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Kvalita samce, mimopárové paternity a rodičovské investice u hýla rudého *Carpodacus erythrinus*

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disertační práce

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Obsah

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Úvod

Sociální monogamie spojená s biparentální péčí o mláďata je u ptáků jedním z nejčastějších párovacích systémů (Lack 1968). Protože samice více investují do rozmnožování, jsou obecně považovány za pohlaví, které si vybírá partnera (Bradbury a Vehrencamp 1998). Jako odpověď samičí preference se cestou pohlavního výběru vyvinula u samců řada znaků, na jejichž základě samice provádějí volbu partnera (viz např. Fisher 1930). Existuje tak řada hypotéz týkajících se vzniku a udržování samčích ornamentálních znaků, které si mohou, ale i nemusí odporovat, a které se snaží problém vysvětlit.

Samičí výběr na základě ornamentálních znaků jako indikátorů kvality samce může samicím přinášet přímé či nepřímé výhody. Přímou výhodou, jakou samice spárování s určitým samcem získá, může být intenzivnější podíl samce na hnízdní péči, dále pak kvalitní teritorium či obrana hnízda (Kikpatrick a Ryan 1991). Tím, že samec například krmí s větší intenzitou, může zvýšit jak životaschopnost mláďat, tak dovolit samici krmit méně a zajistit jí tak lepší kondici pro další přežívání. Podle teorie dobrého rodiče (good parent model, Kikpatrick a Ryan 1991) mohou různé samčí sekundární pohlavní znaky signalizovat úroveň rodičovské péče a umožňovat tak samici předem ohodnotit samčí rodičovské kvality (Hoelzer 1989, Heywood 1989).

Nepřímou výhodu představují kvalitní geny, které může samice získat pro své potomky. Dvě nejčastěji zmiňované hypotézy o evoluci samičího výběru na základě nepřímých výhod jsou Fisherův ‘run-away’ model (Fisher 1930) a hypotéza dobrých genů (good genes model, Bradbury a Anderson 1987). Tyto hypotézy ale nemusí stát proti sobě a existují i přechodné modely vycházející z obou teorií (Kokko 2002).

Podle hypotézy dobrých genů samice používá určité samčí sekundární pohlavní znaky k ohodnocení genetické kvality samce. Předpokladem toho, aby v praxi mohl samičí výběr skutečně fungovat podle těchto hypotéz, je, že tyto znaky jsou dědičné a produkce těchto sekundárních pohlavních znaků je pro samce nákladná. To znamená, že tyto znaky jsou schopni v odpovídající kvalitě produkovat pouze skutečně kvalitní samci.

Jedním z nejvýraznějších a nejčastěji zkoumaných sekundárních pohlavních znaků, které mohou signalizovat samčí kvality a které používají samice při výběru partnera je zbarvení (Andersson 1994, Hill 2002). U velkého počtu druhů pěvců existuje pohlavní dimorfizmus ve zbarvení peří a většinou existuje silný vztah mezi reprodukčním úspěchem samce a kvalitou zbarvení peří samců. Zbarvení peří je dáno jeho strukturou nebo obsahem pigmentů. V ptačím peří se nacházejí tři základní typy pigmentů: karotenoidy, melaniny a porfyriny (Fox 1976, Brush 1978, Hill a McGraw 2006). Strukturální zbarvení způsobuje rozptyl světla na povrchové struktuře peří ptáků, zatímco pigmenty jsou ukládány do struktury pera během jeho růstu. Karotenoidy nejsou živočichové schopni sami syntetizovat. Jsou produkovány výhradně v rostlinách a některých bakteriích a živočichové je přijímají pouze z potravy (Goodwin 1950, Brush 1976). Úroveň červeného zbarvení je ale závislá nejen na schopnosti samce obstarat potravu bohatou na karotenoidy, ale je ovlivněna i genetickými faktory souvisejícími s metabolickými procesy zajišťujícími absorpci, přeměnu a ukládání karotenoidů do peří (Hill 1992). Navíc karotenoidy fungují jako antioxidanty a jsou využívány imunitním systémem (Vershinin 1999). Tudíž je možné předpokládat, že nemocní a parazitovaní samci budou vystaveni vyšší spotřebě karotenoidů pro svou imunitu a méně jim jich zůstane pro tvorbu ornamentálních znaků (Olson a Owens 1998). Karotenoidní zbarvení tak může mimo jiné čestně signalizovat zdravotní stav a kondici jedince (Lozano 1994). Samice by tedy mohla upřednostňováním červených samců získat jak nepřímou, tak přímou výhodu, jako například geny pro vyšší rezistenci proti parazitům (Hamilton a Zuk 1982) a nižší pravděpodobnost přenosů patogenů a parazitů (Poiani a Wilks 2000). Celá problematika karotenoidů a jejich vztahu k zdravotnímu stavu jedince je ale daleko složitější (viz Vinkler a Albrecht 2010).

Síla sexuální selekce je úměrná míře variability reprodukčního úspěchu, za který bývá většinou považován počet zplozených mláďat (Crow 1958, Shuster a Wade 2003). Tradičně býval jako hlavní zdroj variability v reprodukčním úspěchu uváděn počet a kvalita samic, které samec získá, což platí zejména u polygamních ptáků, kde se samci mohou spárovat s více samicemi a naopak několik samic se může spárovat s nejlepším samcem (Darwin 1871, Andersson 1994). Předpokládalo se, že variabilita v reprodukčním úspěchu samců je naopak relativně nízká u sociálně monogamních ptáků. Díky značnému rozšíření molekulárních metod je ovšem dnes známo, že přibližně 86 % ptačích druhů je geneticky polygamních (Griffith 2002), neboli vyskytují se u nich mimopárové paternity (EPP). U monogamních druhů, zvláště pokud hnízdí pouze jednou ročně, jsou mimopárové paternity pro samce jediným způsobem, jakým může zvýšit svůj reprodukční úspěch v daném roce. To znamená, že kvalitní samec, který může mít i díky spárování s kvalitní samicí větší počet mláďat ve svém hnízdě, může zároveň zplodit další mláďata i mimo své hnízdo a zvýšit tak svou reprodukční zdatnost (fitness). Tak se zvýší i variabilita ve fitness mezi jednotlivými samci (Whittingham a Dunn 2005). Na druhou stranu i samice, která se spáruje s nekvalitním samcem, může teoreticky díky EPP získat kvalitnější geny pro své potomky a zvýšit tak svojí fitness (Parker 1979). O tom, jaké dopady mají mimopárové paternity na fitness samců a samic, se ovšem vedou diskuse (viz Arnqvist a Kirkpatrick 2005, Albrecht et al. 2006). Míru mimopárových paternit u daného druhu může ovlivnit řada faktorů, jako např. hustota populace, momentální poměr pohlaví (Hoi 1997), synchronizace hnízdění (Stutchbury a Morton 1995) a motivace samice zúčastnit se EPP, která může být závislá na potřebě samčí rodičovské péče nebo atraktivitě samce (Johnsen a Lifjeld 1995, Griffith et al. 2002).

Samice si ale nemusí vybírat partnera pouze na základě nejlepších sekundárních pohlavních znaků, ale také na základě vhodnosti svého genotypu ke genotypu konkrétního samce (model genetické komplementarity; Tregenza a Wedell 2000, Pialek a Albrecht 2005, Mays et al. 2008). Zatímco model dobrých genů předpokládá, že preference určitých samčích fenotypových znaků jsou u všech samic stejné (e.g. Iwasa et al. 1991), model genetické komplementarity pracuje s hypotézou, že každá samice přizpůsobuje výběr svého partnera s ohledem na svůj vlastní genotyp a hledá takového, aby jejich potomci měli co nejvýhodnější kombinaci genů (Hettyey et al. 2010). Tudíž pro každou samici se jako nejvhodnější jeví odlišný samec.

Přes všechny výše uvedené hypotézy by nebylo správné předpokládat, že úspěšnost samce při EPP je ovlivněna pouze samičím výběrem. Důležitou roli může také hrát kompetice mezi samotnými samci a to například i v podobě kompetice spermií (viz Arnqvist a Kirkpatrick 2005, Immler a Griffith 2009).

Ke studiu některých z výše uvedených hypotéz jsme si já a mí spolupracovníci zvolili jako modelový druh hýla rudého *Carpodacus erythrinus*. Hýl rudý je malý (přibližně 22 g), sociálně monogamní pěvec. Zbarvení dvouletých a starších samců kolísá od žluté po karmínovou a je způsobeno karotenoidní pigmentací. Hýl rudý migruje na dlouhou vzdálenost, na naše území přilétá jako jeden z posledních tažných pěvců v polovině května (Šťastný et al. 2006). Jeho hnízdní sezóna je tak extrémně krátká a hnízdění je tudíž velmi synchronizované. Hýl rudý je neteritoriální a kvalita teritoria tedy nemůže hrát roli v samičím výběru partnera (Björklund 1990). Zpěv je velmi jednoduchý, liší se většinou pouze v počtu strof (Björklund 1990). Červené zbarvení se tedy jeví jako nejpravděpodobnější znak, který by samice mohly při svém výběru preferovat, a jeho úroveň by mohla být zodpovědná za samčí reprodukční úspěch.

Lokalita, na které výzkum probíhal, se nachází v první zóně Národního parku Šumava, ve Vltavském luhu, poblíž obce Želnava (48°49´ N, 13°56´ E). Rozloha lokality je zhruba 1 km^2. Terén je plochý, rovinný a je velmi přehledný. Jedná se o záplavová území, nivní biotop podél meandrující řeky Vltavy. Křovinné porosty zaujímají zhruba 15 % plochy, převládají hlavně tavolník *Spiraea salicifolia*, ve kterém hýl rudý hnízdí, a vrba *Salix sp*.

Disertační práce se zaměřuje zejména na vztah mimopárových paternit k některým z výše uvedených hypotéz. Skládá ze tří publikovaných prací a jednoho rukopisu. V následujícím textu jsem se pokusil stručně shrnout jejich výsledky a okomentovat je v kontextu uvedené problematiky. Jako přílohu jsem přiložil článek, který sice není součástí disertační práce, ale dále rozšiřuje naše znalosti o modelovém druhu.

Mimopárové paternity a jejich vliv na sílu sexuální selekce

Míra mimpárových paternit (EPP) je větší u migrujících druhů, než u stálých (Spottiswoode a Møller 2004). Tento fakt je možné vysvětlit pomocí různých mechanizmů. Několik studií nalezlo pozitivní efekt mezi výskytem EPP a synchronizací hnízdění (Stutchbury a Morton 1995, Stutchbury 1998). Zvýšený poměr EPP v souvislosti se synchronizací může souviset s tím, že pokud je z ekologických důvodů nutno zahnízdit krátce po příletu, je možné, že samice nemá dostatek času pro výběr vhodného partnera a tak se spáruje i s méně kvalitním samcem a výběr genetického partnera probíhá až po spárování se sociálním partnerem (compensatory theory - Westneat et al. 1990, Weatherhead a Yezerinac 1998, Spottiswoode a Møller 2004). Krátká sezona má také za následek, že kvalitní samci nemůžou zvýšit svůj reprodukční úspěch jinou cestou, například vícenásobným hnízděním (e.g. Hill et al. 1994). Navíc, migrace sama může zvyšovat míru EPP (Spottiswoode a Møller 2004) a to tak, že generuje další genetickou variabilitu (Fitzpatrick 1994, Møller 1994, 1998, Spottiswoode a Møller 2004). Pokud se velká genetická variabilita promítne i do velké variability fenotypové, nebo variability ve schopnosti samce obstarat si EPP v kompetici s ostatními samci, může tak být tato variabilita pozitivně korelována s mírou EPP (Petrie et al. 1998). Díky synchronizaci se také v jednu dobu prezentuje samicím daleko více samců a samice má tak lepší šanci porovnat jejich kvality (Stutchbury 1998). Na druhou stranu ale synchronizace hnízdění může pro samce limitovat příležitosti k vyhledávání EPP, protože se musí věnovat hlídání vlastní samice, aby neztratil paternitu ve svém hnízdě (Birkhead a Biggins 1987).

V naší práci jsme se pokusili zjistit, jakou roli hraje EPP ve zvyšování variability v samčím reprodukčním úspěchu pěvců hnízdících v temperátní zóně. Zvláště jsme se zaměřili na vztah mezi mírou jakou EPP přispívají k samčí fitness a délkou migrace. K objasnění tohoto problému jsme použili dva přístupy. Srovnávací mezidruhovou analýzu a studium konkrétního druhu, hýla rudého. Hýl rudý migruje na dlouhou vzdálenost a během velmi krátké hnízdní sezony stihne zahnízdit pouze jednou. Zároveň je jeho hnízdění silně synchronizované. Zjišťovali jsme, zda úspěšnost samce při EPP není vykoupena ztrátou paternity ve vlastním hnízdě. Pokud by tomu tak nebylo, pak malý počet úspěšných samců může zplodit velký počet mláďat a tak silně přispět k variabilitě v reprodukčním úspěchu.

Přestože jsme u hýla rudého nenalezli závratně vysokou míru EPP (18 % mláďat ve 30 % hnízd) ve srovnání s jinými druhy (Griffith et al. 2002), ukázala naše data, že EPP zvyšují variabilitu v reprodukčním úspěchu samců hýla rudého. Samci, kteří byli úspěšní při získávání EPP, neztratili paternitu ve svém vlastním hnízdě a tudíž i jejich celkový reprodukční úspěch byl vyšší, než u ostatních samců. Navíc u blízce příbuzného hýla rudoprsého, který je charakteristický dlouhou hnízdní sezónou (nemigruje) se EPP vyskytují jen zřídka (Hill et al. 1994).

Existuje několik možných vysvětlení této vysoké míry variability v reprodukčním úspěchu mezi samci hýla rudého. První možnost je, že kvalitní samci, kteří jsou úspěšní při EPP, zahnízdí dříve a v době, kdy se věnují EPP nemusí už hlídat vlastní paternitu (Birkhead a Møller 1992). Na druhou stranu jsme nenašli prokazatelný rozdíl v načasování hnízdění mezi různě úspěšnými samci. To ale může být způsobeno malou variabilitou v načasování hnízdění v naší populaci.

Fakt, že samci úspěšní při EPP neztrácejí paternitu ve svých hnízdech, naznačuje, že samice upřednostňují konkrétní samce a to pravděpodobně na základě jejich fenotypových znaků. Samice by pak vyhledávaly EPP pokud by se spárovaly s málo kvalitními samci (e.g. Weatherhead a Yezerinac 1998, Spottiswoode a Møller 2004). U dálkových migrantů, kde je silný tlak na včasné zahnízdění, totiž může být pro samici nevýhodné dlouho vybírat a mít tak opožděné hnízdění. Místo toho raději co nejdříve zahnízdí a svůj případný špatný výběr sociálního partnera kompenzuje cestou EPP. Sociální párování se může jevit nakonec jako náhodné, bez vztahu k samčímu fenotypu, jak například zaznamenal u hýla rudého Bjőklund (1990). Z toho vyplývá nutnost detekce fertilizačního úspěchu samců molekulárními metodami, pomocí nichž lze rekonstruovat jednotlivé komponenty fitness samců.

Dále jsme použili data o hnízdní biologii a migraci 18 druhů pěvců hnízdících v temperátní zóně získaná z literatury, na jejichž základě jsme testovali hypotézu, že příspěvek EPP k variabilitě v reprodukčním úspěchu, a tak síle sexuální selekce obecně, souvisí s délkou migrace. Potvrdilo se, že EPP potenciálně hrají větší roli v sexuální selekci u druhů s krátkou hnízdní sezonou a menší u druhů s dlouhou sezonou, během které může samec zahnízdit vícekrát (e.g. Hill 1994). Ve skutečnosti však délka hnízdní sezony ztratila na míru EPP vliv, pokud byla do analýzy zahrnuta migrace. Tomu by odpovídalo i zjištění, že migrující druhy jsou charakteristické větším sexuálním dichromatizmem (Fitzpatrick 1994, 1998). Migrace ale pochopitelně souvisí s hnízdní synchronizací (e.g. Spottiswoode a Møller 2004, Pitcher et al. 2005). Synchronizace byla dobrým prediktorem míry příspěvku EPP k variabilitě v celkovém reprodukčním úspěchu samců při mezidruhovém porovnání. Avšak naše data, jak se zdá, podporují hypotézu, že efekt migrace je přímý a nezávislý na synchronizaci.

Existuje řada hypotéz pokoušející se vysvětlit, jakým způsobem může migrace ovlivnit míru EPP u ptáků bez ohledu na synchronizaci hnízdění (Spottiswoode a Møller 2004). Bohužel většinu je těžké testovat použitím komparativních metod. Například výše uvedená teorie kompenzace unáhleného špatného výběru sociálního partnera, stejně tak jako teorie předpokládající větší genetickou variabilitu u migrujících ptáků mohou vést ke stejnému nalezenému vztahu mezi zdánlivou a skutečnou variabilitou v reprodukčním úspěchu a migrací, ačkoliv evoluční mechanismy jsou odlišné. Navíc jednotlivé teorie mohou platit současně a nemusí se navzájem vylučovat. Naše pozorování jsou tak v souladu s hypotézami, že délka sezony, délka migrace a vysoká synchronizace hnízdění mohou mít vztah ke zvýšení sexuální selekce cestou EPP.

Mimopárové paternity a jejich příspěvek k selekci sekundárních pohlavních ornamentů

Většina studií spojuje podle teorie sexuální selekce samčí reprodukční úspěch s kvalitou sekundárních pohlavních znaků. V předchozí studii jsme ukázali, že mimopárové paternity (EPP) mohou významně přispívat k variabilitě v samčím reprodukčním úspěchu a tak k síle sexuální selekce. Jinými autory bylo také zjištěno, že výskyt EPP může korelovat s velikostí samce (Yezerinac & Weatherhead 1997), repertoárem zpěvu (Forstmeier et al. 2002, Suter et al. 2009), zbarvením peří (e.g. Foerster et al. 2003, Safran et al. 2005), strukturálními ornamenty (Kleven et al. 2006) nebo kombinací několika faktorů dohromady (Sundberg & Dixon 1996). Ke stanovení role EPP v evoluci ornamentu je nezbytné určit, jak vnitropárová a mimopárová paternita přispívají k celkové variabilitě v samčím reprodukčním úspěchu (viz výše) a následně zda fenotypová kvalita samce ovlivní tyto jednotlivé komponenty samčí fitness.

V naší studii jsme proto zjišťovali, jak EPP můžou přispívat k sexuální selekci červeného karotenoidního zabarvení samců hýla rudého. Důležitou podmínkou pro zpracování této studie byl fakt, že se nám podařilo u většiny mimopárových mláďat stanovit jejich genetického otce. Díky tomu jsme mohli na základě našich dat hledat vztah mezi variabilitou ve zbarvení samců a jednotlivými komponenty jejich reprodukčního úspěchu. Abychom zjistili, zda zbarvení samce ovlivnilo samičí výběr mimopárového partnera, provedli jsme párové srovnání samců, kteří měli mimopárová mláďata ve svém hnízdě se samci, kteří byli genetickými otci jejich mláďat. Nakonec jsme testovali hypotézu, zda karotenoidní zbarvení samců ovlivňuje cestou EPP jejich celkový reprodukční úspěch.

Jako míra celkového zbarvení samce byla v naší studii použita hodnota z analýzy hlavních komponent (PCA) označená jako PC1. Ta odrážela samotnou barvu (hue), ale také sytost (saturation) a světlost (brightness) péřového ornamentu hrudi samců. Lze předpokládat, že jednotlivé komponenty zbarvení jsou různě citlivé na environmentální stres díky odlišným mechanizmům jejich původu a vzniku a dávají tak odlišné informace o svém nositeli (Hill 2006). I když se můžeme pouze domnívat, jaká je signální funkce jednotlivých komponent zbarvení (HSB) u hýla rudého, zdá se, že hue je obecně parametr, který je velmi závislý na kondici a zdravotním stavu jedince u příbuzného hýla rudoprsého, *Carpodacus mexicanus* (Hill 2002).

