide a clear evidence for interspecific parasite-host coevolution. Host egg discrimination may evolve due to selection pressure by conspecific parasites. Under the conspecific parasitism scenario, the rejection of model or real cuckoo eggs is effectively a by-product of adaptations related to conspecific parasitism. Future studies of egg discrimination should consider both IP and CP as viable explanations.

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Table 1. Summary of contrasting predictions of interspecific parasitism (IP) and conspecific parasitism (CP) hypotheses and the results for the two species in this study. S=sympatry, A=allopatry with the interspecific brood parasite (cuckoo).

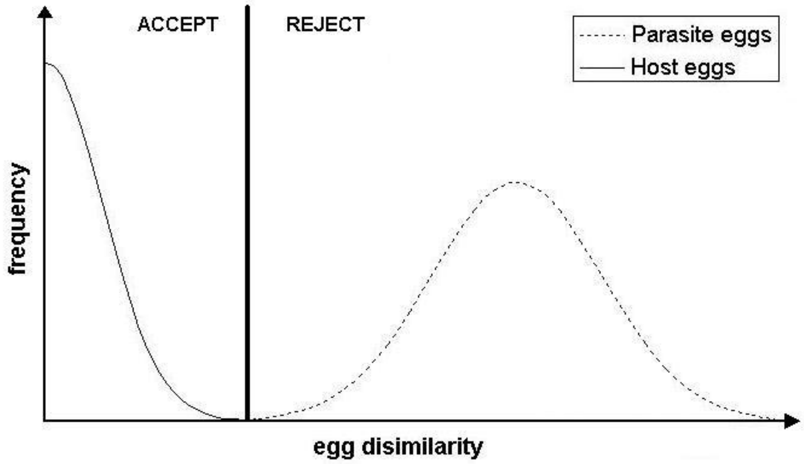
	IP (cuckoo)	CP (thrushes)	Results of this study	
			Song thrush	Blackbird
Conspecific egg rejection	–	+	+	+
Ejection costs/errors	–	+	+	+
Egg rejection frequency	S > A	S ≤ A	S=A	S=A
Egg rejection latency	S < A	S ≥ A	S=A	S=A

Table 2 Egg ejection response and latency to ejection by song thrush and blackbirds. Test statistics and P-values for non-significant terms are from backward elimination procedure just before the particular term (being the least significant) was removed from the model. For effect sizes see Fig. 3. Statistics for fixed effects that are included in significant interactions are not presented (Grafen and Hails 2002, p. 193).

Egg ejection	Song thrush			Blackbird		
	df	F	P	df	F	P
Egg type	2,256	13.26	<0.0001	.	.	.
Geography	2,254	0.31	0.73	.	.	.
Egg type*geography	4,250	1.11	0.35	4,455	3.37	0.0099
Breeding stage	1,256	10.13	0.002	1,455	13.26	0.0003
Breeding season	1,249	2.54	0.11	1,374	0.60	0.44
Breeding season^2	1,157	0.02	0.90	1,373	0.80	0.37
Daytime	1,158	0.06	0.80	1,377	2.39	0.12
Final clutch size	1,248	0.37	0.54	1,375	0.75	0.39
Latency to ejection	df	F	P	df	F	P
Egg type	2,116	3.71	0.03	.	.	.
Geography	2,114	2.05	0.13	.	.	.
Egg type*geography	4,110	0.36	0.84	4,268	6.04	<0.0001
Breeding stage	1,116	11.54	0.0009	1,268	11.07	0.001
Breeding season	1,108	0.06	0.81	1,267	0.54	0.46
Breeding season^2	1,107	0.40	0.53	1,264	0.17	0.68
Daytime	1,68	0.09	0.76	1,222	0.18	0.67
Final clutch size	1,109	0.85	0.36	1,265	0.13	0.72

Figure 1. According to theory (Reeve 1989, Sherman et al. 1997) the optimal discrimination threshold is a) distant from a distribution of host's own egg phenotypes (*permissive*) when mimicry of parasite eggs is poor, and b) closer to the distribution of host's own egg phenotypes (*restrictive*) when parasites evolved better mimicry of host eggs. For detailed explanation see prediction 1 (Introduction).

a)



b)

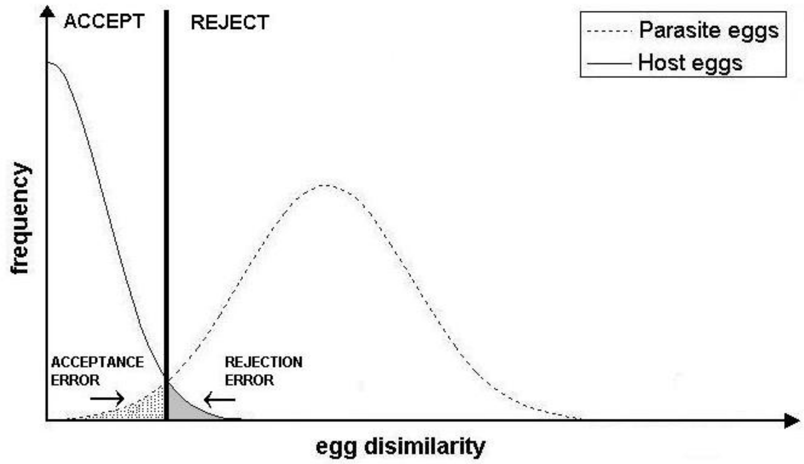
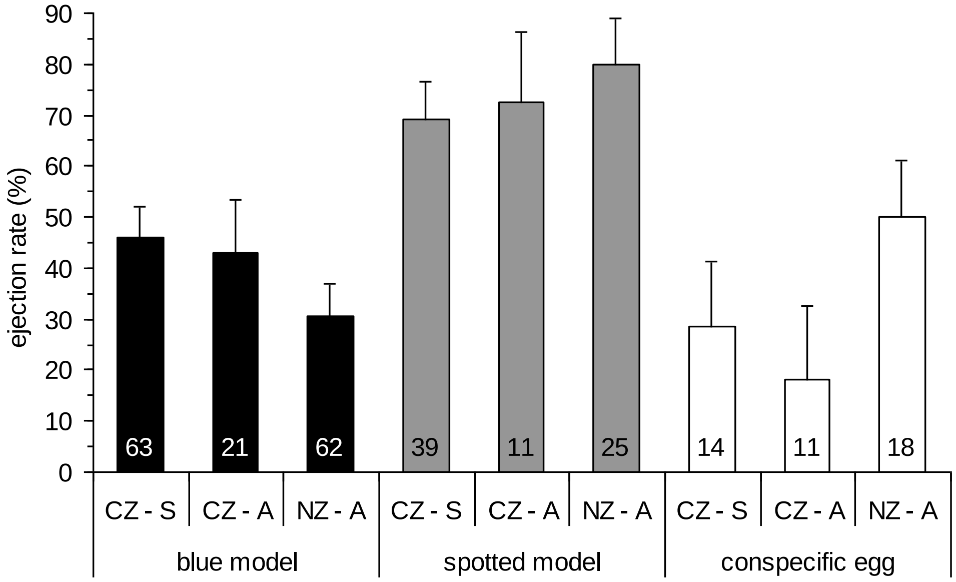


