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Faktory ovlivňující variabilitu v reakcích sýkor (Paridae) vůči nové a aposematické kořisti

Factors influencing variability in behaviour towards novel and aposematic prey in tits (Paridae)

Disertační práce

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Podpis

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Faktory ovlivňující variabilitu v reakcích sýkor (Paridae) vůči nové a aposematické kořisti

"What I am interested in with birds, just as I am with spiders or monkeys, is what they do and why they do it."

- David F. Attenborough

Obsah

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Abstrakt

U sýkor z čeledi Paridae byla zjištěna mezidruhová i vnitrodruhová variabilita v reakcích vůči nové a aposematické kořisti. Tato disertační práce se zabývá různými faktory, které mohou přístup k nové a aposematické kořisti u sýkor ovlivňovat. V několika experimentech rozdělených do přiložených prací jsme testovali, jaký vliv na přístup ptačích predátorů k nové a aposematické kořisti mohou mít rozdíly v jejich exploračním chování, neofobii, potravním konzervatizmu, personalitě, věku a zkušenosti nebo jejich schopnosti učení a generalizace. U dvou vzdálených populací sýkor koňader (*Parus major*) jsme zjistili stejnou míru exploračního chování a různou míru neofobie. Vyšší míra neofobie u finské populace sýkor však neměla vliv na jejich přístup k nové kořisti. Sýkory koňadry z Finska se však od sýkor koňader z Čech lišily v reakcích vůči aposematické kořisti. Finská populace sýkor napadala aposematickou ruměnici (*Pyrrhocoris apterus*) více než populace sýkor ze středních Čech. Tento rozdíl byl způsoben chybějící zkušeností finské populace s aposematickou kořistí z přírody. Dále nás zajímalo, zda je možné prostřednictvím pozitivní zkušenosti s různou potravou snížit neofobii k nové a aposematické kořisti u mláďat tří druhů sýkor z čeledi Paridae (sýkory koňadry *Parus major,* sýkory uhelníčka *Periparus ater,* sýkory modřinky *Cyanistes caeruleus*). Neofobie vůči nové a aposematické kořisti se snížila u sýkor koňader a také u sýkor uhelníčků, sýkory modřinky však projevovaly stále stejně vysokou míru vrozené opatrnosti vůči nové a aposematické kořisti bez ohledu na jejich předchozí zkušenost. U sýkor koňader pocházejících z linií selektovaných na opačný typ personality jsme zjišťovali, zda má věk jedince a typ personality vliv na přístup k aposematické kořisti. Ukázalo se, že rozdíly v přístupu naivních sýkor koňader k aposematické kořisti jsou u jednotlivých typů personalit v průběhu života konzistentní, mění se však způsob a intenzita projevované reakce. Vysoká míra počáteční opatrnosti u dospělých, z chovů pocházejících jedinců, může být ovlivněna jejich chybějící zkušeností s explorací různých podnětů nebo/a absencí zkušenosti s nedostatkem potravy. Dále nás zajímalo, jaký vliv může mít iridescentní zbarvení australské ploštice *Tectocoris diophthalmus* na averzivní učení a generalizaci u mláďat a dospělých sýkor koňader. Obě věkové kategorie sýkor se naučily iridescentně zbarvené kořisti vyhýbat a zkušenost generalizovat na jiný iridescentně zbarvený typ této kořisti. Z našich výsledků vyplývá, že iridescentní zbarvení je důležitou součástí výstražné signalizace ploštic. Tato disertační práce svými výsledky přispívá nejen k pochopení psychologie predátorů, jejich mezidruhové i vnitrodruhové variability v reakcích vůči nové a aposematické kořisti, ale také k samotnému pochopení vznilu a evoluce výstražného zbarvení u hmyzu.

Abstract

Inter-specific and intra-specific variation in reactions towards novel and aposematic prey was found in several species of tits (Paridae). This Ph.D. thesis is focusing on various factors influencing reactions towards novel and aposematic prey in three European species of tits. We tested differences in exploration behaviour, neophobia, dietary conservatism, personality, age and experience as well as ability of avoidance learning and generalisation. We found no difference in exploration behaviour and in reaction towards novel prey in two different populations of great tits (*Parus major*). But the birds from the Finnish population were more neophobic than Czech birds, but they attacked aposematic firebug (*Pyrrhocoris apterus*) more often and faster than Czech birds. The difference can be explained by a different experience with local aposematic prey communities. Than we studied initial wariness in naive juveniles of great tits (*P. major*), coal tits (*Periparus ater*) and blue tits (*Cyanistes caeruleus*), and we tested how the initial wariness towards novel and aposematic prey can be deactivated by experience with palatable prey. Great tits and coal tits from experienced groups significantly decreased their neophobia towards both types of prey while blue tits did not change their strongly neophobic reactions. We also discussed factors constraining rapid neophobia deactivation in blue tits. In next part of this Ph.D. thesis we asked whether the personality differences in reaction towards aposematic prey in great tits artificially selected for two distinct personality lines (fast and slow explorers) are consistent across time and how the age of the birds can affect their reactions. We found differences in reaction towards aposematic firebug in two age categories of naive great tits. Adult great tits showed stronger initial wariness towards aposematic prey than juveniles, which might be caused by the laboratory conditions with unlimited food supply and restricted variety of food types. But the individual differences in reaction of great tits towards aposematic firebug were shown to be consistent across time. And finally we tested whether the iridescent coloration of Australian bug *Tectocoris diophthalmus* affects avoidance learning and generalisation of adults and juveniles of great tits. Both age categories of tested birds learned to avoid iridescent bugs and they also generalized the experience to different type of iridescent coloured bug. These results suggest iridescent coloration and patterning can be an effective aposematic signal. The outputs of this Ph.D. thesis contribute to understanding of predator psychology, its inter- and intra-specific variability in reactions towards novel an aposematic prey as well as to understanding of origin and evolution of the aposematic signal.

Specializované termíny použité v této disertační práci:

Aposematismus	výstražná signalizace
Explorace	prozkoumávání nových podnětů
Neofobie	vyhýbání se novým podnětům
Neofilie	výrazná ochota zkoumat nové podněty
Potravní neofobie	vyhýbání se neznámé potravě
Potravní konzervatizmus	dlouhodobé odmítání určitého typu potravy
Specifická vrozená averze	averze vůči konkrétní vlastnosti kořisti, která nebyla získána učením
Personalita	označení pro soubor spolu souvisejících prvků chování konzistentních v čase za různých podmínek a situací
Behaviorální syndrom	pojem označující skupinu několika chování, která spolu vzájemně korelují, bez ohledu na jejich konzistenci v čase

Úvod do problematiky s komentáři k jednotlivým publikacím disertační práce

Strategie, které ptáci uplatňují při vyhledávání potravy, mohou být velmi variabilní. Setká-li se pták s novou, pro něj doposud zcela neznámou potravou, musí se rozhodnout, zda je pro něj tato potrava bezpečná a plně poživatelná. Chování, které je zodpovědné za rozdílný přístup ptáků k nové potravě, se může lišit jak mezi jednotlivými druhy (Brower 1988; Exnerová et al. 2003; Endler & Mappes 2004; Hotová Svádová et al. 2010), tak mezi jedinci stejného druhu (Marples et al. 1998; Exnerová et al. 2007, 2010, 2015). Mezidruhová variabilita v reakcích ptáků na novou potravu může být způsobena jejich rozdílnou potravní ekologií, smyslovými a kognitivními schopnostmi a také potenciálním rizikem spojeným s konzumací nové potravy (Exnerová et al. 2003). Vnitrodruhově pak mohou být reakce na novou potravu ovlivněny věkem jedince, dosavadní zkušeností, personalitou, pohlavím nebo příslušností ke konkrétní populaci (Jones 1986; Lindström et al. 1999; Exnerová et al. 2007, 2010, 2015; Bókony et al. 2012; Liebl & Martin 2014).

Ze strany kořisti je potom důležitou vlastností její výstražná signalizace (optická, olfaktorická, akustická), jejímž prostřednictvím vysílá příslušným predátorům signál o své potenciální nevýhodnosti, škodlivosti, či jedovatosti (Ruxton et al. 2004). Pro optickou signalizaci nevýhodnosti kořisti jsou důležité především jasně barevné vzory červené, žluté a oranžové v kombinaci s černou barvou. Nápadně zbarvenou kořist predátor lépe rozpozná a naučí se jí vyhýbat snadněji než kořisti kryptické (Gittleman & Harvey 1980; Sillén-Tulberg 1985; Lindström et al. 1999; Riipi et al. 2001). Vizuální signály mohou být navíc kombinovány se signály chemickými a akustickými a vytvářet tak multimodální signalizaci, která může napomáhat lepšímu rozpoznání nevýhodné kořisti (Rowe & Guilford 1999; Rowe & Halpin 2013). Existuje řada druhů aposematického hmyzu, které využívají pestrou škálu nejrůznějších obranných mechanismů, aby je uchránily před predací.

Pro pochopení evoluce výstražného zbarvení je důležitou součástí také studium psychologie predátora, jež je zásadním faktorem, který ovlivňuje vznik a rozšíření výstražně zbarvené kořisti. Mezidruhovou variabilitou v chování ptačích predátorů vůči aposematické kořisti se zabývá **souhrnná práce Exnerová et al. (2008)**, kde byly na základě výsledků z předchozích studií porovnávány reakce několika druhů středoevropských pěvců vůči výstražně zbarvené červeno-černé ruměnici pospolné (*Pyrrhocoris apterus*). Ukázalo se, že různé druhy ptačích predátorů reagují na výstražné zbarvení této ploštice různými způsoby. Drobní hmyzožraví pěvci, jako jsou pěnice a sýkory, aposematickou ploštici odmítají, zatímco zrnožraví pěvci, pěnkavy a strnadi, stejně jako větší hmyzožraví ptáci, kosi a brhlíci, tento typ kořisti ochotně napadají. Práce Exnerová et al. (2008) mimo jiné také shrnuje rozdíly v reakcích vůči aposematické ruměnici mezi blízce příbuznými druhy sýkor z čeledi Paridae. Ukázalo se, že některé druhy sýkor aposematickou ploštici odmítají na základě vrozené averze, jiné druhy se jí naopak musí naučit vyhýbat.

Tato disertační práce se zabývá faktory, které ovlivňují přístup k nové a aposematicky zbarvené kořisti u několika druhů středoevropských sýkor z čeledi Paridae, a to jak rozdíly mezi jednotlivými druhy, tak v rámci druhu stejného. Jaký vliv na reakce ptačích predátorů vůči nové a aposematické kořisti mohou mít rozdíly v jejich exploračním chování, neofobii, potravním konzervatizmu, personalitě, věku a zkušenosti daného jedince nebo ve schopnostech učení a generalizace, jsme testovali v experimentech rozdělených do několika studií, které jsou součástí předkládané disertační práce.

Hlavní otázky, které si klade tato disertační práce, jsou následující:

1) Liší se dvě oddělené populace sýkor koňader (*Parus major*) v exploračním chovaní a neofobii? Může mít míra explorace a neofobie vliv na reakci vůči nové potravě u těchto dvou vzdálených populací sýkor?

2) Jakou mírou se podílí neofobie, potravní konzervatizmus a specifická averze na vrozené opatrnosti vůči nové a aposematické kořisti u tří druhů sýkor z čeledi Paridae? Je možné tuto vrozenou opatrnost snížit pomocí pozitivní zkušenosti s různou potravou?

3) Jsou rozdíly v reakci na aposematickou kořist u jednotlivých typů personalit sýkor koňader (*Parus major*) konzistentní, nebo se mohou v průběhu života měnit? Jaký vliv na přístup sýkor koňader k aposematické kořisti může mít jejich věk?

4) Může iridescentní zbarvení kořisti zvýšit u sýkor koňader (*Parus major*) jejich počáteční opatrnost k aposematické kořisti? Je iridescence efektivním signálem pro averzivní učení a diskriminaci? Jsou sýkory koňadry schopny generalizovat zkušenost na různě zbarvené fenotypy ploštic? A projeví se rozdíly v počáteční opatrnosti, averzivním učení a generalizaci mezi dospělými a naivními jedinci?

5) Jaký vliv na reakci sýkor koňader (*Parus major*) vůči aposematické kořisti může mít jejich dosavadní zkušenost a životní podmínky?

Explorace a neofobie

Explorační chování a neofobie hrají u většiny zvířat významnou roli při shromažďování informací o jejich životním prostředí. Rozdíly v exploraci a neofobii se projevují mezi blízce příbuznými druhy i mezi jedinci v rámci druhu stejného. Nízká míra neofobie a vysoká míra explorace nových podnětů může být výhodnou strategií v prozkoumávání a získávání nových zdrojů potravy. Jedinec se tak ale zároveň vystavuje vyššímu riziku predace, parazitace a konzumace toxické potravy (Greenberg & Mettke-Hofmann 2001).

Pro zjištění míry explorace a neofilie se běžně používá test reakce na nový předmět ve známém prostředí (e.g. Verbeek et al. 1994; Mettke-Hofmann et al. 2002; Drent et al. 2003). Jelikož jedinec není nucen se k novému objektu přiblížit, jakýkoli kontakt s ním je známkou jeho aktivní explorace (Greenberg & Mettke-Hofmenn 2001). Pro zjištění míry neofobie se pak běžně používá test, kdy je nový předmět umístěn v blízkosti známé potravy. Za překonání neofobie se považuje okamžik, kdy se jedinec k novému objektu přiblíží a začne se krmit. Tento test sleduje konflikt jedince mezi potravní motivací a snahou vyhnout se neznámému předmětu (Mettke-Hofmann et al. 2002; Feenders et al. 2011; Mettke-Hofmann 2012).

Pozitivní korelace mezi těmito dvěma testy je považována za součást behaviorálního syndromu. Behaviorální syndrom je pojem, který u zvířat popisuje interindividuální variabilitu a označuje skupinu několika chování, která spolu vzájemně korelují, bez ohledu na jejich konzistenci v čase (Sih et al. 2004; Réale et al. 2007; Bell 2007). Pozitivní korelace mezi explorací a neofobií byla zjištěna u sýkor koňader (*Parus major*) (van Oers et al. 2004), amadin Gouldové (*Erythrura gouldiae*) (Williams et al. 2012), kosů černých (*Turdus merula*) (Miranda et al. 2013), vlhovce karibského (*Quiscalus lugubris*), kněžíka menšího (*Loxigilla noctis*), vlhovce modrolesklého (*Molothrus bonariensis*), hrdličky karibské (*Zenaida aurita*) a holoubka vrabčího (*Columbina passerina*) (Webster & Lefebvre 2001). Zjištěna však byla také negativní korelace mezi těmito dvěma typy chování u pěnice bělohrdlé (*Sylvia melanocephala*) (Mettke-Hofmann et al. 2005). U jiných studovaných druhů nebyla zjištěna žádná korelace mezi testy a chování jednotlivých druhů by mohlo odpovídat spíše jejich specifické ekologii, strategiím ve vyhledávání potravy a celkovému životnímu stylu (Mettke-Hofmann et al. 2002, 2005; Biondi et al. 2010; Feenders et al. 2011).

Některé studie navíc zjistily pozitivní korelaci mezi reakcí na nový předmět u potravy a novou potravou. Tato korelace byla zaznamenána například u sýkor černohlavých (*Poecile atricapillus*) (An et al. 2011) a u jedné populace vrabce domácího (*Passer domesticus*) (Bókony et al. 2012). Explorace nového předmětu pak úzce souvisela s reakcí sýkor koňader na neznámou kořist (aposematickou ploštici *Pyrrhocoris apterus*) v práci Exnerová et al. (2010), kde byla testována reakce ručně odchovaných mláďat sýkor koňader, pocházejících z linií selektovaných na opačný typ personality. Personalita jedince je chápána jako soubor spolu souvisejících prvků chování konzistentních v čase za různých podmínek a situací (Benus et al. 1990; Sih et al. 2004). Podle bodového ohodnocení, které sýkory obdržely z testů explorace nového prostředí a explorace nových objektů, byli jedinci následně spárováni a v zajetí množeni po F4 generaci. V této generaci již byly jednotlivé typy personalit zastoupeny jedinci, kteří obdrželi krajní hodnoty bodovaného skóre z testů na explorační chování (Drent et al. 2004). Takzvaní „pomalý průzkumníci" (slow explorers) prozkoumávali nové podněty pomalu, ale zato důkladně, naproti tomu „rychlý průzkumníci" (fast explorers) se vyznačovali rychlou, avšak povrchní explorací (Verbeek et al. 1994; Drent et al. 2004).

V práci Exnerová et al. (2015) nás zajímalo, zda se dvě vzdálené populace sýkor koňader (*Parus major*) liší mírou explorace a neofobie, zda spolu u jednotlivých populací tato chování vzájemně korelují a zda má míra explorace a neofobie u těchto dvou oddělených populací sýkor koňader vliv na jejich reakci vůči nové kořisti. Testovali jsme dospělé z přírody odchycené jedince pocházející ze dvou geograficky oddělených populací. Ptáci z populace pocházející ze středního Finska se nijak nelišili v latenci explorace nového objektu od ptáků pocházejících z populace odchycené ve středních Čechách, což poukazuje na podobné tendence v exploračním chování u obou populací sýkor koňader. V případě testu neofobie vůči novému předmětu umístěného v blízkosti potravy jsme u finské populace sýkor zaznamenali průkazně delší latence začátku krmení v přítomnosti neznámého předmětu než u populace české. Vyšší míra neofobie u finské populace sýkor by mohla být důsledkem většího rizika predace v přirozené krajině s menším lidským osídlením (Dingemanse et al. 2007; Brydges et al. 2008) nebo větším podílem migrujících jedinců (Cepák et al. 2008), u kterých byla prokázána vyšší míra neofobie, než u ptáků z rezidentních populací, pro které jsou informace o změnách v jejich životním prostředí nezbytné v souvislosti se sezónními výkyvy potravní nabídky (Mettke-Hofmann et al. 2013).

U obou testovaných populací sýkor koňader spolu explorace nového předmětu i neofobie vzájemně pozitivně korelovaly. Pozitivní korelace výše zmíněných testů odpovídá výsledkům většiny předchozích studií a zároveň je naše studie další prací, která dokládá existenci behaviorálního syndromu u divoké populace sýkory koňadry.

Poté, co ptáci absolvovali test na exploraci nového předmětu a test neofobie vůči předmětu umístěného v blízkosti potravy, byla jedincům z obou populací předložena nová zcela poživatelná kořist v podobě larvy banánového cvrčka (*Gryllus asimillis*) s připevněným modrým štítkem na jeho dorsální straně tak, aby byla skryta hlava i celé tělo. Sledovali jsme, zda a jakým způsobem ptáci s předloženou kořistí manipulovali, jestli byla kořist napadena, zabita a konzumována. Měřila se také latence první manipulace s předloženou kořistí.

Ani u jedné z testovaných populací nebyla nalezena korelace latence první manipulace s modrým cvrčkem a latence explorace nového objektu nebo latence konzumace potravy v přítomnosti neznámého předmětu. Naše zjištění neodpovídá výsledkům předchozí studie Exnerová et al. (2010), ve které „pomalí průzkumníci“, kteří se obecně vyznačují vyšší mírou neofobie váhali s útokem na neznámou kořist déle, než „rychlý průzkumníci“, kteří vykazují celkově nižší míru neofobie (Verbeek et al. 1994; Drent et al. 2003). Příčinou těchto rozdílných výsledků může být dosavadní individuální zkušenost dospělých jedinců s různorodou potravou z přírody, stejně jako zkušenost s jejím nedostatkem. Navíc u dospělých z přírody odchycených jedinců může v přístupu k nové potravě hrát významnou roli také jejich nedávné postavení v hierarchii zimního hejna. V případě sýkory černohlavé (*Poecile atricapillus*) například dominantní jedinci projevovali vyšší míru neofobie než jedinci, kteří byli v dominanční hierarchii sledované skupiny postaveni níže (An et al. 2011). Dalším možným vysvětlením může být samotná selekce na extrémní typy jednotlivých personalit (Drent et al. 2003), u kterých se korelace exploračního chování a neofobie k nové potravě projeví lépe než u námi testovaných ptáků z přirozených populací. Nelze však také vyloučit, že explorační chování a neofobie k nové potravě spolu mohou u některých populací vzájemně korelovat (jako je tomu u holandské populace sýkor ze studie Exnerová et al. (2010)), zatímco u jiných populací se vzájemná souvislost explorace a neofobie k nové potravě projevit nemusí (Bókony et al. 2012; Liebl & Martin 2014).

Přestože explorační chování a míra neofobie spolu velmi úzce souvisí u většiny doposud studovaných druhů, vzájemný vztah explorace nového objektu a reakce na novou potravu, stejně jako neofobie testované v přítomnosti nového objektu u potravy a reakce na novou potravu zůstává nejasný a chování ptáků v souvislosti s novou potravou se zdá být daleko komplexnějším behaviorálním projevem, který se nejeví jako součást behaviorálního syndromu.

Celkově z naší studie Exnerová et al. (2015) vyplývá, že míra explorace ani míra neofobie nemají žádný vliv na přístup dospělých sýkor koňader k nové kořisti ani u jedné ze dvou testovaných evropských populací.

Neofobie, potravní konzervatizmus a specifická vrozené averze

U ptáků můžeme často pozorovat různou míru opatrnosti v jejich reakcích na neznámou potravu. Tato opatrnost se projevuje jako váhání přiblížit se k neznámé potravě, následně s ní manipulovat a konzumovat ji. Opatrnost v přístupu k neznámé potravě se může lišit mezi jednotlivými druhy ptáků a odrážet tak jejich percepční, kognitivní a vyhledávací schopnosti (Coppinger 1970; Marples et al. 1998; Marples & Kelly 1999; Exnerová et al. 2003). Opatrnost vůči neznámé potravě může být u ptáků získaná učením (Sillén-Tullberg 1985; Roper & Redstone 1987; Lindström et al. 1999a; Gamberale-Stille & Guilford 2003; Ham et al. 2006; Svádová et al. 2009; Hotová-Svádová et al. 2013) nebo může být vrozená (Smith 1975, 1977; Lindström et al. 1999b; Exnerová et al. 2007). Nedávné studie ukázaly, že vrozená opatrnost k neznámé potravě se liší nejen mezi jednotlivými druhy (Exnerová et al. 2007), ale také mezi jedinci v rámci druhu stejného (Exnerová et al. 2010). Vrozená opatrnost se navíc může projevit až v souvislosti s takzvanou multimodální výstražnou signalizací kořisti, kdy je vizuální složka spojena se složkou chemickou nebo akustickou (Rowe & Guilford 1996, 1999a, 1999b; Jetz et al. 2001; Lindström et al. 2001; Rowe 2002; Kelly & Marples 2004; Rowe & Skelhorn 2005; Skelhorn et al. 2008).