Nalezli jsme pozitivní vztah mezi úrovní samčího zbarvení a jeho celkovým reprodukčním úspěchem. Vybarvenější samci jsou také úspěšnější při získávání EPP. Pokud bychom však hodnotili pouze zdánlivý reprodukční úspěch, neboli počet mláďat v hnízdě, tak podobně jako Björklund (1990) žádný vztah nenalezneme. Absence vztahu mezi samčím zbarvením, načasováním hnízdění a kvalitou samice může být důsledkem rychlého, náhodného a synchronního párování (Stjernberg, 1979; Björklund, 1990; Albrecht et al., 2007). Pokud se kvalitní samice díky rychlému párování spáruje s podprůměrným samcem, může cestou EPP napravit svou chybu. Tuto teorii podporuje i fakt, že samec, který ztratil paternitu ve svém hnízdě, bývá hůře vybarvený, než samec, se kterým jeho samice zplodila mimopárová mláďata.

Jak jsem uvedl výše, u hýla rudého existuje pozitivní vztah mezi vnitropárovým i mimopárovým reprodukčním úspěchem a tudíž EPP přispívají k sexuální selekci. Námi zjištěná skutečná variabilita v reprodukčním úspěchu je zhruba třikrát větší než zdánlivá (Albrecht et al. 2007). V této studii jsme ukázali, že schopnost uhlídat paternitu ve svém hnízdě, stejně jako schopnost samce získat EPP, koreluje se zbarvením samce. Uvedené výsledky naznačují, že sexuální selekce působí na zbarvení a to jak cestou vnitropárového, tak mimopárového reprodukčního úspěchu. Bohužel ale nejsme schopni změřit efekt zbarvení na reprodukční úspěch samce během celého jeho života. Protože samice získá od EP samce pouze spermie, mohli jsme v této studii odfiltrovat alternativní hypotézy vysvětlující samičí preference pro vybarvené samce, jako je například model dobrého rodiče. Avšak jakou má přesně samice výhodu ze spárování s červeným samcem, je otázkou dalších výzkumů.

Mimopárové paternity a variabilita MHC genů

V posledních letech se významně zlepšily metody použitelné pro studium genetických aspektů výběru partnera (Mays a Hill 2004). Ukazuje se tak, že samice si nevybírá partnera pouze na základě nejlepších sekundárních pohlavních znaků (‘good genes’ model, Mays a Hill 2004), ale také na základě vhodnosti svého genotypu ke genotypu konkrétního samce. Dochází tak k disasortativnímu párování známému jako model genetické komplementarity (Tregenza a Wedell 2000, Pialek a Albrecht 2005, Mays et al. 2008).

Zatímco model dobrých genů předpokládá, že si samice vybírají samce na základě určitých fenotypových znaků a preference těchto znaků jsou u všech samic stejné (e.g. Iwasa et al. 1991), model genetické komplementarity pracuje s hypotézou, že každá samice přizpůsobuje výběr svého partnera s ohledem na svůj vlastní genotyp. Hledá tak partnera s genotypem nejvíce vhodném k jejímu vlastnímu tak, aby jejich potomci měli co nejvýhodnější kombinaci genů (Hettyey et al. 2010). Tudíž pro každou samici se jako nejvhodnější jeví odlišný samec, na rozdíl od good genes modelu, kde se všem samicím bude jevit jako nejvhodnější ten samec, který má nejlepší alely genů (v typickém případě signalizované ornamentací). Výsledky různých studií ale jednoznačně nepodpořily jednu nebo druhou hypotézu a výběr partnera tak může být nakonec kombinací obou způsobů (Roberts a Gosling 2003). Existuje však pouze velmi málo studií, které by zkoumaly samičí výběr, zvláště pak pro mimopárové partnery, z hlediska obou modelů (Hettyey et al. 2010).

Z pohledu evoluční biologie je sledování výběru mimopárového partnera obzvláště vhodný způsob, jak studovat nepřímé výhody pro samici. V naší studii jsme se zaměřili na testování hypotézy dobrých genů v podobě úrovně heterozygotnosti samce a hypotézy genové kompatibility a to sledováním variability MHC genů. Geny MHC hrají u obratlovců důležitou roli v adaptivní (specifické) imunitě (Klein 1986). Hypotéza overdominance předpokládá, že čím větší počet alel MHC genů jedinec má, tím většímu spektru patogenů je odolný (Piertney a Oliver 2006). Lze tedy předpokládat, že nejvýhodnější je spárovat se s jedincem, který má MHC alely co nejodlišnější od vlastních, a tak zvýšit heterozygotnost potomků (Milinski 2006, Piertney a Oliver 2006). Nicméně, příliš vysoká variabilita MHC genů může být nevýhodná. Pravděpodobně tak existuje nějaký optimální počet alel MHC genů a tohoto počtu pro potomky by se měli snažit jedinci při párování dosáhnout spíš než maximální variability (Milinski 2006, Woelfing et al. 2009).

Existuje několik domněnek, jak může být samice schopna rozeznat genetickou kvalitu samce. Například u savců nebo ryb může být variabilita MHC genů rozpoznána pomocí pachu (Singh et al. 1987, Penn a Potts 1998). Otázkou ale je, podle čeho by rozpoznávali genetické vlastnosti ptáci, kteří se na rozdíl od většiny savců orientují hlavně zrakem a sluchem. Na druhou stranu jejich čichové receptory se zdají být podobné receptorům ostatních obratlovců (Steiger et al. 2008).

U pěvců je zatím k dispozici poměrně málo studií popisujících vztah MHC a párování a výsledky jsou navíc často neprůkazné (Freeman-Gallant et al. 2003, Westerdahl 2004, Richardson et al. 2005). Ke stanovení variability MHC genů třídy I, konkrétně exonu 3, u hýla rudého jsme použili metodu SSCP (single strand conformation polymorphism; Promerová et al. 2009).

Zjistili jsme, že samci s nižší variabilitou MHC genů ztrácejí paternitu ve svém hnízdě častěji, než samci s větší variabilitou. Avšak mimopárová mláďata nejsou v oblasti MHC genů variabilnější než jejich nevlastní sourozenci zplození sociálním partnerem. Navíc ani samec, který zplodil EP mláďata, není heterozygotnější než samec, kterému hnízdo patří. Stejně tak ve hnízdech se smíšenou paternitou jsme mezi mláďaty nenašli více rozdílných alel MHC ve srovnání s hnízdy, kde jsou všechna mláďata zplozena sociálním partnerem. Nenalezli jsme tak žádný vztah, který by podporoval hypotézu komplementarity, což ale může být dané extrémní diverzitou MHC genů třídy I u tohoto druhu a nemožností získat sekvence všech alel. Domníváme se, že některé alely mohou mít vzájemně podobnější sekvence než jiné a tak, pokud bychom znali všechny sekvence, mohlo by být testování komplementarity daleko přesnější.

Na úrovni variability MHC genů jsme nenalezli žádnou nepřímou výhodu, kterou by samice mohla získat cestou EPP. Naše výsledky ale naznačují, že samci s menší variabilitou MHC ztrácí častěji paternitu ve svém hnízdě a to přesto, že samec který skutečně zplodí mláďata v jejich hnízdě, nemá vyšší heterozygotnost. To může znamenat, že samice nejsou schopné přímo rozeznat heterozygotnějšího samce, ale zůstávají heterozygotnějším (a tedy asi i kvalitnějším) samcům více věrné. Množství alel MHC může souviset i s dalšími kvalitami samce, včetně jeho kompetitivních schopností (viz Zelano a Edwards 2002). Je možné, že velkou míru v determinaci mimopárového a vnitropárového úspěchu samců hýla rudého hrají jiné faktory, nežli přímý výběr samice (viz Arnqvist a Kirkpatrick 2005), například kvalita spermií (Immler a Griffith 2009).

Zbarvení samce, úroveň paternity a intenzita rodičovské péče

Rodičovská péče je obecně považována za velmi nákladnou, protože může snižovat přežívání rodičů (Lessels 1991) a tudíž redukuje budoucí reprodukční úspěch (Gustafsson a Sutherland 1988) nebo omezuje u samců možnost získat další partnerku. Proto je rodičovská péče pravděpodobně kompromisem mezi výhodou zvýšeného reprodukčního úspěchu díky lepšímu přežívání mláďat a náklady, které představují snížení pravděpodobnosti dalšího vlastního přežití a budoucí fitness (Nur 1984, Houston 2005).

Samice spárováním s konkrétním samcem může kromě dobrých genů pro své potomky obdržet i přímou výhodu (direct benefit, Kikpatrick a Ryan 1991). Jedna z přímých výhod spárování s určitým samcem znamená, že se samec podílí intenzivněji na hnízdní péči. Tím může zvýšit jak kondici mláďat, tak dovolit samici krmit s menší intenzitou a zajistit tak samici lepší kondici pro další přežívání. Podle teorie dobrého rodiče (good parent model) mohou různé samčí sekundární pohlavní znaky kromě dobrých genů signalizovat právě i úroveň rodičovské péče a umožňují tak samici předem ohodnotit samčí rodičovské kvality (Hoelzer 1989, Heywood 1989).

Na druhou stranu nejen rodičovská péče, ale i exprese sekundárních pohlavních znaků je pro samce často velmi nákladná. Proto lze naopak očekávat negativní vztah mezi ornamenty samce a úrovní jeho hnízdní péče. Neboli, zvýšené investice do sexuálních ornamentů můžou vést ke snížení hnízdní péče (differential allocation model, Burley 1986). Zatímco tedy model dobrého rodiče předpokládá u samců vznik znaků čestně signalizující úroveň péče (Heywood 1989, Kelly 2009), differential allocation model předpokládá trade-off mezi atraktivitou a hnízdní péčí (Burley 1986, Kokko 1998).

Další faktor, který může ovlivnit míru samčí rodičovské péče je výskyt mimopárových paternit, které jsou mezi ptáky velice rozšířené (Griffith et al. 2002, Birkhead a Møller 1992). Předpokládá se, že existuje negativní vztah mezi časem věnovaným péčí o mláďata a časem, ve kterém se samec věnuje možnosti získat EP samici nebo partnerku na další zahnízdění. Možnost získat mimopárové partnerky tak může hovořit pro existenci negativního vztahu mezi fenotypovou kvalitou samce a úrovní jeho hnízdní péče.

Teorie rodičovských investic (parental investment theory, Trivers 1972) předpokládá, že samci, kteří ztratili ve svém vlastním hnízdě paternitu, krmí méně, protože krmením cizích mláďat by nejenže nezvýšili svoji fitness, ale ještě by snížili svojí kondici a tak šanci na přežití do dalšího hnízdění.

Variabilita v míře samčí péče o mláďata pak může být výsledkem faktorů, které ovlivňují úspěšnost samce při získávání další partnerky, jako jsou fenotypová kvalita samce a dostupnost fertilních samic (Magrath 2003). Atraktivní samci tedy mohou věnovat více času shánění EP partnerky, než krmení (Houston et al 2005). Ve výsledku tak lze očekávat, že samice spárované s atraktivním samcem budou nuceny krmit s větší intenzitou (Witte 1995). Na druhou stranu větší intenzita krmení samic spárovaných s atraktivním samcem může být výsledkem rozhodnutí samice investovat více do mláďat proto, že předpokládá, že tito potomci budou také atraktivní a že tím zvyšuje svojí fitness (Burley 1986).

V naší studii jsme se zaměřili na vztah mezi úrovní rodičovské péče (měřenou jako počet krmení za hodinu), zbarvením samce, výskytem mimopárových mláďat ve hnízdě a úspěchu samce při mimopárových fertilizacích. Krmení jsme sledovali v období stáří mláďat 6-9 dnů a od každého sledovaného hnízda jsme pořídili sedmihodinový kontinuální záznam.

Naše výsledky naznačují, že pouze schopnost samce uspět při EPP koreluje s intenzitou jeho krmení. Zbarvení samce ani výskyt EP mláďat v jeho hnízdě nemá žádný vztah na jeho rodičovské úsilí. To znamená, že samci, kteří ztratili paternitu ve svém hnízdě, neredukují svojí péči. To může být dáno tím, že samec není schopen odhalit mimopárová mláďata. Avšak vzhledem k tomu, že hýl rudý krmí svá mláďata převážně semeny a drobnými členovci (Stejrnberg 1979, Albrecht nepublikovaná data), nemusí být pro něj krmení nijak nákladné. Úspora energie není pro samce tudíž natolik významná, aby vyvážila riziko, že jeho vlastní mláďata by byla postižena redukcí jeho péče.

Zdá se, že u hýla rudého pravděpodobně neplatí model dobrého rodiče. Fakt, že jsme nenalezli žádný vztah mezi zbarvením a hnízdní péčí, může souviset s tím, že párování je díky velmi krátké sezoně u hýla rudého velice rychlé a vzhledem k samčím ornamentům víceméně náhodné (Bjorklund 1990, Albrecht 2009). Zbarvení tak samicím může indikovat jiné samčí kvality, než hnízdní péči.

Na první pohled překvapující zjištění, a to že samci, kteří byli úspěšní při EPP zároveň krmili s větší intenzitou, lze vysvětlit silnou synchronizací hnízdění (Albrecht at al. 2007). V době, kdy jsme sledovali hnízdní péči, se totiž už na lokalitě v podstatě nenacházely žádné fertilní samice (kromě těch, které přišly o hnízdo a pokoušely se o náhradní hnízdění). Proto se samec nemusí rozhodovat mezi hledáním mimopárové partnerky nebo krmením mláďat. Fakt, že úspěšnost samce při EPP koreluje s jeho hnízdní péčí, může tedy souviset s nějakou jinou, v této studii neměřenou kvalitou samce, jako je jeho vitalita a genetická kvalita (Zelano a Edwards 2002).

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I.

Extrapair paternity and the opportunity for sexual selection in long-distant migratory passerines

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Extrapair paternity and the opportunity for sexual selection in long-distant migratory passerines

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Extrapair fertilizations (EPFs) are frequently documented in songbirds; however, the extent to which this reproductive tactic contributes to variance in male reproductive success and hence the strength of sexual selection on males remains little studied. Using 2 approaches, intraspecific and comparative, we test the hypothesis that the contribution of EPFs to variance in male fitness increases with migration distance in north temperate songbirds. Using data on the genetic mating system of the scarlet rosefinch *Carpodacus erythrinus*, a long-distance migrant, we show that the number of extrapair mates and within-pair paternity are the most important components of variance in male reproductive success. There was no evidence of a trade-off between extrapair and within-pair success of individual males as males successful in procuring EPFs were less likely to be cuckolded. Comparative data reveal that the opportunity for sexual selection due to EPFs is positively associated with both migration distance and breeding synchrony in north temperate passerines, and we discuss several mechanisms that could extend these relationships. In general, these data suggest that EPFs have a potential to play an important role in the evolution of sexually selected traits in long-distance migratory songbirds such as rosefinches. *Key words:* comparative analysis, mate choice, parentage assignment, promiscuity, scarlet rosefinch *Carpodacus erythrinus*, within-pair paternity. *[Behav Ecol]*

The strength of sexual selection is proportional to variance in reproductive success, originally defined as the number of mates an individual is able to obtain (Crow 1958; Arnold and Wade 1984; Shuster and Wade 2003) and more widely quoted as the total number of offspring produced by an individual (Webster et al. 1995). Variation in the number and quality of social mates have traditionally been recognized as the 2 main sources of variance in reproductive output among males, leading to the evolution of male secondary ornaments and increased sexual dimorphism (Darwin 1871; Andersson 1994). However, it is now accepted that extrapair fertilizations (EPFs) are widespread in many bird species (Griffith et al. 2002; Westneat and Stewart 2003). Although rates of extrapair paternity appear to be positively correlated with the extent of plumage color dimorphism in birds (Owens and Hartley 1998; but see Dunn et al. 2001), it is not clear to what extent EPFs increase the opportunity for sexual selection (Freeman-Gallant et al. 2005). EPFs would have maximal impact on variance in male fitness if the males that excelled at within-pair paternity also achieve most EPFs at the expense of other males (Webster et al. 1995; Whittingham and Dunn 2005).

Assessing the relative contribution of EPFs to sexual selection requires an accurate assignment of dams and sires to all offspring within the study population. Despite recent advances in molecular tools (e.g., Webster et al. 2001), sufficient data are only available for a limited number of species and only 2 studies assigned paternity to all offspring (Hasselqvist et al. 1995; Whittingham and Lifjeld 1995). In most species analyzed to date, extrapair matings increase variance in male reproductive success, but their relative contribution to variance in male fitness varies considerably across species for unknown reasons (but see Whittingham and Dunn 2005). However, failure to assign sires to offspring in some studies could have a big effect on reported estimates of opportunity for selection due to EPFs (Freeman-Gallant et al. 2005).

Rates of EPFs are higher among migrant species than residents (Spottiswoode and Møller 2004). Several mechanisms have been proposed to explain this relationship. First, migration could be associated with increased breeding synchrony due to strong selection on arrival time in most migratory species. Although a few studies have revealed a positive relationship between breeding synchrony and EPFs at the interspecific level (Stutchbury and Morton 1995; Stutchbury 1998b), there has been an ongoing debate as to the effect of breeding synchrony on extrapair mating strategies (see Møller and Ninni 1998; Stutchbury 1998a, 1998b; Weatherhead and Yezerinac 1998). However, when breeding is synchronized, many males are displaying simultaneously, and thus, females may have a better opportunity to compare their social mate with other males in the population (Stutchbury 1998a, 1998b). Similarly, if female migrants are obliged for ecological reasons to start breeding relatively quickly and synchronously after their arrival at the breeding grounds, choice of genetic mate could only occur after a choice of social mate, as reflected by high rates of EPFs (Westneat et al. 1990; Weatherhead and Yezerinac 1998; Spottiswoode and Møller 2004; but see Stutchbury 1998a). The short breeding season of some long-distance migrants might also prevent high-quality males from increasing the number of sired progeny in a season by mechanisms other than EPFs, such as by producing more than one brood per season with a social mate (e.g., Hill et al. 1994).

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In addition to the effects of the length of the breeding season and breeding synchrony on rates of EPFs, migration itself could increase rates of EPFs in bird populations by one of the evolutionary pathways hypothesized by Spottiswoode and Møller (2004). For example, it has been suggested that migration in birds generates additive genetic variation (Fitzpatrick 1994; Møller 1994, 1998; Spottiswoode and Møller 2004) and that this variation is positively associated with rates of EPFs in birds (Petrie et al. 1998). If genetic variation translates into either high variability in male attractiveness to females or high variance in male ability to obtain EPFs in competition with other males, simultaneous female choice of an extrapair mate and monopolization of EPFs by certain males might be commonplace in migrants. In that case, a significant contribution of EPFs to variance in male fitness should be expected. Surprisingly, however, no study thus far has attempted to evaluate the effects of migration on the opportunity for sexual selection via EPFs in birds.

Here we use 2 approaches, intraspecific and comparative, to examine the role of EPFs in enhancing variance in male reproductive success in songbirds breeding in the north temperate zone, with special emphasis on how long-distance migration affects the contribution of EPFs to male fitness. First, we use the scarlet rosefinch *Carpodacus erythrinus*, hereafter referred to as rosefinch, as a model species to evaluate the effects of EPFs on variance in male fitness in a typical long-distance migratory, single-brooded passerine. We specifically test the predictions that rosefinch males trade within pair for EPF success (e.g., Webster et al. 1995; Whittingham and Dunn 2005). In the absence of this trade-off, monopolization of EPFs by only a few males would strongly increase the contribution of EPFs to variance in male reproductive success. Breeding synchrony could affect the probability that a certain male would engage in EPFs (Webster et al. 2001). We evaluate how this parameter affects the occurrence of extrapair young (EPY) in nests of rosefinches. In a second approach, we perform a comparative analysis that includes rosefinch data from this study to test the prediction that the contribution of EPFs to male fitness is positively linked with migration in north temperate zone breeding passerines. Because breeding synchrony and length of the breeding season can covary with migration distance (Pitcher et al. 2005), these parameters are included in the analyses.