Figure 2. Examples (from left to right) of song thrush and blackbird natural eggs and blue (redstart) and spotted (meadow pipit) models.

Figure 3. Summary of (a) song thrush and (b) blackbird responses to experimental eggs simulating parasitism in sympatric (S) and allopatric (A) populations. Ejection rates (mean±SE) are back-transformed estimates from logistic models controlling for statistically significant covariates and main effects (see Results). CZ=Czech Republic, NZ=New Zealand. Sample sizes (no. of nests) are given inside bars. Statistically significant differences (P < 0.05) of interest (i.e., between geographic groups within egg experiment types) are indicated by *.

(a)



(b)

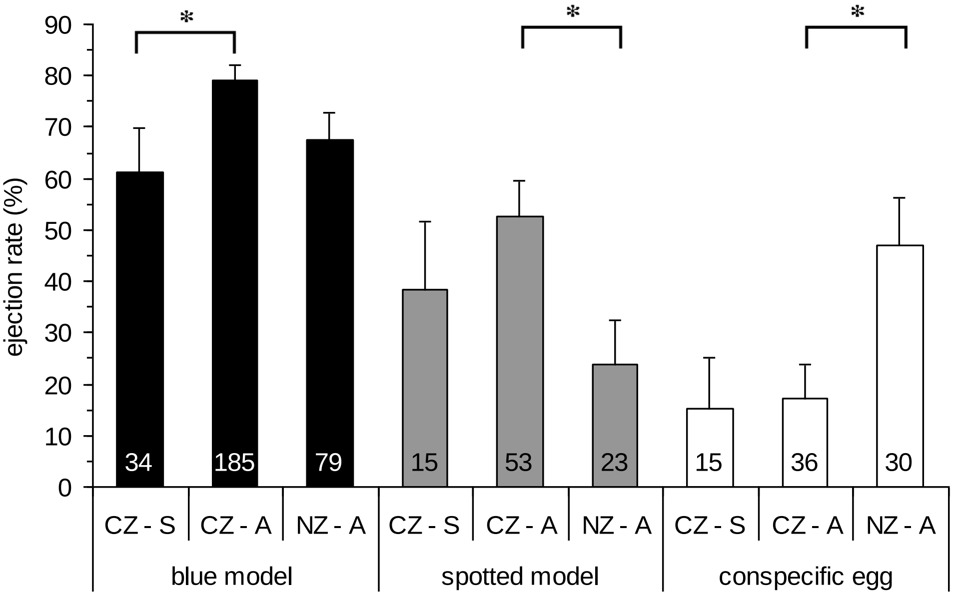
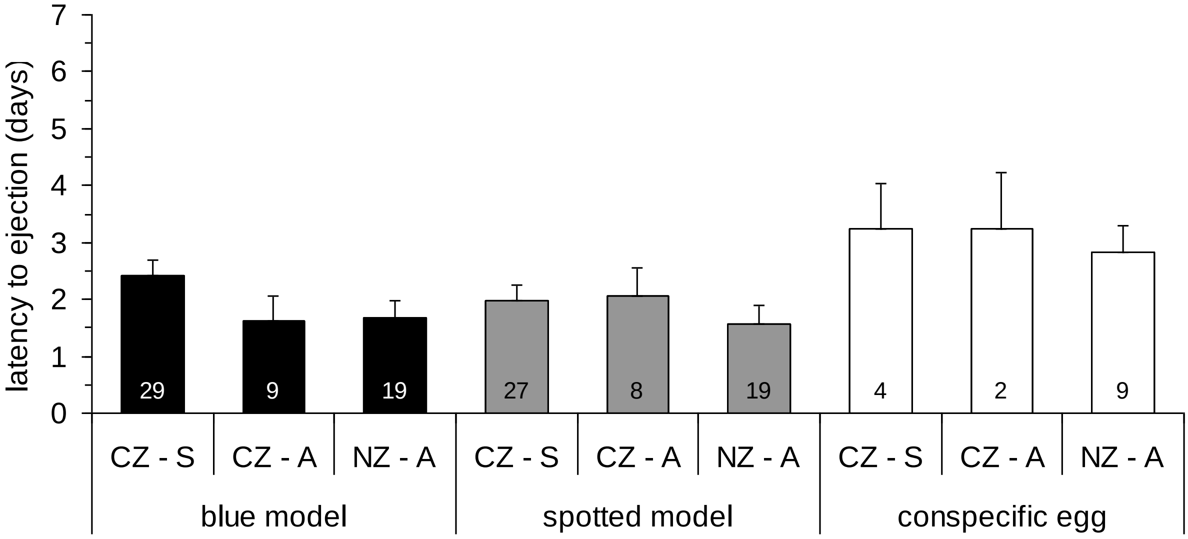
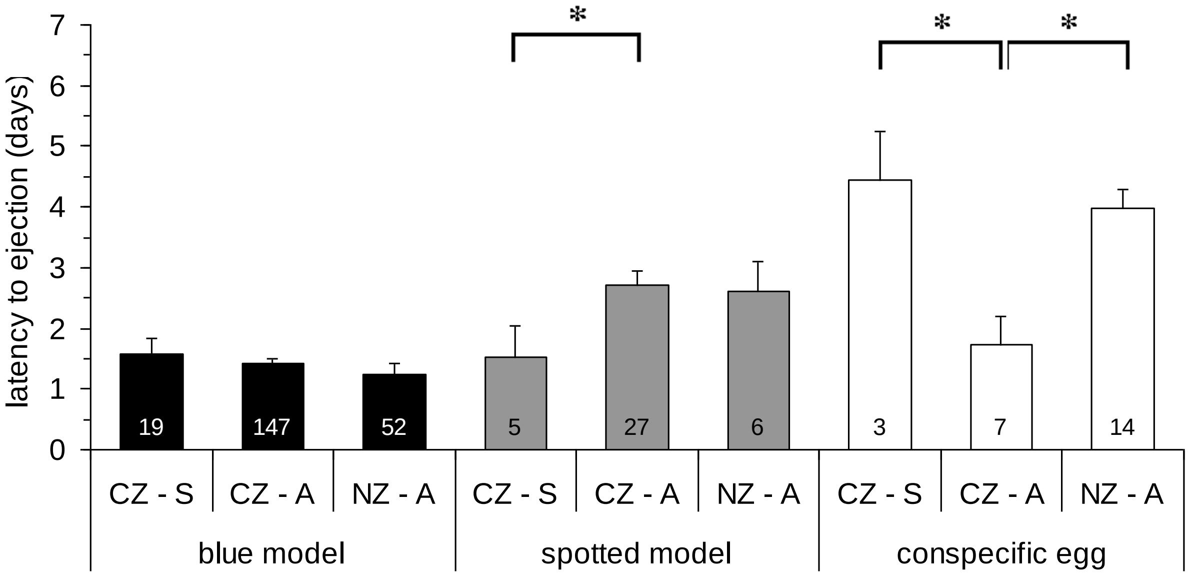