Vrozená opatrnost k neznámé potravě je v současné době považována za komplex několika mechanismů, který zahrnuje tři ne zcela výlučné procesy: 1) neofobii, 2) potravní konzervatizmus a 3) specifickou vrozenou averzi k určité vlastnosti kořisti (Marples et al. 1998; Marples & Kelly 1999; Exnerová et al. 2003; Marples & Mappes 2011).

Neofobie v potravním kontextu byla definována jako váhání přiblížit se k nové potravě a začít s ní manipulovat. Potravní neofobie by tedy měla představovat pouze krátkodobý proces, trvající minuty nebo několik předložení (Marples & Kelly 1999).

Potravní konzervatizmus je oproti neofobii dlouhodobý proces trvající několik týdnů či měsíců (Marples et al. 1998; Marples et al. 2005) nebo také u některých jedinců po celý život (Marples et al. 2007). Vyznačuje se dlouhodobým odmítáním nové potravy i přes to, je-li tato potrava plně poživatelná a zcela neškodná. Dlouhodobě trvající neochota zařadit novou potravu do jídelníčku může být mezidruhově (Marples et al. 1998; Marples & Kelly 2004) i vnitrodruhově variabilní (Marples et al. 1998; Marples & Mappes 2011), přičemž bylo zjištěno, že tato individuální variabilita může mít genetický základ (Marples & Brakefield 1995). Neofobie a potravní konzervatizmus jsou často souhrnně označovány jako tzv. potravní opatrnost neboli „dietary wariness“ (Mappes et al., 2005; Marples et al., 2005;

Marples et al., 2007). Dietary wariness tedy zahrnuje potravní neofobii, která je měřena jako latence prvního taktilního kontaktu s novou potravou a potravní konzervatizmus, což je chování vyjádřené jako doba od prvního kontaktu s novou potravou až po její plnohodnotné zařazení do jídelníčku (Marples et al. 1998; Marples & Kelly 2004). Experimenty s kuřaty a krocany navíc potvrdily existenci dvou odlišných behaviorálních procesů, kdy prostřednictvím předchozí zkušenosti s různorodou potravou deaktivovali u ptáků neofobii k nové potravě, avšak potravní konzervatizmus zůstal u některých jedinců i nadále zachován (Jones 1986; Marples et al. 1998; Lecuelle et al. 2011).

Třetí proces, který se podílí na vrozené opatrnosti k neznámé potravě, je specifická vrozená averze ke konkrétní vlastnosti potravy a nejčastěji se projevuje v souvislosti s typickým výstražným zbarvením aposematické kořisti. Specifická vrozená averze byla zjištěna u celé řady ptačích druhů: naivní kuřata (*Gallus gallus domesticus*) odmítala načerveno nabarvené larvy potemníka moučného (*Tenebrio molitor*) (Roper & Cook 1989; Roper 1990), ale také žlutočerně pruhované larvy (Schuler & Hesse 1985). Naivní jedinci křepela virginského (*Colinus virginianus*) se vyhýbali červenožlutým špendlíkovým hlavičkám (Mastrota & Mench 1995), naivní ručně odchovaní momoti (*Eumomota superciliosa*) a tyrani bentevi (*Pitangus sulphuratus*) se vyhýbali vzoru korálovcovitých hadů (Smith 1975, 1977) a naivní ručně odchovaná mláďata sýkory koňadry (*Parus major*) odmítala atakovat žlutočerně pruhované larvy potemníka moučného (Lindström et al. 1999b). Vizuální signály jsou však u skutečné kořisti často kombinovány se signály olfaktorickými, chuťovými a akustickými, což může u predátorů specifickou vrozenou averzi vyvolat nebo zesílit její projevy (Rowe & Guilford 1999).

V práci Adamová-Ježová et al. (submit.) jsme se zabývali mechanismy, které jsou zodpovědné za vrozenou opatrnost vůči nové a aposematické kořisti u několika druhů sýkor z čeledi Paridae. V předchozí studii Exnerová et al. (2007) bylo zjištěno, že blízce příbuzné druhy sýkor se liší v míře jejich vrozené opatrnosti vůči nové a aposematické kořisti. Zatímco naivní ručně odchovaná mláďata sýkory uhelníčka (*Periparus ater*) a sýkory modřinky (*Cyanistes caeruleus*) odmítala červeno-černou aposematickou ploštici *Pyrrhocoris apterus*, mláďata sýkor koňader (*Parus major*) a sýkor parukářek (*Lophophanes cristatus*) tuto kořist ochotně napadala a konzumovala. Protože mláďata sýkor uhelníčků a sýkor modřinek v téže studii odmítala také nahnědo nabarvenou variantu aposematické kořisti, můžeme předpokládat, že příčinou této reakce by mohla být neofobie vůči neznámé potravě (Exnerová et al. 2007). Jakou měrou se však na vrozené opatrnosti jednotlivých druhů sýkor vůči nové a aposematické kořisti podílí právě neofobie, potravní konzervatizmus a specifická vrozená averze není dosud zcela jasné. V naší práci jsme porovnávali vrozenou reakci tří druhů sýkor (sýkory koňadry, sýkory uhelníčka a sýkory modřinky), vůči dvěma typům nové kořisti: 1) jedlou kořist představovala larva cvrčka (*Acheta domestica*) s nalepeným modrým štítkem na její dorzální straně tak, aby zakrývala hlavu i celé tělo, 2) aposematickou kořist představoval dospělý jedinec červeno-černě zbarvené ruměnice pospolné (*Pyrrhocoris apterus*). Zároveň jsme v rámci každého druhu testovali, má-li předchozí pozitivní zkušenost s novou plně jedlou kořistí (načerveno nabarvenou larvou potemníka moučného, *Tenebrio molitor*) vliv na ochotu naivních ručně odchovaných ptáčat napadat a konzumovat modrého cvrčka a následně aposematickou ploštici.

Zjistili jsme, že zkušenost s načerveno nabarvenou larvou potemníka moučného ovlivnila reakci mláďat sýkor koňader, uhelníčků a modřinek vůči nové poživatelné kořisti různými způsoby. Zatímco sýkory koňadry a sýkory uhelníčci, jež měly pozitivní zkušenost s jedlou kořistí červené barvy, měly signifikantně kratší latence první manipulace s novou kořistí než jedinci bez této předchozí zkušenosti, mláďata sýkor modřinek vykazovala stále stejně vysokou míru vrozené opatrnosti k nové kořisti bez ohledu na jejich předchozí zkušenost. Výsledky zjištěné u sýkor koňader a sýkor uhelníčků jsou v souladu s předchozími studiemi, které prokázaly, že pozitivní zkušenost s potravou jedné nové barvy je postačující pro deaktivaci neofobie k další nově zbarvené potravě (Schlenoff 1984; Jones 1986; Marples et al. 2007; Lecuelle et al. 2011). Naše výsledky navíc ukazují, že pozitivní zkušenost s potravou, která se od známé potravy liší pouze barvou, může deaktivovat neofobii také vůči kořisti, která má zcela neznámý tvar i způsob pohybu.

Přestože ptáci s pozitivní zkušeností s načerveno nabarvenou larvou potemníka moučného ochotněji a rychleji napadali modrého cvrčka než ptáci bez této zkušenosti, pozitivní zkušenost s červeně nabarvenou potravou však nijak výrazně neovlivnila ochotu konzumace modrého cvrčka ani u jednoho z testovaných druhů sýkor. Jak již bylo řečeno v úvodu této kapitoly, překonání neofobie k nové potravě nemusí nutně znamenat také její plnohodnotné zahrnutí do stávajícího jídelníčku. Naše výsledky tedy podporují hypotézu Marples & Kelly (1999) o existenci neofobie a potravního konzervatizmu jakou dvou zcela odlišných procesů.

Různí predátoři se mohou lišit různou mírou vrozené opatrnosti k aposematické kořisti. V práci Exnerová et al. (2007) bylo zjištěno, že naivní ručně odchovaná mláďata našich středoevropských druhů sýkor se liší mírou své vrozené opatrnosti vůči aposematické ploštici *Pyrrhocoris apterus*. Zatímco mláďata sýkor koňader žádnou silnou opatrnost vůči aposematické kořisti neprojevují (Exnerová et al. 2007; Svádová et al. 2009; Hotová Svádová et al. 2013; Fabricant et al. 2014), mladé sýkory modřinky a sýkory uhelníčci vykazují k aposematické kořisti silnou počáteční averzi (Exnerová et al. 2007). Výsledky naší práce dokládají, že pozitivní zkušenost s jedlou potravou červené barvy signifikantně sníží počáteční opatrnost k nové červeno-černě zbarvené aposematické ploštici *Pyrrhocoris apterus* u mladých sýkor uhelníčků, avšak u sýkor modřinek vysoká míra počáteční opatrnosti k této kořisti přetrvá bez ohledu na jejich předchozí zkušenost. U sýkor uhelníčků, stejně jako u nejméně opatrných sýkor koňader, tak pravděpodobně došlo ke snížení počáteční opatrnosti deaktivací neofobie, nebo k široké generalizaci červeně nabarvených jedlých larev potemníka moučného na červeně-černý vzor aposematické ruměnice. Žádný z těchto procesů se však neprojevil u mláďat sýkor modřinek, což nasvědčuje skutečnosti, že jejich vrozená opatrnost je silnější než u ostatních dvou testovaných druhů. Sýkory modřinky nejspíše potřebují opakovanou pozitivní zkušenost s novými typy potravy, protože u dospělých z přírody odchycených jedinců jsme mohli pozorovat celkově nižší neofobii než u mláďat (Adamová-Ježová et al., unpublished).

Dalším zajímavým zjištěním je skutečnost, že mláďata sýkor koňader, která měla pozitivní zkušenost s jedlou potravou červené barvy, také signifikantně zvýšila ochotu konzumovat aposematickou červeno-černou ploštici (Adamová-Ježové et al. submit.). Podobný, avšak neprůkazný trend stejného chování, jsme zaznamenali i u sýkor uhelníčků. Příčinou této zvýšené ochoty ke konzumaci aposematické ploštice může být deaktivace neofobie a zároveň potravního konzervatizmu, díky opakované zkušenosti s jedlou potravou různých barev a tvarů, jak již bylo potvrzeno také u kuřat ve studii Marples et al. (2007), nebo generalizace pozitivní zkušenosti s jedlou potravou červené barvy na jinou podobně zbarvenou kořist. Mnoho prací prokázalo, že barva je pro ptáky hlavním vizuálním podnětem, na základě kterého se učí rozlišovat svou potenciální kořist (Aronsson & Gamberale-Stille, 2008, 2012; Kazemi et al. 2014). Ačkoliv na základě našeho experimentu nejsme schopni rozhodnout, zda to byla pozitivní zkušenost s jedlou potravou červené barvy, která u sýkor koňader a uhelníčků výšila ochotu ke konzumaci červeno-černé ploštice nebo kombinace zkušenosti s červenou barvou a zároveň novým tvarem (modrý cvrček), který se liší od známého tvaru larvy potemníka moučného, je z této studie zcela evidentní, že zkušenost s novou potravou, která je ploštici blízká pouze svým tvarem (modrý cvrček), na snížení míry počáteční opatrnosti vůči aposematické kořisti u studovaných druhů sýkor nestačí.

Rozdíly v chování u tří studovaných druhů sýkor mohou být částečně vysvětleny jejich tělesnou velikostí, například u sýkory koňadry, která jako největší z našich druhů sýkor je zároveň také vůči neznámé potravě nejméně opatrná. Samotná velikost nám však neobjasňuje různou míru neofobie k nové potravě u všech tří studovaných druhů. Velmi silnou neofobii u mláďat sýkor modřinek a naopak ne tak silnou u nejmenšího druhu, sýkory uhelníčka. Dalšími faktory, které mohou hrát roli v této mezidruhové variabilitě, jsou geografická distribuce, habitatová specializace nebo skladba a způsob získávání potravy.

Na rozdíl od sýkory koňadry a sýkory uhelníčka, jejichž geografické rozšíření sahá až do oblasti Sibiře, kde se tyto druhy musí vypořádat s extrémně nízkými teplotami a nízkou nabídkou potravy, sýkora modřinka obývá západní palearktickou oblast s mírným klimatem bez extrémních teplotních výkyvů (Cramp & Perrins 1993; del Hoyo et al. 2007; Tietze & Borthakur 2012). Rozdíly v geografické distribuci studovaných druhů by tudíž mohly částečně vysvětlovat vysokou míru vrozené opatrnosti vůči nové a aposematické kořisti u sýkory modřinky, oproti vyšší ochotě v přístupu k nové a aposematické kořisti u sýkor koňader a sýkor uhelníčků.

Habitatová specializace a skladba potravy jednotlivých druhů sýkor jsou také důležitými faktory, které mohou být příčinou mezidruhových rozdílů naivních sýkor v přístupu k nové a aposematické kořisti. Sýkory koňadry a sýkory modřinky jakožto habitatový generalisté se ve svém životním prostředí setkávají s velkým množstvím různé často i potenciálně škodlivé potravy. Menší sýkory modřinky tak může jejich vysoká míra vrozené opatrnosti chránit před požitím nebezpečné a toxické potravy. Naproti tomu nejmenší ze všech testovaných druhů sýkora uhelníček preferuje jehličnaté lesní porosty, které představují prostředí s monotónní potravní nabídkou a nižším výskytem potenciálně škodlivé potravy. Navíc výraznou část potravy sýkor uhelníčků tvoří mšice (Hemiptera: Sternorrhyncha; Cramp & Perrins 1993; Krištín 1992), které obvykle nejsou nijak chemicky chráněné (Gullan & Craston 2014) a představují tak bezpečný a hojný zdroj potravy. Potravní specializace sýkor uhelníčků jim tedy může dovolovat nižší míru neofobie k neznámé potravě než podobně velkým sýkorám modřinkám.

Sýkora uhelníček je jediným ze tří studovaných druhů, který si ukrývá potravu. Ukázalo se, že někteří ptáci si ukrývají také toxickou potravu, ke které se později vracejí (Yosef & Whitman 1992; Exnerová et al. 2008). Stejnou strategii jsme mohli pozorovat u námi testovaných sýkor uhelníčků, které si v průběhu experimentu zastrkávaly aposematickou ploštici do různých škvír v pokusné kleci a po čase, když její ochranná sekrece vyprchala, tuto kořist i příležitostně konzumovaly. Tato strategie by tudíž také mohla vézt k vyšší ochotě napadat novou a aposematickou kořist u sýkor uhelníčků.

Celkově však můžeme shrnout, že u sýkory koňadry je to její tělesná velikost, která by mohla být spojena s ochotou ručně odchovaných mláďat napadat neznámou kořist, zatímco u dvou nejmenších druhů, omezený areál rozšíření sýkory modřinky v porovnání s areálem výskytu sýkory uhelníčka a jeho habitatová specializace spojená se skladbou potravy, mohou mít zásadní vliv na různou míru neofobie vůči nové a aposematické kořisti u těchto dvou studovaných druhů sýkor.

Personalita a věk

U ptáků byla zjištěna individuální vnitrodruhová variabilita v reakcích na novou a aposematickou potravu (Marples et al. 1998; Exnerová et al. 2003, 2007). Příčinou této individuální variability u dospělých z přírody odchycených jedinců může být jejich dosavadní zkušenost s různou potravou (Marples et al. 1998, 2005; Exnerová et al. 2015). Avšak tato variabilita byla zaznamenána také u naivních ručně odchovaných mláďat, která měla jen omezenou zkušenost s různými zdroji potravy (Sillén-Tullberg 1985; Marples & Brakefield 1995; Exnerová et al. 2007; Svádová et al. 2009).

Individuální rozdíly ve vrozené reakci na neznámou potravu mohou být vysvětleny personalitou jedince. Personalita je definována jako soubor spolu souvisejících prvků chování konzistentních v čase za různých podmínek a situací (Benus et al. 1990; Sih et al. 2004). Personalita byla podrobně studována také u sýkor koňader (*Parus major*). Na základě exploračního chování v neznámém prostředí byli jedinci označeni za tzv. „rychlé průzkumníky" (fast explorers) nebo naopak „pomalé průzkumníky" (slow explorers) (Verbeek et al. 1994; Drent et al. 2003). Rozdíly v rychlosti prozkoumávání nového prostředí korelovaly s rozdíly v přístupu k novým objektům (Verbeek et al. 1994), riskantním chováním („risk-taking behaviour") (van Oers et al. 2004a), agresivitou (Verbeek et al. 1994), potravním chováním (Verbeek et al. 1994), využíváním sociálních informací (Marchetti & Drent 2000; van Oers et al. 2005) nebo také s reakcí na stres (Carere et al. 2003; Carere & van Oers 2004; Fučíková et al. 2009). Personalita jedince je dána z části geneticky (Drent et al. 2003, van Oers 2004b). „Rychlí průzkumníci" jsou popisováni jako odvážní, agresivní, více riskují a často podléhají tvorbě rutinního chování. Nové prostředí prozkoumávají rychle avšak povrchně a mají tendenci kopírovat jiné jedince při vyhledávání potravy. Naproti tomu „pomalí průzkumníci" jsou plaší, nejsou agresivní a riskují méně. Vyznačují se inovativním chováním, nové prostředí prozkoumávají pomalu avšak důkladně a jsou aktivní a nezávislí ve vyhledávání potravy (Verbeek et al. 1994; Drent et al. 2003; van Oers et al. 2004a; Groothuis & Carere 2005).

Vliv typu personality na reakci vůči neznámé kořisti byl zjištěn u naivních ručně odchovaných mláďat sýkor koňader (*Parus major*) ve studii Exnerová et al. (2010). „Pomalí průzkumníci" vykazovali vyšší míru vrozené opatrnosti k červeno-černě zbarvené aposematické ploštici *Pyrrhocoris apterus* a s útokem na ni váhali průkazně déle než „rychlí průzkumníci". „Pomalí průzkumníci" se také naučili této aposematické kořisti vyhýbat rychleji a v průběhu averzivního učení manipulovali s menším počtem ploštic než „rychlí průzkumníci“ (Exnerová et al. 2010). Otázkou však zůstává, zda jsou tyto rozdíly v reakci na aposematickou kořist u jednotlivých typů personalit sýkor koňader konzistentní v čase nebo se mohou s věkem měnit.

V práci Adamová-Ježová et al. (in prep.) jsme stejně jako Exnerová et al. (2010) testovali naivní ručně odchované jedince sýkory koňadry (*P. major*) z linií selektovaných na opačný typ personality - jedinci F4 generace pocházející z křížení jedinců spárovaných podle výsledků jejich bodového ohodnocení, kterého dosáhli v testech na exploraci nového prostředí a nových předmětů. Ve studii Exnerové et al. (2010) byla testována mláďata ve věku 2 až 3 měsíců. V naší práci jsme provedli stejný typ experimentu avšak s dospělými jedinci ve věku 1,5 až 5,5 let. Dospělým sýkorám jsme nabízeli aposematickou ploštici *Pyrrhocoris apterus* v sekvenci se známou potravou, larvou potemníka moučného (*Tenebrio molitor*). Jejich reakci na aposematickou kořist jsme sledovali ve dvou po sobě následujících dnech. V průběhu experimentu jsme zaznamenávali latence prvního přiblížení k aposematické ploštici, počet přiblížení, a zda došlo k manipulaci s ploštici, jejímu zabití a konzumaci.

Ukázalo se, že dospělé sýkory koňadry vykazují vyšší míru vrozené opatrnosti k výstražně zbarvené kořisti než mláďata testovaná v předchozí studii Exnerová et al. (2010). Přestože se dospělí jedinci pravidelně a ochotně k aposematické ploštici přibližovali, velmi často s ní odmítali manipulovat. Celkově se však personalita jedince na manipulaci s aposematickou kořistí projevila. Zatímco pouze 3 z 22 „pomalých průzkumníků“ s aposematickou ploštící v průběhu celého experimentu manipulovali, u „rychlých průzkumníků“ manipulovala s předloženou ploštící přesně polovina jedinců (9 z 18). Dva „rychlí jedinci“ několik ruměnic také zabili a konzumovali. Vliv personality se však u dospělých jedinců odrazil převážně na latenci a počtu přiblížení k aposematické ploštici, to ale až druhý den experimentu. První den jedinci obou typů personalit váhali s prvním přiblížením k aposematické ploštici stejně dlouho a také stejně často si tuto kořist z bezprostřední blízkosti prohlíželi. Personalita jedince však měla vliv na latenci prvního přiblížení druhý den, kdy „pomalí průzkumníci“ s přiblížením k aposematické ploštici váhali signifikantně déle než „rychlí průzkumníci“ a k aposematické ploštici se také přibližovali signifikantně méně často. Vypadá to, že zatímco u „pomalých průzkumníků“ se vrozená opatrnost k aposematické kořisti v průběhu experimentu prohloubila, u „rychlých průzkumníků“ se tato opatrnost vůči aposematické kořisti jejím pravidelným předkládáním snížila. Zdá se tedy, že vlivem věku a životních podmínek v zajetí, které přinášejí ptákům pouze omezenou škálu nových podnětů včetně nedostatku různorodých zdrojů potravy, se u „pomalých průzkumníků“ může vrozená opatrnost k neznámé potravě prohloubit, zatímco u

„rychlých průzkumníků“ může dojít k vytvoření rutinního chování, které se projeví fixací na známou potravu. Stejné projevy chování mohou tedy u různých jedinců vznikat různými způsoby. Přestože chování, které vůči aposematické kořisti projevovaly dospělé ručně odchované sýkory koňadry, se výrazně lišilo od chování ručně odchovaných mláďat z předchozí studie, mohli jsme u jednotlivých typů personalit pozorovat rozdílnou reakci na neznámou aposematickou kořist. Lze tedy shrnout, že rozdíly v přístupu naivních sýkor koňader k aposematické kořisti jsou u jednotlivých typů jejich personalit v průběhu života konzistentní, mění se však způsob a intenzita projevovaného chování.

Mnoho studií dokládá, že v průběhu života jedince se může měnit míra jeho neofobie vůči novým podnětům (Vince 1960; Greenberg 1992; Heinrich 1995; Fox & Millam 2004; Biondi et al. 2010, 2013). Nízkou míru neofobie bychom mohli pozorovat zejména u mláďat, která se vyznačují velkou behaviorální plasticitou spojenou s explorací nových objektů (Greenberg 2003; Biondi et al. 2010). Toto období je pro mladé jedince velmi důležité, protože veškeré nové podněty, se kterými se setkají, mohou následně formovat jejich potravní strategie. Avšak tato ochota mláďat prozkoumávat nové podněty se s narůstajícím věkem snižuje a se vstupem do dospělosti se na základě dosavadních zkušeností mohou stávat při každém dalším střetu s neznámým podnětem opatrnější (Greenberg & Mettke-Hofmann 2001). Heinrich (1995) ve své studii ukázal, že juvenilní krkavci (*Corvus corax*) byli daleko více ochotní prozkoumávat všechny nové podněty a úspěšně mezi nimi najít potenciální potravu než stejní jedinci testovaní ve věku 1 a 1,5 roku (Heinrich 1995). Podobných výsledků dosáhli také Fox & Millam (2004), když testovali neofobii u ručně odchovaných amazoňanů oranžovokřídlých (*Amazona amazonica*), kteří se novým podnětů vyhýbali signifikantně méně ve věku sedmi měsíců než při opětovném testování v jednom roce života. Také mladí z přírody odchycení jihoameričtí sokolovití dravci čimangové šedonozí (*Milvago chimango*) byli více explorativní a méně neofobičtí než dospělí jedinci (Biondi et al. 2010, 2013). Nejnižší míru neofobie u sýkor koňader (*Parus major*) zaznamenal Vince (1960) ve věku mezi osmým až patnáctým týdnem života. Podobně také P. J. Drent (unpublished data) a Verbeek et al. (1994) potvrzují, že strach z novosti se u sýkor koňader v průběhu života mění.