METHODS

Study area and study species

The study was conducted from the end of May to early July in the years 2000–2004 in the Vltava river valley, Šumava Mountains National Park, Czech Republic (48°49′N, 13°56′E). The study plot was an isolated patch of wet shrubby meadow (110 ha) surrounded by agricultural landscape mosaics (for a detailed description of the study site, see Albrecht 2004).

Scarlet rosefinches are small (ca., 20 g), semicolonial, sexually dimorphic cardueline finches with delayed plumage maturation in males (Stjernberg 1979). Second-year males resemble females in that they lack red feather ornament on the breast and rump. Most males do not breed until their third year, second-year males are only present infrequently on breeding grounds (Björklund 1989). Each year, but particularly in the 2001–2004 breeding seasons, we attempted to catch all birds in our study area when they first arrived on the breeding grounds using mist nets, although some birds were caught during the chick-provisioning stage. Each netted individual was banded with a National Museum of Prague aluminum ring and provided with a unique combination of 1 to 3 color rings for individual identification. This enabled us to follow most individuals from their arrival upon the study area in May throughout the entire breeding season. Only resident birds, that is, those seen on the study plot repeatedly in the days following ringing, were considered in the analyses. Every 2 days, we determined the pairing status of resident males. Only a small proportion of resident males (and no resident female) appeared to be unpaired. Only males observed repeatedly feeding the female during the incubation, and feeding chicks, were considered the social fathers of young in a particular nest. Our estimates of realized reproductive success for males could be biased if males frequently sire young outside our study plot; however, this is unlikely because the nearest breeding colony of rosefinches is situated more than 3 km away from our study area. In a 5 year period before this study (1995–1999), a total of 51 male and 33 female rosefinches were color banded in both colonies. No birds banded in one of the colonies were ever recovered from the other, which suggests that rosefinches do not regularly move between the 2 colonies during the breeding season. However, for feeding, both sexes range over large distances and far from breeding areas (Stjernberg 1979); thus, we cannot exclude the possibility that birds from different breeding colonies encounter each other in a third area, where communal feeding takes place (also see Reyer et al. 1997; Westneat and Mays 2005).

Starting in late May, we systematically searched the study area for rosefinch nests. Each potential breeding site was visited at least 3 times per season except of the year 2000 when some places were visited less frequently. Hence, we are confident we were able to find most rosefinch nests on the study area in most years and to genotype the vast majority of young born on our study plot. All nests were found during the egg-laying or incubation stages. A geographic positioning system reading was taken at each nest, and these were used to calculate distances between nests. Rosefinches only rear a single brood per year, although one replacement clutch may be laid in response to the loss of the first (Stjernberg 1979). Relatively few nests were lost before fledging (predation rate 0–25%; Albrecht 2004), and our sample of nests includes predominantly first clutches, not replacements. Nests were checked every 1–4 days to determine the stage of breeding and nesting success. A blood sample (approximately 20 μl) was taken from 7-day-old nestlings and adult birds and stored in 96% ethanol.

Identification of parentage

Blood samples were dried, and DNA was extracted and purified using the DNeasy® Tissue Kit (Qiagen, Hilden, Germany). The parentage analysis was conducted using 3 microsatellite loci (*LOX2*, *LOX7*, and *LOX8*; Piertney at al. 1998) developed for the Scottish Crossbill *Loxia scotica.* Microsatellite loci were amplified individually using an MJ Research PTC-200 thermocycler. One primer of each primer pair was labeled with a different fluorochrome. The reaction conditions were slightly modified from Piertney et al. (1998). The reaction mixture contained the following: approximately 20 ng of genomic DNA, 0.5 units of *Taq* polymerase, 1× reaction buffer, 2 mM $MgCl_2$, 200 μM dNTPs, and 0.5 μM of each primer, in a final volume of 15 μl. The thermal profile consisted of 30 cycles of 92 °C for 30 s, followed by 54 °C for 30 s. No extension step was included in the cycles, except for a 5-min extension at 72 °C after the final cycle.

For each sample, polymerase chain reaction (PCR) products from each of the 3 loci were combined (0.7–1.5 μl of each reaction according to DNA concentration) and added to a denatured mixture of size standard (Genescan®, TAMRA 500, Applied Biosystems, Foster City, CA) and formamide. These mixes were denatured for 5 min at 95 °C, snap-cooled on ice, and loaded on an ABI Prism® 310 Genetic Analyzer (Applied

Biosystems) for separation and detection. DNA fragments were manually compared and analyzed using GeneScan® software (Applied Biosystems). Although the loci were described as tetranucleotide repeats in the Scottish Crossbill (Piertney et al. 1998), we frequently encountered differences smaller than one repeat unit size, which may suggest either indels in flanking regions or a more complex repeat structure (Primmer and Ellegren 1998). To avoid genotyping errors, we considered 2 fragments differing by less than one repeat unit to be different alleles only after very careful analysis. As a final check, we compared the fragment length of each locus for each individual in a family (i.e., the length of an allele did not usually vary within a family but differences were evident between families) and in doubtful cases, we repeated the fragment analysis or PCR.

The average probabilities of excluding a single randomly chosen unrelated individual from being a parent were calculated for each locus and for all 3 loci when the maternal genotype was known using the program Cervus 2.0 (Marshall et al. 1998). These analyses were based on all resident adult individuals genotyped in the course of the study (Table 1). The 3 microsatellite loci examined were extremely variable, had high heterozygosities, and each adult individual possessed a unique genotype. Thus, although we only used 3 loci, the total exclusionary power exceeded 99% for both the first and second parent (Table 1).

We did not find any genetic mismatches between an offspring and its social mother. The offspring's paternal alleles were subsequently compared with the alleles of its putative father (the female's social partner). If alleles did not match at 2 or 3 loci, the putative father was excluded as a sire. One nestling did not match its putative father at just a single locus. We calculated the probability of resemblance for the 2 remaining loci using the method given in Ibarguchi et al. (2004, equations 1 and 3). Because the cumulative probability of resemblance (P_{RaCum}—the probability that the young and the sire match just by chance) is extremely low (1.51×10^{-5}) and the size difference between the offspring allele and that of the putative father was a single repeat unit, we assumed that a mutation is responsible for the difference and we did not exclude the father as the sire. The presence of null alleles (alleles that consistently fail to amplify to detected levels) can cause serious problems in paternity studies (Dakin and Avise 2004). As locus *LOX2* suggests that null alleles may be present at low frequency (Table 1), we paid particular attention to instance where the excluded putative father was homozygous at *LOX2*. In all such cases, the putative father was also excluded at the other 2 loci.

Sires of EPY were determined using the exclusion approach (Jones and Ardren 2003). We compared the paternal alleles of EPFs with the genotypes of all males in the population. The males that did not match the EPY at one or more loci were excluded as sires. If no male matched the particular EPY at all loci, the sire was categorized as "unknown." For the remaining EPY, a single male was found to match at all 3 loci and was therefore assigned as the sire.

Variance in reproductive success of males, breeding synchrony

Apparent reproductive success was estimated by simply counting the number of the young in a male's nest, when the young were 7 days old. By contrast, realized reproductive success for each male was based on the total young sired by him, involving both losses of paternity in his own nest and extrapair offspring sired by him. Chick mortality before the age of 7 days was extremely low (only 4 young out of 270 died due to partial predation or starvation). As for eggs, about 5% remained unhatched in successful nests, but we were unable to extract DNA from any of them. Only the data from the years 2001–2004 were used to calculate the opportunity for sexual selection due to EPFs as we were not able to catch all males in 2000. We calculated standardized variances of realized and apparent success (I_{s}, I_{sapp}; Arnold and Wade 1984) and used the ratio between I_{s} and I_{sapp} as an estimate of the relative contribution of EPFs to male fitness (Webster et al. 1995; Freeman-Gallant et al. 2005; Whittingham and Dunn 2005). The components of standardized variance in reproductive success among males were calculated following the method outlined in Webster et al. (1995). The total variance in male reproductive success ($T\mathrm{m}$) can be expressed as

$$\mathrm{var}(T\mathrm{m}) = \mathrm{var}(\mathrm{W}) + \mathrm{var}(\mathrm{E}) + 2\mathrm{cov}(\mathrm{W}, \mathrm{E}), \qquad (1)$$

where W and E denote the variance in within-pair and extrapair success, respectively. Both W and E can be further partitioned into variance in number of mates (within [M_{w}] and extrapair [M_{e}]), proportion of young sired in a nest (P_{w} and P_{e}), and mate quality expressed as mate productivity (N_{w} and N_{e}; Webster et al. 1995, equation 17), with the effects of extrapair terms being additive to those of within-pair terms. All means, variances, and covariances were calculated using only individuals with a defined value for the fitness component of interest (nonzero fitness in the previous episode of selection; see Webster et al. 1995). To avoid pseudoreplication, data were only used from the first breeding season of each male in the period 2001–2004

Table 1

Summary statistics of the microsatellite loci used to determine parentage in scarlet rosefinches

Locus	n^{a}	k^{b}	Allele size range	Het (exp)^{c}	Het (obs)^{d}	P (excl) 1^{e}	P (excl) 2^{f}	Estimated frequency of null alleles
LOX2	98	22	166–282	0.905	0.867	0.665	0.799	0.0204
LOX7	93	70	130–357	0.983	0.968	0.916	0.956	0.0055
LOX8	98	73	200–439	0.980	0.949	0.904	0.949	0.0134
Combined						0.997	>0.999	

Number of individuals tested.

Number of alleles.

Expected heterozygosity.

Observed heterozygosity.

Probability of maternal exclusion.

Probability of paternal exclusion.

(see also Whittingham and Dunn 2005). Three nests with known social parents were lost before we took blood samples from the young. Males from these nests as well as 3 EPY sired by one of them were excluded from calculations of variance in male reproductive success.

A breeding synchrony index (SI) for each breeding female in breeding population was calculated following Kempenaers (1993). We did not distinguish between "population level" and "local" breeding synchrony sensu Chuang et al. (1999) and treated the breeding colony as a unit where all birds are equally likely to encounter each other, as suggested by Martens and Kessler (2000). "Fertile period" was defined as the period starting 5 days before the first egg in a clutch was laid and ending with the penultimate egg in a clutch being laid (Stutchbury et al. 1997; Birkhead 1998), which reflects the potential for stored sperm to fertilize ovulated eggs later (Birkhead and Møller 1992). This fertile period in rosefinch females is also indicated by intensity of mate guarding because this appears to be high and stable over the whole period of 5 days before the first egg in a clutch appears (Albrecht T, unpublished data).

Statistical analysis

We performed multiple logistic regressions (general linear model procedures, S-Plus 6.0; Mathsoft 2001) with binary response variable to analyze how the timing of breeding (standardized around the median laying date) and breeding synchrony with other pairs in local population affects the likelihood of a male being cuckolded and losing paternity in his own nest. We repeated the analysis with the number of within-pair young as a dependent variable and the total number of young in the nest as the binomial denominator to account for the fact that different proportions of young in a nest were sired by social male parents. However, this second analysis led to the same conclusions and is not shown. All significance values of multiple tests are based on the Type III sum of squares (Crawley 2002). Numbers of EPY in nests were compared with the expected random values estimated from a prediction of multivariate hypergeometric distribution of EPY among nests (Neuhauser et al. 2001; Byers et al. 2004). Sufficient data were only available for broods of 5 young (the modal brood size). Differences in sample sizes between analyses were the result of incomplete data for some males or nests. Means are presented ± standard error throughout.

Comparative analysis

We first compared the contribution EPFs make with the variation in male reproductive success in species with similar breeding phenology as rosefinches (single brooded) and 2-brooded species. To do this, we used nonparametric Mann–Whitney statistics (see also Whittingham and Dunn 2005), with the I_s/I_{sapp} ratio as a dependent variable. Means were used when more than one I_s/I_{sapp} ratio was available for a single species. Single-brooded species were defined as those in which a second breeding attempt (not replacement clutch) in one season has never been recorded, or is rare. The data on the number of breeding attempts per season were obtained from Cramp et al. (1977–1994) and from The Birds of North America Online (Poole 2005). It has been argued that the data published on I_s/I_{sapp} ratios should be considered preliminary because studies where the paternity was assigned to only a low proportion of EPY tend to overestimate variance in realized male reproductive success (Freeman-Gallant et al. 2005). Therefore, we evaluated whether the groups of interest differed in this respect. When possible, we estimated the typical length of the main egg-laying period (an approximation of the length of the breeding season) to the nearest week from the pie charts reported in Cramp et al. (1977–1994) and Poole (2005). We did not consider the tails signaling exceptionally early and exceptionally late breeding attempts. Data on mean migration distance (to the nearest 1000 km) and breeding synchrony were obtained from Spottiswoode and Møller (2004) and using the compendia cited above. We realize that our sample is restricted to northern temperate zone breeding passerines, in contrast to some previous comparative studies (e.g., Spottiswoode and Møller 2004; Pitcher et al. 2005). Hence, we did not include latitude as an explanatory variable; species vary relatively little for this parameter (range 38.96–52.07, sensu Spottiswoode and Møller 2004; ca., 1500 km along the meridian), and there is no relationship between mean latitude and the length of breeding season in our sample of species ($r_s = -0.25$, $P = 0.32$, $n = 18$).

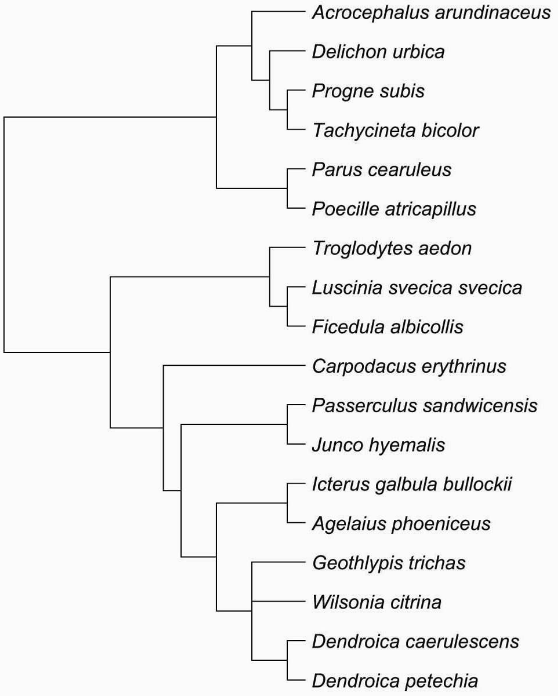

Figure 1
Phylogeny for passerine species used in the comparative analysis. Relationships among higher taxa were based on the phylogeny published in Barker et al. (2004). We used the phylogeny presented in Spottiswoode and Møller (2004) for Hirundinidae and Parulidae.

We employed methods based on phylogenetically independent contrasts (Felsenstein 1985) to evaluate how the breeding season length, breeding synchrony (arcsin-root transformed), and migration distance affect the contribution of EPF to the opportunity for sexual selection across species. Relationships among higher taxa were based on molecular phylogeny presented in Barker et al. (2004), which is consistent with other phylogenies based on nucleotide sequence data (e.g., Ericson and Johansson 2003). For familes Hirundinidae and Parulidae, detailed phylogenies presented in Spottiswoode and Møller (2004) were used (Figure 1). We assessed arbitrary branch lengths in our analysis assuming both a punctuated mode of evolution (equal branch length, hereafter PUNCT; Harvey and Pagel 1991) and a gradual mode of evolution (branch lengths proportional to the number of species in the clade, hereafter GRAD; Grafen 1989). Because the I_s/I_{sapp} ratio deviated from normality (Shapiro-Wilk's test,

$P < 0.005$), we performed a Box-Cox transformation (Crawley 2002) in which the power transformation λ was set to -0.60, that is, to the value equal to the minimum residual sum of squares. After this transformation, the I_s/I_{sapp} ratio was normally distributed (Shapiro-Wilk's test, $P = 0.55$). Phylogeny contrasts were calculated for the I_s/I_{sapp} ratio (transformed) as the dependent variable and the length of the breeding season, breeding synchrony, and migration distance as predictors, using COMPARE 4.6 (Martins 2004). In all regressions, results were statistically controlled for the variation among the proportion of EPY that had assigned sires (arcsin-root transformed). No correlation between the absolute values of independent contrasts and their standard deviation was detected for any variable under both modes of character evolution (PUNCT and GRAD); this indicates that the branch lengths successfully standardized the contrasts and, therefore, are reasonable for use in our analysis (Garland et al. 1992). Because the null expectation for a contrast at any given node is zero, all regressions in comparative analyses were forced through the origin (Harvey and Pagel 1991).

RESULTS

Rates of extrapair paternity

Over the 2000–2004 study period, 62 rosefinch nests were found to contain a total of 266 young of which 48 in 21 nests were EPY (mean: 2.29 ± 0.244 EPY per nest containing EPY). The proportion of nests containing EPY varied from 14% in 2000 ($n = 7$ nests) to 40% in 2004 ($n = 15$ nests). EPY were nonrandomly distributed in broods of 5 young, with zero and >3 EPY in a brood occurring more frequently than would be expected by chance ($\chi^2_5 = 33.59$, $P < 0.001$, $n = 23$ broods; Figure 2). Between 1 and 3, males sired EPY in broods with a mixed paternity (mean = 1.19 ± 0.136).

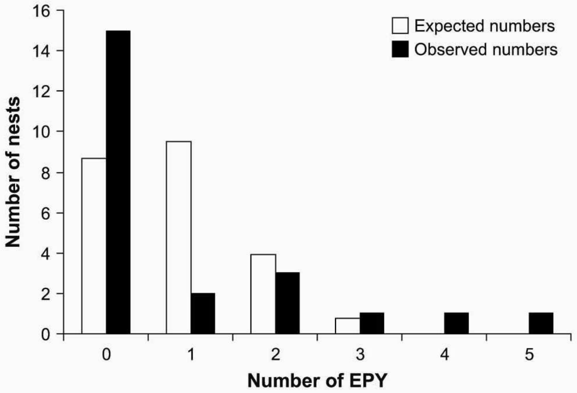

Figure 2
Expected and observed numbers of EPY in nests of scarlet rosefinches ($n = 23$) containing 5 young. Expected numbers of EPY were estimated from a prediction of multivariate hypergeometric distribution of EPY among nests (Neuhauser et al. 2001).

Effects of breeding synchrony and geography on extrapair paternity

Breeding synchrony as well as the geographical distribution of male–female interactions could affect the probability of cuckoldry. Over the 5 study years, the egg-laying period in rosefinches lasted 14 ± 2.4 days (range 10–22), and only few nests ($n = 11$) were established very late in the season (commencing ≥10 days after the first clutch of the season, hereafter described as late nests; also see Stjernberg 1979). Breeding synchrony (SI) averaged 57 ± 2.8% (range 2–96, $n = 62$). The probability a nest would contain at least one EPY was unrelated to standardized timing of breeding (partial effect: $\chi^2_1 = 0.01$, $P = 0.98$, $n = 62$) or breeding synchrony (SI) with other pairs on the study plot (partial effect: $\chi^2_1 = 0.082$, $P = 0.77$, $n = 62$). EPY occurred with equal probability in the nests of early and late breeders (15 of 51 vs. 6 of 11 nests, Fisher's exact test, $P = 0.17$). However, this result should be treated with caution because the power of the analysis is low (0.22).

The difference in the timing of breeding of males who sired EPY in nests and males they cuckolded was 6.6 ± 1.1 days (range = 1–14, $n = 12$), but there was no evidence that the cuckolders bred earlier or later than the males that lost paternity (Wilcoxon matched pairs test, $z = 1.02$, $P = 0.31$, $n = 12$). Similarly, there appeared to be no difference between cuckolded and extrapair sire in the value of SI (Wilcoxon matched pairs test, $z = 0.71$, $P = 0.48$, $n = 12$). In only one case was a male cuckolded by his closest neighbor. In all other cases, there was at least one other nest (median = 6, range 1–11) located closer to the nest of the cuckolded male than was the nest of the extrapair sire. In fact, nests of the extrapair sire and cuckolded male were sometimes situated at considerable distances apart, in one case over the length of the study plot (mean 144 ± 28.3 m; range 25–920 m).