Figure 4. The latency to ejection (mean+S.E.) by (a) song thrush and (b) blackbirds to experimental parasitism in sympatric (S) and allopatric (A) populations. CZ=Czech Republic, NZ=New Zealand. Sample sizes are given inside bars. Statistically significant differences (P < 0.05) between groups are indicated by *.

(a)



(b)



Příspěvek VI.

Hauber M.E., Samaš P., Anderson M.G., Grim T. & Cassey P. (rukopis): Life-history theory predicts alternative egg rejection strategies of introduced *Turdus* thrushes in New Zealand.

Life-history theory predicts alternative rejection responses to foreign eggs of introduced *Turdus* thrushes in New Zealand

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Abstract

Life history theory posits that the evolutionary responses of hosts to reject avian brood parasitism will be shaped by the extent of its costs, which are positively related to both parasitism rate and the virulence of the parasite. Specifically, theoretical modelling predicts that hosts of more virulent parasites (e.g. evicting cuckoos) should eject parasite eggs, irrespective of clutch size, while hosts with smaller clutch sizes that are impacted by less virulent parasites (e.g. conspecifics) should desert parasitized clutches. We demonstrate the application of these predictions of host behavioural responses to evaluate the extent of brood parasitism's costs under which egg rejection behaviours in two species of *Turdus* thrushes may have evolved. We induced the egg rejection behaviour of European blackbirds, *T. merula*, and song thrushes, *T. philomelos* by manipulating the colour of one of the birds' own eggs. Eggs painted entirely black were more often rejected than eggs painted with 20 black spots but with the background colour left visible. Rejections of black eggs occurred mainly through nest desertion in blackbirds compared to mainly through egg ejection in song thrush. These data are consistent with a scenario that egg rejection evolved in response to low virulent brood parasitism in blackbirds but are not decisive as for the evolutionary cause of rejection of dissimilar eggs in song thrush. Such a life history theory informed experimental approach can be generally useful to determine what the relative roles of different types brood parasitism are in the evolution of egg rejection behaviours across avian lineages.

Keywords: clutch size, life history theory, nest abandonment, virulence.

Introduction

Avian brood parasites vary in the extent to which they reduce the reproductive success of hosts (Hauber, 2003). For example, obligately interspecific *Cuculus* cuckoos and other virulent parasite chicks typically eliminate all of the host's breeding success by evicting nestmates soon after hatching (Hauber & Moskát, 2008). In contrast, less virulent *Molothrus* cowbirds and intraspecific brood parasite chicks, often tolerate host nestmates to grow up together (Kilner et al., 2004). As an evolutionary response, to reduce or to eliminate the costs of avian brood parasitism, hosts may reject parasitism by ejecting foreign eggs and chicks from nests and/or by abandoning (deserting) parasitized broods and initiate a new breeding attempt (Davies, 2000; Hauber, 2003; Hosoi & Rothstein, 2000; Rutila et al., 2006).

From a recognition systems perspective, experimental research on egg rejection in hosts of brood parasites requires the assessment of the hosts' behavioural responses as either egg ejection or nest desertion, or the relative frequencies of these displays. This is necessary to identify whether egg ejection and/or nest desertion is the evolutionarily evolved adaptation of hosts in response to coevolutionary histories with brood parasitism (Moskát et al., 2011). Alternatively, ejection/desertion may be a generalized response to cease costly investment into reduced clutch and brood sizes following partial clutch predation-like egg removal by female brood parasites and hosts (Kosciuch et al., 2006), or they represent an experimental artefact following the disturbance of manipulating host egg content by researchers (Rutila et al., 2006).