V práci Adamová-Ježová (in prep.) jsme se zabývali srovnáním reakcí vůči neznámé aposematické kořisti u dvou věkových kategorií ručně odchovaných sýkor koňader: sýkor koňader testovaných ve věku 1,5 až 5,5 let s reakcemi sýkor koňader z předchozí studie Exnerová et al. (2010) testovaných ve věku 64 až 90 dní. Obě tyto studie byly prováděny v totožných podmínkách za stejného experimentálního uspořádání. Naivním ručně odchovaným mláďatům i dospělcům byla předkládána pro obě věkové skupiny zcela neznámá aposematická kořist, ruměnice pospolná (*Pyrrhocoris apterus*) v sekvenci se známou kontrolní kořistí, larvou potemníka moučného (*Tenebrio molitor*). Zaznamenávali jsme latence a počet přiblížení k aposematické ploštici, četnost a způsob manipulace s plošticí, zda byla ploštice zabita a konzumována.

Jelikož u naivních ručně odchovaných mláďat sýkor koňader nebyla doposud pozorována vrozená opatrnost vůči nové či aposematické kořisti (Exnerová et al. 2007; Svádová et al. 2009; Hotová Svádová et al. 2013; Fabricant et al. 2014), přináší naše studie velmi překvapivé výsledky. Oproti mláďatům testovaným v práci Exnerová et al. (2010), která velmi ochotně napadala, zabíjela a konzumovala aposematickou ploštici, dospělí jedinci ze studie Adamová-Ježová et al. (in prep.) projevovali vůči tomuto typu kořisti silnou počáteční averzi. Ptáci aposematickou ploštici napadali jen velmi zřídka a ve většině případů se k ní pouze přiblížili a z bezprostřední vzdálenosti si předloženou kořist důkladně prohlédli, aniž by se jí jakkoliv dotkli. Takové chování může být u dospělých sýkor koňader způsobeno neofobií nebo jiným procesem, který u dospělých v zajetí chovaných ptáků vyvolal tuto silnou počáteční opatrnost k aposematické kořisti.

Neofobie k potravě je proces, který zvířata chrání před požitím toxické nebo jinak nebezpečné potravy, na druhou stranu je však explorace nových potravních zdrojů velmi důležitá, zejména u mladých jedinců, protože formuje jejich budoucí potravní strategie (Greenberg & Mettke-Hofmann 2001). Řada studií prokázala, že ptáci prozkoumávají ochotněji nové podněty v raných fázích svého života (Vince 1960; Heinrich 1995; Fox & Millam 2004; Biondi et al. 2010). Zatímco Smith (1980) ve své studii s naivními ručně odchovanými sojkami chocholatými (*Cyanocitta cristata*) a vlhovci červenokřídlými (*Agelaius phoeniceus*) testovanými ve věku 40 dní, nezaznamenala žádnou vrozenou opatrnost k výstražně zbarveným modelům jejich potenciální kořisti, Coppinger (1970) zjistil vrozenou opatrnost vůči aposematickým druhům motýlů (Nymphalinae) u ručně odchovaných jedinců stejných druhů, které však testoval ve věku 9 až 10 měsíců. Také Mastrota & Mench (1994) prokázali větší opatrnost k červeně a oranžově zbarvené potravě u 64 až 67 týdnů starých samiček křepela virginského (*Colinus virginianus*) než u o polovinu mladších samiček testovaných ve stáří 31 týdnů. Hypotézu, že neofobie vzrůstá s věkem, však nepotvrdila studie Langhama (2006), ve které starší z přírody odchycení jedinci leskovce neotropického (*Galbula ruficauda*) napadali novou formu aposematicky zbarveného motýla rodu *Heliconius* častěji než taktéž z přírody odchycení mladší jedinci. K podobným závěrům došli také Lindström et al. (1999b) testováním tří věkových kategorií sýkor koňader (*Parus major*). Ručně odchovaná mláďata a dospělí odchycení ptáci atakovali černožlutě nabarvenou larvu potemníka moučného častěji než odchycené jednoleté sýkory. Stejně tak v práci Exnerová et al. (2006) jednoleté sýkory koňadry napadaly a zabíjely uměle odchované homozygotní oranžovo-černé, žluto-černé a bílo-černé mutanty ruměnice pospolné (*Pyrrhocoris apterus*) méně než víceleté sýkory a dospělé z přírody odchycené sýkory koňadry z finské populace ochotně napadaly neznámou přirozeně zbarvenou červeno-černou formu této ploštice (Exnerová et al. 2015).

Zdá se tedy, že nízká míra neofobie u mláďat může být důležitá zejména v době jejich osamostatňování, kdy jsou nucena naučit se sama shánět potravu. Poté, co se již mladí jedinci v přírodě uživí, se stávají opatrnějšími, a to zvláště v případě, mají-li s nevhodnou potravou teprve nedávnou zkušenost. Starší jedinci z přírody, kteří mají již bohaté zkušenosti s různorodou potravou, jsou pak v přístupu k neznámé kořisti odvážnější.

Lépe než samotný věk jedince, tedy variabilitu v chování vůči neznámé kořisti, která se může měnit v průběhu života, vysvětluje dosavadní zkušenost. Možnými příčinami neochoty prozkoumávat novou potravu u dospělých sýkor koňader pocházejících z chovů, ve srovnání s dospělými jedinci z přírody, může být například jejich chybějící zkušenost s nedostatkem potravy nebo celková absence různorodých podnětů, se kterými se ptáci v chovech během svého života setkají (Adamová-Ježová et al. in prep.).

Učení, generalizace a zkušenost

Aposematická kořist dává svým potenciálním predátorům najevo nepoživatelnost a/nebo toxicitu nápadnými a snadno zapamatovatelnými signály (Ruxton et al. 2004). Někteří predátoři se vyznačují vrozenou averzí k určité vlastnosti aposematické kořisti jako je například její zbarvení nebo specifický pach (Smith 1975; Schuler & Hesse 1985; Roper 1990; Rowe & Guilford 1996), jiní se však musí této nevýhodné kořisti s určitým varovným signálem naučit vyhýbat (Järvi et al. 1981; Sillén-Tullberg 1985; Exnerová et al. 2007; Svádová et al. 2009; Hotová Svádová et al. 2013). Ptáci by se měli rychleji naučit odmítat nepoživatelnou kořist, je-li nápadná a snadno odlišitelná od jedlé, většinou krypticky zbarvené kořisti. Velké množství studií potvrzuje, že ptáci se při učení orientují převážně na základě vizuálních signálů, jako je barva kořisti (Exnerová et al. 2006; Ham et al. 2006; Svádová et al. 2009), kontrast s podkladem (Gamberale-Stille 2001; Gamberale-Stille & Guilford 2003), vnitřní kontrast a vzor (Aronsson & Gamberale-Stille 2008, 2009; Hegna et al. 2011) nebo pravidelnost a symetrie vzoru (Forsman & Merilaita 1999; Stevens et al. 2009). Aposematická kořist se vyznačuje především jasně barevnými vzory červené, žluté a oranžové v kombinaci s černou barvou (Endler & Mappes 2004, Ruxton et al. 2004). Avšak existuje také několik potenciálně aposematických druhů, jejichž součástí výstražné signalizace je modrá barva (Umbers 2013). Doposud se zabývalo efektivitou modré a zelené barvy jako součásti výstražné signalizace pouze několik studií, které však přinášejí rozdílné výsledky (Rowe & Guilford 1996; Marples et al. 1998; Gamberale-Stille & Guilford 2003; Rowe & Skelhorn 2005; Aronsson & Gamberalle-Stille 2008). Podobně také iridescentnímu zbarvení jako součásti výstražné signalizace aposematického hmyzu nebylo doposud věnováno příliš pozornosti. Přitom iridescentní a jiná strukturní zbarvení mohou vytvářet jasně zářivé odstíny modré a ultrafialové (Doucet & Meadows 2009; Umbers 2013), které ve spojení s pigmentovým zbarvením vytváří nápadný kontrastní vzor, který může napomáhat při rozpoznávání aposematického signálu predátorem (Endler 1992; Doucet & Meadows 2009). Existují studie, které se zabývaly funkcí iridescentního zbarvení jako součásti výstražné signalizace u brouků (Schultz 2001) a u motýlů (Bowers & Larin 1989; Rutowski et al. 2010; Pegram et al. 2013), avšak u ploštic (Heteroptera) se tomuto tématu doposud mnoho pozornosti nevěnovalo.

Jakmile je proces učení dokončen, může určitá vlastnost aposematické kořisti napomáhat, aby byla predátorem správně rozpoznána (Guilford 1986; Gamberale-Stille 2001). Generalizace je reakce na nový podnět, přičemž vychází z předchozí zkušenosti s jiným podobným podnětem (Lieberman 2000). Schopnost predátora generalizovat svou zkušenost s aposematickou kořistí je velmi důležitá pro evoluci výstražného zbarvení (Leimar et al. 1986; Yachi & Higashi 1998).

Jaký vliv může mít na počáteční opatrnost, averzivní učení, diskriminaci a generalizaci u naivních ručně odchovaných mláďat a dospělých z přírody odchycených jedinců sýkor koňader (*Parus major*) strukturní iridescentní zbarvení ve vzoru australské ploštice *Tectocoris diophthalmus* (Heteroptera: Scutelleridae), jsme testovali **v práci Fabricant et al. (2014).** *T. diophthalmus* je aposematický druh ploštice, který se vyznačuje matným oranžovým zbarvením s modro-zelenými kovově lesklými skvrnami. Velikost těchto skvrn může být značně vnitrodruhově variabilní, přičemž mohou pokrývat až celý dorsální povrch těla jedince nebo mohou zcela úplně chybět (Fabricant et al. 2013). Tento druh ploštice je díky svému polymorfismu ideální pro studium významu iridescence, jako signálu důležitého pro rozpoznání aposematické kořisti predátorem a následného učení se jí vyhýbat. Ručně odchovaná mláďata v tomto experimentu postrádají jakoukoli zkušenost s nepoživatelnou kořistí a z předchozích studií navíc vyplývá, že mláďata sýkor koňader neprojevují vůči výstražně zbarvené kořisti žádnou specifickou vrozenou averzi (Exnerová et al. 2007, Svádová et al. 2009, Hotová Svádová et al. 2013). U dospělých z přírody odchycených jedinců je naopak velmi pravděpodobné, že dosavadní zkušenost s aposematickými druhy ploštic již mají (Hotová Svádová et al. 2010), nikoli však s iridescentními.

Zajímalo nás, jestli může iridescentní zbarvení *T. diophthalmus* zvýšit u sýkor koňader jejich počáteční opatrnost k tomuto typu kořisti. Zda je iridiscence efektivním signálem pro averzivní učení a diskriminaci od jedlé varianty kořisti. Jestli budou námi testovaní ptáci schopni svou zkušenost generalizovat na různě zbarvené fenotypy těchto ploštic. A projeví-li se rozdíly v počáteční opatrnosti, averzivním učení a generalizaci mezi dospělými a naivními jedinci.

Kořist byla vytvořena za pomocí suchých exemplářů *T. diophthalmus*, kterým byla vypreparována ventrální a vnitřní část abdomenu a nahrazena polovinou larvy potemníka moučného (*Tenebrio molitor*) namočené ve vodě (jedlá varianta) nebo v 6 % roztoku chininu (nejedlá varianta). Pro jednotlivé fáze experimentu bylo použito 5 typů kořistí, které se lišily svým zbarvením: (1) iridescentní typ kořisti, jehož povrch byl z 50 % tvořen iridescentními zeleno-modrými skvrnami s oranžovým zbarvením; (2) jednobarevně oranžový typ kořisti; (3) celá černá kořist; (4) iridescentně-černá kořist s načerno zabarvenými oranžovými částmi a (5) oranžovo-černá kořist s černě zabarvenými iridescentními skvrnami.

V prvním testu jsme prostřednictvím preferencí mezi dvěma přirozenými formami *T. diophthalmus* (iridescentní typ a oranžový typ) zjišťovali, zda má iridescentní zbarvení vliv na počáteční opatrnost sýkor koňader vůči tomuto neznámému typu kořisti. Zaznamenávali jsme pořadí, ve kterém bylo s jednotlivými formami ploštice manipulováno a také latence této manipulace. Porovnávali jsme chování naivních ručně odchovaných mláďat a reakce dospělých z přírody odchycených jedinců. Zjistili jsme, že v průběhu preferenčního testu ptáci přednostně napadali oranžový typ kořisti a vůči iridescentnímu typu projevovali průkazně vyšší míru počáteční opatrnosti jak mláďata, tak i dospělí jedinci. Dospělí jedinci měli navíc signifikantně delší latence útoku na iridescentní než na oranžovou formu kořisti. Určitá míra opatrnosti k iridescentní formě ploštice u mláďat se jeví jako vrozená, což je ale v rozporu s předchozími pracemi, ve kterých naivní mláďata žádnou vrozenou averzi vůči aposematické kořisti neprojevovala (Exnerová et al. 2007; Svádová et al. 2009; Hotová Svádová et al. 2013; ale Lindström et al. 1999b). Zdá se, že vrozená opatrnost u sýkor koňader je spíše otázkou možnosti výběru než projevu silné averze, a proto u nich můžeme vrozenou opatrnost pozorovat pouze v případě, má-li jedinec současně na výběr mezi několika alternativními typy kořisti, jako tomu bylo například v práci Lindström et al. (1999b). Dospělé sýkory v našem experimentu váhaly s výběrem kořisti celkově déle než naivní mláďata a také latence manipulace s iridescentní formou ploštice byly signifikantně delší než s oranžovou formou, přičemž obě dvě formy kořisti pro ně představovaly nový typ potravy. Možným vysvětlením by mohla být například vyšší míra neofobie a potravního konzervatizmu dospělých jedinců. To se však zdá být nepravděpodobné, protože v předchozí práci Exnerová et al. (2006) dospělé sýkory ochotně napadaly nové různě zbarvené formy aposematické kořisti. Zvýšená opatrnost dospělých sýkor koňader vůči iridescentní formě ploštice *T. diophthalmus* spíše ukazuje na schopnost z přírody odchycených jedinců generalizovat svoji zkušenost s červeno-černými aposematickými druhy mimetického komplexu středoevropských ploštic (Exnerová et al. 2006; Hotová Svádová et al. 2010).

Dále jsme u sýkor testovali průběh jejich averzivního učení. Ptáci byli rozděleni do tří skupin 1) odchycení dospělci, kteří se učili odmítat iridescentní formu ploštice *T. diophthalmus* 2) odchycení dospělci, kteří se učili odmítat její oranžovou formu a 3) naivní mláďata, která se stejně jako první skupina učila odmítat iridescentní formu ploštice. Sýkorám byla nejedlá varianta kořisti (iridescentní nebo oranžová forma) předkládána v sekvenci s kontrolní jedlou načerno nabarvenou variantou této ploštice.

U dospělých sýkor koňader se ani jeden typ kořisti (iridescentní vs. oranžový) neukázal jako efektivnější podnět pro averzivní učení. Latence manipulace s oranžovou ploštticí se však na rozdíl od latencí manipulace s iridescentní ploštticí v průběhu jednotlivých kol averzivního učení prodlužovaly. Vzrůst latencí napadení oranžových ploštic mohl být ovlivněn celkově menší počáteční opatrností k oranžové formě ploštice. Otázkou zůstává, jestli by delší sekvence averzivního učení vedla i nadále k zvyšujícím se latencím manipulace s nepoživatelnou formou ploštice až do úplného odmítání této kořisti nebo by bylo zapotřebí použití silnější chemické ochrany, přestože přirozená chemická ochrana *T. diophthalmus* byla popsána spíše jako slabá (Staddon et al. 1987). Stejně jako u dospělých jedinců ani u naivních mláďat nedocházelo v průběhu učení k výraznějšímu odmítání iridescentní kořisti. Mláďata však váhala signifikantně déle při napadání iridescentní kořisti než při napadání její černě zbarvené jedlé varianty. Takové chování svědčí pro diskriminační učení mezi jedlým a nejedlým typem kořisti u naivních mláďat sýkor koňader.

Abychom dokázali zjistit, jak dobře se ptáci naučili rozlišovat mezi jedlou a nejedlou variantou kořisti, předložili jsme jim následně oba dva typy kořisti současně. Zaznamenávali jsme pořadí, v jakém byly jednotlivé typy kořisti napadeny. Všechny experimentální skupiny sýkor signifikantně více preferovaly jedlou černě zbarvenou plošttici nad její iridescentní/oranžovou variantou. Z výsledků tedy vyplývá, že proces učení u ptáků probíhá, přestože při sekvenčním uspořádání předkládání kořisti nemusí být zcela zřejmý. Zdá se tedy, že pro přežití kořisti s málo efektivní chemickou obranou je současná přítomnost alternativního typu kořisti velmi důležitá.

Nakonec byla u všech skupin ptáků testována schopnost generalizace jejich dosavadní zkušenosti na pět různě zbarvených variant ploštice *T. diophthalmus*: 1) iridescentní 2) oranžová 3) černá 4) iridescentně-černá a 5) oranžovo-černá. Všechny typy kořisti byly ptákům předloženy v jejich jedlé variantě a současně. Zaznamenávali jsme latenci a pořadí jejich napadení. Chování sýkor v průběhu generalizačního testu ukázalo, jakým způsobem se může iridescence uplatňovat při výstražné signalizaci aposematické kořisti. Bez ohledu na to, jakou barevnou variantu se dospělé sýkory učily odmítat, oranžovo-černá ploštice pro ně představovala vysoce averzivní podnět. Iridescentní plošttici se pak vyhýbali i ptáci, kteří byli učeni odmítat oranžovou plošttici a s iridescentní formou měli pouze pozitivní zkušenost z preferenčního testu. Naopak pro ptáky, kteří byli učeni odmítat iridescentní formu, zůstala oranžová kořist tou nejatraktivnější (dokonce více než černá kontrolní kořist z testu na averzivní učení). Ačkoliv je vzor australské ploštice *T. diophthalmus* značně odlišný od vzoru mimetického komplexu středoevropských červeno-černých ploštic (Exnerová et al. 2008), kontrastní vzor oranžové a černé barvy zřejmě způsobil vysokou míru averze u ptáků, kteří mají zkušenost s aposematickými červeno-černými ploštticemi z přírody.

Naproti tomu naivní mláďata, která se učila odmítat nejedlou iridescentní formu ploštice, se jí také v generalizačním testu nejvíce vyhýbala. Iridescentně-černá forma se ukázala jako druhá nejvíce averzivní, což naznačuje, že naivní mláďata si iridescenci spojila s nepoživatelností kořisti a tudíž by iridescence samotná mohla fungovat jako aposematický signál. Pro naivní mláďata byla překvapivě druhou nejvyhledávanější kořistí oranžovo-černá forma ploštice (hned po černé kontrolní kořisti z averzivního tréninku).

Celkově z naší studie Fabricant et al. (2014) vyplývá, že iridescentní zbarvení je u ploštic důležitou součástí jejich aposematické signalizace.

Přístup k nové a aposematické kořisti se u ptáků může v průběhu života měnit v závislosti na věku a získaných zkušenostech. Reakci vůči aposematické kořisti však může ovlivnit nejen přímo zkušenost s danou (nebo podobnou) kořistí, ale také prostředí, ve kterém zvíře žije, včetně rozmanité nabídky potravy a dalších podnětů, se kterými se v průběhu života setká.

Studie, která potvrdila vliv předchozí zkušenosti jako jednoho z faktorů, který ovlivňuje variabilitu v reakcích sýkor vůči aposematické kořisti, je **práce Exnerová et al. (2015)**. V této studii byly porovnány reakce dospělých odchycených sýkor koňader (*Parus major*) ze dvou vzdálených populací, které se lišily svou dosavadní zkušeností s aposematickou kořistí. Testovali jsme dospělé sýkory z finské populace, které mají díky svému geografickému výskytu pouze velmi omezené zkušenosti s výstražně zbarvenou kořistí a populaci sýkor koňader ze středních Čech, u kterých byla již opakovaně prokázána averze k aposematické kořisti díky jejich zkušenosti z přírody (Exnerová et al. 2003, 2006, 2007; Hotová Svádová et al. 2010). Abychom vyloučili vliv neofobie, testovali jsme obě populace sýkor se třemi typy kořisti: 1) pro obě populace novou palatabilní kořistí, larvou banánového cvrčka (*Gryllus asimillis*) s připevněným modrým štítkem na jeho dorsální straně 2) pro obě populace novou nepalatabilní kořistí, nahnědo nabarvenou ruměnicí pospolnou (*Pyrrhocoris apterus*) a 3) s aposematickou kořistí, přirozeně červeno-černě zbarvenou ruměnicí (*P. apterus*). Z našich výsledků vyplývá, že obě populace sýkor koňader napadaly stejně ochotně novou kořist ve formě modrého cvrčka i nahnědo nabarvené ploštice. Rozdílné chování jsme však zaznamenali v reakcích sýkor na aposematickou červeno-černou ruměnici. Zatímco sýkory koňadry z české populace tento typ kořisti opakovaně odmítaly, sýkory ze středního Finska ruměnici ochotně napadaly a v některých případech také konzumovaly. Z našeho experimentu tedy vyplývá, že rozdíl v přístupu k aposematické ploštici u dvou vzdálených populací sýkor koňader neodráží celková míra jejich neofobie, ale jejich dosavadní zkušenost s výstražně zbarvenou kořistí z přírody. Celkově tedy můžeme shrnout, že dosavadní zkušenost je jedním z nejdůležitějších faktorů, které ovlivňují reakce sýkor na aposematickou kořist.