Variance in male reproductive success due to extrapair paternity

Over the restricted period 2001–2004, we examined the annual reproductive success of 46 male rosefinches, of which 2 were second-year males. Together, these males sired 24 of a total of 33 EPY uncovered in their nests. Each male sired 0–5 EPY (mean 0.54 ± 0.178, $n = 46$), and males that sired EPY achieved this in either 1 or 2 nests. Variance in total reproductive success (T) was 7.02 greater for paired males than for paired females (variance ratio test: $F_{42,42} = 0.158$, $P < 0.001$; $I_{smales} = 0.344$, $I_{sfemales} = 0.049$). When we partitioned the variance in overall male reproductive success (Tm) into the variance due to the number of mates, the average number

Table 2
Standardized variance in male reproductive success in scarlet rosefinches attributable to within (W) and extrapair (E) success and a covariance between them

Variance in male reproductive success	total variance (%)
W	67.3
E	22.9
2 × Cov (W, E)	9.8
Within-pair terms	
Due to variance in no. of mates (M_w)	17.4
Due to variance in no. of young/mate (N_w)	9.2
Due to variance in proportion of young sired (P_w)	28.7
Extrapair terms	
Due to variance in no. of mates (M_e)	18.9
Due to variance in no. of young/mate (N_e)	0.2
Due to variance in proportion of young sired (P_e)	1.3
Covariances + D	16.0

This variance is further partitioned into variance due to the number of mates (M), the average number of young produced per mate (N), and the proportion of young sired by male in mate's nest (P). The table shows also the proportion of variance in male fitness attributable to all covariance terms and D (remainder term that reflects multivariate skewness; Webster et al. 1995).

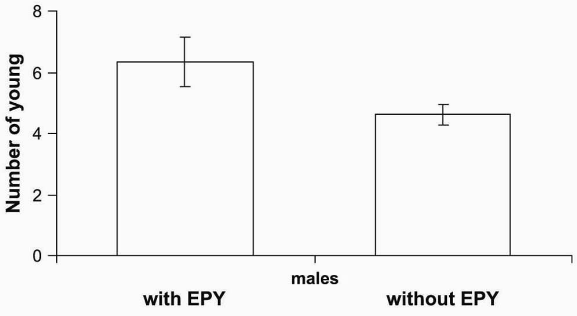

Figure 3
Mean annual reproductive success (number of young sired) of scarlet rosefinch males that were able to sire EPY (left column; $n = 9$ males) and those that avoided cuckoldry but did not sire any EPY (right column; $n = 22$). Only paired males are included. Vertical bars denote standard error.

of young per mate and the proportion of young sired through both within and extrapair mates. The major source of variance in male fitness was within-pair paternity (P_w) in combination with the number of extrapair mates (M_e) (Table 2), both positively correlated with *Tm* (only paired males; Spearman rank, $r_s = 0.74$, $P < 0.001$ and $r_s = 0.44$, $P < 0.001$, respectively, $n = 43$). The number of social mates (M_w) also contributed to variance in male fitness and was correlated with *Tm* (all males: $r_s = 0.39$, $P < 0.01$, $n = 46$). Three bachelor males occurred on the study plot, one of which sired 3 young in 2 nests, and we recorded one case of simultaneous polygyny (1 male with 2 social mates).

There was no evidence that males faced a trade-off between achieving EPFs and ensuring paternity within their own nests because the correlation between P_w and M_e was positive ($r_s = 0.31$, $P = 0.040$, $n = 43$), and, interestingly, no male that gained EPFs was cuckolded (comparison with other males, Fisher's exact test, $P = 0.044$, $n = 9$ and 34). As a result, males that were able to sire EPY achieved greater reproductive success than males that avoided cuckoldry but were not able to sire young outside their pair-bond (analysis of variance, $F_{1,29} = 6.13$, $P = 0.019$; Figure 3). The covariance term between within-pair and extrapair success of males was positive and amounted to 10% (Table 2). Standardized variance in realized reproductive success of males (I_s) was 3.31 times greater than variance in apparent reproductive success (0.402 vs. 0.122). Realized reproductive success of paired males (*Tm*) was unrelated to the timing of breeding ($r_s = -0.23$, $P = 0.13$, $n = 43$).

The opportunity for sexual selection due to extrapair paternity in north temperate zone passerines

In single-brooded species, such as rosefinches, EPFs tended to contribute relatively more to male fitness, defined as the I_s/I_{sapp} ratio, than in those with 2 breeding attempts per season ($n = 6$ and 12; Mann–Whitney *U* test, $Z = 2.43$, $P = 0.015$; Table 3). However, assignment success was higher in 2-brooded

Table 3
The contribution of EPFs to the opportunity for sexual selection expressed as the I_s/I_{sapp} ratio for temperate zone breeding songbirds

Species	I_{sapp}	I_s	I_s/I_{sapp}	%EPY	%EPY assigned	Mating system[a]	Breeding attempts/ season	Migr[b]	SI[c]	Length[d]	Source[e]
Acrocephalus arundinaceus	1.00	1.02	1.0	3	100	Poly	2	8	30	8	1
Agelaius phoeniceus 1	0.25	0.39	1.1	26	78.5	Poly	2	5	36	11	2
Agelaius phoeniceus 2	0.34	0.49	1.4	25	60.0	Poly	2	5	36	11	3
Agelaius phoeniceus 3	0.25	0.39	1.6	28	93.0	Poly	2	5	36	11	4
Carpodacus erythrinus	0.12	0.40	3.3	18	73.0	Mono	1	6[e]	57	4	5
Delichon urbica	0.06	0.31	5.2	19	100	Mono	2	8	60	10	6
Dendroica caerulescens	0.49	0.71	1.4	21	62.3	Mono	2	2	27	7	7
Dendroica petechia	0.04	0.53	13.3	37	35.4	Mono	1	6	47	3	8
Ficedula albicollis	0.03	0.14	4.7	16	53.8	Mono	1	7	—	5	9
Geothlypis trichas	0.28	0.48	1.7	26	83.0	Mono	2	5	25.5	5	10
Icterus galbula bullockii	0.07	0.17	2.4	32	44.6	Mono	1	3	—	8	11
Junco hyemalis	0.55	0.72	1.3	28	54.7	Mono	2	3	—	10	12
Luscinia svecica svecica	0.08	0.37	4.6	29	56	Mono	1	5	66	4	13
Parus caeruleus	0.16	0.27	1.7	11	72.3	Mono	2	0	57	12	14
Passerculus sandwicensis	0.27	0.48	1.8	47	92.3	Poly	2	4	34	8	15
Poecile atricapillus	0.04	0.10	2.5	9	46.9	Mono	2	0	53	6	16
Progne subis	0.05	0.33	6.6	19	53.8	Mono	2	7	28	6	17
Tachycineta bicolor	0.09	0.99	11.0	52	47.2	Mono	1	5	46	3	18
Troglodytes aedon	0.18	0.22	1.2	10	88.0	Poly	2	3	—	8	9
Wilsonia citrina	0.18	0.46	2.6	27	54.7	Mono	2	2	33	5	19

[a] poly, socially polygynous, mono, socially monogamous.

[b] Values for migration distance (migr; to the nearest thousand kilometer) and breeding SI obtained from Spottiswoode and Møller (2004) and from Cramp et al. (1977–1994).

[c] Length in weeks of the main laying period from Cramp et al. (1977–1994) and Poole (2005): if possible, estimated to the nearest week from pie charts, without considering the tails signalizing exceptionally early or exceptionally late breeding attempts.

[d] Distance (to the nearest thousand kilometer) between breeding areas in Central Europe and wintering grounds in India (Cramp et al. 1977–1994).

[e] Source for realized variance in male reproductive success (I_s, I_{sapp}), percentage of EPY in a population (%EPY) and %EPY for which extrapair sires were assigned (%EPY assigned): 1, Hasselqvist et al. (1995); 2, Weatherhead and Boag (1997); 3, Webster et al. (1995); 4, Gibbs et al. (1990); 5, this study; 6, Whittingham and Lifjeld (1995); 7, Webster et al. (2001); 8, Yezerinac et al. (1995); 9, Sheldon and Ellegren (1999); 10, Whittingham and Dunn (2005); 11, Richardson and Burke (2001); 12, Ketterson et al. (1997); Whittingham and Dunn (2005); 13, Johnsen et al. (2002); 14, Kempenaers et al. (1992); 15, Freeman-Gallant et al. (2005); 16, Otter et al. (1998); Whittingham and Dunn (2005); 17, Wagner et al. (1996); Møller (1998); 18, Kempenaers et al. (2001); 19, Stutchbury et al. (1997).

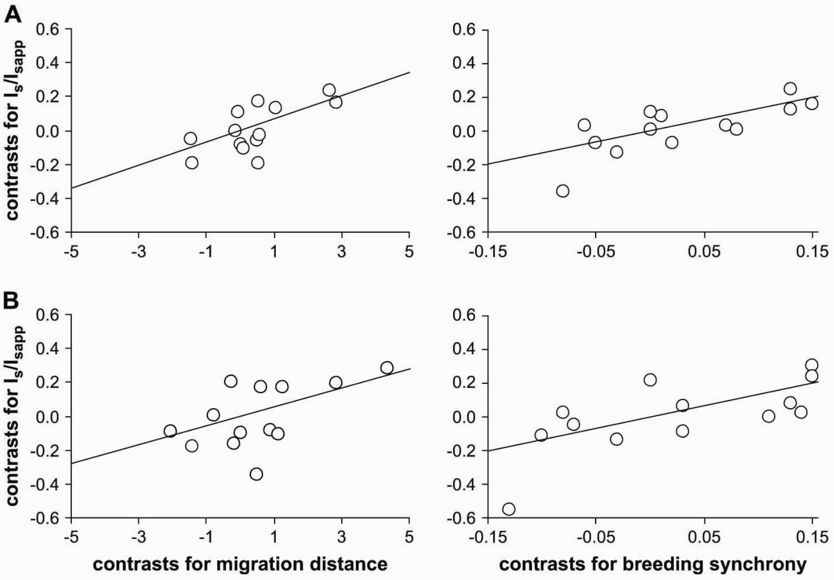

Figure 4
Migration distance (left column) and breeding synchrony (right column) as predictors of the opportunity for sexual selection due to EPFs (I_s/I_{sapp} ratio) in temperate zone breeding passerines. Effects controlled for percentage of young with assigned sire, the length breeding season, and migration distance or breeding synchrony, respectively. (A) Assuming gradual mode of evolution (Grafen 1989). (B) Assuming punctuated mode of evolution (Harvey and Pagel 1991). Regression lines are forced through the origin.

compared with single-brooded species (Mann–Whitney *U* test, $Z = 2.20$, $P = 0.023$) and for polygynous compared with socially monogamous species ($n = 4$ and 14; Mann–Whitney *U* test, $Z = 2.50$, $P = 0.012$). When the analysis was restricted to socially monogamous species, the difference in I_s/I_{sapp} ratio between single and 2-brooded species approached significance ($n = 6$ and 8; Mann–Whitney *U* test, $Z = 1.81$, $P = 0.07$) with no difference in assignment success between the groups (Mann–Whitney *U* test, $Z = 1.48$, $P = 0.13$).

The length of the breeding season tended to be inversely correlated with the opportunity for sexual selection to operate through extrapair paternity after being statistically controlled for percentage of EPY assigned (GRAD: $F = 6.20$, $P = 0.025$; PUNCT: $F = 4.16$, $P = 0.059$; $n = 18$). However, this effect was lost ($P = 0.057$ and 0.21, respectively) when migration distance was added into the model. Migration distance tended to be a better predictor of I_s/I_{sapp} ratio than the length of the breeding season (partial effect of migration—GRAD: $F = 5.38$, $P = 0.036$; PUNCT: $F = 2.82$, $P = 0.11$). This pattern was even stronger when the analysis was restricted to socially monogamous species ($n = 14$; partial effect of migration—GRAD: $F = 13.59$, $P < 0.005$; PUNCT: $F = 8.14$, $P = 0.018$; partial effect of breeding season length—GRAD: $F = 3.46$, $P = 0.09$; PUNCT: $F = 2.53$, $P = 0.14$). In a more complex model, using a set of 14 species for which the effects of breeding season length, migration distance, and breeding synchrony could be simultaneously evaluated (Table 3), the length of breeding season again was no longer significant ($P > 0.40$ in both cases) and both migration distance (GRAD: $F = 8.36$, $P = 0.018$; PUNCT: $F = 2.32$, $P = 0.16$) and breeding synchrony (GRAD: $F = 15.13$, $P < 0.01$; PUNCT: $F = 10.03$, $P = 0.011$) tended to be positively correlated with the I_s/I_{sapp} ratio (Figure 4).

DISCUSSION

We show that EPFs considerably increase variance in reproductive success among rosefinch males despite only a moderate level of extrapair paternity (18% young in 30% nests were extrapair) in comparison with other passerines (reviewed in Griffith et al. 2002). Consistent with this result, EPYs were nonrandomly distributed across broods of 5 young. Because we were able to identify genetic sires to a relatively large proportion of EPY in our study population over the period of 2001–2004, the relatively high value of the I_s/I_{sapp} ratio (a measure of the opportunity for sexual selection due to EPFs) is probably not due to bias resulting from an artificial overestimation of variance in realized reproductive success of males (e.g., Freeman-Gallant et al. 2005). Moreover, other lines of evidence highlight the contribution EPFs make in enhancing the reproductive success of certain males at the expense of others. There was a positive relationship between an individual male's success regarding within- and extrapair paternity, suggested also by a positive covariance term between these components of male fitness. Similarly, realized reproductive success was higher for males that achieved EPFs than in males that did not and was positively related to the number of extrapair mates.

There are several explanations that may account for high variance in male fertilization success in rosefinches. If, for example, high-quality males successful in EPFs were those that started to breed earlier than the rest of males, those males may not have to trade-off pursuing extrapair copulations against investments to protect within-pair paternity (Birkhead and Møller 1992). However, in this study, no systematic difference in the timing of breeding or breeding synchrony was found between males that lost paternity and those that sired EPY in their nests. Moreover, neither breeding synchrony nor timing of breeding influenced occurrence of EPY in rosefinch nests. It is possible that too few nests in our sample were asynchronous for any intraspecific effects of breeding synchrony to be detectable. Whether breeding synchrony affects extrapair paternity remains an area of debate (Westneat and Stewart 2003). Consistent with other within-species studies, we failed to detect any significant effects, either positive or negative, of breeding synchrony on extrapair paternity (reviewed in Griffith et al. 2002; see also Møller and Ninni 1998). Territoriality is another factor that may limit access of high-quality males to extrapair females, as well as sampling among an adequate number of males by females (Webster et al. 2001). However, Indeed, we found that even males up to 920 m away could sire EPY. This indicates that choice of extrapair mates may not be restricted to a local spatial scale (closest neighbors) in rosefinches, a finding reported in other songbirds (e.g., Reyer et al. 1997; Westneat and Mays 2005; Woolfenden et al. 2005; Kleven et al. 2006).

The fact that males that achieve EPFs were not cuckolded themselves could be a result of congruent female preference for a particular male phenotype combined with the willingness of females paired with low-quality males to accept extrapair mates of higher quality. It has been argued that high rates of extrapair paternity in long-distance migrants, such as rosefinches, indicate that females in these species accept an extrapair male to offset their hasty or inappropriate choice of social mates (e.g., Weatherhead and Yezerinac 1998; Spottiswoode and Møller 2004). Females may be less "choosy" during social pairing if any delay in breeding incurs fitness costs to them (Westneat et al. 1990), and these costs of delay can be expected high in long-distance migratory species. Whether this explanation applies to rosefinches remains unclear. However, in line with this "compensatory" hypothesis, social pairing in rosefinches seems to be random with respect to morphological and plumage characters of males, possibly as a result of time constraints that appear to limit female choice (Björklund 1990). To date, however, no data are available on extrapair mating preferences. Monopolization of EPFs by certain males could also be a result of male–male competition and male pursuit tactics (Westneat and Stewart 2003).

Using comparative methods, we tested the prediction that patterns of contribution of EPFs to the opportunity for sexual selection in rosefinches can be explained by explicit hypotheses related to migration. Although effects of migration on rates of EPFs and/or the level of sperm competition in birds have been already evaluated (e.g., Spottiswoode and Møller 2004; Pitcher et al. 2005), our analysis extends previous studies by focusing on variance in fertilization success among males due to EPFs. We found that the potential role of EPFs in sexual selection is higher in species with short breeding seasons than in species with a prolonged breeding season where high-quality males could increase their number of annual progeny by multiple breeding with high-quality females (e.g., Hill 1994). However, a more detailed analysis reveals that migration may be a confounding factor in this relationship. In fact, breeding season length lost its effect when migration was added to the model. This suggests, all else being equal, that EPFs contribute more to variance in male reproductive success and thus result in a greater opportunity for selection in migratory species compared with sedentary species. This result is consistent with the finding that migratory species tend to exhibit more sexual dichromatism (Fitzpatrick 1994, 1998).

Migration can covary with breeding synchrony (e.g., Spottiswoode and Møller 2004; Pitcher et al. 2005). Although breeding synchrony seemed to have no effect on the fertilization success of rosefinch males, it was an important predictor of variance in male success due to EPFs in the interspecific comparison. Such a discrepancy between the results of intra- and interspecific analyses seems to be relatively common in evolutionary ecology (e.g., Martin et al. 2001) and was also reported for the effect of breeding synchrony on rates of EPFs in socially monogamous passerines (slight negative intraspecific effect, Møller and Ninni 1998 vs. positive interspecific trend, Stutchbury 1998b). Here we demonstrate a positive interspecific effect, independent of migration, of breeding synchrony on variance in fertilization success among males. These results are in line with the prediction that synchrony allows females to compare potential extrapair males that are competing and displaying for EPFs at the same time (Stutchbury and Morton 1995) and simultaneously provides high-quality males more EPF opportunities. However, data seem to support the idea that the effect of migration is direct and independent of breeding synchrony, as breeding synchrony did not significantly changed a positive relationship between migration and I_s/I_{sapp} ratio at least under one (gradual) mode of evolution.

A set of non–mutually exclusive hypotheses has already been invoked to explain the direct effects of migration on rates of EPFs in birds (Spottiswoode and Møller 2004), all of them also applicable to the relationship between opportunity for sexual selection due to EPFs and migration demonstrated in our study. Unfortunately, most are difficult to distinguish using comparative methods. For example, the above-mentioned "compensatory" hypothesis as well as "higher variance in male quality in migrants" hypothesis (Fitzpatrick 1994) would lead to the same relationship between I_s/I_{sapp} ratio and migration, despite evolutionary mechanisms being different. The latter would attribute either higher fertilization success of certain males to their superior genetic and phenotypic qualities (that could involve, e.g., quality of ejaculate and the ability to gain EPFs in competition with other males) or higher attractiveness of certain males to all females in a population and greater benefits from EPFs to females. It specifically demands that migration generates additive variation in genetic quality among individuals, a prediction that has biological relevance (Fitzpatrick 1994, 1998). However, whether long-distance migration in birds is associated with higher genetic variability deserves further investigation.

Using a large set of species from several geographic regions, Pitcher et al. (2005) found that strength of sperm competition, as measured by relative testes size, is related to social mating system and breeding density rather than to migration in birds. However, these authors treated migration as a 2-level categorical variable used also as an index of breeding synchrony. We demonstrate that EPFs have the potential to increase the opportunity for sexual selection particularly in long-distance migratory songbirds such as rosefinches, and analyses should distinguish between sedentary species, short-distance, and long-distance migrants. Moreover, our data indicate that migration and breeding synchrony could promote sexual selection via EPFs through independent pathways, at least in passerine species from the north temperate zone. Our findings agree with the prediction that at a given latitude and implicit level of seasonality, long-distance migration and high breeding synchrony could be associated with increased strength of sexual selection through extrapair paternity.

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II.