Furthermore, it remains to be addressed empirically what proximate and ultimate factors determine which mechanisms of rejection behaviours hosts display? Egg ejection, for example, eliminates the immediate the costs of incubating and hatching genetically unrelated eggs, providing parental care for parasitic nestlings, and experiencing loss of fitness due to competition of own chicks with the parasite or the elimination of reproductive success altogether as the parasitic chick evicts all host eggs and nestmates (Grim et al. 2009; Hauber & Moskát, 2008; Kilner et al., 2004). Still, egg ejection is not necessarily cost free for the potential foster parents, as it does not emancipate the host from its nest already having been discovered by the parasite, from the parasitic female typically removing a host egg when laying her own egg, or the return of the parasite to lay additional eggs in the clutch (Hauber et al., 2006; Moskát et al. 2009). In contrast, nest desertion and renesting elsewhere frees the host from providing costly incubation and provisioning to unrelated progeny, but renesting also incurs the costs of locating new nest sites, the time and energy required to build a new nest, and the resources required to laying a new clutch (Servedio & Hauber, 2006). Accordingly, previous theoretical work, based on the extent of phenotypic similarity between host and parasite eggs, the cost of host reproductive investment per breeding bout, the fitness reduction in relative breeding success owing to parasitism, and the rate of parasitism, predicted that hosts may evolve consistently different mechanisms and displays of rejection behaviours to reduce the cost of caring for foreign eggs following in response to brood parasitism (Davies et al., 1996; Reeve, 1989; Servedio & Lande, 2003; Takasu et al., 1993). The critical factor in these models was the absolute and relative clutch size of egg ejector versus nest deserter host species: in response to moderately costly (less virulent) parasitism, species with larger clutch sizes were predicted to eject eggs and species with smaller clutch sizes were predicted to abandon nests, whereas in response to severely costly parasitism, all species are predicted to respond with egg ejection (Servedio & Hauber, 2006). Comparative data are largely consistent with these predictions of egg rejection methods (of egg ejection/nest desertion) across hosts of several avian brood parasitic lineages (Hauber, 2003; Hosoi & Rothstein, 2000; Langmore et al., 2005; Servedio & Hauber, 2006). The central aim of this study is to provide a critical empirical test of this theoretical model, as applied to two sympatric species of egg rejecter thrush species in New Zealand. The predictions of the model, as applied to these species are summarized in Table 2.1.

Table 2.1. Predictions of predominant rejection behaviour methods by *Turdus spp.* from life history theory modelling (Servedio & Hauber, 2006).

Virulence (cost of parasitism)	**Clutch size: lower blackbird**	**Clutch size: higher song thrush**
Lower	***Desert nest***	***Eject egg***
Higher	***Eject egg***	***Eject egg***

Throughout the global distribution of the thrush genus *Turdus,* individual species have been reported to be variably impacted by brood parasites, including both highly virulent, nestmate evictor interspecific parasites (Grim et al., 2011; Honza et al., 2005) and less virulent nestmate-tolerant inter- (Friedman, 1929; Lichtenstein, 1998) or intraspecific parasites (Grim and Honza, 2001; Moskát et al., 2003). Specifically, previous work on European blackbirds (*T. merula*) and song thrush (*T. philomelos*) documented variation rejection rates in response to foreign (natural or experimental) eggs of heterospecifics or conspecifics, when studied in the context of evaluating the evolved responses of these species to virulent brood parasitism by the common cuckoo *Cuculus canorus* (Hale & Briskie, 2007; Honza et al., 2007). Accordingly, within their European range, in a specific comparison of rural versus urban blackbirds, this species was reported to have rejected non-mimetic (cuckoo-like) eggs at higher rates in rural areas where they bred in sympatry with the cuckoo, compared to nearby urban areas where blackbirds bred in allopatry from the cuckoo (Moskát et al., 2003). These covarying differences in rejection rates between areas of sympatry versus allopatry with the cuckoo imply an evolutionary history and selection pressure on blackbirds to recognize and reject interspecific brood parasitism. In contrast to these conclusions, other reports of blackbirds and song thrush in areas of both in sympatry with the cuckoo, including Britain (Davies & Brooke, 1989a,b), the Czech Republic (Grim & Honza, 2001), and Hungary (Moskát et al., 2003), and also in allopatry in New Zealand (breeding stock introduced from Britain; Hale & Briskie, 2007), recorded consistently high rejection rates of non-mimetic, model cuckoo eggs. These latter reports on the lack of covariation in rejection rates between areas of sympatry or allopatry, in turn, support an evolutionary scenario whereby egg rejection in blackbirds has evolved in a context other than interspecific brood parasitism (Grim et al., 2011).

With respect to highly mimetic (natural conspecific or artificially painted) eggs, rejection rates of experimental intraspecific brood parasitism by blackbirds and song thrush were low in Europe (Davies & Brooke, 1989; Honza et al., 2007; Moskát et al., 2003) but high in New Zealand (Hale & Briskie, 2007). However, natural parasitism on various *Turdus* species in Europe by common cuckoos is extremely rare (Møller, 1976), these thrushes are generally unsuitable cuckoo hosts, as the parasitic chicks survive poorly in their nests (Grim et al., 2011), and intraspecific brood parasitism has been reported repeatedly for both blackbirds (Moskát et al., 2003) and song thrush (Grim & Honza, 2001) within their European range (Grim et al., 2011). Thus, another possible interpretation of these combined findings from the literature is that egg rejection behaviours blackbirds and song thrush are owing to an evolutionary selection pressure from intraspecific, rather than interspecific, brood parasitism on these species (Grim et al., 2011).

Critically, for the purposes of this study, both nest desertion and egg ejection are part of the behavioural repertoires of these species in response to experimental parasitism (Grim & Honza 2001; Moskát et al., 2003). Within the context of life history theory, interspecific brood parasitism by common cuckoos in general represents a high cost, high virulence trait, because cuckoo chicks evict host eggs and nestmates (Hauber, 2003). In contrast, intraspecific brood parasitism represents a low cost, low virulence trait because host and foreign chicks grow up together in parasitized broods (Kilner et al. 2004; Servedio & Hauber, 2006). Specifically, modelling work, using egg recognition mechanisms based on host-parasite egg phenotype discrimination, predicted that hosts of more virulent parasites would reject parasitism by the method of ejecting foreign eggs from clutches, while hosts of less virulent parasites with smaller clutch sizes would desert (or abandon) parasitized clutches (Servedio & Hauber, 2006) (Table 2.1).

Given that clutch size of blackbirds is consistently smaller than that of song thrush, and that clutch sizes are even smaller in these *Turdus* species in their introduced range of New Zealand than in their native European range (Evans et al., 2005; Cassey et al., 2006), life history theory specifically predicts that less virulent parasitism would select for *more frequent nest desertion in blackbirds* compared to *more frequent egg ejection in song thrush* (Servedio & Hauber; 2006). Alternatively, more virulent interspecific brood parasitism would select for egg ejection in both species, irrespective of clutch size (Hauber, 2003). These predictions were tested using experimentally manipulated egg phenotypes in New Zealand, where interspecific parasitism is absent on *Turdus spp.* (Hale & Briskie, 2007).