Nejen zkušenost s konkrétními typy potravy, ale také absence dostatečného množství různorodých podnětů, se kterými se ptáci v průběhu svého života setkají, může způsobovat rozdílný přístup sýkor koňader k aposematické kořisti. Stejně jako dospělé sýkory koňadry pocházející z finské populace, ani ručně odchované dospělé sýkory koňadry v práci **Adamová-Ježová et al. (in prep.)** neměly žádnou dosavadní zkušenost s aposematickou kořistí. Porovnáme-li však velmi rozdílné reakce těchto dospělých sýkor žijících v rozdílných podmínkách (finská populace z přírody vs. holandská populace v chovech), je zřejmé, že jednou z možných příčin silné vrozené opatrnosti dospělých ručně odchovaných sýkor koňader může být právě jejich chovu v laboratotních podmínkách. Několik studií potvrzuje, že prostředí, ve kterém byli ptáci vychováni, následně ovlivnilo jejich budoucí přístup k novým podnětům (Jones 1986; Meehan & Mench 2002; Fox & Millam 2004, 2007). Například u kuřat (*Gallus gallus domestica*) a amazoňanů oranžovokřídlých (*Amazona amazonica*) obohacené životní prostředí v chovech signifikantně snížilo strach z nových objektů a u kuřat se díky komplexnějším životním podmínkám, obohaceným o množství různorodých předmětů a barevných obrazců na zdech jejich ubikací, zvýšilo také přijetí nové potravy do jejich jídelníčku (Jones 1986; Meehan & Mench 2002; Fox & Millam 2004, 2007). Ke snížení neofobie vůči neznámé potravě a její následné konzumaci přispívá také předchozí pozitivní zkušenost s různou potravou (Jones 1986; Marples et al. 2007; Leculle et al. 2011; Adamová-Ježová et al. submit.). Z naší předchozí studie (Adamová-Ježová et al. submit.), která se zabývá vrozenou opatrností tří druhů sýkor k nové a aposematické kořisti vyplývá, že u naivních ručně odchovaných sýkor koňader snižuje předchozí pozitivní zkušenost s potravou červené barvy neofobii k aposematické kořisti a následně zvyšuje také ochotu k její konzumaci. Protože u většiny ptáků z holandské v laboratorních podmínkách chované populace ze studie Adamová-Ježová et al. (in prep.) nedošlo k taktilnímu kontaktu s předloženou ploštici, nemůžeme jednoznačně stanovit, zda je u nich příčinou averze k neznámé kořisti silná míra neofobie nebo určitá forma konzervatizmu. Podle Marples & Kelly (1999) je neofobie k potravě popisována jako krátkodobý proces, který trvá pouze několik minut či několik předložení. To však neodpovídá relativně dlouhému a opakovanému odmítání nové kořisti u námi testovaných sýkor. Můžeme se tedy domnívat, že životní podmínky v chovech, které ptákům přinášejí pouze omezenou škálu nových podnětů, nedostatek různorodých zdrojů potravy a neomezenou potravní nabídku, by mohly být příčinou jejich neochoty prozkoumávat nové podněty. Fixace na známou potravu, která je dána opakovanou pozitivní zkušeností s ní a zároveň nedostatek zkušeností s potravou jinou, stejně jako absence zkušenosti s jejím nedostatkem by mohlo být u testovaných sýkor koňader příčinou jejich odmítavého přístupu k novým typům potravy.

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European birds and aposematic Heteroptera: review of comparative experiments

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Souhlasím, aby společně dosažené výsledky byly použity v její disertační práci.

Alice Exner

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European birds and aposematic Heteroptera: review of comparative experiments

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Abstract

The efficiency of defensive mechanisms in 11 European aposematic species of Heteroptera against various passerine predators was analysed. Bird species differed in their reactions to aposematic preys: small insectivorous birds generally avoided aposematic bugs, but granivorous birds as well as large insectivorous birds frequently attacked them. The ability to overcome heteropteran chemical defences appears to be connected with the larger body size of birds and with their food-storing behaviour. From the bird's point of view, various red-and-black aposematic species of Heteroptera form a mimetic complex. However, antipredatory defence properties of individual species differ substantially in their efficiency against bird predators, and the nature of the mimetic complex is rather quasi-Batesian than Müllerian.

Key words: antipredatory defences, warning signals, Heteroptera Pentatomomorpha, Passeriformes.

Introduction

Aposematism is a type of antipredatory strategy, when the prey signals its own unprofitability by a signal understandable to predators (Ruxton *et al.*, 2004). There is considerable evidence that prey defences as well as warning signals may be multimodal, i.e. may consist of visual, behavioural, acoustic, olfactory and gustatory components. Two or more warning signals either reinforce themselves or act synergistically, and the effect may lead to predator's unlearned avoidance or phobia, may accelerate avoidance learning, or enhance memorability of the warning signal (Rowe and Guilford, 1999; Ruxton *et al.*, 2004). With few exceptions (Marples *et al.*, 1994) the evidence is based mainly on experiments with artificial prey items and model predator species (usually domestic chicks). Therefore, the function of multimodal warning signals and defences of real prey species against a variety of their natural predators remains largely unknown.

The Heteroptera possess multimodal antipredatory defences whose main components are (a) visual signals (warning coloration or cryptic coloration), (b) acoustic signals (warning stridulation), and (c) allelochemicals (signalling the unpalatability, or directly repellent to toxic; synthesized by exocrine glands or taken over from hostplants and sequestered). This complex array of antipredatory defences makes the Heteroptera an excellent model group for studying aposematism and mimicry. Our studies were focused mainly on following problems (1) universality of warning function of aposematic signals against different avian predators, (2) abilities of predators to overcome bug defence system, (3) comparison of the efficiency of defensive mechanisms of various heteropteran species, (4) role of individual species in the potential mimetic complex of red-and-black pentatomomorphan true bugs, (5) importance of various components of heteropteran warning signals for learning and discrimination in bird predators.

Materials and methods

Heteroptera

We tested the reactions of birds to adults of the following species: *Pyrrhocoris apterus* (L.), its white, yellow, and orange mutants, and brown-painted individuals; *Pyrrhocoris marginatus* (Kolenati); *Scantius aegyptius* (L.); *Lygaeus equestris* (L.)/*simulans* Deckert; *Spilostethus saxatilis* (Scopoli); *Tropidothorax leucopterus* (Goeze); *Horvathiolus superbus* (Pollich); *Corizus hyoscyami* (L.); *Graphosoma lineatum* (L.); *Eurydema oleraceum* (L.); *Eurydema ornatum* (L.). We included ladybirds *Coccinella septempunctata* L. and *Propylaea quatuordecimpunctata* (L.), and froghopper *Cercopis vulnerata* Rossi as possible non-heteropteran members of mimetic complexes.

Birds

Wild-caught passerine birds of the following species were tested: (1) mainly insectivorous species - *Turdus merula* L., *Erithacus rubecula* (L.), *Phoenicurus ochruros* (S. G. Gmelin), *Parus major* L., *Cyanistes caeruleus* (L.), *Periparus ater* (L.), *Lophophanes cristatus* (L.), *Poecile montanus* (Conrad), *Poecile palustris* (L.), *Aegithalos caudatus* (L.), *Sitta europaea* L., *Sylvia atricapilla* (L.); (2) partly granivorous species - *Passer montanus* (L.), *Passer domesticus* (L.), *Fringilla coelebs* L., *Carduelis chloris* (L.), and *Emberiza citrinella* L.. Hand-raised great tits (*P. major*) were used as naive predators in learning, memory, and discrimination experiments.

Experimental set-up

Experiments were carried out in the cage equipped with one-way glass, perch, and rotating feeding tray. Cage illumination simulated the full daylight spectrum. Bird's behaviour was scored as a continuous record in Observer Video-Pro (Noldus) and recorded by videocamera. Each bird was deprived of food about two hours before starting the experiment. Experiment consisted of a sequence of several consecutive five-minute trials, during which the birds were offered individual bugs. The trials followed one after another and alternated with presentations of a standard prey, *Tenebrio molitor* L. larvae, to check bird's foraging motivation.

In the experiments with wild-caught birds, the sequence consisted of 5 trials with heteropterans. In experiments focused on learning in naive hand-reared birds, the sequence of five-minute trials continued until the birds reached the learning criterion. Memory or discrimination tests were performed next day after the learning session. The birds were subjected either to the sequence of trials with the same prey they learned to avoid, or with another prey, or to the preference test with several different types of prey.

Results and discussion

Universality of warning function of aposematic signals against different predators

Warning coloration of a certain species of Heteroptera does not have a universal function among passerine predators (Exnerová *et al.*, 2003). Smaller and insectivorous birds (chats, warblers and tits) generally avoided aposematic bugs, but granivorous birds (finches and buntings) as well as larger insectivorous birds (blackbirds and nuthatches) frequently attacked them. Even closely related bird species (family Paridae) can differ in the way they acquire the avoidance; it appears to be innate in some species and learned in others (Exnerová *et al.*, 2007). Surprisingly, firebugs (*P. apterus*) were avoided also by tree sparrows (*P. montanus*), which readily attacked and consumed unpalatable and poisonous ladybirds (*C. septempunctata*) during the experiments.

Abilities of predators to overcome bug defence system

Generally, it appears that body size (weight) of the bird affects its cautiousness in encounters with potentially noxious aposematic bugs. Out of nine passerine species tested with *P. apterus*, the smallest species (*A. caudatus* and *C. caeruleus*) were most cautious and the largest species (*T. merula*) most prone to attacking and consuming the firebugs (Exnerová *et al.*, 2003). Similar trend was observed in the experiments with *L. equestris/simulans*, *S. saxatilis*, and *G. lineatum*. Some predators may possess physiological or behavioural adaptations to overcome the prey defences (Yosef and Whitman, 1992). Nuthatches (*S. europaea*) and crested tits (*L. cristatus*) frequently used slits in the experimental cage for storing the bugs (*P. apterus*), then checked them time from time, and eventually consumed them, after the repellent secretion vanished. Both species store the food regularly; food-storing behaviour seems to be a general exaptation for consuming noxious prey.

Importance of heteropteran visual warning signals for learning and discrimination in bird predators

The colour is especially important cue for passerine predators among the various components of the visual warning signal of the true bugs. Majority of wild-caught birds (*P. major, C. caeruleus, E. rubecula, S. atricapilla*) experienced with red-and-black wild type of *P. apterus*, did not recognize its yellow and white colour mutants as the same prey, even though they had the same shape, size, and black pattern (Exnerová *et al.*, 2006). Similarly, naive great tits (*P. major*) primarily attended to colour and not pattern, when they learned to avoid *P. apterus* and its colour mutants. Moreover, their ability to generalize among various aposematic colours seems to be limited – birds generalized their experience with yellow form to the red one but not in the opposite direction. On the contrary, typical warning coloration (red-and-black) did not accelerate the avoidance learning when compared with the non-aposematic (uniformly brown) coloration. However, great tits remembered their experience with red-and-black *P. apterus* even after three months, while their memory for brown-painted form vanished.

Comparison of the efficiency of defensive mechanisms of various species of Heteroptera

Effectiveness of defensive mechanisms was assessed in the experiments with naive great tits (*P. major*), in which the rate of avoidance learning and memory for the experience with various species of Heteroptera was measured. *L. equestris/simulans* and *S. saxatilis* were strongly defended, the birds usually avoided them after one or two encounters, and even the attacked bugs usually survived. *G. lineatum* appeared similarly well defended, and was protected also by its strongly sclerotised cuticle. *P. apterus* was rather weakly defended; the birds usually took several encounters to develop the avoidance reaction and the encounters were usually lethal for the bug. *Eurydema* species seemed to be weakly defended, and they are probably quasi-Batesian mimics of other heteropterans and ladybirds; this hypothesis may explain their otherwise surprising colour polymorphism.

Role of individual species in the potential mimetic complex of red-and-black pentatomomorphan true bugs

Majority of red-and-black Pentatomomorpha from Central Europe form a mimetic complex. Wild-caught great tits (with a few exceptions) avoided all the heteropterans studied. Similarly, naive great tits generalized their experience with one of the red-and-black species to any other one, even if it was of quite different appearance. Nevertheless, protection of the individual species does not fit the simple Müllerian concept of all the species sharing the costs and benefits equally. Individual species play rather different roles in the complex: *L. eguestris/simulans* and *S. saxatilis* being effective models, and *P. apterus* rather quasi-Batesian mimic. The complex includes also non-heteropteran members – *C. septempunctata* and *C. vulnerata*, which both can play a role of models.

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Different reactions to aposematic prey in 2 geographically distant populations of great tits

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Original Article

Different reactions to aposematic prey in 2 geographically distant populations of great tits

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Variation in predator behavior toward aposematic prey was frequently studied at interspecific and individual levels, but interpopulation differences have been neglected. Geographic differences in prey fauna offer an opportunity to test their implications for predator behavior. It can be expected that 1) predator populations inhabiting environments with high diversity of aposematic prey are more neophobic than those living in areas where aposematic prey are scarce, and 2) different levels of neophobia jointly with avoidance learning affect selection on aposematic prey. We compared the behavior of wild-caught great tits (*Parus major*) from Bohemia and Central Finland toward aposematic firebugs (*Pyrrhocoris apterus*), nonaposematic firebugs, novel objects and novel palatable nonaposematic prey. Finnish and Bohemian birds did not differ in their novel-object exploration, but Finnish birds hesitated longer than Bohemian birds before resuming feeding next to a novel object. Latencies to attack novel palatable prey did not differ and were not correlated with the attitude toward novel objects. Tits from the Bohemian population mostly avoided aposematic firebugs and attacked nonaposematic ones. Finnish birds were more likely to attack both firebug color forms, and their attack latencies were correlated with latencies of attacking novel palatable prey. Thus, Bohemian birds avoided the aposematic prey, but were not more neophobic than Finnish birds. These results suggest that differences between Finnish and Bohemian birds in behavior to aposematic prey do not follow differences in exploration strategy and neophobia. The observed differences can be explained by a different experience with local aposematic prey communities.

Key words: **aposematism, exploration, geographic differences, neophobia, *Parus major, Pyrrhocoris apterus*.**

INTRODUCTION

Avoidance of aposematic prey usually involves several cognitive mechanisms that affect the behavior of predators (Ruxton et al. 2004). Reaction toward prey may be influenced by neophobia (e.g., Coppinger 1969, 1970; Exnerová et al. 2010) or dietary conservatism (Marples et al. 1998; Marples and Kelly 1999), and by inherited (Smith 1975; Lindström et al. 1999a) or learned (e.g., Lindström et al. 1999b; Exnerová et al. 2007; Aronsson and Gamberale-Stille 2008; Barnett et al. 2012) aversions against certain warning signals and their combinations (Marples and Roper 1996; Rowe and Guilford 1996; Lindström et al. 2001). The response of a predator to warning signals is affected by associative learning, the degree of which is influenced by memorability, prey recognition, discrimination, and generalization (Roper and Redston 1987; Gamberale-Stille and Tullberg 1999; Speed 2000; Ham et al. 2006; Svádová et al. 2009). Given the complexity of the cognitive processes, which contribute to the formation of the avoidance of aposematic prey, it is not surprising that there exists a considerable variation in behavior of different predators toward a defended prey species (Brower 1988; Exnerová et al. 2003; Endler and Mappes 2004; Valkonen et al. 2012; Nokelainen et al. 2014).

Predators from different taxa may react differently to a particular prey species, and several mechanisms have been discussed as potential factors responsible for the variation: the energetic requirements of a predator (i.e., body size or hunger level) are important in determining whether or not the predator decides to attack and consume a defended prey (Exnerová et al. 2003; Barnett et al. 2007; Halpin et al. 2014). Also, sensory and cognitive abilities of predators can be highly variable (Hart 2001; Sol et al. 2005) influencing

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their ability to learn to avoid aposematic prey (Endler and Mappes 2004). Closely related species also frequently differ in their reactions to novel environments, objects and food; the neophobia level may be correlated with the degree of habitat and foraging specialization (Greenberg 1989; Mettke-Hofmann et al. 2009, 2012; Tebbich et al. 2009).

Variation in reactions to aposematic prey also exists among conspecific predators, where the initial wariness and rate of avoidance learning may be correlated with personality traits (Exnerová et al. 2010). On the other hand, the rate of incorporation of a novel food item into an individual's diet represents another process: dietary conservatism (Marples et al. 1998; Marples and Kelly 1999), which is independent of personality and not correlated with neophobia (Marples and Mappes 2011). The behavior of predators toward aposematic prey may also be correlated with their age (Lindström et al. 1999a; Exnerová et al. 2006; Langham 2006; Mappes et al. 2014), and due to the importance of learning also highly affected by individual experience (Exnerová et al. 2007; Ihalainen et al. 2008; Barnett et al. 2012; Hotová Svádová et al. 2013).

In contrast to interspecific and individual differences in behavior toward aposematic prey, the potential differences between conspecific populations, namely those living in geographically distant areas and different habitats, have not been studied. Despite being interesting per se, the knowledge of potential geographical differences may be important for the generalization of results based on studies of different populations of a particular species of predator. Individuals from geographically distant populations may react differently to aposematic prey simply because of different individual experience with local aposematic prey. Alternatively, the behavior toward aposematic prey may reflect population-specific differences in neophobia (see Liebl and Martin 2014) and exploration strategies evolved for living in different conditions such as prey diversity and frequency of noxious prey. Individuals from populations living in different conditions (e.g., different predation pressure and environment stability) differ in their exploration of a novel environment, and their reactions to novel objects and novel food (Martin and Fitzgerald 2005; Brydges et al. 2008; Echeverría and Vassallo 2008; Korsten et al. 2010; Liebl and Martin 2014). Likewise, individuals from migratory populations may be more neophobic than their resident conspecifics (Mettke-Hofmann et al. 2013).

In this study, we investigated geographical differences in response to novel stimuli and reaction to aposematic prey in the great tit (*Parus major* L., 1758), a small passerine which is mainly insectivorous during spring and summer, although in autumn and winter, when insect prey become scarcer, adds berries and seeds to its diet (Cramp and Perrins 1993). The great tit is a resident species inhabiting a wide range of woodland habitats in the Palaearctic region, and its distribution covers the whole Europe including the far North (Cramp and Perrins 1993). In recent years, the great tit has become a model species in studies on aposematism and mimicry. Because such studies are based on experiments with birds from various localities across Europe (e.g., Sillén-Tullberg 1985; Lindström et al. 1999a, 1999b; Exnerová et al. 2006), it is worth testing whether birds from different populations behave in the same way. As a model aposematic prey we used the firebug (*Pyrrhocoris apterus*), which is conspicuously red-and-black colored and unpalatable for small passerine birds (Exnerová et al. 2003). The firebug is widespread in the Palaearctic, but it is absent in Britain and most of Northern Europe.

By comparing the behavior of wild-caught great tits from 2 geographically distant areas, Bohemia and Central Finland, we tested the following hypotheses concerning the reaction of these birds toward aposematic prey: 1) Differences in behavior toward aposematic firebugs follow differences between the 2 populations in their behavior toward novel palatable prey and other types of novel objects, that is, they reflect the levels of individual exploration and neophobia. 2) The 2 populations exhibit specific differences concerning the aposematic firebugs. The Finnish birds are expected to be more willing to attack aposematic firebugs because of their lack of experience with this type of the aposematic prey in their natural environment. 3) Birds from both populations avoid attacking firebugs regardless of their experience. This may happen if the avoidance of aposematic prey has a strong genetic basis or the avoidance learning of prey with a given warning signal is generalized to other prey whose signal is similar enough. Because the local diversity of aposematic prey is important for the interpretation of the behavior of birds, we also analyzed data on the distribution of aposematic and nonaposematic species of Heteroptera between the 2 compared areas.

MATERIALS AND METHODS

Experiments were carried out at Konnevesi Research Station, University of Jyväskylä (Central Finland) and in Prague at the Faculty of Science, Charles University (Bohemia) during autumn 2012. In order to standardize the phenological differences between the 2 localities, the experiments were conducted during October in Central Finland and during November in Bohemia.

Comparison of occurrence of aposematic species of Heteroptera in Central Europe and Central Finland

In order to compare the composition of the fauna of true bugs from both areas, we gathered data on the occurrence of heteropterans, particularly of the aposematic species, in Central Europe and Central Finland. Data for Central Europe were obtained combining the whole heteropteran faunas of Germany (species included in Wachmann et al. 2004, 2006, 2007, 2008) and Czech Republic (Štys P, unpublished data). As for Central Finland, we covered species occurring in Finland at the latitude of Konnevesi (62°38′N), or likely to occur there (known to occur slightly more to the South or to the North), as shown by distributional maps (Rintala and Rinne 2010). Purely coastal species were excluded. Taxonomically, we considered only the terrestrial Heteroptera (Leptopodomorpha, Cimicomorpha, and Pentatomomorpha *s. lat.*). In their adult stage, the dorsum of those species regarded as aposematic is uniformly colored with bright white, yellow, orange or red, or with a combination of any of those and a contrasting dark pattern. For the purpose of this analysis, we also classified as aposematic those species having nonaposematic morphs as well. The dull whitish, yellowish to reddish taxa/morphs were not taken as aposematic (e.g., some Miridae: Phylinae). All terrestrial true bugs are known to have a chemical defense (Schuh and Slater 1995).

Predators

Altogether we tested 100 wild-caught great tits, 50 from each of 2 geographically distant populations: 1) Konnevesi in Central Finland (62°38′N, 26°19′E) and 2) Prague in Bohemia (Czech Republic, 50°04′N, 14°26′E). The words "Finnish" and "Finland," whenever used without qualification in the text, always refer to Central Finland at about Konnevesi latitude. The sex and age of both experimental groups were balanced (Central Finland: 30 males and

20 females, 19 yearlings and 31 adults; Bohemia: 29 males and 21 females, 17 yearlings and 33 adults). The birds from both populations had similar body weight (Bohemian birds—mean 16.5 g ± 0.9 g, $N = 50$; Finnish birds—mean 17.1 g ± 1.0 g, $N = 50$).

The habitats around Konnevesi include mainly sparse mixed forest with a low undergrowth of moss, grasses and sedges, herbaceous plants and shrubs; the forest is interspersed with large clearings including also buildings and human settlements; a brook and meadows along lake sides are present as well. The Prague locality consists mainly of large city parks with coniferous and broad-leaved trees; the parks are surrounded by roads and residential houses with small patches of ruderal vegetation interspersed. The major park is an old botanical garden, which represents various Bohemian habitats, and also includes several pools and a small brook. The birds were trapped in the autumn, when overwintering birds typically move around (Cepák et al. 2008). Thus, birds in each locality were likely to be coming from surrounding areas as well.

Birds were caught using food-baited traps (Central Finland, see Ham et al. 2006 for details) or mist nets placed near the feeders (Bohemia) during autumn 2012. They were housed individually in cages (50 cm × 40 cm × 50 cm in Prague; 65 cm × 50 cm × 80 cm in Konnevesi) under natural light conditions and were kept on a diet consisting of mealworms (larvae of *Tenebrio molitor* L., 1758), peanuts, sunflower seeds and water ad libitum. The birds were allowed to habituate to the laboratory conditions for 5–7 days before the experiment. Each bird was used only once in each experiment. After the experiment they were ringed individually and released in the locality of their capture.

Prey

As aposematic prey we used brachypterous adult firebugs [*Pyrrhocoris apterus* (L., 1758); Heteroptera], which possess a conspicuous red-and-black coloration. The species' defensive secretion from metathoracic glands containing mainly aldehydes (Farine et al. 1992) makes this insect distasteful for small passerine birds including great tits (Exnerová et al. 2003). The firebugs live on the ground and partly also on trees, and feed mainly on seeds of Malvaceae (herbaceous species and linden tree, *Tilia*) and locust tree, *Robinia pseudacacia* (Kristenová et al. 2011; Hotová Svádová et al. 2014). They are widespread in the Palaearctic but absent in most of Northern Europe; their range does not exceed the latitude of 60°N (Aukema and Rieger 2001; Rintala and Rinne 2010).