Extra-pair fertilizations contribute to selection on secondary male ornamentation in a socially monogamous passerine

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Extra-pair fertilizations contribute to selection on secondary male ornamentation in a socially monogamous passerine

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Abstract

Despite considerable research effort, it remains unclear whether extra-pair fertilizations (EPF) drive the evolution of male secondary ornamentation in socially monogamous systems. In this study, we test the hypothesis that EPF contribute to the evolution or maintenance of male feather ornamentation in a sexually dichromatic passerine, the Scarlet Rosefinch, *Carpodacus erythrinus*. We show that the colouration of ornamental breast feathers is a good predictor of basic sources of variation in male annual reproductive output in rosefinches and that the annual realized reproductive success of males is positively associated with measures of ornamental colouration only when gains and losses because of EPF are considered. The results indicate that EPF in rosefinches may rely on absolute (good genes) rather than self referential (genetic complementarity) criteria of mate choice. Our study corroborates the potentially important role of EPF in the evolution and/or maintenance of elaborate male ornaments in socially monogamous taxa.

Introduction

Sexual selection has long been proposed as an explanation for the existence of elaborate male ornaments (Darwin, 1871). The opportunity (and strength) of sexual selection has traditionally been attributed to variance in the number of social mates among males, or the variance in the fecundity of social mates pairing with these males (Andersson, 1994; Shuster & Wade, 2003). However, with the advent of molecular tools, it has become apparent that there are other sources of variation in reproductive success. For example, it has been shown that female promiscuity occurs in mammals (Wolff & Macdonald, 2004), reptiles (Uller & Olsson, 2008) or amphibians (Liebgold *et al.*, 2006), and both males and females frequently mate outside their pair bonds in socially monogamous birds (Griffith *et al.*, 2002; Westneat & Stewart, 2003). The resulting extra-pair fertilizations (EPF) may represent an important process leading to an increased variance in the reproductive success of males and the strength of sexual selection; if distributed unequally over individuals, EPF may contribute to the evolution of secondary ornaments even in strictly socially monogamous species (Webster *et al.*, 1995; Owens & Hartley, 1998).

Previous studies have demonstrated that in birds, extra-pair mating can contribute to variance in male reproductive success, or to the opportunity for sexual selection (OSS). However, whereas some intra-specific studies have suggested that this reproductive tactic significantly adds to variance in male fitness (reviewed in Whittingham & Dunn, 2005; see also Albrecht *et al.*, 2007), others reported slight or no increase of OSS because of EPF (e.g. Kraaijeveld *et al.*, 2004; Freeman-Gallant *et al.*, 2005). The contribution of EPF to OSS seems to be affected by inter-specific migration distance (Albrecht *et al.*, 2007) and mating system (polygyny or monogamy; Freeman-Gallant *et al.*, 2005; Whittingham & Dunn, 2005), but may also vary depending on the mechanism of mate choice, i.e. whether the extra-pair mate choice is mainly driven by self-referential or absolute criteria (Mays *et al.*, 2008). Similarly, rates of

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extra-pair paternity across species seem to correlate with degree of sexual dimorphism (Owens & Hartley, 1998) and testes size (Pitcher *et al.*, 2005), but apparently not with the OSS itself (Freeman-Gallant *et al.*, 2005).

Empirical studies that have attempted to evaluate the basic predictions of sexual selection theory, linking fertilization success of males with ornamental expression, have found that the occurrence of extra-pair paternity correlates with male size (Yezerinac & Weatherhead, 1997), song repertoire (Forstmeier *et al.*, 2002; Suter *et al.*, 2009), feather colouration (e.g. Foerster *et al.*, 2003; Safran *et al.*, 2005), structural ornaments (Kleven *et al.*, 2006), or a combination of several factors (e.g. Sundberg & Dixon, 1996). Yet other studies have found no selection via EPF acting on secondary male ornaments despite high contribution of EPF to OSS (Westneat, 2006). Thus, understanding the role of EPF in the process of evolution/maintenance of elaborate male ornamentation remains a challenging issue of evolutionary biology (Westneat & Stewart, 2003), particularly important for understanding the signalling function of various ornaments given the mainly indirect benefits that females gain from extra-pair mating (Griffith *et al.*, 2002).

Variance in total reproductive success of males [var (T_m)] can be partitioned between within- and extra-pair components (Webster *et al.*, 1995). To determine the evolutionary significance of EPF, it is necessary to identify (1) how the terms describing the ability of a male to avoid being cuckolded (within-pair fertilization success of males), and his ability to obtain extra-pair mate(s) contribute to overall variation in male fitness (Webster *et al.*, 2007) and (2) how phenotypic traits affect these components of male fertilization success. Although this information is crucial for understanding the mechanism by which promiscuity contributes to the process of sexual selection (Webster *et al.*, 2007), obtaining it is particularly challenging because to track the entire reproductive output of males, most if not all sires of extra-pair young (EPY) in nests must be identified (Freeman-Gallant *et al.*, 2005).

In this study, we evaluate the hypothesis that extra-pair mating drives selection on orange-to-red carotenoid-based feather male ornamentation in sexually dichromatic Scarlet Rosefinches (*Carpodacus erythrinus*, Pallas) by relating data on within- and EPF success of males with the extent of their sexual ornamentation in a population where most EPY can be associated with their biological fathers (Albrecht *et al.*, 2007). As in other related species, redness was expected to indicate a high level of expression of carotenoid-based ornamentation (for review see Hill, 2002). The data allowed us to link variation in sexual ornamentation to several components of fertilization success of males, in particular (1) the proportion of within-pair young (WPY) sired and (2) the number of extra-pair mates a male is able to obtain. Variation in these two fitness components (P_w and M_e *sensu* Webster *et al.*, 1995) has been shown to explain most (29% and 19%) variance in total annual reproductive output of male rosefinches (Albrecht *et al.*, 2007). We also performed a pair-wise comparison of extra-pair males and males they cuckolded to elucidate whether sexual ornamentation affects female extra-pair mate choice. Finally, we test the hypothesis that sexual ornamentation *via* EPF success determines annual reproductive output in rosefinch males. While costly carotenoid-based sexual ornamentation plays a crucial role in mate choice decision in House Finches (*Carpodacus mexicanus*), where yellow males tend to be ignored by females and red males are preferred as mates (Hill, 2002; Oh & Badyaev, 2006), the speed of pairing between social mates in rosefinches appear to be unrelated to many male traits, including feather colouration (Björklund, 1990). However, thus far, data linking various components of EPF success and ornamentation have not been available for these species.

Methods

General procedures

Field work was carried out from May to July during the years 2001–2007 in the Šumava Mountains National Park, Czech Republic (48°49′N, 13°56′E). A detailed description of the study site and field procedures is presented in Albrecht *et al.* (2007). Briefly, the study was conducted in a shrubby wetland meadow of about 1 km^2 surrounded by a mosaic agricultural landscape hosting a colony of 10–20 breeding pairs of rosefinches per year (see also Albrecht, 2004; Albrecht *et al.*, 2007). The study site was searched systematically several times over each breeding season where there is vegetation suitable for nesting (mainly *Spiraea* bushes) so that nearly all nests were found during the egg-laying or incubation stages. Nests were visited regularly during the field season to estimate the exact day of egg hatching. Blood samples ($\sim$20 μL) were taken by venipuncture from adults and in 7-day-old chicks. Since nest survival in the study area was high (Albrecht, 2004), only a few broods (< 20%) were lost before it was possible to sample the chicks for blood. Adult birds were trapped using mist-nets either upon their arrival to the study area in May, or during the provisioning of chicks later in the season. Each adult bird was ringed with one aluminium ring (N MUSEUM PRAHA) and with an individual combination of 1–3 coloured plastic rings (AVINET). Individual females were assigned to a nest having either been flushed from that nests during the incubation period, or observed feeding and/or brooding the chicks in that nest. Males observed repeatedly feeding the female during incubation, and/or feeding chicks, were considered the social fathers of young in a particular nest.

Since the colour of ornamental patches in itself appears to be the most important feature in mate choice decision in several avian species (e.g. Hill, 2002; Safran *et al.*, 2005), and breast characteristics of rosefinch males seem to be of a particular importance during mate attraction (Stjernberg, 1979), we decided to concentrate on breast patch colour as the most relevant ornamentation in this species. In the analyses (see below), we thus omitted other potentially important parameters that might each convey further individual information, such as pigment symmetry, patch size or patch symmetry (Badyaev *et al.*, 2001) and also omitted ornamentation on other parts of the male body. Where possible, the breast patch of males in their 3rd year or older were photographed using a digital camera (Olympus Corp., Tokyo, Japan). Only photographed males were included in the analyses. All photographs were taken under standard lighting conditions in the dark room of a nearby field station using an electronic flash 40 cm from the colour patch, and colour swatches (grey card GC 18 and colour & grey chart Q 14; Danes-Picta, Praha, Czech Republic) were used to standardize measurements (Montgomerie, 2006). Each bird was placed directly on the grey card in a standardized position. Photographs were analysed using ADOBE PHOTOSHOP™ software (Adobe Systems Inc., San Jose, California). Hue, saturation and brightness (HSB colour space) were measured on 10 5 × 5 pixels points randomly distributed over the breast colour patch of males (also Kilner, 1997). There was a high repeatability in estimates for all three parameters within individual males (hue: $r = 0.996$; saturation: $r = 0.946$; brightness: $r = 0.987$). Increasing values of hue [range −9.1 (hue angle 350.9) to 23.17] indicate a yellower (less red) plumage, increasing values of saturation (range 42.75–85.45%) indicate more intensive colouration, and increasing values of brightness (range 35.08–95.30%) indicate lighter colouration (Montgomerie, 2006). Digital image analysis of avian ornaments and other methods based on human perception have been criticized (Bennett *et al.*, 1994) since birds are able to recognize reflectance in UV (320–400 nm) spectra. However, it has been argued that these methods can capture biologically relevant colour signal under certain conditions (reviewed in Andersson & Prager, 2006; Montgomerie, 2006). We compared measures obtained using the above method (not sensitive to UV wavelengths) with those received from spectrometer using a sample of 20 males trapped on the study plot or in nearby areas in the 2007 field season. Spectra were obtained using an Avantes Avaspec 2048 spectrometer with light source Avalight XE (Avantes BV, Eerbeek, The Netherlands). Standardized measurements were taken on ornaments with reflection probe (2 mm diameter). Colorimetric measures of the colour of the breast patch [spectral intensity (brightness), spectral location (hue) and spectral purity (saturation)] were assessed following equations given in Andersson & Prager (2006) using the average of five measurements per bird. There was a significant correlation between HSB obtained by both methods (hue: $r^2 = 0.847$, $F_{1,18} = 99.915$, $P < 0.0001$; saturation: $r^2 = 0.509$, $F_{1,18} = 18.687$, $P < 0.001$; brightness: $r^2 = 0.224$, $F_{1,18} = 5.197$, $P = 0.037$), indicating that our estimates based on digital photography can be used to describe colouration of ornamental feathers in rosefinches.

Delayed plumage maturation occurs in rosefinches, with males in their 2nd calendar year (SY males) resembling females (Stjernberg, 1979). Only five SY males (9%) were found to be breeding on our study plot, a situation also reported from other areas (Björklund, 1989). As there is no sexual ornamentation in SY males (Stjernberg, 1979), these males were excluded from analyses. Only six after-second-year (ASY) males on the study plot were identified as being unpaired (for criteria see Albrecht *et al.*, 2007). Sexual ornamentation was scored in five of these males, but these were excluded from all analyses since no data on within-pair fertilization success were by definition available for them (and in any case, inclusion of these males in potentially relevant analyses did not change the results). However, we were able to use one unpaired male in pair-wise comparisons of cuckolded and cuckolding males. Males trapped as ASY individuals in more than one season ($n = 13$) were used to evaluate how sexual ornamentation changes with age.

Identification of parentage

Parentage analysis was conducted using 15 microsatellite loci developed by screening genomic libraries of two *Carpodacus* finches and by cross-species amplification of loci from related species: CE207, CE165, CE150, CETC215, CE152, CE147, CM026, CM014, CM001 and CM008E (Poláková *et al.*, 2007), Hofi 17, Hofi 52, Hofi 24, Hofi 5 (Hawley, 2005) and LOX 1 (Piertney *et al.*, 1998). Primer details, PCR amplification conditions, fragment detection and analysis are described in Poláková *et al.* (2007). The loci used for parentage analyses were remarkably variable (4–95 alleles per locus; median = 18), had high heterozygosities for particular loci within adult individuals ($H_e = 0.39–0.98$, median = 0.86), and each adult individual possessed a unique genotype. The average probabilities of excluding a single randomly chosen unrelated individual from being a parent were calculated for all 15 loci (when the maternal genotype was known) using the program CERVUS 3.0 (Kalinowski *et al.*, 2007). These analyses were based (1) on all resident adult individuals genotyped in the course of the study and (2) separately for each year. The total exclusionary power exceeded in all cases 99%.

We did not find any genetic mismatches (i.e. evidence of a mutation) between an offspring and its social mother. The offspring's paternal alleles were subsequently compared with the alleles of its putative father (the female's social partner). Some offspring did not match their social father at 5–14 loci (mean = 9.44) and they were considered EPY. Sires of EPY were determined using the exclusion approach (Jones & Ardren, 2003); that is, we compared the paternal alleles of EPY with the genotypes of all males in the population using the CERVUS 3.0 software. Because we found no mismatches between WPY and their parents, we also used the same strict criteria when attempting to identify the EPY sires. If no male matched a particular EPY at all loci (in fact, social fathers either matched at all loci or differed at least at three loci), the sire was categorized as unknown.

Estimating reproductive success

Apparent reproductive success for a given male was measured as the total number of chicks (including possible EPY) that survived until age 7 days in his nest, corresponding to the age when chicks are able to leave the nest unaided in response to a perceived threat (Björklund, 1990). As some mortality of young occurs after this age, the above method of measuring reproductive success may lead to an over-estimate; however, these random mortality events will not bias our estimates of relative reproductive output of males. Realized reproductive success of a particular male was measured as a sum of offspring sired in his nest and in nests of other males in the study area surviving to 7 days post-hatch. Although our estimate of realized reproductive success could be biased if males frequently sire young outside the study plot, and we cannot rule out the possibility that birds from the different breeding colonies encounter each other on a third site, this seems unlikely to occur with high frequency in our study animals considering the relative isolation of our study area (the nearest rosefinch colony is situated 3 km away; for further discussion see Albrecht *et al.*, 2007). Although the incomplete sampling of nests (e.g. because of predation, see above) may also affect estimates of EPF success in males, this would not significantly change the directions of ornamentation–reproductive success correlations as males that monopolized fertilizations in sampled nests are also those more likely to fertilize eggs in nests we missed. Variation in male fitness can be partitioned into several components (Webster *et al.*, 1995), such as the variance in number of mates [within (M_w) and extrapair (M_e)], proportion of young sired in a nest (P_w and P_e), and mate quality expressed as mate productivity (N_w and N_e; Webster *et al.*, 1995). In this study, we relate variation in male ornamentation to components associated with EPF success of males that were previously shown to account for significant portion of variance in male fitness in rosefinches (M_e, P_w; Albrecht *et al.*, 2007).

Statistical analyses

All analyses were based on data from ASY males for which we had photographs of the breast patch to score sexual ornamentation ($n = 54$ males, 75 observations). To reduce the number of explanatory variables describing the sexual ornamentation to a minimum, we performed a principal component analysis (PCA) on tri-stimulus HSB measures of all resident males for which we had adequate data. First, we checked the distribution of HSB for normality using a Kolmogorov–Smirnov test which revealed a normal distribution in all cases (all $\chi^2 > 0.10$). The single PCA axis (PC1, eigenvalue = 1.66) was strongly associated with hue ($r = -0.91$) and, albeit only to a lesser extend, also with saturation ($r = -0.67$) and brightness ($r = -0.64$), and explained 55.36% of variation in data. We interpreted scores from the PC1 axis (hereafter ‘composite colouration’ or ‘overall sexual ornamentation’) as the combined measure of ornamentation and assumed that the expression of sexual ornamentation (degree of carotenoid-based colouration, redness) in rosefinches was positively associated with PC1 values. However, we also present analyses based raw measures of HSB of ornamental feathers.

As some males were scored in more than 1 year ($n = 13$), in most analyses we used generalized linear-mixed effect model approach (GLMM) with male identity ($n = 54$) treated as a random effect to avoid pseudoreplications (Faraway, 2006). We chose link functions in the models following the nature of dependent variables. When modelling numbers (the number of offspring sired, the number of extra-pair mates obtained) we assumed a Poisson distribution of error terms and used log-link function. When modelling probabilities and proportions (the likelihood of being cuckolded, the proportion of young sired in a nest), logit-link function was applied instead, assuming a binomial distribution of error terms (Faraway, 2006). The onset of breeding, if included in the analysis, was standardized over years (1, the day a first egg in a season was laid) and log transformed to achieve normality. The significance of a particular term in models was based on the change in deviance between the full and reduced/null models, distributed as χ^2 with degrees of freedom equal to the difference in the degrees of freedom between the models with and without the term in question (Faraway, 2006). Minimal adequate models, i.e. models with all terms significant, are presented (Crawley, 2007). When modelling change in colouration of individual males over years, GLMM with an identity link function was applied, with year of male occurrence treated as the ordered categorical predictor, measures of sexual ornamentation as the dependent variables, and male identity as a random effect. Pair-wise comparisons of cuckolding and cuckolded males at the same nest were conducted using standard *t*-tests for dependent samples. In cases when more than one male was cuckolding in the nest of a single social male we considered these as independent events in the analysis. However, results (not shown) remained similar even when nests cuckolded by only a single male were evaluated. Analyses were performed using R 2.8.1 (http://www.r-project.org/) and STATISTICA 6.0 statistical packages. Estimates are presented ± SE unless stated otherwise.

Results

Patterns of paternity

Extra-pair young were identified in 24 of 75 (32%) broods for which we also obtained phenotypic traits of social fathers. In most cases, broods with mixed paternity contained two EPY (mean 2.17 ± 0.214, range 1–5, $n = 24$), and three broods consisted exclusively of EPY. A total of 52 EPY were sired in these nests and the biological father was assigned to 39 (72.2%) of them. The number of genetic fathers siring young in nests varied from one (no EPY detected) to four (mean 2.30 ± 0.092, $n = 75$), and paired males obtained between zero and two extra-pair mates (mean 0.22 ± 0.052, $n = 75$) and sired zero to four EPY (mean 0.48 ± 0.12, $n = 75$). The overall probability of occurrence of at least one EPY in nests was not associated with the onset of breeding and year, though the slope of the relation between the occurrence of EPF and the onset of breeding in particular years differed (Table 1). Similarly, the onset of breeding was not correlated with the overall sexual ornamentation of males (Table 1).

Extra-pair paternity and male ornamentation

We found no systematic effect of age on hue (GLMM, $\chi^2_5 = 0.604$, $P = 0.989$) or brightness (GLMM, $\chi^2_5 = 9.639$, $P = 0.089$), but a positive effect of age on saturation (GLMM, $\chi^2_5 = 11.864$, $P = 0.038$) in a subset of males scored in more than 1 year ($n = 13$ males, 34 repeats in total). However, no effect of age on the composed measure of sexual ornamentation (PC1) was detectable (GLMM, $\chi^2_5 = 0.819$, $P = 0.976$). In models describing within-pair and EPF success of males ($n = 54$ individuals and 75 observations), the occurrence of at least one EPY in nests was not significantly associated with the composite colouration of social fathers (PC1: GLMM, binomial errors; $\chi^2_1 = 1.994$, $P = 0.158$, Fig. 1a), but was associated with hue (Table 2). On the other hand, overall sexual ornamentation was identified as an important predictor explaining variation in the proportion of young sired by a male in his nest [GLMM, binomial errors; $\chi^2_1 = 6.274$, $P < 0.05$ (slope: 0.701 ± 0.296)]; was strongly associated with the probability of obtaining at least one EPF [GLMM, binomial errors; PC1: $\chi^2_1 = 12.690$, $P < 0.001$ (slope: 1.303 ± 0.435); Fig. 1b]; and was also a good predictor of the total number of EPY a male obtained in a season [GLMM, Poisson errors; $\chi^2_1 = 10.764$, $P < 0.001$ (slope: 0.867 ± 0.278)]. Again, specific components of colouration contributed unequally to these associations, with hue and brightness being the most important predictors of male fertilization success (Table 2). Finally, composite colouration was a parameter which allowed the discrimination between cuckolding and cuckolded males in nests containing EPY where both the social and cuckolding males were scored for sexual ornamentation ($n = 19$ male couples), indicating that cuckolding males were redder than the males they cuckolded (*t*-test for dependent samples, $t_{18} = -2.404$, $P = 0.027$, Fig. 2). Cuckolding and cuckolded males, however, did not differ in hue, saturation or brightness when these were treated separately (hue: $t_{18} = -1.931$, $P = 0.069$, saturation: $t_{18} = -1.698$, $P = 0.107$, brightness: $t_{18} = -0.820$, $P = 0.423$).