Methods

General procedures: To document behavioural tactics of egg rejection in response to experimentally manipulated coloration, *Turdus* nests were located during the laying and incubation stages to alter the appearance of one of the eggs already laid in a clutch. For this thesis chapter, I present a novel contextual framework and analyses of unpublished experimental data collected during the 2005-8 austral breeding seasons (September – January) for both species while I was in residence in New Zealand. Introduced European blackbirds and song thrush are widespread across New Zealand and occur sympatrically in both urban and rural habitats at high densities. The study sites included urban and rural parkland within a 50 km radius of Auckland and Hamilton cities, North Island, New Zealand. *Turdus* nests are conspicuously bulky structures that can be easily noticed when searching wooded vegetation near open grasslands and lawns. Each nest was included in a single experimental procedure. Although breeding birds were not colour marked in this study, pseudoreplication was considered to be minimised by conducting experiments typically within periods of 1-2 weeks at each site, followed by a move to another study site 7+ km away, thus reducing the chance of using two nests of the same parents.

Egg manipulation protocols: At each nest, all eggs already in the nest were marked for individual identification with a black felt pen at the blunt end (to control for scent). In addition, one egg was manipulated by either (1) dyeing it with 20 black spots of approximately 4 mm in diameter, leaving the background colour visible (Fig. 2.1), or (2) dyeing it all black, covering the background colour entirely. After manipulation, the egg was returned to the clutch. This procedure avoided the use of artificial egg materials (Martin-Vivaldi et al., 2002; Moskát & Honza, 2008) by manipulating the phenotype of the hosts' own eggs (Honza et al., 2007; Moskát et al., 2008), therefore varying only the single parameter of egg coloration (Hauber et al., 2006). However, as cuckoos typically remove a host egg when laying their own egg (Davies, 2000), and so this protocol did not alter the clutch size for these experiments.

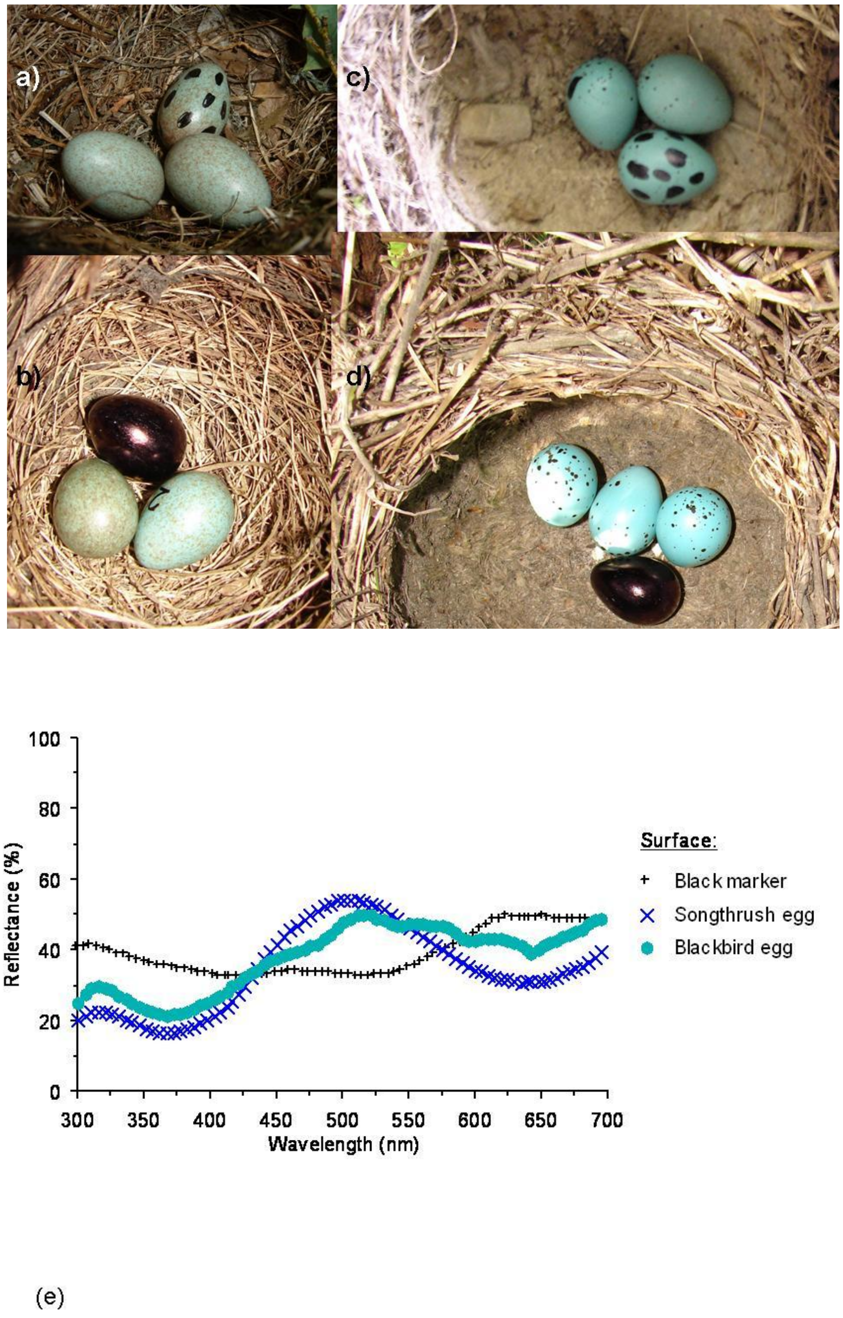

Fig. 2.1. Clutches with manipulated eggs of European blackbirds (a,b) and song thrush (c,d), with representative avian-visible reflectance spectra (taken following Cassey et al., 2009) of black marker covered shell, natural blackbird egg background, and natural song thrush egg background (e). Photo credits: Mark E. Hauber.

The nest contents were then checked up to 5 days after manipulation to assess whether the manipulated egg was present or missing. Clutches in which new egg(s) appeared were considered to be during the laying stage for the experiment while all other clutches were considered to be during the incubation stage.

Eggs that disappeared in $\leq$ 5 days (experiment = day 0) were considered ejected (Hale & Briskie, 2007) and clutches with cold or wet eggs within this period were considered deserted. Confirmed nest desertions were recorded during at least one subsequent visit to the nest. Nests with evidence of predation (i.e., broken eggs, shell remains within a cold clutch, and entirely missing clutch contents) were excluded from the analysis.