A nonaposematic variant of the firebugs lacking the red-and-black color pattern was obtained by painting their upper parts with dark brown watercolor dye and chalk. We used these color-manipulated bugs to test the specificity of birds' reaction toward the firebugs' warning coloration, as we needed prey that did not differ from the aposematic prey in any other trait (size, body shape, composition of defensive secretion, and so forth). The dye used to modify the visual part of the firebug warning signal was odorless and nontoxic, and the chemical defense of these artificially made nonaposematic firebugs was unchanged (see Exnerová et al. 2003). The firebugs were collected in Prague (Czech Republic). They were kept at a temperature of 24 ± 1 °C and a light: dark cycle of 16:8 h, reared on linden seeds (*Tilia cordata*) and provided with water ad libitum.

Mealworms (larvae of *Tenebrio molitor*) were used as a palatable control prey to check the foraging motivation of birds before starting a trial with experimental prey. We used nymphs of Jamaican field crickets [*Gryllus assimilis* (Fabricius, 1775)] carrying a bright-blue paper sticker attached to their dorsal side as a novel, edible, nonaposematic prey to test the level of birds' food-specific neophobia. The size of crickets offered in experiments matched the average size of tested firebugs (i.e., 10–12 mm). The sticker covered most of the cricket's dorsum, leaving its antennae and legs visible. In a preliminary experiment (involving 2 other groups of 20 wild-caught birds, both from Bohemia), great tits hesitated longer before attacking crickets with a blue sticker (mean 179.2 s ± 28.2, $N = 20$) than before attacking those without the sticker (mean 40.9 s ± 12.4, $N = 20$; Mann–Whitney U-test: $Z = -3.92$, $N = 40$, $P < 0.001$), which they attacked with similar latencies as familiar mealworms (mean 24.6 s ± 10.5; Wilcoxon matched pairs test: $Z = 1.49$, $N = 20$, $P = 0.135$). These results indicate that crickets with the blue sticker represent a stimulus sufficiently novel to increase attack latency.

Experimental design and equipment

Experiments were designed to compare the exploration behavior, levels of neophobia, and specific reactions to aposematic and novel palatable prey between 2 populations of great tits. Each bird was tested individually in 4 separate tests in the following order, which was identical for all the birds: 1) exploration test with a novel object, 2) neophobia test with a novel object placed near the food bowl, 3) test of reaction toward novel palatable prey, and 4) test of reaction toward aposematic firebug or its nonaposematic brown-painted variant. This way the recent aversive experience with firebugs did not affect the reactions of birds toward novel objects and palatable prey, and the order per se did not influence the comparison between populations.

Exploration and neophobia tests

To study exploration behavior and neophobia level we carried out 2 types of novel-object tests: 1) a novel object presented in a neutral location, and 2) a novel object attached to the food bowl. A novel object presented in a neutral location is frequently used to measure exploration behavior and neophilia, because the bird is not forced to approach the novel object, and when it does, it indicates its interest in exploring the object (e.g., Verbeek et al. 1994; Mettke-Hofmann et al. 2002; Drent et al. 2003). The presentation of a novel object close to the food bowl is regarded as a measure of neophobia, because it creates a conflict between foraging motivation and motivation to avoid a novel object; the bird has to overcome the neophobia to come close to the object and feed (Mettke-Hofmann et al. 2002; Feenders et al. 2011; Mettke-Hofmann 2012). Although exploration and neophobia may be correlated as they represent personality traits (van Oers et al. 2004), they are considered to be 2 distinct responses to novel stimuli (Greenberg and Mettke-Hofmann 2001).

In the exploration test, we used a bright-blue pen attached to one of the perches close to the front wall of the home cage. We measured the latency to peck at the novel object. The test lasted 10 min (maximum) and was terminated earlier if the bird pecked at the novel object. Food and water were freely available at all times.

In the neophobia test, we used a pink plastic clothes-peg attached to the food bowl placed on the home-cage floor. The birds were deprived of food for 1 h before the test to increase their foraging motivation. We recorded the latency to feed near the novel object. The test lasted 10 min and was terminated earlier if the bird started to feed in presence of the novel object. To control for potential differences in foraging motivation, we also carried out a control test under the same conditions but with the peg absent.

Novel and aposematic prey tests

Tests with novel palatable prey and firebugs were performed in experimental cages and followed one after another. Experimental cages used in Bohemia and in Central Finland were of similar size (70 cm × 70 cm × 70 cm in Bohemia and 50 cm × 50 cm × 70 cm in Central Finland); they were made of plywood and a wire mesh, and equipped with a perch and a water bowl. The front wall of cages used in Bohemia was made of 1-way glass; the birds in Central Finland were observed through a small mesh-covered window in the cage wall. Illumination of the cages simulated the natural daylight spectrum (including UV wavelengths). The tested prey was put into the cage in a glass Petri dish placed on a sliding food tray on the cage floor. Despite of difference in the cage sizes, the distance between the food tray and the closest perch was identical (35 cm). All the prey types appeared conspicuous on the light beige background of the plywood food tray. Prior to the experiments, the birds were habituated to the experimental cages, and they were deprived of food for 2 h to increase their foraging motivation. The experiments were video-recorded and the birds' behavior was continuously registered using Observer XT 8.0 software.

Novel palatable prey test

The novel, palatable, nonaposematic prey was represented by a Jamaican field cricket with a blue-colored paper sticker attached to its dorsal side. The experiment consisted of a sequence of 5-min (maximum duration) trials. At the beginning of the sequence, we offered the bird a mealworm as a control prey to check its foraging motivation. When the bird consumed a mealworm, it was offered a cricket in the subsequent trial. If the bird did not attack the cricket within the time limit, the sequence continued with another mealworm trial followed by another cricket trial up to a maximum of 3 cricket presentations. We measured the latency to attack (touch, peck, or seize) the cricket and recorded whether the cricket was killed and eaten.

Firebug test

The birds from both Bohemian and Finnish populations were subdivided into 2 experimental groups of 25 birds each, with similar proportion of yearlings and adults, and males and females. One group was tested with aposematic red-and-black firebugs, and the other one with manipulated (brown-painted), nonaposematic (but still unpalatable) firebugs.

The test consisted of a sequence of alternating trials in which the birds were presented either with a control prey (mealworms) as a check of foraging motivation or with a firebug. Each trial lasted 5 min at most, and was terminated earlier if the bird attacked the prey. The sequence always started with a mealworm trial. When the bird consumed a mealworm, it was offered a firebug in the subsequent trial. If the bird did not attack the firebug within the time limit, the sequence continued up to a maximum of 20 firebug presentations. We measured the latency to approach and attack (to touch, peck, or seize) the firebug and recorded whether it was killed, thrown away, or eaten. If the bird attacked one of the firebugs, we kept offering them (alternating with mealworms) until the bird left untouched 3 firebugs in a row, which was considered an avoidance-learning criterion.

Data analysis

A Cox hazard regression was used to analyze the differences between Bohemian and Finnish great tits in exploration behavior toward a novel object (the latency to peck at the blue pen) and in neophobia (the latency to feed in presence of a novel object, the pink clothes-peg). Locality of bird origin, and bird's sex and age were used as explanatory variables. In the analysis of neophobia, we included the control (peg absent) latencies in the model as a covariate. The model selection procedure started from the model including all possible 2-way interactions of locality, age and sex, and was subsequently simplified. Model selection was conducted in a hierarchical manner based on the significance of the terms in the model.

A Cox hazard regression was also used to analyze the behavior of birds to novel palatable prey (cricket with blue sticker), aposematic or nonaposematic firebugs, and control familiar prey (mealworm offered at the beginning of the experiment). As a response variable we used latency to attack each prey type (cricket, firebug, or mealworm). Locality, bird's sex and age, and in case of firebugs also their coloration (aposematic, nonaposematic), were used as explanatory variables. The model selection procedure was similar to that used for analyzing reaction to a novel object.

We computed Spearman rank correlations between latency to peck at the novel object in the exploration test, latency to feed in the presence of novel object in the neophobia test, and latencies to attack the novel palatable prey and the firebugs. To check whether the birds considered crickets with blue stickers a novel prey, we compared attack latencies between crickets and mealworms offered in the trial preceding the cricket test; the latencies were compared by Wilcoxon matched pairs test.

We used the number of attacked firebugs before a bird stopped attacking them as an indicator of learning. A generalized linear model with Poisson distribution was used to analyze the data. The model selection was based on significance of the terms in the model. Bird sex and age, as well as locality of bird origin and firebug coloration, and all possible 2-way interaction terms were used as explanatory variables. All analyses were conducted using R 2.11.1 and the lme4 package.

RESULTS

Comparison of occurrence of aposematic species of Heteroptera in Central Europe and Central Finland

Fauna of terrestrial Heteroptera of Central Europe comprises 922 species, 766 (83%) of which are nonaposematic, and 156 (17%) aposematic; in Central Finland 262 (90%) out of 292 species of Heteroptera are nonaposematic, whereas 30 (10%) are aposematic. Thus, the less speciose fauna of Central Finland (32% species as compared with Central Europe) has not only a smaller absolute number of aposematic species (19% as compared with Central Europe) but also the proportion of aposematic species in the fauna is 0.59 times smaller than it should be in accordance with an uniform decrease in species diversity with increasing latitude (chi square = 7.75, df = 1, and $P = 0.006$).

Exploration and neophobia

We fitted Cox hazard regression model to explain the variation in exploration of a novel object (blue pen). However, no significant differences between the Bohemian and Finnish birds were detected (Table 1, Figure 1). Most birds approached the object and pecked at it within the time limit. The average pecking latency was 325 ± 21.5 s ($N = 100$).

Table 1

Fitting Cox hazard regression describing exploration of a novel object (latency to peck at a blue pen) in Bohemian and Central Finnish great tits

Model	Term removed	df	*P*
Sex*age + age*locality + sex*locality	Sex*age	1	0.9036
Age*locality + sex*locality	Locality*age	1	0.5712
Age + locality + sex + sex*locality	Sex*locality	1	0.4664
Age + locality + sex	Age	1	0.5506
Locality + sex	Sex	1	0.6572
Locality		1	0.0897

When an interaction is indicated (*), the main effect of the term is also included in the model. Degrees of freedom and significances are given for the excluded term in the model.

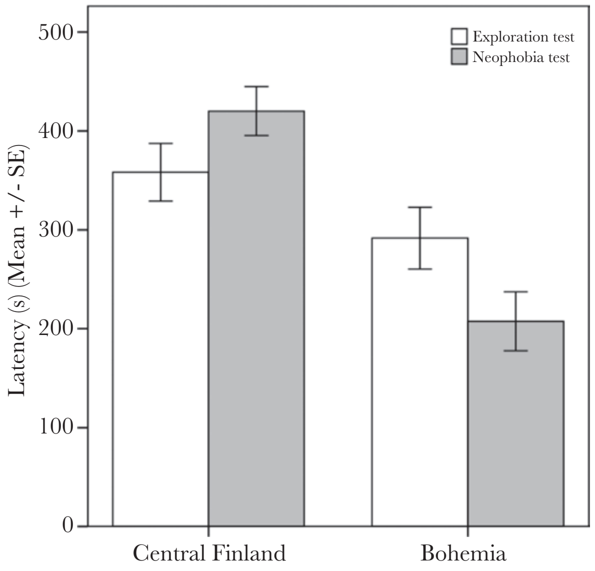

Figure 1

Latency of Bohemian and Central Finnish great tits to peck at a novel object (blue pen; exploration test; open bars) and their latency to start feeding after a novel object was attached to the food bowl (pink peg; neophobia test; gray bars).

Our final Cox hazard regression model explaining the birds' latency to feed in proximity of a novel object (pink peg) included locality, bird age, and the interaction of both (Figure 1, Table 2). In general, Finnish birds hesitated longer compared with those from Bohemia. Among all the birds, Bohemian adults were the least neophobic, which caused the effect of the interaction between locality and age of birds (Figure 1, Table 2).

Latencies measured in exploration and neophobia tests were significantly correlated in birds from both populations (Finnish birds: $r_s = 0.60$, $t = 5.15$, df = 48, and $P < 0.05$; Bohemian birds: $r_s = 0.29$, $t = 2.10$, df = 48, and $P < 0.05$).

Reaction to novel palatable prey

Most birds attacked the novel palatable prey (blue cricket) in the first or second trial (mean 357 ± 37.2 s, $N = 100$). The birds from both localities hesitated longer before attacking the blue crickets than before attacking familiar mealworms (Wilcoxon matched pairs test: Finnish birds, $Z = 5.56$, $N = 50$, and $P < 0.01$; Bohemian birds, $Z = 5.23$, $N = 50$, and $P < 0.01$).

Table 2

Cox regression model explaining latency of Bohemian and Central Finnish great tits to start feeding after a novel object was attached to the food bowl (pink peg; neophobia test)

Source	Coef.	SE	*z*	*P*
Locality (Bohemia)	1.336	0.293	4.565	<0.001
Age (juv.)	−0.039	0.377	−0.103	0.918
Sex (males)	−0.002	0.998	−0.010	0.992
Locality (Bohemia): age (juv.)	−0.950	0.519	−1.827	0.068

Interaction terms of the sex and age, and the locality and sex were removed from the model because they were not significant ($z < \pm 1$, $P > 0.36$).

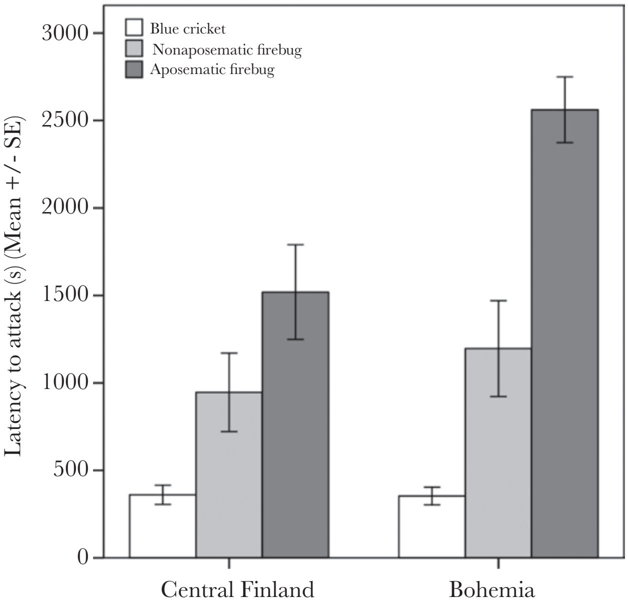

Figure 2

Latency of Bohemian and Central Finnish great tits to attack a novel prey (cricket with a blue sticker; open bars), nonaposematic firebug (brown-painted; light gray bars), and aposematic red-and-black firebug (wild-type; dark gray bars).

No significant effect of locality of bird origin was found explaining the variation in attack latencies (Figure 2, Table 3). However, nonsignificant trend for adult birds to be less hesitant than yearlings was detected (Table 3). We did not find a significant effect explaining the variation in latency to attack the mealworm offered just before the novel-prey test either (all *P* values of main effects (locality, age, sex) and their interactions were > 0.230); this indicates that all the birds entered the test with similar foraging motivation.

Attack latencies did not correlate with the latencies measured in exploration (Finnish birds: $r_s = 0.04$, $t = 0.27$, df = 48, NS; Bohemian birds: $r_s = 0.02$, $t = 0.12$, df = 48, NS) and neophobia (Finnish birds: $r_s = 0.06$, $t = 0.40$, df = 48, NS; Bohemian birds: $r_s = 0.16$, $t = 1.10$, df = 48, NS) tests.

Reaction to aposematic and nonaposematic firebugs

A similar proportion of Finnish and Bohemian birds attacked nonaposematic firebugs (chi square = 1.75, df = 1, and $P = 0.185$). Aposematic firebugs were mostly attacked by Finnish birds, and mostly avoided by Bohemian birds (chi square = 8.33, df = 1, and $P = 0.004$).

Table 3

Fitting Cox hazard regression describing latency of Bohemian and Central Finnish great tits to attack a novel palatable prey (blue cricket)

Model	Term removed	df	*P*
Sex * age + age * locality + sex * locality	Age * locality	1	0.9049
Sex * age + sex * locality	Sex * locality	1	0.3953
Age + sex + locality + sex * age	Locality	1	0.9588
Age + sex + sex * age	Sex * age	1	0.2273
Age + sex	Sex	1	0.3675
Age		1	0.1283

When an interaction is indicated (*), the main effect of the term is also included in the model. Degrees of freedom and significances are given for the excluded term in the model.

Table 4

Model fitting of Poisson GLM explaining the number of firebugs (either aposematic or nonaposematic) attacked by Bohemian and Central Finnish great tits

Model	Term removed	df	*P*
Locality * sex + locality * age + locality * color + age * color + sex * color	Age * color	1	0.9811
Locality * sex + locality * age + age * color + sex * color	Locality * age	1	0.8621
Locality * sex + age * color + sex * color	Locality * sex	1	0.8452
Age + locality + sex + color + locality * color + sex * color	Age	1	0.5427
Locality + sex + color + locality * color + sex * color	None		

If interaction is indicated (*), the main effect of the term is also included in the model. Degrees of freedom and significances are given for the excluded term in the model. Our final model (see details in Table 5) is highlighted in bold.

Cox hazard regression model explaining the variation in latencies to attack the firebugs revealed a significant interaction between firebug coloration and the locality of bird origin (chi square = 3.95, df = 1, and $P = 0.047$). Other terms included in the model were: locality, age and sex of the bird, and firebug coloration. Bohemian birds were more hesitant to attack aposematic firebugs than Finnish birds, but there was no difference in latencies to attack nonaposematic firebugs (Figure 2).

In Finnish birds, the latencies to attack both aposematic and nonaposematic firebugs correlated with the latencies to attack novel palatable prey (aposematic bugs: $r_s = 0.46$, $t = 2.46$, df = 23, and $P < 0.05$; nonaposematic bugs: $r_s = 0.61$, $t = 3.65$, df = 23, and $P < 0.05$); for Bohemian birds no such correlation was found (aposematic bugs: $r_s = 0.10$, $t = 0.49$, df = 23, NS; nonaposematic bugs: $r_s = 0.26$, $t = 1.27$, df = 23, NS).

General linear model explaining the number of attacked firebugs comprised locality of bird origin, bird sex, and firebug coloration and the interactions between locality and firebug coloration, and bird sex and firebug coloration (Table 4, Figure 3). The significant interaction between firebug coloration and locality arose from the fact that Bohemian birds attacked more nonaposematic firebugs than aposematic ones, whereas Finnish birds attacked similar numbers of both color forms (Table 5, Figure 3). Interestingly, in both localities, males tended to attack more aposematic firebugs than females. Individual females usually did not attack more than a single firebug (mean 1.2 ± 0.6, $N = 41$), whereas the males frequently attacked 2 and more (up to 4) individuals (mean 1.6 ± 0.7, $N = 59$).

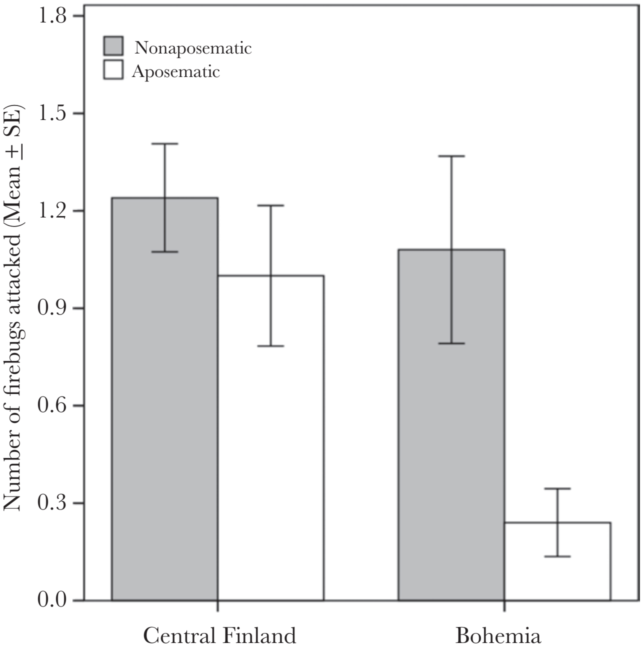

Figure 3

Difference between Bohemian and Central Finnish great tits in the number of nonaposematic and aposematic firebugs attacked.

Table 5

Poisson GLM explaining number of firebugs attacked by Bohemian and Central Finnish great tits

Source	Estimate	SE	*z*	*P*
Intercept	0.3400	0.2228	1.526	0.1270
Locality	−0.1476	0.2635	−0.560	0.5753
Sex	−0.2353	0.2630	−0.895	0.3710
Color	−0.9606	0.4733	−2.029	0.0424
Locality * color	−1.2795	0.5254	−2.435	0.0149
Sex * color	1.0870	0.5252	2.070	0.0385

Effects of factor levels locality (Central Finland), sex (female), and firebug color (nonaposematic) are included in the intercept.

DISCUSSION

Environmental factors, particularly food availability and predators, are suggested to be the main drivers selecting for differences in foraging behavior among local bird populations (e.g., Shochat et al. 2004). However, we still have very limited understanding of how such differences arise, and whether certain behavioral traits are selected together or independently. We compared several foraging-related traits in 2 geographically distant populations of great tits. Those populations have presumably experienced different selective environments in terms of habitats, diversity and abundance of noxious prey. Although our experiments are not able to discern whether the observed differences already reflect local adaptations or whether they reflect differences in experience, our results raise some interesting points regarding the nature of differences in the reaction of wild-caught birds to aposematic prey and how they are correlated with exploration behavior and neophobia.

Exploration and neophobia—comparison of Finnish and Bohemian birds

Finnish and Bohemian great tits did not differ in their tendency to explore a novel object placed in a neutral location in their home cage. However, Finnish birds appeared more neophobic, and hesitated longer than Bohemian birds before starting to feed next to a novel object attached to their food bowl. Our results suggest that environmental conditions both in Bohemia and Central Finland may, in spite of their differences, favor similar exploration tendencies in great tit populations. Similarly, Miranda et al. (2013) did not find any differences in novel object exploration between 2 populations of European blackbirds (*Turdus merula*) living in different habitats.