Table 1 (a) Correlates of the occurrence of EPF, i.e. of at least one extra-pair young (EPY) found in nest ($n = 75$) as a function of the standardized timing of breeding (log transformed) and year, and (b) the onset of breeding as a function of male sexual ornamentation (PC1) and year.

(a)	d.f.	χ^2	*P*	(b)	d.f.	*F*	*P*
Timing	1	1.619	0.203	PC1	1, 67	0.129	0.721
Year	6	5.000	0.544	Year	6, 67	1.144	0.347
Year:timing	6	13.277	0.039	PC1:year	6, 61	0.651	0.689

Analyses are based on logistic regression (a) and general linear (b) models, respectively. Significances are based on Type III sums of squares. The total numbers of nests analysed in respective years are as follows (year in parenthesis): 10 (2001), 9 (2002), 10 (2003), 13 (2004), 13 (2005), 9 (2006) and 11 (2007).

Sexual ornamentation and annual reproductive success of males

There was no relationship between apparent reproductive success of males and their overall sexual ornamentation

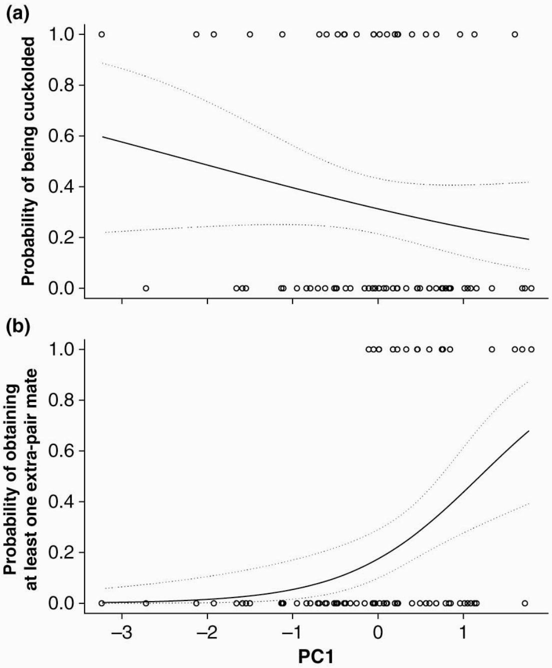

Fig. 1 Predicted probability of (a) being cuckolded and (b) obtaining at least one extra-pair mate for rosefinch males as a function of composite male ornamentation (PC1; higher values of PC1 indicate higher degree of ornamentation). Dotted lines are 95% confidence limits. Estimations on the figures are based on simple logistic regression models not adjusted for repeated sampling for several males (see the main text for results based on mixed-model effect approach).

Table 2 Results of mixed-effect models (GLMM) evaluating the effects of hue, saturation and brightness on (a) the probability of having at least one EPY in a nest (0 – no EPY, 1 – EPY); (b) the proportion of young sired by a male in his nest; (c) the probability of obtaining at least one extra-pair mate (0 – no mate, 1 – EP mate); and (d) the number of extra-pair offspring sired.

MAM	Term	Estimate	SE	χ	d.f.	*P*
(a) **Hue**	Intercept	−1.452	0.435			
	Hue	0.179	0.0932	4.311	1	0.038
(b) **Hue**	Intercept	3.327	0.481			
	Hue	−0.164	0.061	8.108	1	0.004
(c) **Hue + Brightness**	Intercept	2.720	1.899			
	Hue	−0.270	0.103	8.675	1	0.003
	Brightness	−0.069	0.034	4.895	1	0.027
(d) **Hue + Brightness**	Intercept	3.440	1.531			
	Hue	−0.205	0.079	8.101	1	0.004
	Brightness	−0.087	0.028	12.544	1	< 0.001

Minimal adequate models (MAM), i.e. models for which all terms are significant are presented in bold. Values of significance for particular terms are based on Type III sum of squares (adjusted for effects of other terms in a particular MAM). Analyses a, b and c are logistic regressions (logit link function), while analysis *D* is a Poisson regression (log link function).

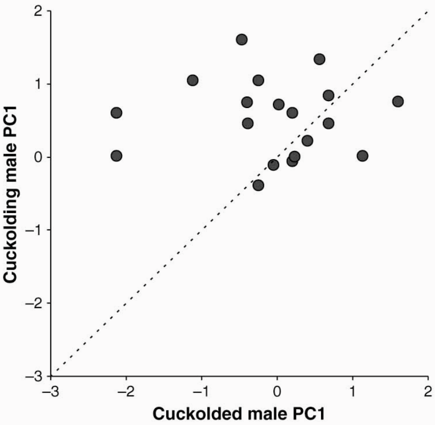

Fig. 2 The relationship between the PC1 scores (higher values of PC1 indicate higher degree of ornamentation) for cuckolded and cuckolding males at the same nests. Diagonal dashed line represents identical colouration of cuckolded and cuckolding males.

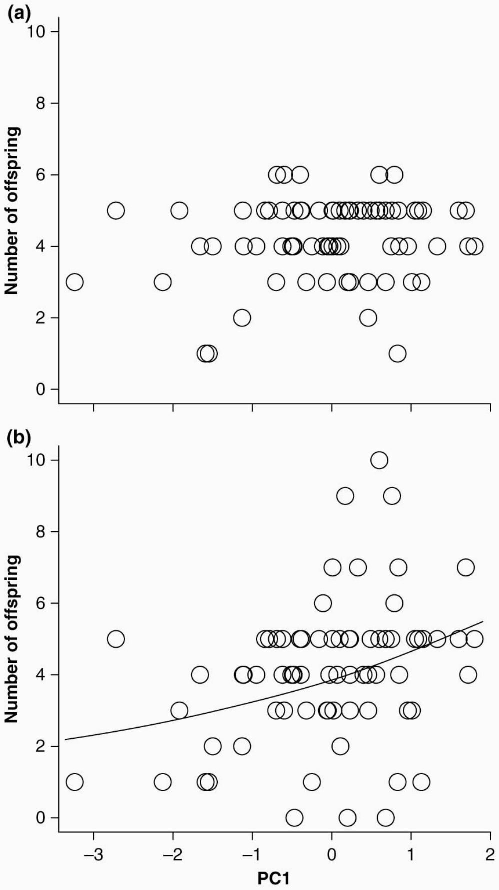

Fig. 3 The relationship between PC1 (higher values of PC1 indicate higher degree of ornamentation) and (a) apparent annual reproductive success of paired Scarlet Rosefinch males when no gains and losses because of extra-pair fertilizations (EPF) were considered; and (b) realized reproductive success for paired males with the effect of EPF taken into account. The line on the bottom graph (b) is based on the predictions from a simple Poisson regression model with the number of offspring as dependent variable. Calculations based on mixed-effect model (male identity included as random effect) assuming Poisson distribution of dependent variable showed that only realized reproductive success of males was associated with male colouration (see the main text for further details).

(GLMM, Poisson errors; $\chi^2_1 = 0.705$, $P = 0.401$, $n = 54$ individuals and 75 observations; Fig. 3a), and the same was true for particular components of sexual ornamentation (GLMM, Poisson errors; hue: $\chi^2_1 = 0.507$, $P = 0.476$; saturation: $\chi^2_1 = 0.607$, $P = 0.436$; brightness: $\chi^2_1 = 0.013$, $P = 0.910$). However, an increased number of extra-pair mates also increased annual reproductive success of males (Spearman Rank Correlation, $r_s = 0.508$, $P < 0.0001$, $n = 75$), and standardized variance in fitness (*sensu* Arnold & Wade, 1984) was 3.36 higher for realized than for apparent reproductive success (0.234 and 0.069, respectively) in a sample of males ($n = 54$ males, 75 observations, all years combined) used in the analyses. When the analysis accounted for gains and losses because of EPF, composite colouration was a significant predictor of annual reproductive success of males [GLMM, Poisson errors; PC1: $\chi^2_1 = 8.167$, $P < 0.01$ (slope: 0.172 ± 0.061), $n = 54$ individuals and 75 observations; Fig. 3b]. Of the three components of colouration, only hue was a significant predictor of total male reproductive output

[GLMM, Poisson errors; hue: $\chi^2_1 = 7.712$, $P < 0.01$ (slope: -0.036 ± 0.014); saturation: $\chi^2_1 = 0.874$, $P = 0.350$; brightness: $\chi^2_1 = 3.133$, $P = 0.077$].

Discussion

We found a positive association between the expression of ornamental feather colouration in rosefinch males and their annual reproductive success. Similarly, colouration was a good predictor of male ability to obtain extra-pair mates. Sexual ornamentation was only important as a predictor of male fitness when gains and losses because of EPF were taken into account; that is, when we considered the realized reproductive success. The fact that apparent reproductive success was unrelated to male ornamentation indicates that drab and bright males did not differ in their ability to attract high quality (fecund) females. We have shown elsewhere (Albrecht *et al.*, 2007) that variation in fecundity of social mate (N_w) contributes some 9% to the OSS in rosefinches, but this variation is apparently not related to male characteristics we measured. The onset of breeding, which may reflect pairing date and hence male attractiveness, was also not associated with male ornamentation. The lack of relationship between male secondary ornamentation, the onset of breeding, and female quality could be the outcome of the rapid, random and synchronous pairing in this long-distance migratory passerine (Stjernberg, 1979; Björklund, 1990; Albrecht *et al.*, 2007). Female promiscuity in rosefinches might thus have evolved as a 'compensatory' mechanism through which high-quality females paired socially to inferior (drab) males engage in extra-pair copulations to adjust for their inappropriate choice of social mate. In fact, a comparison of cuckolded and cuckolding males at the same nest showed that the latter were more colourful.

The idea of EPF as the outcome of female behaviour correcting for inappropriate or hasty choice of social males has been already advocated by several studies (e.g. Weatherhead & Yezerinac, 1998), and seems to be supported by some comparative studies. For example, long distance migration may be associated with hasty and apparently inappropriate choice of social mate. Correspondingly, rates of EPF are higher in migratory than sedentary species (Spottiswoode & Møller, 2004), and the OSS because of EPF is positively associated with migration distance in north-temperate zone breeding passerines (Albrecht *et al.*, 2007). An implicit assumption in this scenario is, however, that EPF represent a female strategy, a view that is not shared by all authors (e.g. Arnqvist & Kirkpatrick, 2005; but see Griffith, 2007). Moreover, other factors, such as breeding synchrony, can independently contribute to high contribution of EPF to OSS across species (Albrecht *et al.*, 2007; Macedo *et al.*, 2008).

Male sexual ornamentation could also serve as a dominance signal in male–male competition, or may signal the intensity of mate guarding, factors that may affect the variation among males in the ability to obtain EPF or loose paternity in own nests independently of direct female preferences (Qvarnstrom, 1997). However, in house finches, pale males tend to be dominant over red males and seem to invest more in obtaining mates, yet red males have a greater ability to attract females (McGraw & Hill, 2000). Similarly, attractive males devote less time guarding their mates in bluethroats, despite being more successful in both within- and EPF than drab males (Johnsen *et al.*, 1998). This indicates that, at least in some passerines, mate guarding is not a very effective paternity-assurance strategy (Johnsen *et al.*, 2003; but see Chuang-Dobbs *et al.*, 2001), and a negative relationship between male ornamentation and the intensity of mate guarding could even be the rule (Kokko & Morrell, 2005). Ornament colouration might also reflect quality of males not directly assessed by females. For example, health and oxidative state of an individual could affect both the male ornamentation (e.g. Alonso-Alvarez *et al.*, 2004) and ejaculate quality (Tremellen, 2008). Higher fertilization success of superiorly ornamented males in the process of extra-pair-mating-induced sperm competition may thus not be under direct behavioural control of females (Pizzari *et al.*, 2008), although post-copulatory cryptic female choice (e.g. Birkhead & Møller, 1998) cannot be ruled out. Although this possibility was not evaluated in our study, both within- and extra-pair success were associated with various components of sexual ornamentation of rosefinch males.

Traits associated with life history but not sexual selection *per se* could be another important factor affecting the distribution of EPF in a population. Young males are typically more prone to cuckoldry than older males, and age is associated positively with the ability of males to obtain EPF outside their pair bonds in birds (e.g. Richardson & Burke, 1999; Bouwman *et al.*, 2007). However, age itself is unlikely to bias the relationship between male ornamentation and reproductive success reported in our study. First, we have restricted our analyses to ASY males, thus decreasing the variance in age of males in the sample. In addition, there was either no (for hue and brightness) or only moderate (for saturation) association between male ornamentation and age in a group of males sampled repeatedly over several breeding seasons. It seems that hue in particular represents only a poor indicator of male age. We therefore conclude that this parameter of male ornamentation is a good predictor of annual realized success for rosefinch males independent of age.

The variation in realized reproductive success of males is affected by at least two processes: male ability to protect paternity in his own nest, and his ability to attract extra-pair mates (Webster *et al.*, 1995, 2007). These components have either a synergistic positive effect on male fitness, or there could be a trade-off when males loose paternity in own nests when seeking for EPF

(Webster *et al.*, 1995). High contribution of EPF to the OSS is typically associated with positive covariance between within-pair paternity and EPF, and seems to indicate absolute (good genes) rather than self-referential (genetic complementarity) criteria of female extra-pair mating preferences (Mays & Hill, 2004; Pialek & Albrecht, 2005; Mays *et al.*, 2008). We have demonstrated elsewhere that variance in realized reproductive success is about three times higher than in apparent reproductive success in rosefinches, with EPF accounting for a significant portion of variance in male fitness (Albrecht *et al.*, 2007). This figure corresponds to that reported for other long-distance migratory passerines (Albrecht *et al.*, 2007). In this study, we evaluated the effect of male sexual ornamentation on basic components of variation in reproductive output in male rosefinches. First, we demonstrate that variation in male ability to sire offspring in their own nests (P_w) is explained by ornamental expression. However, sexual ornamentation was also associated with the variance among males in the number of extra-pair mates obtained (M_e). These results imply that the selection on sexual colouration in rosefinches operates simultaneously through both within- and EPF success, in line with predictions of absolute criteria of female extra-pair mating preferences (e.g. Mays & Hill, 2004). Unfortunately, we were unable to measure the effect of colouration on the contribution of within- and extra-pair paternity to the life-time reproductive success of males, a common pitfall of many similar studies (but see Webster *et al.*, 2007).

Orange to red ornamentation of cardueline finches is caused by carotenoids (e.g. Hill, 2006), and although there have been other studies suggesting an important role of EPF in the evolution of carotenoid-based ornamentation in passerines (e.g. Sundberg & Dixon, 1996; Oh & Badyaev, 2006; Reudink *et al.*, 2009; but see Hill *et al.*, 1994), our study provides to our knowledge one of the first evidence of a link between carotenoid-based colouration and various components of male extra-pair and within-pair fertilization success in this taxon. Because female birds only obtain sperm from extra-pair mates, we can eliminate some alternatives often used to explain female preferences for brightly coloured males (e.g. the 'good parent' model). Similarly, the quality of territory and/or spatial distribution of nests were unlikely to affect our results (see discussion in Albrecht *et al.*, 2007). However, whether (and how) female rosefinches benefit from preferring coloured males as extra-pair mates awaits further study. Carotenoid-based colouration honestly reflects health status and condition of the bearer in a wide array of taxa, including fish (Magurran, 2005) and birds (reviewed in Hill, 2006), therefore, females might gain both direct and indirect benefits by preferring ornamented extra-pair mates, either through decreased probability of parasite/pathogen transfer (Poiani & Wilks, 2000), or through genetic quality reflected by ornamentation that can be inherited by offspring (e.g. good immunocompetence genes are reflected by ornaments; Hamilton & Zuk, 1982). The expression of carotenoid-based ornamentation could be linked with immunocompetence genes via several pathways (e.g. Zelano & Edwards, 2002), but the evidence for any 'good genes' related to carotenoid-based colouration in birds, although substantial, is still only indirect (Hill, 2006). However, it has been unequivocally established that the expression of carotenoid-based colouration reflects the ability of an individual to cope with and liberate itself from experimentally-induced infection (Hill & Farmer, 2004); in addition, it has been suggested that the intensity of carotenoid-based colouration has a heritable component (Birkhead *et al.*, 2006).

Our measure of overall sexual ornamentation (PC1) was mainly based on hue, but was also positively correlated with saturation and brightness. It has been suggested that different colour parameters have different sensitivity to environmental stress given their different mechanisms of origin and development, and thus convey different information about the bearer (reviewed in Hill, 2006). For example, hue may be a function of the proportion of yellow xanthophylls and costly red keto-carotenoids deposited in feathers, whereas the saturation will be determined by total carotenoid concentrations (Inouye *et al.*, 2001; Andersson & Prager, 2006) and brightness reflects the structural properties of the feather surface (e.g. bacterial damage; Shawkey *et al.*, 2007). We can only speculate about the signalling function of HSB in rosefinches. However, it seems that hue of the ornament (redness) is a parameter extremely sensitive to condition and health status in related house finches (see Hill, 2002 for review). Hence, hue (perhaps better than other colour components) reflects either the indirect qualities males provide via EPF to females, or at least mirror variance in the general vigour of male rosefinches. Although hue was the most important predictor of male fertilization success in rosefinches, it is worth noting that compared with saturation and brightness, our photography-based estimates of hue were the least different from measurements taken by a spectrometer (also Hill, 1998). This may indicate that there was some variation among individuals in saturation and brightness that was not measurable using simple digital photographs. In any case, the brightness we measured was still a good predictor of male abilities to obtain an extra-pair mate, independent of hue.

In summary, our study demonstrates the propensity of brightly coloured males to gain both high within-pair paternity and EPF at the expense of drab males, resulting in a link between ornamental colouration of males and their total annual reproductive success. As our study is correlative, we cannot distinguish between alternative explanations of the observed pattern and to fully exclude the possibility that ornamentation and fertilization success of males may be correlated with a third, unmeasured variable, such as overall male competitive abilities. Most importantly, the interpretation of the results is based on the understanding of the adaptive value of extra-pair behaviour for female birds (e.g. Westneat & Stewart, 2003; Arnqvist & Kirkpatrick, 2005; Albrecht et al., 2006). In any case, we show that EPF may significantly contribute to the evolution and/or maintenance of sexual ornamentation in rosefinches, and conclude that EPF not only represent an important process contributing to the increased OSS, but also promote the evolution of male secondary traits in socially monogamous systems, and at least in some passerine species.

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III.

Occurence of extra-pair paternity is connected to social male´s MHC-variability in the scarlet rosefinch *Carpodacus erythrinus*

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Genes of the Major Histocompatibility Complex (MHC) represent an essential component of the vertebrate acquired immune system. In the last decades, the role of MHC genes in mate choice has been subject of particular scientific interest. However, results of studies dealing with this topic in different species are equivocal and mechanisms conducting MHC-based mate choice are still puzzling. We investigated the impact of MHC class I variability on within-pair and extra-pair fertilisation success in a wild population of a socially monogamous passerine bird with considerable rates of extra-pair paternity, the scarlet rosefinch *Carpodacus erythrinus.* We found some support for the 'good-genes-as-heterozygosity model', as social males of high MHC-heterozygosity were cheated by their females less frequently than less MHC-heterozygous males. However, cuckolding males were not more MHC-heterozygous than the cheated social males, nor were extra-pair young more MHC-heterozygous than within-pair young. We did not find any evidence for mating preferences according to the complementarity model.