Statistical analyses: I first I used nominal logistic mixed models to examine potential differences between years, study sites, and breeding stages with respect to the outcome of these experiments with either *Turdus* species. In the absence of an overall statistical effect of these predictors (see below), all data were combined into contingency analyses for each species separately.

The combined data sets were based on sample sizes which varied between 27 – 34 per taxon and per treatment, comprising some of the most extensive datasets using the same experimental methodology for egg rejection studies in any passerine bird (Grim, 2007). I then specifically examined (i) what the effects of treatment were on either bivariate and or detailed rejection responses and (ii) whether there were species-specific differences in the detailed rejection responses.

Results

Multivariate analysis: The nominal logistic fit models showed no consistent relationships between any of predictor variables of year of experimentation, study site, and breeding stage and either the bivariate outcomes (acceptance/rejection) of the experimental treatments (all Wald test < 0.51, P > 0.91), or when examining the rejection responses (ejection vs. desertion) within the black dyed egg treatment only (all Wald test < 1.6, P > 0.44). Therefore, univariate analyses are presented below for each species separately.

Rejection rates: European blackbirds rejected black eggs (67%) more often than 20 spotted eggs (13%) (Fisher's exact test [Fet]: P < 0.001, n = 57). Song thrushes also rejected black eggs (50%) more often than 20 spotted eggs (6%) (Fet: P = 0.001, n = 64). There were no species differences in either the higher rejection rates of black eggs (Fet: P = 0.21) or the lower rejection rates of 20 spotted eggs (Fet: P = 0.42) (Fig. 2.2).

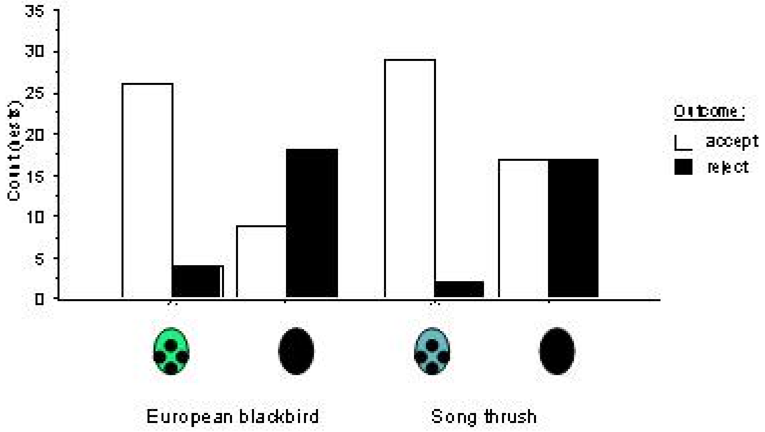

Fig. 2.2. Bivariate outcomes of experiments of European blackbird and song thrush clutches in response to manipulation of single eggs

Methods of rejection: Regarding the detailed methods of egg rejection, blackbirds ejected all (n = 4) and song thrush either ejected (1) or deserted (1) those eggs that were rejected following the 20 spotted manipulation (sample sizes too small for a statistical test). Blackbirds most often deserted nests with black eggs (14 of 18 rejections), whereas song thrush most often ejected black eggs (14 of 17 rejections) (Fet: P = 0.0006) (Fig. 2.3).

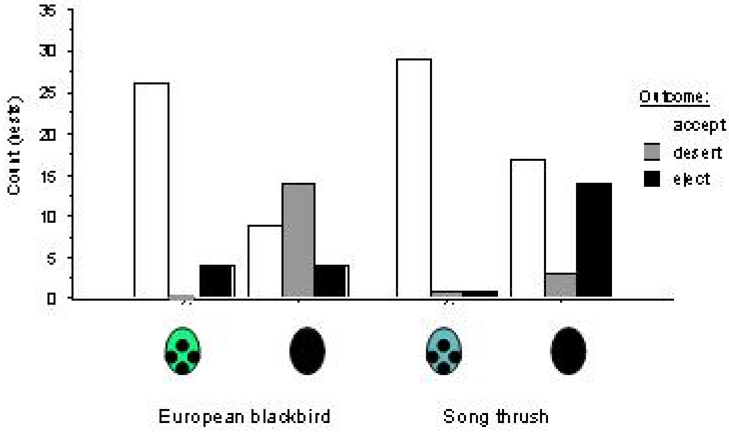

Fig. 2.3. Detailed behavioural responses of European blackbirds and song thrush in response to colour manipulations of single own eggs per clutch.

Discussion

These data (Fig. 2.2) support previous work on European blackbirds, song thrush, and several other hosts of brood parasites by demonstrating that eggs whose background coloration matches that of host eggs are more likely to be accepted than eggs whose background coloration is altered dramatically (Rothstein, 1975; Davies & Brooke, 1989; Hauber et al., 2006; Rutila et al., 2006; Moskát et al., 2008). Yet, in contrast to previous work on *Turdus spp.* in both their native (Grim & Honza, 2001; Grim et al., 2011; Honza et al., 2007; Moskát et al., 2003) and introduced ranges (Hale & Briskie, 2007), here it was found that blackbirds and song thrush at the New Zealand study sites responded to non-mimetic eggs by different egg rejection methods: blackbirds typically deserted experimental clutches with black eggs while song thrush ejected black eggs and continued to incubate (Fig. 2.3). In the absence of repeated observations on the same individuals, however, we cannot conclude whether egg ejection and nest desertion are alternative strategies or tactics (*sensu* Gross, 1996) within individuals' antiparasitic behavioural repertoires of these species. We can, however, reject the hypothesis that differences in details of egg rejection methods were owing to experimental or observational variation in reproductive stage or seasonality, as neither of these predictors explained significant proportions of behavioural variability in these data. Critically, for the recognition system analysis of host-parasite coevolution in this thesis, it can also be concluded that the behavioural response of these two *Turdus* thrushes do and must include nest desertion as an alternative parasitically induced response behaviour to egg ejection in future experimental and observational studies.