The extent of object neophobia has been frequently found to differ between conspecific populations (e.g., Martin and Fitzgerald 2005; Mettke-Hofmann et al. 2013; Miranda et al. 2013, but see Echeverría and Vassallo 2008; Bókony et al. 2012). Several factors may explain the greater object neophobia of Finnish great tits in our experiment. First, Finnish birds may be more cautious to approach novel objects near their feeding place due to greater vigilance caused by higher frequency of potential predators as can be expected in a less populated and more natural landscape. In sticklebacks (*Gasterosteus aculeatus*), individuals living in areas with greater predation pressure are less bold and more neophobic than those inhabiting areas with less predators (Bell 2005; Dingemanse et al. 2007; Brydges et al. 2008). Second, Finnish birds may exhibit greater object neophobia because of a larger proportion of migrating individuals (Cepák et al. 2008). Although the great tit is mostly a resident species, some individuals (mostly yearlings) undergo short-distance autumnal migration (Cramp and Perrins 1993). Greater neophobia of birds from migratory populations than from the resident ones has been found in 2 New World blackbird species (red-winged blackbird *Agelaius phoeniceus* and Brewer's blackbird *Euphagus cyanocephalus*); the difference may have been caused by residents having higher costs of missing new opportunities in the seasonally changing environment (Mettke-Hofmann et al. 2013). Lesser neophobia of Bohemian birds may be also partly explained by their more urbanized locality. Birds from urban populations may be bolder and less neophobic than their rural conspecifics, as is the case in European blackbirds (*Turdus merula*; Miranda et al. 2013), and song sparrows (*Melospiza melodia*; Evans et al. 2010).

Behavior of Finnish and Bohemian birds toward novel palatable prey

Finnish and Bohemian great tits did not differ in their behavior toward a novel palatable prey, the cricket with a blue sticker attached. They attacked the crickets with similar latencies, and mostly killed and consumed them. This suggests that the hesitation behavior toward novel prey does not reflect any general difference in local prey communities and frequency of potentially dangerous prey.

Surprisingly, we did not find any correlation between the behavior of birds toward novel prey and their reaction to novel objects (exploration and neophobia) in the 2 populations studied. In juvenile Dutch great tits coming from lines selected for opposed personality traits (Drent et al. 2003), the generally more neophobic "slow explorers" hesitated longer before attacking novel prey (red-and-black firebugs) than the less neophobic "fast explorers" (Exnerová et al. 2010). There are several mutually nonexclusive factors possibly responsible for the difference between the results of our present and previous studies (Exnerová et al. 2010): 1) The inherited correlation between food neophobia and object neophobia may be prominent in naive juvenile birds. In contrast, adult wild-caught individuals may not show such a correlation due to their experience with various types of both palatable and unpalatable prey, as well as experience with food shortage periods. In wild-caught tits living outside the breeding season in small flocks, the food neophobia may also be influenced by the position of an individual in the flock hierarchy (Farine et al. 2015). In black-capped chickadees (*Poecile atricapillus*), for instance, subordinate individuals are less neophobic than dominant ones (An et al. 2011). 2) The correlation may be more apparent in birds coming from the lines selected for the opposed personalities (Drent et al. 2003), than in birds from natural populations with possibly less extreme values of personality traits. 3) Food neophobia may be correlated with other personality traits in some populations (Dutch; Exnerová et al. 2010), but not in others (Finnish and Bohemian, this study). Bókony et al. (2012) found that food neophobia in house sparrows (*Passer domesticus*) correlated with activity, risk-taking, and object neophobia only in 1 of 4 Hungarian populations studied. In Kenya none of the 8 populations of house sparrows tested showed any relationship between exploration of a novel object and consumption of novel food (Liebl and Martin 2014).

Reactions of Finnish and Bohemian birds toward firebugs

The behavior of birds toward firebugs was affected by the firebug coloration. In contrast to behavior toward novel palatable prey, the Finnish birds partly differed from the Bohemian birds in their reactions. Nonaposematic firebugs were attacked in similar proportions by Finnish and Bohemian birds. The birds from both populations also hesitated about the same time before attacking nonaposematic firebugs, and learned to avoid them at a similar rate. In Finnish birds, the attack latencies for nonaposematic firebugs correlated with those for novel palatable prey, whereas in Bohemian birds the latencies were not correlated. These results indicate that nonaposematic, brown-painted firebugs were novel for Finnish birds, and that the reaction to them followed the general behavior of birds toward a novel prey. Noncorrelated latencies of Bohemian birds suggest that these birds may be experienced with some similar, nonaposematic but unpalatable true bugs from the wild (e.g., some species of Rhyparochromidae), and that they partly generalized their experience. On the other hand, the Bohemian birds attacked nonaposematic firebugs more frequently than the aposematic ones, which confirms the results of previous studies (Exnerová et al. 2003, 2006) suggesting that the characteristic red-and-black color pattern of firebugs facilitates their recognition by avian predators.

The Finnish and Bohemian great tits significantly differed in their behavior toward aposematic firebugs. Most Bohemian birds avoided them on sight, whereas most Finnish birds attacked at least 1 individual. Finnish birds generally behaved toward aposematic firebugs in a similar way as to the nonaposematic ones, and their initial reactions (before the first contact with firebug defense chemicals) were correlated with their reactions to palatable prey of novel appearance (blue crickets). The differences in behavior toward the aposematic firebugs did not follow the differences between the 2 populations in exploration and neophobia. Birds from both populations behaved similarly in response to a novel object and to a novel palatable prey, and the only difference—hesitation to feed in presence of a novel object—does not correspond with behavior toward the aposematic prey, because the Finnish birds were more neophobic. Therefore, the difference in avoidance between the wild-caught Finnish and Bohemian great tits is likely to be the result of individual learning and their different experiences in the wild.

Although the Finnish birds attacked aposematic firebugs more frequently than the Bohemian birds, it is interesting that the

Finnish birds stopped attacking them on average after only 1 trial. This means that the Finnish wild-caught birds learned to avoid the novel aposematic firebugs considerably faster than the conspecific naive hand-reared birds from Bohemia, which attacked on average 5 firebugs (Svádová et al. 2009) before learning to avoid them. This difference suggests that the Finnish wild-caught birds may have generalized their previous experience with some unpalatable defended prey, even of a different appearance than firebugs, and this may have increased their avoidance learning rate.

Alternatively, the difference may suggest an innate bias against conspicuous aposematic prey (Lindström et al. 1999a), which can speed up avoidance learning. Avoidance learning against an aposematic prey that is evolutionarily novel needs typically several unpleasant experiences before the novel prey is learned to be avoided (e.g., Mappes and Alatalo 1997; Lindström et al. 1999b). In previous studies, naive juvenile great tits did not hesitate longer before attacking aposematic than nonaposematic prey (Svádová et al. 2009), but they showed an innate bias against aposematic prey, when the nonaposematic (Lindström et al. 1999a) or less conspicuous (Fabricant et al. 2014) alternative prey was present. These 2 alternatives are not mutually exclusive, and the reactions observed in wild-caught birds are likely the result of an interaction between an innate bias and individual experience. Assessing the effect of both processes would, however, require further experiments with naive predators and the use of the prey novel for both populations.

For the nonaposematic firebugs, the learning rates were similar in Bohemian and Finnish birds, indicating that the populations do not differ in their ability to learn avoiding unpalatable prey. The Finnish birds, for which both firebug color forms were novel, learned to avoid red-and-black and brown-painted firebugs at a similar rate. This agrees with previous studies with great tits and defended conspicuous prey where learning rate between "typical" warning colors versus gray or brown did not differ (Ham et al. 2006; Svádová et al. 2009). Because our experiment was designed mainly to test the attack willingness toward the aposematic prey, rather than learning abilities, we are unable to make any strong conclusions about the general differences in learning abilities between populations. Furthermore, we compared an experienced population to a naive one. In the future, it would be interesting to compare learning abilities between populations by using a completely novel aposematic prey and/or naive birds.

Our results indicate a stronger avoidance of aposematic bugs by female great tits than by males. Similar results were obtained in a study where female bobwhites (*Colinus virginianus*) showed more aversion than males toward red- and orange-dyed food (Mastrota and Mench 1994). A different study with the same species however, found no differences between the sexes in color aversion, presumably because the individuals used in both experiments differed in age (Mastrota and Mench 1995). Color aversion in females, but not in males, seems to increase with age (Mastrota and Mench 1994). A possible explanation is that females consume more insects than males, and they teach chicks to avoid toxic prey. Whether this is the case for great tits is a matter of future research.

Aposematic Heteroptera and other insects of Central Finland as potential models

Wild-caught great tits are potentially experienced with an unknown number of both palatable and unpalatable species of insects, both in Central Finland (around the 62°N latitude) and Bohemia. Unfortunately, there are no data available to compare the abundance and diversity of potential insect prey between the 2 areas. However, because the reactions of the birds toward the firebug were specific, we consider the evaluation of heteropteran faunas informative, as it compares the numbers of potential models the birds may be experienced with from the wild.

We have documented that the fauna of aposematic terrestrial Heteroptera of Central Finland is much less diverse than that of Central Europe both in absolute and relative number of species (30 vs. 156 species, 10% vs. 17% of total faunas, respectively). The Bohemian great tits are certainly experienced with *Pyrrhocoris apterus* due to the frequent occurrence of the bug, its aggregations and its ubiquitous host plants (Exnerová et al. 2006); these birds can be potentially experienced with other chemically protected red-and-black true bug species as well (Hotová Svádová et al. 2010). Although *P. apterus* does not occur in Central Finland (Rintala and Rinne 2010), we cannot exclude a priori the local occurrence of insect species that the birds would generalize with. However, the other Finnish similarly colored aposematic insects are either too rare (*Corizus hyoscyami*, Rhopalidae), or look too different (plant bugs, Miridae; burnet moths, Zygaenidae; ladybird beetles, Coccinellidae) to function as models for generalization with red-and-black firebugs. Moreover, recent analysis of Finnish Lepidoptera showed that only less than 5% of caterpillars are aposematic (Mappes et al. 2014). It is still possible that the negative experience with some of the above taxa may have played a role in the generalization of the firebug color patterns as great tits have been shown to generalize their learned avoidance among colors (red, yellow, and orange; Ham et al. 2006), among aposematic species (Hotová Svádová et al. 2013) and between bi-chromatic symbols (Ihalainen et al. 2008).

CONCLUSIONS

Even though a broader generalization of our results is limited by the study of only 2 populations, we have shown that conspecific birds from 2 geographically distant populations may express similar reactions to a prey which is novel for both of them. In our case, food neophobia was low in both populations, whereas the avoidance of aposematic prey was different between the populations, and could be explained by differences in experience. Bohemian birds have plenty of opportunities to meet firebugs in their natural environment and learn about their unpalatability; Finnish birds do not have such opportunity. Our results emphasize that naive birds' tendency to attack novel aposematic prey may have important implications for range extensions of prey species. We can not tell whether the difference in avoidance was a result of Finnish birds' lower experience with any aposematic prey, or whether the avoidance learning is prey specific. Our results indicate, however, that studies on aposematism and mimicry based on geographically distant conspecific populations can be compared and generalized.

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Through experience to boldness? Deactivation of neophobia towards novel and aposematic prey in tits (Paridae)

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Through experience to boldness? Deactivation of neophobia towards novel and aposematic prey in three European species of tits (Paridae)

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Abstract

European tits (Paridae) exhibit species-specific levels of initial wariness towards aposematic prey, which may be caused by neophobia, dietary conservatism or specific innate bias against particular prey traits. We assessed contribution of the three mechanisms to behaviour of juvenile tits towards novel palatable prey and novel aposematic prey. We compared levels of initial wariness in naive juvenile great tits (*Parus major*), blue tits (*Cyanistes caeruleus*) and coal tits (*Periparus ater*), and tested how the initial wariness can be deactivated by experience with a palatable prey. One group of birds was pre-trained to attack familiar naturally coloured mealworms, the other novel red-painted mealworms. Then we offered all the birds a novel palatable prey of different colour and shape – cricket with blue sticker *Acheta domestica*, and then a novel aposematic firebug *Pyrrhocoris apterus*. The initial reactions of birds differed according to species and experience with red mealworms. Great tits and coal tits from experienced groups have significantly decreased their neophobia towards both palatable prey and aposematic prey while blue tits have not changed their strongly neophobic reactions. Experience did not affected willingness to consume novel prey in any species. We discuss factors constraining rapid neophobia deactivation blue tits.

Keywords

Aposematism, dietary conservatism, innate wariness, interspecific difference, neophobia, Paridae

1. Introduction

Aposematic prey signals its noxiousness or unprofitability to its potential predators by some understandable signal (reviewed in Ruxton et al., 2004). Bird predators usually learn to avoid aposematic prey, and many experimental studies were focused on factors affecting the mechanisms of avoidance learning, memory and generalization (e.g. Sillén-Tullberg, 1985; Roper and Redston, 1987; Gamberale and Tullberg, 1996; Lindström et al., 1999a; Riipi et al., 2001; Gamberale-Stille and Guilford, 2003; Ham et al., 2006; Exnerová et al., 2010; Aronsson and Gamberale-Stille, 2008; Svádová et al., 2009; Barnett et al., 2012). An avoidance of aposematic prey may also have an inherited component, which causes even inexperienced individuals to avoid aposematic prey or at least to hesitate longer before attacking it (Smith, 1975, 1977; Lindström et al., 1999b; Exnerová et al., 2007) and is therefore important for the evolution of aposematic prey (Marples et al., 2005; Marples and Mappes, 2011). Recent studies of the phenomenon of innate wariness have shown that it may vary between bird species (Exnerová et al., 2007) and individuals (Exnerová et al., 2010), may often include hidden biases that are manifested only when combination of visual and chemical signals of a prey are involved (Rowe and Guilford, 1996, 1999a; Jetz et al., 2001; Lindström et al., 2001; Kelly and Marples, 2004; Rowe and Skelhorn, 2005), and that the wariness may in fact be a complex of several, partly independent mechanisms (Marples et al., 1998; Marples and Kelly, 1999; Exnerová et al., 2003; Marples and Mappes, 2011). Innate wariness towards aposematic prey may include three mutually not exclusive processes: 1) food neophobia, 2) dietary conservatism and 3) specific innate biases against warning signals of aposematic prey. All these processes may contribute by various degree to the predator's response and in natural situation it is usually difficult to distinguish them.

Neophobia, usually defined as a tendency to avoid novel objects and situations (Barrows, 2011), is a widespread phenomenon first described in rats tested with novel objects (Barnett, 1958). It has since been observed in many animal taxa also as a response to novel food (Honey, 1990; Galef, 1993; reviews in Kelly and Marples, 2004; Mappes et al., 2005; Marples et al., 2005). Food neophobia has been defined as a hesitation to approach a new food and come into a physical contact with it, which lasts usually only several minutes, and is followed by investigation of the novel food (Marples and Kelly, 1999). In some bird species, food neophobia may be correlated with object neophobia (e.g. blacked-capped chickadees (*Poecile atricapillus*) - An et al., 2011), whereas in others the correlation may be absent or present only in some populations (e.g. great tits (*Parus majo*r) - Exnerová et al., 2010, 2015; house sparrows (*Passer domesticus*) - Bókony et al., 2012). After the first contact with novel food, birds are no longer showing neophobia, but they may still refuse to consume the novel food. This reaction was termed dietary conservatism, and is usually measured as a time from initial contact with the novel food item to its incorporation into the diet (Marples et al., 1998; Kelly and Marples, 2004). Dietary conservatism (DC) has been described as a relatively long lasting refusal (persisting for days to months) of some individuals to accept novel food into their diet (Marples et al., 1998; Thomas et al., 2003; Thomas et al., 2004). Individual variation in dietary conservatism has been shown to have genetic basis (Marples and Brakefield, 1995). Neophobia and dietary conservatism together are usually referred to as dietary wariness (Mappes et al., 2005; Marples et al., 2005; Marples et al., 2007) and the evidence that neophobia but not dietary conservatism can be reduced through experience with food of various colours in chicks (Jones, 1986; Marples et al., 1998) and turkeys (Lecuelle et al., 2011) supports the idea of an existence of two distinct processes.

The third process contributing to innate wariness of aposematic prey are specific innate biases against particular warning signals and their combinations. This type of inherited avoidance of warning signals has been found in birds from several taxa (Galliformes, Momotidae and Passeriformes) and is usually connected with conspicuous aposematic colour patterns. Naive domestic chicks (*Gallus gallus domesticus*) avoided red painted mealworms (larvae of *Tenebrio molitor*) (Roper and Cook, 1989; Roper, 1990) and mealworms with black-and-yellow stripes (Schuler and Hesse, 1985), naive northern bobwhites (*Colinus virginianus*) avoided red-and-yellow pinheads (Mastrota and Mench, 1995), naive hand-reared turquoise-browed motmots (*Eumomota superciliosa*) and great kiskadees (*Pitangus sulphuratus*) avoided the coral snake pattern (Smith, 1975, 1977) and hand-reared juveniles of great tits (*Parus major*) avoided black-and-yellow striped mealworms (Lindström et al., 1999b). Furthermore, warning signals are often multimodal and innate biases may emerge only when visual signals are combined with olfactory, gustatory or acoustic cues (Rowe and Guilford, 1999a). These hidden biases have been intensively studied mainly in domestic chicks, in which a combination of warning coloration and smell (Rowe and Guilford, 1996, 1999b; Jetz et al., 2001), taste (Rowe and Skelhorn, 2005; Skelhorn et al., 2008) or sound (Rowe and Guilford, 1999a; but see Siddall and Marples, 2011) triggers a manifestation of innate biases.

Degree of innate wariness may differ even between closely related bird species (Exnerová et al., 2007), and also the mechanisms responsible for the wariness may be species specific. Moreover, predator species may differ not only in their initial degree of innate wariness, but also in how easily it is modified by further experience with palatable or unpalatable prey. Considerable variation in innate wariness exists among European species of tits (Paridae).

Whereas naive hand-reared great tits (*Parus major*) and crested tits (*Lophophanes cristatus*) readily attack aposematic red-and-black firebugs, *Pyrrhocoris apterus*, naive hand-reared coal tits (*Periparus ater*) and blue tits (*Cyanistes caeruleus*) avoid them (Exnerová et al., 2007). Since in the same study, naive coal tits and blue tits avoided also non-aposematic brown-painted firebugs, these results indicate effect of neophobia, but the role, which neophobia, dietary conservatism and specific biases against warning colours play in innate wariness of different species of tits is unknown.

In this study we compared the degree of innate wariness towards novel prey in three European species of tits and attempted to assess the role of neophobia, dietary conservatism and specific bias against aposematic coloration in an overall wariness. Specifically, we have compared reactions of hand-reared juveniles of great tits (*Parus major*), coal tits (*Periparus ater*) and blue tits (*Cyanistes caeruleus*) towards two types of novel prey: 1) novel palatable prey (a cricket *Acheta domestica* with a blue sticker) and 2) novel aposematic prey (red-and-black firebug *Pyrrhocoris apterus*). Within each species we tested, whether the previous positive experience with another novel palatable prey (red-painted mealworms) affects willingness of the birds to attack and consume the two novel prey types.

2. Material and methods

2.1. Birds

Hand-reared juveniles of three European tit species were tested: 85 great tits (*Parus major* L. 1758), 65 coal tits (*Periparus ater* (L. 1758)) and 85 blue tits (*Cyanistes caeruleus* (L. 1758)). All three species are sedentary, and their diet includes mainly small arthropods, supplemented with seeds and berries (Cramp and Perrins, 1993). Preferred prey-length of all the three tit species is around 1 cm, with coal tits preferring slightly smaller prey than the other two species (Cramp and Perrins, 1993). Coal tits hoard their food regularly, whereas blue tits and great tits do not (Štorchová et al., 2010). Great tits and coal tits are widespread thorough the whole Palaearctic (Cramp and Perrins, 1993). Great tits inhabit wide range of woodland habitats, as well as urban and suburban areas, whereas coal tits are restricted to coniferous and mixed woodlands (del Hoyo et al., 2009). Blue tit are endemic to West Palaearctic (Harrap and Quinn, 1996), inhabiting mostly lowland and submontane deciduous woodlands (Cramp and Perrins, 1993; del Hoyo et al., 2009). Great tit is the largest of the three species (weight: 14.0-22.0g, body length: 140mm), followed by blue tit (weight: 7.5-14.7g, body length: 115mm) and coal tit (weight: 7.2-12.0g, body length: 115mm) (Cramp and Perrins, 1993; del Hoyo et al., 2009). Juveniles tested in our experiments had following body weights: great tits – mean 16.2g ± 0.8g (range: 14 – 18g); blue tits – mean 11.1g ± 0.8g (range: 9.5 – 13g); coal tits – mean 9.5g ± 0.5g (range: 8.5 – 10.5g).

The nestlings were taken from nest boxes at the age of 12-16 days, when they had only very limited visual experience with prey brought to the nest by their parents. The nest boxes were placed in large parks at the outskirts of Prague (50°04'N, 14°26'E) and in mixed woods near Hradec Králové (50°12'N, 15°50'E). Not more than two nestlings were taken from a single brood. Nestlings were kept in artificial nests and were fed every two hours from 6 AM to 10 PM for several days, until they were able to feed themselves. Their diet consisted of mealworms, boiled eggs, handmix (Orlux), egg mixture Oké-bird (Versele-Laga), mixtures for insectivorous birds Uni patee (Orlux), Nutribird (Versele-Laga), Insect patee (Orlux) and vitamins Roboran (Unisvit), Vitamin plus V (Sera) and Activ plus W (Sera). After fledging, the birds were housed individually in plastic home cages (50 x 40 x 40 cm) with wire-mesh front wall. Each cage was equipped with three perches, two water bowls with drinking and bathing water *ad libitum* and with two feeders situated on the bottom of the cage. Birds were kept under natural light conditions (16:8 h light/dark) and were daily provided with fresh water and food. All birds were tested when they were fully independent, most of them at the age of 38–65 days (minimum 35 days and maximum 73 days).

2.2. Prey

Naturally coloured mealworms (larvae of *Tenebrio molitor*), which were familiar to the birds, were used to check their foraging motivation during the experiment and for training the birds from non-experienced experimental group (see below). We used three types of novel prey: (1) red-painted mealworms, which were dyed with non-toxic finger paints (Jovi S.A. Barcelona, red); the dye covering the whole body of the mealworm, (2) late-instar larvae of house cricket, *Acheta domestica* with head and body covered with oval-shaped bright blue-coloured (Faber-Castell, textliner 1548 blue) paper sticker attached to their dorsal side, and (3) adults of red-and-black aposematic firebug, *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae). Unlike mealworms and crickets, the firebugs possess a chemical defence, which makes them unpalatable for small passerine birds (Exnerová et al., 2003, 2007; Svádová et al., 2009). The defensive secretion is produced in metathoracic glands and it contains mostly short-chained aldehydes (Farine et al., 1992). Firebugs are widespread in various habitats of the Palaearctic region, mainly in deciduous woodlands, parks and gardens. They feed on seeds of linden trees (*Tilia* spp.), various herbaceous Malvaceae, and locust tree (*Robinia pseudacacia*). The firebugs were collected on linden trees at several localities in Prague. Cricket nymphs and adult firebugs were of similar body length (10-12 mm), mealworms were about 15 mm long. All prey used in the experiments were live.