In recent years, the tools for studying genetic aspects of mate choice have improved significantly (Mays and Hill 2004). It has been proved that females may optimize their choice not only by choosing males exhibiting the most elaborate ornaments (the 'good genes' model, Mays and Hill 2004) but also according to their own genotype by disassortative mating, which is referred to as the genetic complementarity model (also known as compatibility, see e.g. Pialek and Albrecht 2005, Mays et al. 2008). According to the 'good genes' model, males with certain phenotypic traits should be generally preferred by females in a population as they might confer advantageous alleles increasing offspring quality (e.g. Iwasa et al. 1991), assuming that there is additive genetic variation in fitness. According to the complementarity model female preferences depend on their own genotype and they aim at the best possible combination of maternal and paternal genes to create optimal offspring genomes gaining non-additive genetic benefits for their progeny (reviewed in Hettyey et al. 2010). However, evidence shows that in some species the pattern is not unequivocal, but mate choice might be a complex of both of these (Roberts and Gosling 2003). As reviewed in Hettyey et al. (2010) there is a lack of studies examining mate choice (mainly extra-pair mating) in the frame of both 'good genes' and 'complementarity' models in relation to particular genes.

Via their choosiness females may obtain direct benefits, e.g. male's territory, nuptial food gifts or male's ability to fertilize ova, or indirect benefits, i.e. genes that confer increased offspring viability (Andersson 1994). In some mating systems, for example in lekking birds or in socially monogamous avian species where females engage in extra-pair copulations (EPC), exclusively indirect benefits are obtained by females since all that males or extra-pair males, respectively, contribute to the offspring are genes (reviewed in Griffith et al. 2002).

From the perspective of evolutionary biology, extra-pair mating offers a particularly useful model for the investigation of the indirect benefits rising from mating with males differing in quality. Extra-pair mating has been recorded in approximately 90% of avian species and if we take into account only the socially monogamous species (in which extra-pair paternity [EPP] is twice as common as in the polygynous species, Hasselquist and Sherman 2001) then the level of EPP is estimated on average to 11% of offspring and approximately 19% of broods (Griffith et al. 2002). Thus far, we do not understand EPPs enough to fully comprehend the EPC behaviour. For instance, as proposed by Arnqvist and Kirkpatrick (2005) EPCs may represent a solely male offensive strategy bringing no sufficient benefits to females. However, this conclusion was exposed to severe criticism (Griffith 2007), and as there is evidence suggesting that females may directly search for EPCs and initiate them (Kempenaers et al. 1992, Birkhead and Møller 1993, Strohbach et al. 1998, Bouwman et al. 2006, Dunn and Whittingham 2007), the question concerning female benefits from EPFs remain unresolved. In socially monogamous species EPCs might be the only way how females could get offspring with chosen males when these are already paired (Westneat et al. 1990, Birkhead and Møller 1993) or when their previous social-partner choice showed to be inappropriate (reviewed in Jennions and Petrie 2000), but we clearly need more evidence concerning these potential indirect benefits of extra-pair fertilisations (EPFs) to females.

Genes of the major histocompatibility complex (MHC) play a crucial role in the vertebrate acquired immunity (Klein 1986). They encode glycoproteins which bind antigen peptides and present them on cell surfaces to T cells. If the antigen peptide is recognised by a T cell, an immune response is triggered (Abbas et al. 1994). The MHC genes are under strong positive selection, acting mainly on amino-acid sites involved in antigen binding (peptide-binding region). In the last decades they have been subject of particular interest as they were shown to influence mate choice in several non-model species of mammals (Schwensow et al. 2008), fish (Eizaguirre et al. 2009), amphibians (Bos et al. 2009), reptiles (Miller et al. 2009), and also in birds (Richardson et al. 2005, Bonneaud et al. 2006).

Evidence shows that resistance to a specific parasite is ensured mostly by one or only few MHC alleles (e.g. Bonneaud et al. 2005, Loiseau et al. 2008, Mankowski et al. 2008, Fraser and Neff 2010; reviewed in Jeffery and Bangham 2000). Overdominance hypothesis (reviewed in Piertney and Oliver 2006) assumes that the more MHC alleles an individual has, the higher should be its resistance to a wide spectrum of pathogens. It might be difficult to distinguish which alleles are advantageous, therefore it has been suggested that as ‘the best of a bad job’ it might be convenient for individuals to mate with MHC-dissimilar mates to produce the most MHC-heterozygous offspring (Milinski 2006, reviewed in Piertney and Oliver 2006). Also mating with highly MHC-heterozygous mates might be beneficial, because it has been shown that highly MHC-heterozygous parents produce highly MHC-diverse young (Bonneaud et al. 2006). On the contrary, theoretical models (Nowak et al. 1992) suggest that too high heterozygosity on MHC might be disadvantageous. This is because of increased loss of T-cell variability due to negative selection of autoreactive T-cell clones in thymus. When there are too many MHC molecules, too many peptide variants are generated from self proteins leaving less peptide variants to be recognised as non-self by the T cells. Therefore it was suggested that an individual should possess an optimal rather than maximal number of MHC alleles (Milinski 2006, Woelfing et al. 2009). This was evidenced by some experimental studies (Hill et al. 1991, Ilmonen et al. 2007, Bos et al. 2009). If individuals optimize mate-choice to achieve an optimal level of MHC-heterozygosity, rather than maximal, then mates of an intermediate level of MHC-dissimilarity should be preferred (Milinski 2006, Eizaguirre et al. 2009). Here, we evaluate the hypotheses of ‘good genes as heterozygosity’ and complementarity in a mating system of social monogamy with considerable rates of extra-pair fertilisations, studying MHC class I diversity. We examined the variation of exon 3, which encodes parts of the peptide-binding region. Our model species, the Scarlet rosefinch, is a sexually dichromatic long-distance migratory passerine with delayed plumage maturation and high levels of plumage ornament variability in males (Stjernberg 1979). It is a socially monogamous species with moderate rates of extra-pair paternity (almost 40% of nests contained extra-pair young; Albrecht et al. 2007). It breeds once a year and the breeding season is extremely short (Björklund 1990). Females build social pairs with males immediately after arrival on the breeding site, but later some of them have young also with males outside the pair-bond (Albrecht et al. 2007).

Materials and methods

Study population and field procedures

We studied a population of Scarlet rosefinches nesting in the Šumava Mountains National Park, Czech Republic (48°49′ N, 13°56′ E, ~750 m a.s.l.). A detailed description of the study site and field procedures is given in Albrecht et al. (2007). The dataset included samples of 614 individuals (108 nests, 70 females, 91 males and 453 nestlings) collected during breeding seasons of 2000–2008. Adult birds were captured upon their arrival, weighed and their tarsus length was measured. Males in their 3rd year or older were photographed for colour analysis of the breast ornament, which is a secondary sexual trait in this species: hue, saturation and brightness (HSB colour space) were measured (for a detailed description of ornament analysis see Albrecht et al. 2009). In all adults and 7 day old chicks a blood sample (20–30 μl) was collected and stored in 96% ethanol at −20° C until DNA extraction.

Genetic analyses

Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to manufacturer’s instructions. A previous study on the structure and diversity of MHC in the Scarlet rosefinch revealed that there are 82 MHC class I variants (hereafter called ‘alleles’ for simplicity) in this population, with individuals displaying between three to nine alleles (mean: males = 5.15 ± 0.12 (SE), females = 5.1 ± 0.12 (SE)). Most of the adult birds displayed unique MHC class I genotypes (Promerova et al. 2009). We used single-strand conformation polymorphism (SSCP) analysis in a capillary to assess allelic diversity of the exon 3 region in MHC class I genes, which encodes parts of the peptide-binding region of the protein (for more details see Promerova et al. 2009). Alleles were visualized using GeneMapper v3.7 (Applied Biosystems). To assess paternity, we genotyped all individuals at 15 polymorphic microsatellite loci (amplification conditions used in this study are described in Poláková et al. (2007), for more details on parentage analysis see Albrecht et al. 2009). The genotypes were analysed using GeneMapper v3.7 (Applied Biosystems). The same panel of microsatellites and two additional loci were used for calculating Internal relatedness (IR; Amos et al. 2001) and standardized heterozygosity (Het_{ST}; Coltman et al. 1999) using 'IR macroN3' (<www.zoo.cam.ac.uk/zoostaff/amos>).

Statistical analyses

Pairs were considered social if the mates took care of the young together (feeding, nest defence). Extra-pair (EP) were considered males with which females had young outside the pair-bond, and these males apparently did not contribute to parental care. Generalized linear mixed models (GLMM; R 2.8.1 <http://www.r-project.org/>) were used to assess the effect of allelic diversity (number of identified MHC class I alleles) of fathers on the occurrence of extra-pair paternity (EPP), since several males were sampled repeatedly over years. To find out whether females can gain indirect benefits from EP matings via increasing offspring MHC variability, the number of alleles per chick in within-pair (WPO) and extra-pair offspring (EPO) was compared, using nest identity as a random effect in the analysis (GLMM; R 2.8.1). We also tested if females might increase the number of different alleles in their broods via EPC, by comparing the overall number of different MHC class I alleles for the whole broods with and without EPP. To account for pseudoreplication arising from repeated inclusion of particular females in successive breeding seasons, female identity was included as a random effect in the analysis (GLMM; R 2.8.1).

MHC-similarity between males and females was calculated as MHC allele-sharing: the proportion of allele-sharing in a pair is twice the sum of alleles the individuals share divided by the sum of alleles of both individuals – ($D = 2F_{ab}/(F_a + F_b)$; Wetton et al. 1987). We tested whether females were more dissimilar in MHC from EP-males than from their social mates by comparing MHC allele-sharing between social and extra-pair mates, respectively (t-test, STATISTICA 6.0).

We tested for correlation between individual standardized heterozygosity on microsatellites (using both IR and Het_{ST}) and number of MHC alleles using Spearman's correlation test (STATISTICA 6.0).

Results

Effect of MHC variation on extra-pair mating

EPO were found in 37.8% of nests. We found significant negative effect of the number of MHC alleles in the social male on the occurrence of EPP in his own nest ($N = 104$ nests, GLMM, $\chi^2 = 7.3$, $DF = 1$, $p \leq 0.01$; slope: -0.573 ± 0.216 (SE); Fig. 1) independent of female MHC variability (GLMM, female MHC: $\chi^2 = 0.47$, $DF = 1$, $p = 0.49$; slope: 0.164 ± 0.230 (SE), interaction between M- and F-MHC: $\chi^2 = 1.19$, $DF = 1$, $p = 0.28$; slope: -0.210 ± 0.200 (SE); random factor = male identity). However, the cuckolded social male was not less MHC heterozygous than the male that had cuckolded on him (paired *t*-test: $p = 0.29$, $n = 36$ male couples). We compared MHC allele-sharing between females and their social and EP mates, respectively. There was no evidence supporting the idea of higher MHC dissimilarity of EP than social males ($p = 0.6$, $n = 34$ pairs of mating events). There was no correlation between MHC similarity of social pairs and occurrence of EPP (Spearman; $p = 0.8$, $n = 104$ mating events).

Finally we compared the number of MHC class I alleles of EPO and WPO for each nest with mixed paternity, and we found no difference in allelic diversity between the nestlings (GLMM, $p = 0.8$, $\chi^2 = 0.064$, $DF = 1$, slope: -0.021 ± 0.082; random factor = brood identity). To address the possibility that females increase the total number of MHC class I allels in their broods via EPP, we compared the number of alleles in nests containing EPO with nests without EPO in an analysis assuming Poisson distribution of the dependent variable (total number of different alleles). However, in a model containing brood size, total number of alleles in social partners and nest type (containing or not containing EPO) as explanatory variables, the latter was a poor predictor of the number of different alleles in broods ($n = 102$ nests, GLMM, effect of EPO occurrence in nest: $\chi^2 = 2.329$, $p = 0.127$, slope: 0.118 ± 0.078; effect of brood size: $\chi^2 = 2.736$, $p = 0.098$, slope: 0.056 ± 0.034; effect of the number of parental alleles: $\chi^2 = 14.234$, $p < 0.001$, slope: 0.099 ± 0.026).

Association of MHC diversity and phenotypic traits

We analysed whether some of the measured phenotypic traits could be affected by the number of MHC class I alleles to reflect male genotype. However, we failed to show any relation between individual MHC diversity and body weight (Spearman; F: $p = 0.4$, $n = 66$; M: $p = 0.16$, $n = 91$) or tarsus length (Spearman; F: $p = 0.7$, $n = 65$; M: $p = 0.09$, $n = 88$), neither in males nor in females. Moreover, there was no association between the number of MHC alleles and the level of expression of the carotenoid-based feather ornamentation in males (Spearman; hue: $p = 0.3$, saturation: $p = 0.6$, brightness: $p = 0.12$; $n = 91$).

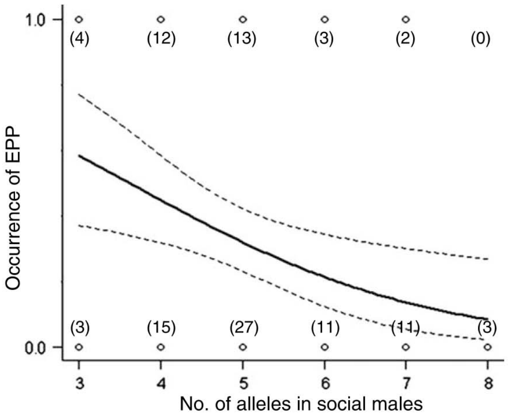

Figure 1. Predicted effect of MHC class I allelic diversity of social males on the occurrence of extra-pair paternity (EPP) in their nests. Counts of observations per each category of MHC class I allele numbers are given in parentheses. Dashed lines represent 95% CI. The lines are based on GLM, with occurrence of EPP in nests treated as binary variable (0 – no EPP detected, 1 – at lest one extra-pair offspring detected).

Comparing overall heterozygosity and MHC diversity

To exclude the probability that the individual MHC variability could be only a reflection of the overall heterozygosity, we tested if the number of MHC class I alleles in an individual is correlated with the individual's IR or Het_{ST}. We found no such pattern in the scarlet rosefinches (Spearman; IR: $p = 0.83$, $n = 614$; Het_{ST}: $p = 0.86$, $n = 614$).

Discussion

We tested for female extra-pair mate choice mechanisms in a socially monogamous songbird with biparental care. We found evidence that more MHC heterozygous males lose paternity in their own nest less frequently than males with low MHC variability. However, EP males were not more MHC-heterozygous than cuckolded social males, and EPO were not more MHC-heterozygous than WPO. Neither did the broods with EPO contain a higher number of different alleles than broods of entirely within-pair chicks. There was no support found for the complementarity hypothesis, but this might be partly due to the extreme diversity of MHC class I in this species and the impossibility of obtaining nucleotide sequences for all alleles. We think there is quite a high probability that some alleles are more similar in sequence to each other than others, thus testing of the complementarity hypothesis would be more robust with the data on sequences.

Hence, at least based on MHC class I variability there is no evidence for females gaining any indirect advantage from mating outside the pair-bond in the scarlet rosefinch. We also tested if these results might not only reflect a preference for overall heterozygosity, but the individual MHC-variability is not correlated to genome-wide heterozygosity in our dataset.

Our findings imply that the more MHC class I alleles a male has, the higher is the probability of protecting paternity in his own nest. Males which obtained extra-pair copulations were not more MHC-heterozygous than the males they cuckolded on, thus we suppose that females are unable to directly discriminate for more MHC-heterozygous males; they only seem to remain faithful to males with high MHC diversity. This might be due to better mate-guarding in the more MHC-heterozygous males (Zelano and Edwards 2002), but we cannot exclude the posibility that sperm-sperm and sperm-ova interactions contribute to the observed pattern. In any case, our study provides one of the first evidences for the effect of number of MHC class I alleles on within-pair fertilization success of males in birds.

In songbirds, there are so far only few studies reporting the impact of MHC genes on mating (Freeman-Gallant et al. 2003, Westerdahl 2004, Richardson et al. 2005, Bonneaud et al. 2006) and the results are largely equivocal. Moreover, only two of them focus on extra-pair mating. In their study in Seychelles warblers *Acrocephalus sechellensis.* Richardson et al. (2005) showed that EPP occurred when the social male was of low MHC diversity. However, unlike in the Scarlet rosefinches, in the Seychelles warblers the MHC diversity of the EP male was significantly higher than that of the cuckolded social male indicating a female preference for high MHC-heterozygosity. In Savannah sparrows *Passerculus sandwichensis*, Freeman-Gallant et al. (2003) found that yearling females (but not older) were more likely to obtain EPP if mated to a male with similar MHC to their own. Similar studies were conducted also in mammals. For example in the socially monogamous fat-tailed dwarf lemur *Cheirogaleus medius* females engaging in extra-pair copulations shared more MHC supertypes (allelic lineages grouped by functionality) with their social males than faithful females (Schwensow et al. 2008). In this study, nevertheless there was also evidence for 'good-genes-as-heterozygosity' hypothesis predicting mate choice in general for both social and extra-pair males, as the genetic fathers of offspring had more MHC supertypes than randomly chosen males (Schwensow et al. 2008).

The polygamous mating system precludes existence of any extra-pair copulations per se. Despite different scheme of pair forming and successive parental care the evidence obtained in polygamous fish may help to investigate female strategy concerning MHC variability in multiple mating. In the three-spined sticklebacks *Gasterosteus aculeatus* Eizaguirre et al. (2009) found that females preferentially mated with males with whom they shared an intermediate level of MHC diversity to produce offspring with optimal MHC heterozygosity (see also Milinski et al. 2005). At the same time males with certain MHC haplotypes ensuring resistance against common parasites were preferred (Eizaguirre et al. 2009).

Females may assess the genetic quality of males according to different cues. In mammals or fish, for instance, MHC has been shown to affect odour (Singh et al 1987, reviewed in Penn and Potts 1998), and through odour also sexual selection (in humans, Thornhill et al. 2003; mice, Penn and Potts 1998; fish, Milinski et al. 2010; lizards, Olsson et al. 2003). However, birds are presumed to rely more on visual cues and hearing than on olfaction (Roper 1999, but see also Balthazart and Taziaux 2009), although their olfactory receptors seem to be similar as in other vertebrates (Steiger et al. 2008). Alhough the relationship between condition-associated phenotypic traits and certain MHC genotype has been found in birds (von Schantz et al. 1997, Ekblom et al. 2004, Hale et al. 2009), in the Scarlet rosefinch we failed to find any correlation between the number of MHC class I alleles and condition-dependent traits such as body mass and tarsus length or expression of a secondary sexual ornamentation in males. This is despite the fact that the carotenoid-based feather ornament has already been proved to govern reproductive success in this species (Albrecht et al. 2009).

The molecular methods we used in this study are routinely used for analysing MHC in non-model species (e.g. Binz et al. 2001, Bryja et al. 2005, Alcaide et al. 2010, Baratti et al. 2010). When using the method of CE-SSCP, although the outcome is reliable genotyping, the particular nucleotide sequences remain unknown. Nevertheless, considering the impact of MHC on mate choice and phenotypic traits, it is possible that not only the number of alleles and identity of alleles is important, but also the actual nucleotide sequences and hence the structural differences among alleles. To conclude, in the future, new methods like 'next generation sequencing', which produce huge sets of sequence data (Babik et al. 2009) might elaborate our understanding of the role of MHC in mate choice.

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IV.

Male ornamentation and within-pair paternity do not predict offspring provisioning rates in Scarlet Rosefinches

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Male ornamentation and within-pair paternity do not predict offspring provisioning rates in Scarlet Rosefinches

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Abstrakt

Offspring provisioning by male is typical in socially monogamous passerines. As proposed by the good Good parent model of evolution of secondary male ornamentation, secondary ornaments may signalize male provisioning rates and therefore the direct benefits to females. On the other hand, the intensity of male parental care can be affected by ocurence of extrapair young ones in its nest. According to the Parental investment theory, males that lost paternity in their nests should reduce their parental care. In this study we evaluate potential relationships between the intensity of parental care, male ornamentation, the occurrence of extra-pair paternity and male extra-pair fertilization success in the Scarlet Rosefinch *Carpodacus erythrinus*. Our results indicate no effect of paternity loss on the rate of food provisioning to young in Scarlet Rosefinches. Simultaneously, we found no evidence for a linkage between male ornamentation and male feeding rates. The only male trait associated

with feeding was its ability to sire extra-pair offspring, indicating no evidence for a trade-off between investments into parental care and extra-pair mating in this species.