These results are consistent with several scenarios for the evolution of blackbird and song thrush egg rejection behaviours. First, these data are consistent with a moderately virulent level of parasitism selecting for the species-specific egg rejection strategies of blackbirds and song thrush. Given that blackbirds have a smaller clutch size than do song thrush in New Zealand (Evans et al., 2005), life history theory predicts that in response to moderately costly nest parasitism blackbirds should be more likely to reject parasitism by nest desertion rather than egg ejection (Table 2.1, Servedio & Hauber, 2006). In fact, life history theory would also predict that any moderately costly circumstance which reduces the breeding success of nesting birds (i.e. the presence of detritus, broken shells, foreign objects, etc.) should select for nest abandonment by blackbirds compared to song thrush with their relative differences in clutch sizes (Hoover et al. 2006; Servedio & Hauber, 2006), and so behaviours unrelated to parasitism and connected with, for example, nest hygiene of broken eggshells (Hauber, 2003) and nest cleaning of non-egg materials, too, could explain the observed species-specific differences in responses to black eggs from these experiments (Moskát et al., 2002).

An alternative is that egg rejection in blackbirds evolved in response to less virulent brood parasitism compared to more virulent parasitism on song thrush. For example, intraspecific brood parasitism in blackbirds versus common cuckoo parasitism in song thrush would too predict the same alternative pattern of egg rejection behaviours that was observed in this study. That successful common cuckoo parasitism has only been recorded on song thrush and not in blackbirds in museum collections is consistent with this scenario (Grim et al., 2011).

Finally, it is possible that interspecific parasitism has less costly consequences for blackbirds than for song thrush, even when caused by virulent brood parasites. For example, cuckoo chicks do not always succeed in host nests, owing to nest architecture, host foraging regimes, and discrimination (Grim et al., 2009). Accordingly, experimental work (Grim, 2006; Grim et al., 2011) recently demonstrated that common cuckoo chicks do not survive in broods of blackbirds, thus imposing time-limited costs on the foster parents. In contrast, cuckoo chicks grow rapidly and can fledge successfully from experimental broods of song thrush, thus potentially imposing high and longer-lasting costs on the fosterers. Once again, these relative levels of virulence would predict more nest desertion by blackbirds and more egg ejection by song thrush, as documented by this study (Table 2.1). Given these parallel predictions of the alternative evolutionary scenarios, future experimental and comparative work should focus on predicting additional differences in the egg rejection behaviours of *Turdus spp.* in response to more or less virulent parasitism.

Irrespective of the evolutionary history, these data confirm that the use of birds' own eggs, together with experimental manipulation of egg appearance, can be used to effectively test alternative predictions of sensory mechanisms (Honza et al., 2007; Moskát et al., 2008) as well as life history (Servedio & Hauber, 2006) and optimal acceptance threshold theory (Hauber et al., 2006). Specifically, future work should address the range of behavioural responses and rejection repertoires of individuals and populations, following natural or experimental intraspecific brood parasitism within and outside the native ranges of blackbirds and song thrush. In addition, these data also call for further experimental work, using matched methodologies, to compare and contrast the egg rejection behaviours of potential hosts within and outside areas of sympatry with avian brood parasites.

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Prohlašuji, že tato práce je mým původním autorským dílem, které jsem vypracoval samostatně. Výjimku tvoří přiložené články, na nichž se podíleli uvedení spoluautoři. Můj autorský podíl je uveden v přiložených potvrzeních od spoluautorů.

Práce nebyla využita k získání jiného nebo stejného titulu.

Contributions of Peter Samaš to following publications:

I. **Samaš P.**, Hauber M.E., Cassey P. & Grim T.: High repeatability of foreign egg rejection by brood parasite hosts. Ethology 117(7): 606–619. (doi: 10.1111/j.1439-0310.2011.01917.x)

- Contributed to design of the research project
- Collected vast majority of data
- Performed all statistical analyses
- Wrote the first draft, finalized the MS

II. Grim T., **Samaš P.**, Moskát C., Kleven O., Honza M., Moksnes A., Røskaft E. & Stokke B.G. 2011: Constraints on host choice in parasitic birds: why do parasitic birds rarely exploit some common potential hosts? Journal of Animal Ecology 80(3): 508–518. (doi: 10.1111/j.1365-2656.2010.01798.x)

- Collected majority of experimental data from the blackbird and song thrush in CZ
- Performed crucial experiments with cross-fostering (cuckoo nestlings in thrush nests)
- Performed almost all statistical analyses
- Contributed to writing of second, subsequent, and final drafts of the MS

III. Igic B., Cassey P., **Samaš P.**, Grim T. & Hauber M.E. 2009: Cigarette butts form a perceptually cryptic component of song thrush (*Turdus philomelos*) nests. Notornis 56(3): 134–138.

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- Contributed to writing of second, subsequent, and final drafts of the MS

IV. **Samaš P.**, Grim T., Hauber M.E., Cassey P. & Evans K.L. (manuscript): Ecological predictors of reduced avian reproductive investment in the southern hemisphere.

- Contributed to design of the research project
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V. **Samaš P.**, Cassey P., Hauber M.E. & Grim T. (manuscript): Host responses to interspecific brood parasitism: a by-product of adaptations to conspecific parasitism?

- Contributed to design of the research project
- Collected vast majority of data
- Performed all statistical analyses
- Wrote the first draft, finalized the MS

VI. Hauber M.E., **Samaš P.**, Rutila J., Anderson M.G., Grim T. & Cassey P. (manuscript): Life-history theory predicts alternative egg rejection strategies of introduced *Turdus* thrushes in New Zealand.

- Collected part of data (~50 %)
- Contributed to writing of second, subsequent, and final drafts of the MS

VII. **Samaš P.** & Grim T. (manuscript): Jak urbanizace ovlivňuje hnízdní a mimohnízdní disperzi kosa černého? (How urbanisation influences breeding and non-breeding dispersal of the blackbird?)

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I. **Samaš P.**, Hauber M.E., Cassey P. & Grim T.: High repeatability of foreign egg rejection by brood parasite hosts. Ethology 117(7): 606–619. (doi: 10.1111/j.1439-0310.2011.01917.x)

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Contributions of Peter Samaš to following publications:

Grim T., **Samaš P.**, Moskát C., Kleven O., Honza M., Moksnes A., Røskaft E. & Stokke B.G. 2011: Constraints on host choice in parasitic birds: why are thrushes rarely parasitized by common cuckoos? Journal of Animal Ecology 80(3): 508–518. (doi: 10.1111/j.1365-2656.2010.01798.x)

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Název práce:
Ekologie a chování drozdů (*Turdus* spp.) v původních a introdukovaných populacích

Typ práce: disertační

Pracoviště: Katedra zoologie a Ornitologická laboratoř, Univerzita Palackého

Vedoucí práce: Doc. RNDr. Tomáš Grim, PhD.