2.3. Experimental cages

Experiments were carried out in wooden cages (70 x 70 x 70 cm) with wire-mesh walls and front wall made of one-way glass. Cages were equipped with a wooden perch, a water bowl and a rotating feeding tray with six cups (see Exnerová et al., 2003 for details). The perch was placed 30 cm from the feeding tray. Cage illumination (Biolux Combi 18W, Osram) simulated the daylight spectrum. Birds were trained to search for mealworms (*Tenebrio molitor* larvae) in one of the cups of the feeding tray and then deprived of food for two hours before the experiment.

2.4. Experimental design

Within each species, we divided the birds into two experimental groups. (1) Birds from the first group were trained to attack red-painted mealworms (experienced group henceforth), while (2) birds from the second (control) group were trained to attack naturally coloured mealworms (non-experienced group henceforth). The training consisted of a sequence of five-minute trials; the bird was offered a single mealworm in each trial. For the experienced group the red mealworms were alternating with naturally coloured mealworms to check the foraging motivation of the birds. Birds from the non-experienced group were offered a sequence of naturally coloured mealworms. Birds from the experienced group had to attack and consume one red mealworm to be considered having a positive experience with the novel prey. The maximum number of mealworm presentations for the experienced group was 30 five-minute trials (15 red mealworms and 15 naturally coloured mealworms). Ten birds from the experienced group (4 great tits and 6 blue tits) did not meet our criterion during the entire training session. These birds were not included into the analyses. The non-experienced group had the same training setup, but with naturally coloured mealworms only (Tab. 1).

To test whether the experience with the red mealworms affects the behaviour of birds towards a prey of another novel colour and unfamiliar body shape, all birds were immediately after the training phase offered a blue cricket in a single five-minute trial. To check the foraging motivation of birds, we offered a naturally coloured mealworm before the blue-cricket trial (Tab. 1). We measured the latency to approach and attack (touch, peck or seize) the blue cricket and recorded whether the cricket was killed and eaten. If the bird did not attack the blue cricket within the five minutes, it was assigned a maximum latency of 300 seconds.

In the subsequent five-minute trial all birds were offered the aposematic firebug. In this trial we tested whether the reaction of birds to red-and-black aposematic prey can be affected by their positive experience with palatable red-coloured prey of different body shape (Tab. 1). Again, the latency to approach and attack (touch, peck or seize) the firebug was recorded as well as whether the firebug was killed and eaten. If the bird did not attack the firebug within five minutes it was assigned a maximum latency of 300 seconds.

All trials were recorded using Observer XT 8.0 © Noldus and by digital videocamera.

2.5. Ethical Note

We obtained permissions for taking juvenile tits from nest boxes in Prague (MHMP-043585/2009/OOP-V-26/R-8/Pra) and Hradec Králové (MMHK/10568/2009/ŽP/Han17386), and for laboratory experiments with the birds (29532/2006-30, 150/99 and CZ01059) issued by Central Commission for Animal Welfare of the Czech Republic. The permissions cover all the necessary methodological details, i.e. housing and feeding conditions, experimental design, and release after the experiment. All birds were individually ringed (licence No. 1087, Czech Ringing Centre, Praha) and released in healthy condition on days of fair weather at their origin. The releasing sites were equipped with feeders and provided with supplementary food for several days after releasing.

2.6. Statistical analyses

A Cox hazard regression was used to analyze the interspecific differences and an effect of experience with red mealworms on latencies to attack (a) novel palatable blue crickets, and (2) aposematic firebugs. Training group and species were used as explanatory variables.

Using a generalized linear models (GLM ANOVA) with binomial distribution and logit link function we tested, whether the probability to consume (a) novel palatable blue crickets, and (b) aposematic firebugs differed between the tit species and was affected by the previous experience with palatable red mealworms. Training group and species were used as explanatory variables.

All calculations were made in S-Plus 4.0 (MathSoft, 1997).

3. Results

3.1. Reaction to blue cricket (*Acheta domestica*)

We found higher proportion of birds that attacked blue crickets among the birds from the experienced group (62%) than in the non-experienced group (44%). The latency of attacking blue crickets was affected both by tit species and experience (Cox hazard regression model: Wald test=21.9, $p<0.001$, Fig. 1). Great tits differed significantly in their latencies to attack blue crickets from the other two species ($Z=3.34$, $p<0.001$) and coal tits differed from great tits and blue tits as well ($Z=2.7$, $p<0.01$). Birds lacking positive experience with red mealworms hesitated longer before attacking the blue crickets than the birds from the experienced groups ($Z=-3.35$, $p<0.001$, Fig. 1).

The biggest intraspecific difference in reactions to blue crickets was observed in great tits. The latencies to attack blue crickets were significantly shorter in birds from the group experienced with palatable red mealworms than in the non-experienced group ($Z=- 4.74$, $p<0.001$, Fig. 1). In coal tits, the positive experience with red mealworms also influenced the latencies to attack blue crickets. Coal tits from the experienced group attacked blue crickets significantly faster than the birds from the non-experienced group (Z=- 3.21, p=0.02, Fig. 1). In contrast to great tits and coal tits, positive experience with palatable red mealworms did not affect the reactions of blue tits; latencies to attack blue crickets did not differ between experienced and non-experienced groups (Z=1.13, p=0.26, Fig. 1).

Overall proportion of birds that have consumed blue cricket was almost the same in experienced (33%) and in non-experienced group (31%). Probability of consuming the blue crickets differed between the tit species (GLM ANOVA: Chi-square=15.7, df=2,222, p<0.001), but was not affected by the previous experience with red mealworms (GLM ANOVA: Chi-square=0.02, df=1,221, p =0.89); the effect of interaction of both factors was marginal (GLM ANOVA: Chi-square=5.3, df=2,219, p=0.07).

In great tits, there was a non-significant tendency to consume blue crickets more frequently among the birds experienced with palatable red mealworms (GLM ANOVA: Chi-square=2.87, df=1,79, p=0.09) than among the birds from the non-experienced group. In the experienced group, blue crickets were consumed by 54% of birds, and in non-experienced group by 35% of birds. The probability of consuming blue crickets was not affected by the previous experience with red mealworms in coal tits (GLM ANOVA: Chi-square=0.26, df=1,63, p=0.61) and in blue tits (GLM ANOVA: Chi-square=2.20, df=1,77, p=0.14).

3.2. Reaction to aposematic firebug (*Pyrrhocoris apterus*)

The overall proportion of birds that attacked the firebugs was higher among the birds with (57%) previous experience with palatable red mealworms than without (38%) this experience. The latency to attack the firebugs differed between the tit species and also between the training groups (Cox hazard regression model: Wald test=63.6, p<0.001, Fig. 2). Great tits attacked the firebugs significantly faster than the two other species (Z=7.12, p<0.001). Coal tits hesitated longer than great tits, but not as long as blue tits before attacking the firebugs

(Z=4.3, p<0.001). Birds from the experienced groups showed significantly shorter attack latencies than the birds from non-experienced groups (Z=-3.81, p<0.001, Fig. 2).

The positive experience with palatable red mealworms resulted into significantly shorter attack latencies of birds from experienced group than from non-experienced group in great tits (Z=-2.71, p<0.01, Fig. 2) and coal tits (Z=-2.29, p=0.02, Fig. 2). Blue tits showed strong initial wariness of the firebugs, and their attack latencies were not affected by experience with palatable red mealworms (Z=-1.26, p=0.21, Fig. 2).

Higher overall proportion of birds willing to consume aposematic firebugs were among the those experienced with red palatable mealworms (26%) than among the birds for which the red coloured prey was entirely novel (14%). Probability of consuming the aposematic firebugs differed between the tit species (GLM ANOVA: Chi-square=52.8, df=2,222, p<0.001) and was also affected by previous experience with red mealworms (GLM ANOVA: Chi-square=8.01, df=1,221, p<0.01). The interaction of the two factors was not significant (GLM ANOVA: Chi-square=0.85, df=2,219, p=0.65).

The proportion of great tits that consumed the firebug was higher in the group experienced with palatable red mealworms than in the non-experienced group (GLM ANOVA: chi-square=4.61, df=1,79, p=0.03). After positive experience with red mealworms 56% of great tits consumed the aposematic firebug, in comparison with 33% of birds from non-experienced group. Although coal tits remained cautious about consuming the firebugs, we observed an increasing trend in the willingness to consume them in the group experienced with palatable red mealworms (GLM ANOVA: chi-square=2.84, df=1,63, p=0.09). Only one coal tit from non-experienced group consumed the aposematic firebug, compared to nine coal tits from group experienced with palatable red mealworms. Additionally, four coal tits which consumed the firebug, cached the firebug into the slits in experimental cage before consuming it. The number of blue tits that consumed aposematic firebugs did not differ between the groups experienced and non-experienced with palatable red mealworms (GLM ANOVA: chi-square=1.42, df=1,77, p=0.23). Only one of the experienced blue tits consumed the firebug and blue tits from the non-experienced group neither killed nor ate any single firebug.

4. Discussion

The fear of novelty may be seen as an adaptive strategy for avoiding potentially dangerous and toxic food (Barnett, 1958; Heinrich, 1988). Under some circumstances, however, especially when the food becomes scarce, overcoming the neophobia and sampling new types of food can be advantageous (Greenberg, 1990; Greenberg and Mettke-Hofmann, 2001). Reducing the neophobia may be particularly important for resident bird species, which are confronted with food supply changes throughout the year, and frequently explore new sources of food and invent new foraging methods (Sol et al., 2005). In the present study we tested, whether positive experience with a novel palatable prey can increase the willingness of juveniles of three resident species of European tits to attack and consume another types of novel prey.

4.1. Reaction towards novel palatable prey

Great tits, coal tits, and blue tits differed in how the experience with a novel palatable prey (red mealworms encountered during the training) affected their behaviour towards another palatable novel prey (blue crickets). In great tits and coal tits the latencies to attack novel palatable prey were significantly shorter in those birds that had the positive experience than in those that lacked it. These results are in accordance with previous studies, which have shown that the positive experience with food of novel colour was sufficient for deactivation of neophobia towards another food of a new colour in blue jays (*Cyanocitta cristata*) (Schlenoff, 1984), domestic chicks (Jones, 1986; Marples et al., 2007) and turkeys (Lecuelle et al., 2011).

Moreover our results show that the positive experience with the food, which differs from the familiar one in a single trait (colour) may deactivate (or considerably decrease) neophobia towards food, which is novel also in other characters (shape, way of movement).

Our results with great tits and coal tits also support the findings of previous studies carried out with domestic chicks (Marples and Kelly, 1999; Marples et al., 2007) that a food neophobia may be deactivated by just a single experience with a novel food. Marples et al. (2007) offered two alternative explanations for the cognitive mechanisms involved in a single-experience deactivation of neophobia. Either the chicks used novelty itself as a cue and generalised their experience to another types of novel food or they first formed a mental representation of a palatable food (chick crumb of a familiar colour) and after the experience with novel-coloured chick crumbs they omitted the colour as an unimportant trait of a palatable food (Marples et al., 2007). Because the great tits and coal tits in our study decreased considerably their neophobia towards the food novel not only in colour but also in shape and way of movement, it is likely that they used the novelty itself as a cue for recognizing palatable food.

In blue tits, however, the hesitation to attack a novel palatable prey was not affected by their previous positive experience with another type of novel prey. This indicates that the food neophobia in blue tits is considerably stronger and more persistent than in the other two species. The juvenile blue tits remain cautious when attacking a prey of novel colour and shape regardless their previous experience.

In contrast to its effect on attack latencies, the positive experience from training with novel palatable prey did not affect the willingness to consume another novel palatable prey in any of the three tit species tested. Although the birds from experienced groups were more likely to attack the blue crickets and shortened their attack latencies significantly, their willingness to consume the prey was the same as that of the birds from non-experienced groups.

Consequently, to overcome neophobia towards novel and fully palatable prey does not necessarily mean an acceptance of this prey into the diet. These results suggest that the birds decided about the food in two rather independent steps, one concerning attacking and the other consuming the prey. This is consistent with the hypothesis that neophobia and dietary conservatism are two distinct processes (Marples and Kelly, 1999), and in tits, only the neophobia was deactivated by the positive experience with the novel prey during the training. Contrastingly, in domestic chicks, the exposure to a coloured palatable prey in the training deactivated both components of dietary wariness: neophobia and dietary conservatism (Marples et al., 2007). There are three possible explanations of this difference between the results of Marples et al. (2007) and this study. (1) It may be connected with the duration of the exposure to the training novel food. Marples et al. (2007) have shown that while neophobia in domestic chicks was significantly reduced after one-minute exposure to novel food, to deactivate both components of dietary wariness required longer time (25 to 40 minutes). In our experiments the exposure usually took 15 to 30 minutes, and it is therefore possible that longer and more intensive experience could deactivate both components of dietary wariness also in the tits. (2) It may be easier for the birds to overcome dietary conservatism towards the novel food which differs only in colour from the training food (coloured chicken crumbs in Marples et al., 2007) than towards the food which differs also in shape and way of movement (red mealworms versus blue crickets in our experiment). (3) There may be an overall difference in strength and duration of dietary conservatism between domestic gallinaceous and passerine birds, as Marples et al. (1998) observed the dietary conservatism in robins (*Erithacus rubecula*) and blackbirds (*Turdus merula*) to last for weeks or even for months. Moreover, the strength of dietary conservatism in domestic chicks might have been reduced by the artificial selection for rapid weight gain of the breeds used for the experiments (Marples and Kelly, 1999).

4.2. Reaction towards aposematic prey

Predator species may differ in their innate wariness against aposematic prey, and these differences may concern specific biases against warning coloration (Smith, 1975, 1977) or general degree of food neophobia (Exnerová et al., 2007). Initial innate wariness may, however, be modified by experience, and even the species with similar degree of innate wariness may potentially differ in how much it is susceptible to further changes. Several studies have shown that innate wariness may be decreased or deactivated by a positive experience with novel palatable food (Coppinger, 1970; Marples et al., 2007), but comparative data are still lacking. On the other hand, the wariness may also be increased or reactivated by a negative experience with unpalatable food (Schlenoff, 1984; Marples et al., 2007). Among the European species of Paridae, juvenile blue tits and coal tits exhibit high degree of food neophobia and innate wariness against aposematic firebugs (Exnerová et al., 2007). In this study, we have found that whereas in coal tits the positive experience with red-coloured palatable mealworms significantly reduced initial wariness, in blue tits the wariness persisted irrespectively of experience. In coal tits (and similarly, in the least wary great tits) the decreased wariness may be caused either by deactivation of neophobia or by very broad generalisation between plain red mealworms and red-and-black patterned firebugs. Whatever the case, none of these processes appeared to take place in blue tits, which indicates that their innate wariness is considerably stronger than in the other two species. To overcome their innate wariness, blue tits may require repeated positive experience with novel palatable prey, because adult (wild-caught) blue tits are less neophobic than the juveniles (Adamová-Ježová et al., unpublished).

In contrast to blue tits and coal tits, the naive juvenile great tits usually do not show any strong wariness against aposematic prey (Exnerová et al., 2007; Svádová et al., 2009; Hotová

Svádová et al., 2013; but see Lindström et al., 1999b). Individual birds, however, differ in their degree of hesitancy to attack novel aposematic prey, and the differences are connected with the differences in personality (Exnerová et al., 2010). In this study we have found that a positive experience with red-coloured palatable mealworms decreased the hesitancy of great tits to attack red-and-black firebugs even farther. In contrast to the reactions towards blue crickets, the experience also made the great tits more willing to consume the aposematic firebugs, and a similar though not significant trend was found also in coal tits. The explanation may be in deactivated wariness of novel prey (both neophobia and dietary conservatism) by repeated positive experience with palatable food of various colours and shapes, similarly to the results found in the domestic chicks (Marples et al., 2007). An alternative explanation may be that the birds generalised their experience with palatable red-coloured mealworms to the red-and-black firebugs. Several studies have shown that for birds the colour is the most salient visual stimulus in associative and discrimination learning about prey palatability (Aronsson and Gamberale-Stille, 2008, 2012; Kazemi et al., 2014), and it is therefore possible that the birds from an experienced group associated the red colour with palatability. Even if we cannot decide whether the experience with the red colour alone was responsible for making the birds more willing to eat the firebugs or it was combined experience with prey of a similar colour (red mealworms) and shape (blue crickets), it is evident that experience with palatable prey of only shape similar to the firebugs did not have a similar effect. In the group experienced with red mealworms, 17 out of 23 birds that had consumed the blue crickets also consumed the firebugs, whereas in the group non-experienced with red mealworms it was only 5 birds out of 13.

4.3. Comparison between species

The same positive experience with a novel palatable prey had a different effect on decisions of birds from three closely related species, whether to attack another novel palatable prey and novel aposematic prey. Great tits and coal tits which had a positive experience with a novel prey (palatable red mealworms) were more willing to attack another novel palatable prey (blue crickets) and a novel aposematic prey (red-and-black firebug). On the contrary, blue tits remained highly aversive towards the both types of novel prey irrespective of their experience. There are several factors that may be connected with these interspecific differences.

(a) Size. We may expect larger species to be less neophobic towards the food than smaller ones, because the larger body size would contribute to easier physiological overcoming the potential adverse effects of noxious food (Lamanna and Hart, 1968; Anderson and Weber, 1975; Smith and Phillips, 2006). Larger passerine species attack aposematic prey more frequently than species with smaller body size (Exnerová et al., 2003). This assumption would explain the differences between the large great tit and much smaller blue tit (and partly also the differences between great tit and the smallest species, the coal tit) but not the fact, that the coal tit is considerably less hesitant to attack novel prey than the blue tit.

(b) Dominance in mixed winter flocks. Kawamori and Matsushima (2012) found in their study of risk-taking in three sympatric tit species in Japan that the varied tit *(Poecile varius*), which is the largest and dominant over two smaller species (marsh tit, *Poecile palustris* and great tit, *Parus major*), is also the most neophobic. Smaller and submissive marsh tits and great tits have probably been more prone to risk being driven by the dominant species to exploit variable and risky food. Our results, however, do not support this hypothesis, because the far less neophobic species in our study was the great tit, which is the largest of the three species, and is also dominant in winter foraging flocks (Cramp and Perrins, 1993; Morse, 1978). The difference between the results of Kawamori and Matsushima (2012) and our results may be simply due to the different species studied (according to the nowadays mostly accepted taxonomy (Kvist et al., 2007; Päckert et al., 2005; Kvist and Rytkönen, 2006), the Japanese great tits belong to the Eastern-Palaearctic species *Parus minor* Temminck and Schlegel, 1848) or due to the fact that while Kawamori and Matsushima (2012) tested wild-caught adults, we have tested naive juvenile birds, and the innate degree of neophobia may be modified by experience, especially during the first winter period.

(c) Geographic range. The range is another explanatory factor though its delimitation depends considerably on the taxonomy accepted (see Kvist et al., 1996; Gill et al., 2005). The blue tit (sensu stricto; Salzburger et al., 2002; Kvist et al., 2004) inhabits the Western Palaearctic, an area with largely moderate climate without regular temperature excesses (particularly without too low temperatures in winter). On the other hand, the ranges of great tit (regardless whether conceived in restricted or broad taxonomic sense) and coal tit include broad and long part of Siberia (Cramp and Perrins, 1993; del Hoyo et al. 2007; Tietze and Borthakur, 2012) in which harsh climate and scarcity of food occur regularly. This may explain the „affordable“ neophobia in blue tit and „enforced“ neophilia in great and coal tit enhanced by long-lasting postglacial selection. However, more exact development of this hypothesis would need detailed phylogeographic assessment of postglacial biogeographical history of the species concerned.

(d) Habitat. The great tit and the blue tit are habitat generalists, encounter therefore a wide range of different (and potentially noxious) insect prey, and this may have enhanced the neophobia of the small-sized blue tit. On the other hand, the coal tit is a habitat specialist preferring coniferous woods (Cramp and Perrins, 1993; del Hoyo et al., 2007) with more monotonous food supply. Therefore, even with the smallest body size, coal tits can (or have to) be less neophobic than blue tits, which have a more frequent chance to encounter a novel noxious prey.

(e) Diet composition. This factor is closely related with habitat. All the three species feed in the spring and summer (when our experiments have been carried out) on various invertebrates, but large proportion of their diet consists of small spiders and caterpillars (Cramp and Perrins, 1993). Unlike the other two species, coal tits also included into their diet large proportion of aphids (Hemiptera: Sternorrhyncha; Cramp and Perrins, 1993; Krištín, 1992), which are usually not chemically defended (Gullan and Cranston, 2014) and represent a safe and abundant source of food. This foraging specialization might allow for the less neophobic reactions of coal tits in comparison with blue tits, which are similarly small but more generalist foragers.

(f) Food caching. Of the three species, only coal tits cache the food. This behaviour can be associated with greater food neophobia, since the food-caching species may be at lesser immediate risk of starvation than the non-caching species. On the other hand, some food caching bird species are known to cache toxic prey and return to it later, when the toxins have degraded (Yosef and Whitman, 1992; Exnerová et al., 2008), a strategy that may lead to lesser hesitation to attack the novel prey and partly explains the difference between the coal tits and the blue tits. In our experiments we observed this behaviour in coal tits. They used slits in experimental cage for storing the firebug and after several minutes, when the repellent secretion has probably evaporated, they occasionally consumed them.

5. Conclusions

We have considered those factors that can potentially be of importance for the species-specific differences between the three tit species studied, particularly explaining the strong food-neophobia in the blue tit, and lack of it in the smallest species, the coal tit. The great tit is in all the respect the boldest of the three species and this is most likely associated with its large size. But the size itself does not explain differences in the degree of neophobia in all the three species. However, the more restricted range of the blue tit in comparison against the coal tit, and the habitat specialization associated with the different food composition of the latter species are in accordance with their degree of neophobia. The potential role of food caching in the coal tit remains ambiguous.

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Legends

Fig. 1: Latency to attack novel prey (cricket with blue sticker) by tits without and with experience with red coloured mealworms (white bars – non-experienced group, black bars – experienced group). Circles = median, box = lower and upper quartile, whiskers = non-outlier range, * = outliers.

Fig. 2: Latency to attack aposematic prey (red-and-black firebug) by tits experienced or non-experienced with red coloured mealworms (white bars – non-experienced group, black bars –

experienced group). Circles = median, box = lower and upper quartile, whiskers = non-outlier range, * = outliers, + = extremes.

Tab. 1. Sample size of tested birds, which met the criteria in training session (consuming of red mealworm) and were used in next experiments with novel prey (blue cricket) and aposematic prey (red-and-black firebug).