Introduction

Social monogamy, closely associated with bi-parental care, is the most frequent pairing system found in birds (Lack 1968). In general, parental care bears certain costs, since this investment may reduce survival of parents (Lessels 1991) and limit future reproductive success (Gustafsson a Sutherland 1988) or affect ability to gain another sexual partner. However, the intensity of parental care definitely affects offspring fitness and survival (Williams 1966, Lee et. all 2010). Parental investment is therefore a compromise between investments into the current reproductive attempt (in terms of improved quality of offspring) and future reproduction (Nur1984, Houston 2005).

Extra-pair paternity (EPP), resulting from female copulations with other than social mates, leads to the occurrence of extra-pair offspring (EPO) in nests. Extra-pair matings are widespread in socially monogamous passerines (Griffith et al. 2002, Westneat and Stewart 2003). Whereas (EPO) are genetically related to mother, they are not related to its social mate. According to parental investment theory, those males that lost paternity in their nests should invest less into the current reproductive attempt, since investment in non-related offspring will not lead to increase in their fitness and costs associated with investment will reduce the probability of their future reproduction (Trivers 1972). However this mechanism requires males to be able to assess the levels of female promiscuity. Although the ability to respond behaviorally to reduced paternity has been confirmed in some passerine species (e.g., Ewen 2000, Møller 2000), where males tend to reduce parental care in response to female infidelity, this reduction parental investments in response to paternity los is weak or has not been observed in others (see Arnqvist and Kirkpatrick 2005 for a review). There seems to be variation in response to reduced paternity even between populations of the same species. For example, in highly promiscuous Reed Bunting, no reduction of male parental care in response to the occurrence of EPP was found in one study (Bouwman et al. 2005) but was demonstrated in another one (Dixon et al. 1994). By reducing parental care, social male may also reduce its own fitness. Strong reductions of parental care cannot be expected in situation when the assessment of the level of social mate's promiscuity is difficult and nests contain unknown proportion of male's and extra-pair offspring (Kokko 1998). The ability of males to detect the occurrence of extra-pair offspring in their own nests is still a matter of discussion (Peterson 2001).

In promiscuous systems, where males may increase their own fitness by siring offspring outside their pair bonds, the male ability to obtain extra-pair mate may affect its investments into the parental care. If males trade parental care against seeking for extra-pair copulations, a negative relationship between male phenotypic quality and parental care can be expected (Magrath 2003, Houston et al. 2005). It follows that females mated to attractive social partner may invest more into parental care to compensate for the lack of investment of their social mate (Witte 1995, Burley 1986).

Regarding the mate choice male ability to invest into parental care may represent an important trait affecting female mating preferences. As proposed by the Good parent model of evolution of secondary male ornamentation, secondary ornaments signalize direct benefits to females (Hoelzer 1989, Heywood 1989, Préault 2005). By pairing with good-parent males, females increase their own fitness in terms of better offspring quality and simultaneously save energy for future reproductive attempts. Genetic quality of a male may affect both male ornamentation and parental investments (Zelano and Edwards 2002). Females may thus prefer ornamented males as social mates to obtain both direct and indirect (genetic) benefits (Sundberg J. et al. 1994, Senar JC et al. 2002, Préault M. et al. 2005)

The expression of secondary male ornamentation is, however, costly (Bradbury and Anderson 1987) and a trade-off between male investments into secondary male ornamentation and parental care may appear, as proposed in differential allocation model (Burley 1986, Kokko 1998). The good parent and differential allocation models thus differ in the expected direction of association between male ornamentation and parental care, with the positive relationship being expected in the former, and a negative one being expected in the latter (Kokko 1998, Kelly 2009). Based on the different allocation model, females may even prefer drab males as mates, if low expression of ornamentation reflects male parental investments and when male parental care is important (Kikpatrick et al. 1990).

The flexibility of adopted reproductive strategies can be demonstrated in House finches, socially monogamous bird in which males express variable, carotenoid-based ornamental feathers on breast (Hill 2002). While in some studies and some populations a positive relationship between male ornamentation and parental care has been demonstrated (Hill 1990), other studies found an opposite pattern (Duckworth 2003). In this study we evaluate potential relationships between the intensity of parental care measured as a feeding effort devoted to 6-9-days-old offspring and (1) male ornamentation, (2) the occurrence of EPP and (3) male extra-pair fertilization success in the Scarlet Rosefinch *Carpodacus erythrinus*. This species is characterized by moderate levels of EPPs that significantly contribute to variation in male fitness (Albrecht et al. 2007). Moreover, male rosefinches express carotenoid based ornamentation, associated with within- and extra-pair fertilization success (Albrecht et al. 2009) and male condition (Vinkler, unpublished data).

METHODS

General procedures

The study was conducted in 8 breeding seasons, from 2001-2008 in the Vltava river valley, Šumava Mountains National Park, Czech Republic (48_49#N, 13_56#E). A detailed description of the study site is given in Albrecht et al. (2004).

Adult birds were captured upon their arrival or during breeding season. Each adult was ringed with one aluminium ring (N MUSEUM PRAHA) and with an individual combination of 1–2 coloured plastic rings (AVINET).

Males in their 3rd year or older were photographed for colour analysis of the ornamental patches. All photographs were taken in standart condition in the dark room of a nearby field station. Grey card and colour & grey chart were used to standardize measurements. Photographs were analysed using ADOBE PHOTOSHOP software (Adobe Systems Inc., San Jose, California). Hue, saturation and brightness (HSB colour space) were measured (for a detailed description of ornament analysis see Albrecht et al. 2009). Previous analyses indicated good correlation between measures taken from photographs and with the use of AVANTES spectrophotometer (Albrecht et al. 2009).

The study site was searched systematically several times over each breeding season where there is vegetation suitable for nesting (mainly Spiraea bushes) so that nearly all nests were found during the egg-laying or incubation stages. Nests were visited regularly during the field season to estimate the exact day of egg hatching.

Identification of parentage

Males observed repeatedly feeding nestlings, were considered the social fathers of young in a nest. For the parentage analysis in all adults and 7-day old nestlings a blood sample (20-40 µl) was collected and stored in 96% ethanol at – 20 °C until DNA extraction. The analysis was conducted using 17 highly polymorphic microsatellite loci (for a detailed description of the parentage analysis see Polákova et al. 2007, Albrecht et al. 2009).

Parental food-provisioning care

Rate of food provisioning to young was used as a measure of male and female parental care. Observations were made when nestlings were 6 – 9 days old. The rate was recorded using micro-cameras positioned in a close proximity of a nest, connected with hidden video recorder located ca 5-10 m from the nest. The cameras did not appear to affect parental behaviour, females typically returned to nests in few minutes after the installation the cameras. Seven hours of a continuous record were analysed for each nest. Following paramteres were later obtained for both the feeding male and female at each nest: total number of visits to the nest, feeding frequency per hour and interval of provisioning (mean interval between two feeding visits to the nest for male of female). However, since these parameters used to describe feeding effort were highly intercorrelated ($r = 0.749$, $r = -0.586$, $r = -0.691$ for males and $r = 0.828$, $r = -0.506$, $r = -0.754$ for females respectively, $p << 0.001$ in all cases), and results remained qualitatively and quantitatively unchanged irrespective of the parameter used, later we used in analyses only the number of feeding per hour (MFED for male, FFED for female) as a measurement of the parental feeding effort.

Statistical analyses

Male and female feeding per hour were normally distributed. Since several males were followed repeatedly over several years, general linear mixed effect models were applied to find variables associated with MFED. In the analysis, ornament saturation, brightness, and hue, together with FFED, number of young in nest, timing of breeding, the proportion of extra-pair young in nest and male ability to engage in extra-pair fertilizations were used as explanatory variables and male identity (n = 38 males with a total of 50 observed nests) as random effect. Minimal adequate modelss (MAM, sensu Crawley 2007), i.e. models with all terms significant, were selected based on backward elimination of full model containing main effects, comparing changes of deviance of the model with the term of interest included and the model where the term was removed. The significance of a particular term in models was based on the change in deviance between the full and reduced ⁄ null models, distributed as χ^2 with degrees of freedom equal to the difference in the degrees of freedom between the models with and without the term in question (Faraway, 2006). All analyses were performed with R 2.9.2.

RESULTS

Male fed 0.77 times/hour, while female fed 0.91 times/hour (n=50). Over the 2001-2008 study period, 50 nests were observed. Extra-pair offspring occurred in 21 cases (42 % of evaluated nests), 45 EPO of total 226 youngs were sired. In general, nest with EPO contained between 1 and 5 EPO (25 - 100% of young in the nest). Of variables considered as predictors for MFED, only male ability to gain extra-pair offspring was associated with male feeding rates (Table 1) and was thus the only variable in the MAM. The MAM was significantly different from the null model ($\chi 2 = 6.64$, $\Delta Df = 1$, $P = 0.010$). The model indicated that males able to gain EPP fed the offspring with higher intensity than males not able to gain EPPs (Fig

2). Interestingly, neither the proportion of EPP in nest nor male ornamentation traits was associated with MFED (Table 1). Although no association between the expression of male ornamentation and MFED was found, males able to obtain EPP had lower brightness than other males, but were similar in hue and saturation of ornamental feathers (general linear models with binomial error term and male ability to get EPP as binary dependent variable; hue: $\chi 2 = 0.003$, $\Delta Df = 1$, $P = 0.96$; saturation: $\chi 2 = 0.526$, $\Delta Df = 1$, $P = 0.468$; brightness: $\chi 2 = 8.129$, $\Delta Df = 1$, $P = 0.004$, Fig ..B).

Table 1. Significance of variables used to explain male provisioning per hour (MFED) in Scarlet Rosefinches, based on general linear mixed effect models with male identity threated as a random effect. Estimates are based on Type I Sum of squares, i.e. by comparing the model involving the term of interest with the null model. The minimum adequate model (MAM, see the main text) only involved male ability to obtain extra-pair fertilizations.

	Estimate	Std. Error	Chi	P
female provisioning per hour	0.205	0.167	1.503	0.220
rate of EP young/ WP young	0.002	1.144	0.002	0.960
EP young in own nest	0.031	0.085	0.130	0.710
male ability to gain EPP	0.340	0.130	6.640	0.009
onset of breeding	0.002	0.010	0.040	0.842
hue	0.011	0.012	0.925	0.336
saturation	-0.001	0.004	0.015	0.902
brightnes	-0.003	0.003	1.013	0.314

Fig. 1 Male provisionig per hour in relation to the presence of extrapair offspring in their broods.

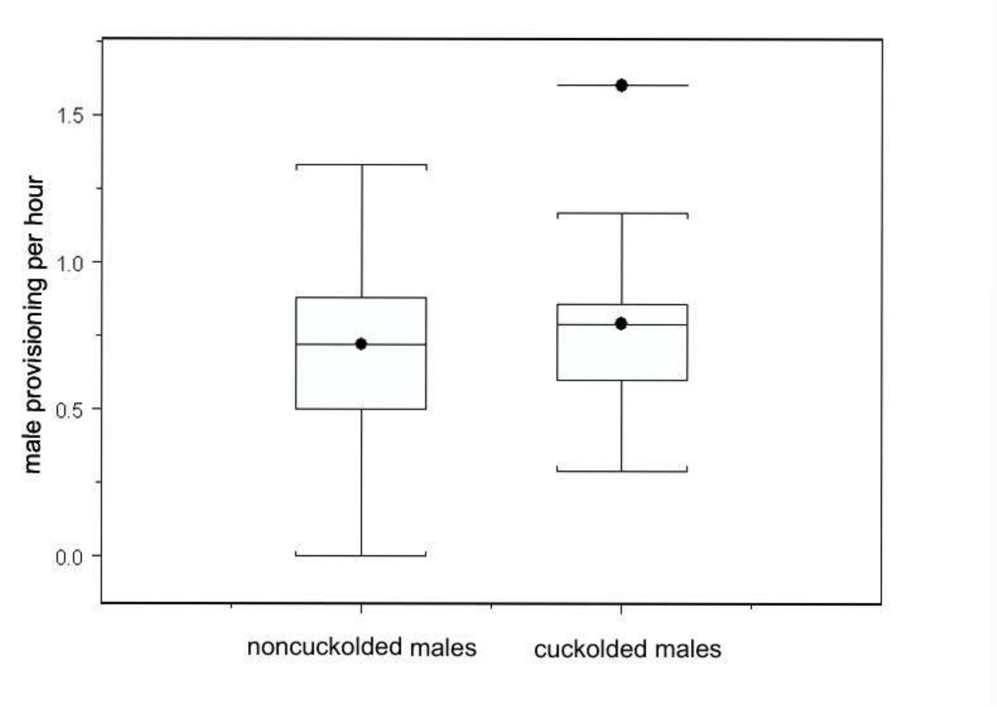
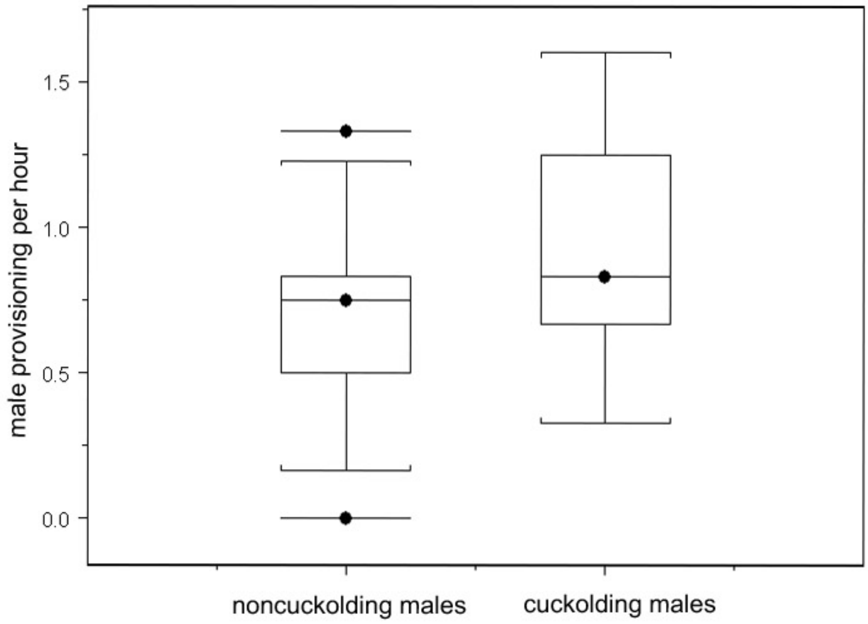


Fig. 2 The differnece in male provisionig per hour between males that achieve EPP and those that did not sired any extra-pair offspring.



DISCUSSION

Our study indicates no effect of paternity loss on provisioning rates in Scarlet Rosefinches. Simultaneously, we found no evidence for any linkage between male ornamentation and male feeding rates. Surprisingly, the only male trait associated with feeding was the ability of a male to sire extra-pair offspring.

Based on prediction from parental investment theory, cheated males should reduce offspring provisioning in response to female infidelity (Trivers 1972). However, this change of behavior requires that several criteria are met. First, males must able to recognize female cheating (Peterson 2001). While male ability to recognize female infidelity has been demonstrated in some passerines (e.g., Ewen 2000), and reduction in male provisioning rates observed in response to female cheating (reviewed in Arnqvist and Kirkpatrick 2005), other studies have failed to find any evidence for this behavioural response from males (Kempenaers 1998, Vigon 2009) despite suggested strong selection in terms of frequent occurrence of extra-pair young in nests (Bouwman et al. 2005). Mechanism of male inspection of female fidelity may involve mate guarding (Birkhead 1982), and male rosefinches tend to intensively guard their mates during the period of egg-laying (Bjorklund 1990, Schnitzer, unpubl). It has been observed that males in some species punish females for their short term absence in the territory (Mota and Hoi - Leitner 2003) or reduce feeding in response to female removal during the fertile period of egg-laying (Møller and Birkhead 1993). However, this experimental reduction of male control over female behaviour during the critical period of egg-laying has resulted in no reduction in male feeding in other cases (Kempenaers 1998). Despite intense mate guarding, rosefinch males may not be able to assess female promiscuity accurately (also see Kokko and Morrell 2005). Second, female extra-pair forays should result in the occurrence of EPP, otherwise reduction in parental care affects offspring sired by a social male. This may not be the case in all occasions (Birkhead 2009) and males than risk reduction of own reproductive success by not investing into their own offspring. Third, investment into feeding must be costly for male to respond strongly to the perceived risk of cuckolding by reductions in offspring feeding (Møller and Birkhead 1993). In the case of rosefinches, species holding no territories and feeding offspring with a mixture of seeds and small passively collected arthropods (Stjernberg 1979, Albrecht, unpublished data) that are abound around the nests, the cost of feeding unrelated offspring might be relatively low compared to potential cost of reducing feeding in response to inapropriately assessed paternity.

Apparently, species tend vary in male responses to female cheating in terms of reduced parental investment, and this seems to be associated with species-specific levels of promiscuity (Albrecht et al. 2006). From this perspective, relatively high levels of extra-pair paternity found in rosefinches (Albrecht et al. 2007, 2009) accompanied with the lack of response to the loss of paternity from males fits well into this trend. Our study was only correlative, since it was impossible to follow individual males with the same partner over more than one breeding attempt (e.g., Bouwman et al. 2005, Dixon et al. 1994, Moller and Cuervo 2000). However, our analyses involved repeated observations of individual males with different females, suggesting no change in provisioning rates of males with paternity loss.

Our data suggest that male ornamentation may be not used by females as a signal of male parental abilities. This is in line with the observations in House finches, where carotenoid-based male ornamentation was not associated with provisioning rate at least in one population, but contradicts finding from another populations of the same species (Hill 1990, 2002, Duckworth 2003). Based on the results, we conclude that the ‘good parent’ model of evolution of secondary male ornamentation does not apply to rosefinches. In fact, as suggested previously, social pairing is rapid in this long-distance migratory passerine (Björklund 1990) and random with respect to many male attributes, including song rate and plumage colouration. Since colouration is not associated with male parental care, we suspect that male colouration may signalize other benefits to females. These may include indirect benefits, such as higher genetic quality of offspring (Fossøy et al. 2006, Foerster et al. 2003). We have indeed shown that the male ability to obtain extrapair mates is associated with plumage hue and brightness (Albrecht et al. 2009), and results from a restricted dataset used in this study partly corroborates our previous findings since male brightness was positively associated with male ability to obtain extra-pair mates in this study. Plumage colouration was associated with male fertilization success, but this was only due to extra-pair fertilization – apparent reproductive success and the number of young found in nests, were not associated with any male colour attribute (Albrecht et al. 2009). Male colouration thus determines male extra-pair attractiveness, but not its parental investments or female reproductive investemets in terms of clutch size.

The only male trait associated with feeding was its ability to obtain extra-pair offspring. Contrary to expectations, males siring offspring in other nests were those investing more into parental feeding. These males did not differ from the rest of males in their within-pair paternity. This finding may indicate the nonexistence of a trade-off between seeking extra-pair paternity and parental investments. In species like rosefinches, where breeding is highly synchronized (Albrecht et al. 2007), the occurrence of such a trade-off is unlikely, however. In fact, at the time of offspring provisioning (second half of June in Sumava Mts.) only few females are in fertile phase (usually in pairs that had lost their regular first nesting attempt). Hence extra-pair copulations mostly occur earlier, well before the feeding of offspring takes place. The fact that the male ability to sire extra-pair offspring positively correlates with his provisioning rate may indicate that both are determined by a third unmeasured factor, such as general male vigor and/or genetic quality (also Zelano and Edwards 2002). This idea requires further evaluation.

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Příloha

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