Studijní program: P1527 Biologie

Obor: Zoologie

Rok obhajoby práce: 2011

Abstrakt:

V této disertační práci jsem se zaměřil na výzkum dvou modelových druhů, kosa černého (*Turdus merula*) a drozda zpěvného (*T. philomelos*). Zkoumal jsem původní evropské populace ze severní polokoule a populace, které byly před přibližně 140 lety introdukované na Nový Zéland na jižní polokouli. Větší část výzkumu je věnovaná hnízdnímu parazitismu, jmenovitě protiparazitickému chování a změnám v tomto chování mezi původními a introdukovanými populacemi. V další části využívám introdukovaných populací ke studiu změn v hnízdních charakteristikách.

Oba druhy se dokážou velmi účinně bránit proti mezidruhovému parazitismu především díky vysoce vyvinutým schopnostem rozlišit a vyhodit cizí vejce. Zjistili jsme, že tato schopnost je u každého jedince vysoce konzistentní a proti parazitaci obvykle reaguje opakovaně vyhozením nebo přijetím cizího vejce. Přes existující protiparazitické schopnosti je prokázaný hnízdní parazitismus u obou druhů výjimečný, a proto jsme v další práci hledali příčiny stojící za absencí parazitismu a za evolucí protiparazitických schopností. Ve srovnávací průzkumné části jsme porovnali life-history znaky deseti nejběžnějších hostitelů kukačky obecné (*Cuculus canorus*) se šesti evropskými drozdy rodu *Turdus* spp., abychom nejdříve určili kandidátní znaky, které by mohly být příčinou absence parazitismu kukačkou (např. srovnání velikosti těla, hnízda, vajec, mláděcí potravy drozdů a hostitelů). Poté jsme experimentální části ukázali, že tyto příčiny jsou druhově specifické a navíc jsou výsledkem působení interakce několika faktorů, které se uplatňují v mláděcí periodě (např. velikost a/nebo design hnízda, neschopnost kukačky konkurovat hostitelským mláďatům). V další experimentální práci jsme na populacích žijících v sympatrii a alopatrii s kukačkou ukázali, že schopnost odmítat cizí vejce u obou druhů je výsledkem evolučního tlaku ze strany vnitrodruhového parazitismu. To také podporují námi zjištěné případy vnitrodruhového parazitismu ve studovaných populacích.

Evropské a novozélandské populace modelových druhů vykazují několik rozdílů v hnízdních parametrech, přičemž nejvíce zkoumanou změnou je menší velikost snůšek u populací na jižní polokouli. Přesto jsou příčiny takového rozdílu nejasné a stále chybí komplexní experimentální studie, které berou v úvahu více potenciálních faktorů. V naší experimentální studii se jako nejvýznamnější faktor jevila sezonalita, zatímco hnízdní predace a hustoty nedokázaly pokles snůšky vysvětlit. Dále jsme zjistili, že pokles velikosti snůšky introdukovaných populací se již stabilizoval a netrval déle než 100 generací. K lepšímu pochopení příčin by bylo vhodné vzít v úvahu i míru přežívání dospělců, která může úzce souviset s mírou sezonality. Trade-off velikosti snůšky a vajec predikovaný klasickou life-history teorií jsme prokázali pouze u kosa. Jiným významným faktorem ovlivňujícím snůšku i míru predace je urbanizace, ale naše studie provedená na několika prostorových replikátech a dvou druzích její vliv nepotvrdila.

Klíčová slova: introdukce, vnitrodruhový hnízdní parazitismus, mezidruhový hnízní parazitismus, odmítání vajec, kukačka obecná, drozd zpěvný, kos černý, velikost snůšky

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Abstract:

The aim of this project was to explore behaviour and selected life-history traits in two model species, the blackbird (*Turdus merula*) and the song thrush (*T. philomelos*). I studied native European populations in the northern hemisphere and introduced populations to New Zealand in the southern hemisphere. A major part of my research was devoted to avian brood parasitism, examining antiparasitic strategies and differences in such strategies between native and introduced populations in particular. Further, I studied causes of changes in breeding characteristics between these populations.

Both species have evolved effective antiparasitic strategies against interspecific parasitism. They have developed especially good abilities to recognize and eject an alien egg. We found that rejection behaviour was higly repeatable at the individual level and that host birds responded highly consistently by ejection or acceptance. While antiparasitic strategies are shown to exist, the evidence of interspecific parasitism is very scarce. Thus, we posed the questions ‘why is parasitism rare in these species‘ and then ‘how did their rejection abilities evolve‘. We compared life-history traits of ten most common cuckoo (*Cuculus canorus*) hosts in Europe with six European *Turdus* thrushes. We subsequently identified candidate traits that could be responsible for the absence of cuckoo parasitism in thrushes (e.g. comparisons of body size, nest size, egg size, chick diet). Our results showed experimentally that multiple potential factors and their interactions, which are species-specific and restricted to nestling period, are responsible for absence of parasitism (e.g. nest size and/or design, poor cuckoo chick competition abilities with host nestlings). In another experimental study we explored populations occuring in allopatry and sympatry with cuckoo to show that intraspecific parasitism is responsible for the evolution of rejection abilities in both the blackbird and the song thrush. This result further support few documented cases of intraspecific parasitism in our study populations.

The breeding parameters of the New Zealand populations of our study species differed from those of their European conspecifics. The most documented pattern is that clutch size is smaller in the southern hemisphere than in the northern one. However, potential causes are still debated and complex experimental studies considering more factors are lacking. We showed in our experimental study that seasonality partly explained variaton in clutch size, whereas predation rates and breeding densities are insufficient to explain this pattern. We also showed that reduction of clutch size had stabilized in introduced populations and took place in less than 100 generations . Future studies should also include the analysis of adult survival rates which can be closely related to seasonality. Avian life-history theory predicts a trade-off between clutch size and egg size which we demonstrated only in the blackbird. Urbanisation is another significant factor influencing clutch size and predation rates but we did not confirm such effects in either of the two study species.

Keywords: introduction, intraspecific brood parasitism, interspecific brood parasitism, egg rejection, common cuckoo, blackbird, song thrush, clutch size

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