NON-EXPERIENCED			EXPERIENCED		
Great tits	Coal tits	Blue tits	Great tits	Coal tits	Blue tits
40	20	40	41	45	39
naturally coloured mealworms	naturally coloured mealworms	naturally coloured mealworms	red mealworms	red mealworms	red mealworms
↓	↓	↓	↓	↓	↓
blue cricket	blue cricket	blue cricket	blue cricket	blue cricket	blue cricket
↓	↓	↓	↓	↓	↓
aposematic firebug	aposematic firebug	aposematic firebug	aposematic firebug	aposematic firebug	aposematic firebug

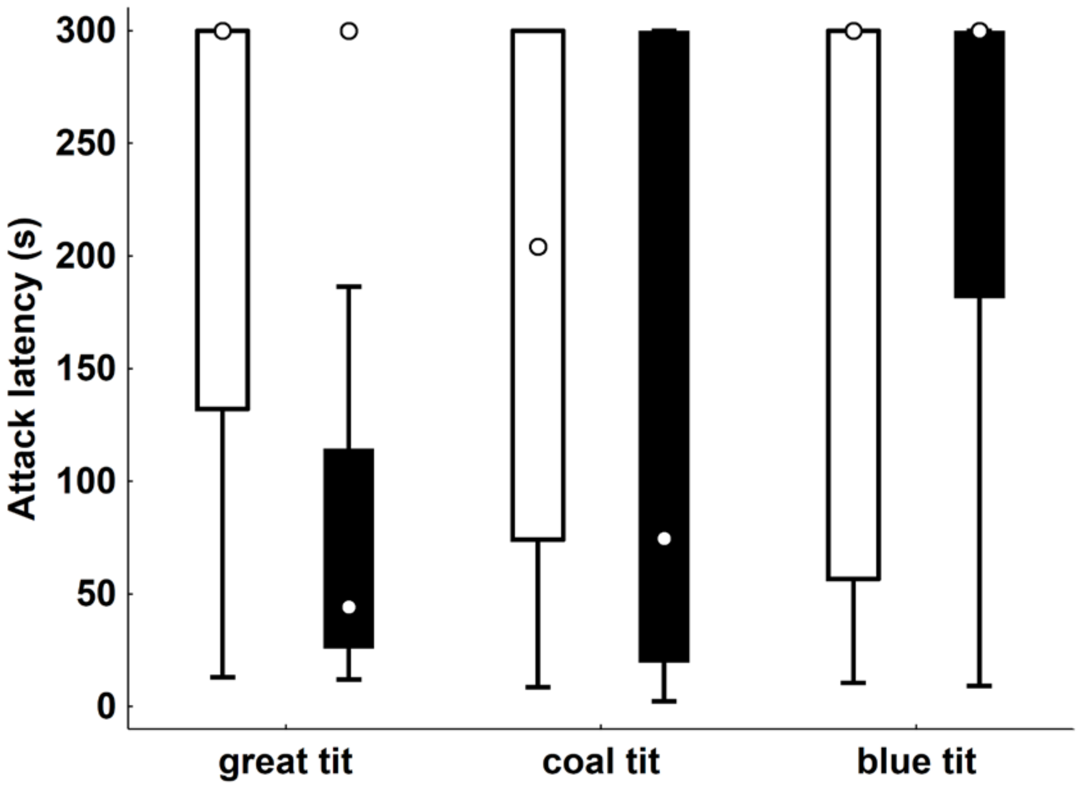

Figure 1

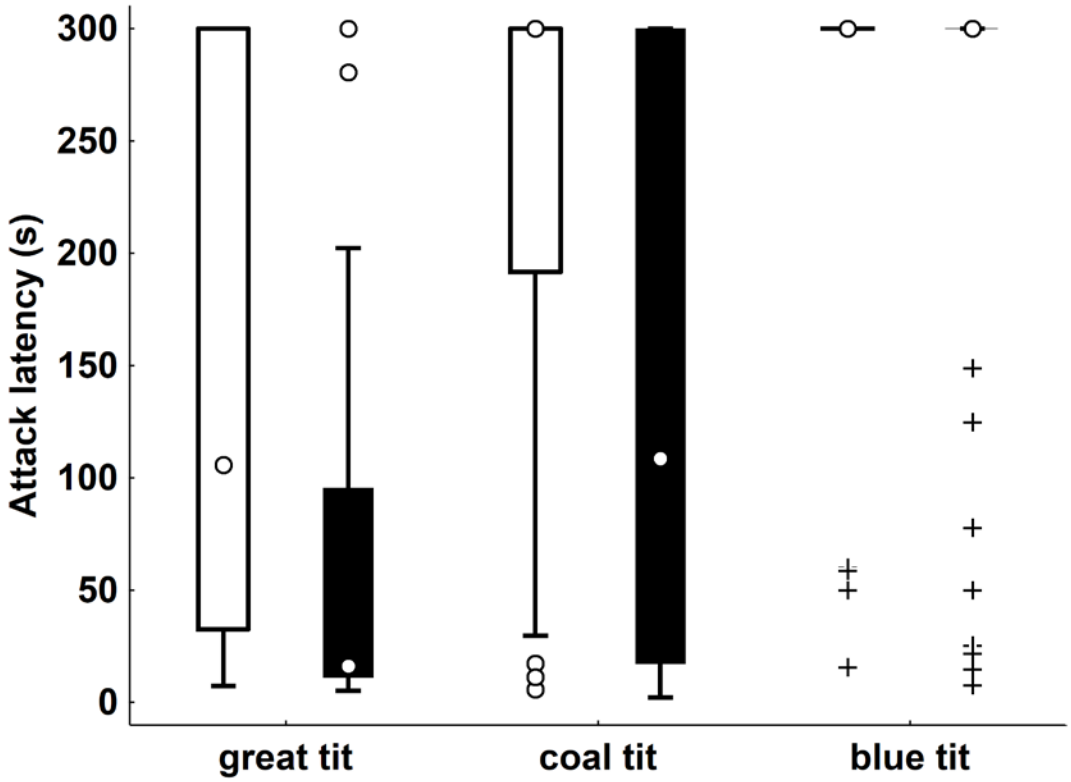

Figure 2

Manuscript in prep.

Growing out of personality? Reactions of adult great tits towards an aposematic firebug

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Growing out of personality? Reactions of adult great tits towards an aposematic firebug

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ABSTRACT

Variation in the reaction to novel and aposematic prey is common among conspecific individuals of avian predators. In wild-caught adults this variation may be caused by individual experience with various types of prey, but similar variation is present also among naive hand-reared juveniles. This variation may be explained by the effect of personality – a complex of correlated, heritable behavioural traits consistent across contexts. Two extreme personality types were defined in great tits (*Parus major*): the "fast explorers" are bold, aggressive and routine-forming; the "slow explorers" are shy, non-aggressive and innovative. We have tested the influence of personality type on innate wariness towards aposematic prey in adult hand-reared great tits from two opposite lines selected for exploration ("fast" against "slow"). The birds were offered aposematic firebugs (*Pyrrhocoris apterus*) in a sequence of trials in two following days. Birds from both personality types showed similar innate wariness towards the firebugs on the first day, but on the second day the "fast" birds approached firebugs significantly faster and more frequently than "slow" birds. Whether a bird attacked the firebug was also dependent on its personality. On the second day, half of the "fast" birds approached and also attacked the firebugs. While the "slow" birds showed the same level of wariness towards aposematic prey during the whole experiment, the initial wariness of "fast" birds decreased. However, the overall proportion of birds that attacked aposematic firebugs was much smaller than in the previous study of similarly reared hand-reared juveniles. The personality-related individual differences in reactions of great tits towards the aposematic prey were consistent across time, but the overall willingness to attack such a prey has decreased in older birds. We suggest that laboratory conditions with unlimited food supply and restricted variety of food types might have enhanced the food neophobia in "slow" birds, leading at the same time to fixation on the familiar food in routine-prone "fast" birds.

KEYWORDS

Age, aposematism, naive adult predators, *Parus major*, personality, *Pyrrhocoris apterus*

INTRODUCTION

Aposematic prey advertises its unprofitability and noxiousness by the conspicuous coloration (for review see Ruxton et al. 2004). The initial reactions of bird predators towards aposematic prey may vary among species (Brower 1988; Exnerová et al. 2003; Endler and Mappes 2004; Valkonen et al. 2012; Nokelainen et al. 2014) as well as among the conspecific individuals (Exnerová et al. 2007, 2010, 2015). Encountering novel aposematic prey can elicit neophobic reaction, which differ individually in wild birds with previous experience with a variety of food types (Marples et al. 1998, 2005; Exnerová et al. 2015), but also in naive hand-reared juveniles (Sillén-Tullberg 1985; Marples and Brakefield 1995; Exnerová et al. 2007, 2010; Svádová et al. 2009). As has been shown in previous studies, hand-reared juveniles of great tits (*Parus major*) do not exhibit any strong innate bias against aposematic prey (Sillén-Tullberg 1985; Exnerová et al. 2007, 2010; Svádová et al. 2009; Hotová Svádová et al. 2013;

Fabricant et al. 2014), but there is a variation in neophobia, rate of avoidance learning, and memory for aposematic signals among the individuals (Svádová et al. 2009; Exnerová et al. 2010; Hotová Svádová et al. 2013). This individual variability can be correlated with personality types (Exnerová et al. 2010). Personality is recognized in a variety of animal species (Gosling 2001) and it is described as “a complex of correlated behavioural traits that are consistent across time and ecological situations” (Benus et al. 1990; Sih et al. 2004). In this study we tested great tits (*Parus major*) coming from two lines selected for the opposite types of personality (for more see Verbeek et al. 1994; Drent et al. 2003). ”Slow” individuals are cautious, innovative, less aggressive and more thorough explorers, ”Fast” individuals are more aggressive, more prone to risk-taking behaviour, are superficial explorers and routine formers (Verbeek et al. 1994; Drent et al. 2003; van Oers et al. 2004; Groothuis and Carere, 2005). In our previous study (Exnerová et al. 2010), the "slow" juvenile great tits were more neophobic and more cautious in attacking aposematic firebugs (*Pyrrhocoris apterus*), and were more successful in avoidance learning than the "fast" individuals. But whether these differences are consistent across time has not yet been studied.

Neophobia was shown to be non-consistent during the life of an individual (Vince 1960; Greenberg 1992; Heinrich 1995; Fox and Millam 2004; Biondi et al. 2010, 2013). Low neophobia in young birds makes this period important in shaping their foraging niche and is mainly restricted to the juvenile period, the phase when individuals show the greatest plasticity associated with object exploration (Greenberg 2003; Biondi et al. 2010). Neophobia may increase during the life (Greenberg and Mettke-Hofmann, 2001). Heinrich (1995) showed that juvenile common ravens (*Corvus corax*) are highly explorative and curious, but at the age of one and half year they started to be more hesitant in contacting novel items. Hand-reared juveniles of Orange-winged amazon parrot (*Amazona amazonica*) exhibited lower avoidance of novel objects at the age of 7 months than at 12 months (Fox and Millam

2004), and young wild-caught Chimangos Caracara (*Milvago chimango*) were more explorative and less neophobic than their adult conspecifics (Biondi et al. 2010, 2013). Nevertheless the correlation between neophobia to novel objects and neophobia to novel food is still ambiguous. While some studies found strong correlation between exploration of novel objects (Exnerová et al. 2010) or neophobia (An et al. 2011) and acceptance of novel food, others did not show such a relationship (Bókony et al. 2012; Exnerová et al. 2015).

The aim of our study was to test whether the personality type of naive hand-reared adults of great tits (*Parus major*) affects their reaction to a novel aposematic prey - the firebug (*Pyrrhocoris apterus*). In our previous study (Exnerová et al. 2010) we found that naive hand-reared juveniles from lines selected for opposite personalities ("slow" versus "fast") showed different responses to aposematic prey. The juvenile birds were tested between 64 – 90 days of age, and almost all of them attacked the aposematic firebugs. Nevertheless, "slow" birds showed a greater degree of unlearned wariness and learned to avoid the firebugs faster than did the "fast" birds (Exnerová et al. 2010). In the present study we tested naive hand-reared adults (1,5 – 5,5 years old) from the same two personality lines, and compared their reactions towards aposematic firebugs with the behaviour of juvenile birds (Exnerová et al. 2010) to find out , whether the personality-related differences are consistent across time.

MATERIAL AND METHODS

The experiments were carried out in the Netherlands Institute of Ecology, Heteren.

(a) ***Birds***

We tested hand-reared juveniles of great tits (*Parus major*) from the F4 generation of artificial selection for two distinct personality lines: fast and slow explorers (Drent et al. 2003). Birds were tested at the age of 35 days for early exploratory behaviour by standard tests (reaction to novel objects and behaviour in novel environment (for details see Verbeek et al. 1994; Drent et al. 2003). Birds were housed individually in plastic home cages (90 x 40 x 50 cm) with front wire mesh wall, three perches, three feeders and water bowl with *ad libitum* drinking and bathing water. Birds were daily provided with mixture of seeds, dried egg yolk, dried beef heart and vitamins, supplemented with mealworms every second day. Mealworms (*Tenebrio molitor* larvae) were used in our experiments as a control familiar prey. We tested altogether 40 individuals, balanced in terms of personality and sex - 18 fast explorers (8 females and 10 males) and 22 slow explorers (12 females and 10 males). Within fast group we had six pairs of siblings and in slow group five pairs of siblings (2 birds from one nest, in one case we had 3 birds from one nest). Birds had no previous experience with aposematic or any other noxious prey and were between 1,5 and 5,5 years old when the experiments with aposematic firebug were carried out. After the experiments all birds were returned to breeding facilities.

(b) *Prey*

Adult brachypterous firebugs (*Pyrrhocoris apterus*; Heteroptera: Pyrrhocoridae) were used as novel aposematic prey. Firebugs are 7 – 12 mm long and possess red-and-black coloration with contrasting pattern. They produce defensive secretions from methathoracic glands which contain, beside others, short-chained aldehydes (Farine et al. 1992). Previous studies showed that firebugs are unpalatable for small passerine birds and their consuming may result in nausea and vomiting in some birds (Exnerová et al. 2003, 2007; Svádová et al. 2009). Firebugs are widespread in Palaearctic region and can be found all over the Europe (excluding Ireland, Norway and Finland) (Aukema and Rieger 2001). The firebugs feed mainly on seeds of *Tiliaceae*, *Malvaceae* and *Robinia pseudoacacia* (Socha 1993). We have collected adult firebugs on linden trees at two localities in Czech Republic (Prague, 50°04'N, 14°26'E and

Uherské Hradiště, 49°04'N, 17°26'E) and kept them under a long-day photoperiod (18:6h) at a temperature of 24 ± 1°C. The firebugs were provided with linden seeds and water *ad libitum*.

(c) *Experimental design*

The experimental cages were made of plywood and wire-mesh with one-way glass front wall (70 x 70 x 70cm). They were equipped with water bowl, beige wooden rotating feeding tray containing 6 glass dishes, l and a perch placed 30cm from the feeding tray. The simulation of daylight condition was achieved by the lamps Biolux Combi, Osram (see Exnerová et al. 2003, 2010 for details). The birds were deprived of food for 2 hours before an experiment and trained to search for mealworms in one of the dishes.

The experiment consisted of (1) avoidance learning session and (2) memory test. (1) The first day birds learned to avoid firebugs in a sequence of 5 min trials, in which the control palatable mealworms were alternating with aposematic firebugs. The learning session was considered successful when the bird refused to attack three firebugs in a row. If the bird did not attack the first firebug, the sequence continued until a maximum of 10 firebugs was presented, and the learning session was repeated next day. (2) If the bird attacked the firebugs, and then learned to avoid them, the memory test was carried out on the following day. The memory test consisted of a sequence of 5 firebug trials. We recorded whether the firebug was approached, attacked, killed and consumed as well as the approach and attack latency. Behaviour of birds was recorded in Observer Video-Pro © Noldus and on video recorder.

(d) *Data analysis*

We compared latencies to attack the mealworms offered at the beginning of each-day sessions to check that birds from both personality groups entered the experiment with similar foraging motivation and habituation to the experimental cage. The latencies were log-transformed, and then analysed using ANOVA with personality type as a factor. Latencies of the first approach to the firebug were log-transformed and analysed by ANCOVA with personality as fixed factor, bird's sex as random factor, and age as covariate. Numbers of approaches per trial were square-root transformed and tested by ANCOVA with personality as fixed factor, bird's sex as random factor, and age as covariate. Within-group changes in approach latencies between day 1 and day 2 were tested by t-tests for dependent samples. We also compared counts of birds that attacked at least one firebug during the experiment using GLM ANCOVA model for binomial distribution and logit link function, with personality as fixed factor, bird's sex as random factor, and age as covariate. The calculations were made using Statistica 6.1 (StatSoft Inc.).

RESULTS

(a) *Reaction to familiar prey*

There was no difference between the two personality groups in latencies to attack the mealworm offered in the first trial of avoidance learning (ANOVA: $F_{1,38} = 2.121$, $p = 0.154$), as well as in latencies to attack the first mealworm in the memory test (ANOVA: $F_{1,38} = 0.961$, $p = 0.333$). All birds killed and ate the mealworms they were offered.

(b) *Reaction to aposematic prey*

All the birds but five (three slow explorers and two fast explorers) approached the firebugs and inspected them from a close distance at least once during the first-day learning session. The latencies of the first approach to the firebug and the numbers of approaches per trial were not influenced by personality (ANCOVA: latency - $F_{1,31} = 0.003$, $p = 0.960$; number: $F_{1,31} = 0.024$, $p = 0.879$), sex (ANCOVA: latency - $F_{1,30} = 3.149$, $p = 0.086$; number: $F_{1,30} = 0.091$, p

= 0.765) and age of the birds (ANCOVA: latency - $F_{1,29} = 0.851$, $p = 0.364$; number: $F_{1,29} = 0.239$, $p = 0.629$).

In the second-day test, there was a difference between the two personality groups in both the latencies of approaching the firebugs (ANCOVA: $F_{1,31} = 18.394$, $p < 0.001$) and in the number of approaches per trial (ANCOVA: $F_{1,31} = 10.430$, $p = 0.003$). Fast birds hesitated shorter before first approaching the firebugs, and also inspected the firebugs at close distance more often than slow birds. The sex and age of the bird affected neither the latencies (ANCOVA: effect of sex - $F_{1,30} = 2.561$, $p = 0.120$; effect of age - $F_{1,29} = 2.727$, $p = 0.109$) nor the number of approaches (ANCOVA: effect of sex - $F_{1,30} = 0.183$, $p = 0.672$; effect of age - $F_{1,29} = 1.377$, $p = 0.250$). Fast birds shortened their latencies to approach the firebugs from the first-day to the second-day session (t-test: $t = 2.283$, df = 13, $p = 0.040$), whereas the approach latencies of slow birds remained the same (t-test: $t = -1.214$, df = 18, $p = 0.240$).

Although the birds repeatedly approached the firebugs and inspected them from close distance, most of them refused to attack any firebug during both days of learning sessions. Nevertheless, whether the birds attacked at least one firebug was influenced by their personality (GLM ANCOVA: Chi-square = 6.390, df = 1, 38, $p = 0.011$); half of the fast birds and only three of the slow birds attacked the firebugs. There was no effect of sex and age of the birds on the probability of attacking the firebugs (GLM ANCOVA: effect of sex - Chi-square = 0.076, df = 1, 37, $p = 0.783$; effect of age - Chi-square = 0.442, df = 1, 36, $p = 0.506$). The birds learned to avoid the firebugs very quickly, attacking mostly only one individual during the process of learning. They usually only slightly pecked the firebug or seized it by the bill and dropped it. The firebugs almost always survived the attacks. Only two fast birds attacked, killed, and consumed several firebugs before reaching the learning criterion. None of the birds that learned to avoid the firebugs attacked any firebug during the memory test.

DISCCUSSION

(a) *Effect of age*

There is a considerable difference in behaviour towards aposematic prey between naive hand-reared juvenile great tits and their experienced wild-caught conspecifics (Exnerová et al. 2007). Since the previous studies did not show any strong innate wariness of aposematic prey in hand-reared great tits (Exnerová et al. 2007, 2010; Svádová et al. 2009; Hotová Svádová et al. 2013), it is surprising that in our experiment hand-reared adults frequently avoided the aposematic prey. Birds in this study rarely attacked, pecked or seized the aposematic firebugs (*Pyrrhocoris apterus*), and they mostly only came to a close proximity and inspected the prey by sight. In the previous study (Exnerová et al. 2010), juvenile great tits reared in the same conditions and same location as birds tested in this study exhibited greater willingness to attack, kill and consume the firebugs. The difference between behaviour of juvenile and adult birds might have been caused by changes in neophobia or dietary conservatism during the life.

Neophobia is a process which protects animals from toxic and dangerous food, but on the other hand, the exploration of novel resources is important for young individuals when their foraging behaviour is being shaped (Greenberg and Mettke-Hofmann 2001). Several studies have shown that birds are more willing to explore novel stimuli, when they are young (Vince 1960; Heinrich 1995; Fox and Millam 2004; Biondi et al. 2010). Whereas Coppinger (1970) found innate avoidance of novel aposematic butterflies (Nymphalinae) in hand-reared blue jays (*Cyanocitta cristata*) and red-winged blackbirds (*Agelaius phoeniceus*) tested at the age of 9 – 10 months, no such avoidance was recorded by Smith (1980) testing hand-reared juveniles of the same bird species with novel and warningly coloured stimuli when they were 40 days old. Mastrota and Mench (1994) observed greater aversion of female bobwhites (*Colinus virginianus*) towards red- and orange-dyed food, when tested in 64 – 67 weeks, than in younger, 31 weeks old females (Mastrota and Mench 1995). No such an effect was observed in the conspecific males. The aversion of novel-coloured food in female bobwhites thus appears to increase with age, and a possible explanation may be a different role of sexes in parental care in bobwhites, where the females teach their chicks to avoid toxic prey. Considering the role of both parents in post-fledging care in great tits, we may expect the same level of wariness in both sexes.

The hypothesis of increasing neophobia with age was not supported by the study of Langham (2006), who found older individuals of wild-caught rufous-tailed jacamars (*Galbula ruficauda*) to be more willing to attack novel aposematic butterflies (*Heliconius* sp.) than their younger (and also wild-caught) conspecifics. It is therefore likely that wild-caught experienced adult birds are bolder to attack novel prey, because they have already learned to avoid unpalatable food, whereas wild-caught younger birds may be more cautious due to a recent experience with unpalatable or toxic prey. This hypothesis is supported by Lindström et al. (1999), who compared reactions towards warningly coloured yellow-and-black prey in three age categories of great tits: hand-reared juveniles, wild-caught yearlings and wild-caught adults. While both hand-reared juveniles and wild-caught adults readily attacked warningly coloured prey, wild-caught yearlings appeared to be the most neophobic age group (Lindström et al. 1999). Similarly, wild-caught yearlings of great tits attacked and killed more individuals of novel colour forms of the firebug (*Pyrrhocoris apterus*) than did the wild-caught adults (Exnerová et al. 2006).

Even though the great tits in this experiment were inexperienced with aposematic prey and 1,5 – 5,5 years old at the time of testing, they were very cautious to attack unfamiliar aposematic firebugs. Contrastingly, wild-caught great tits from the Finnish population, which lack any experience with aposematic firebugs (due to their absence in Finland), showed no hesitation to attack them. There might be another factor possibly responsible for the cautious behaviour of birds in the present study. It is likely that the environment and rearing conditions play an important role in shaping the reactions towards novelty in birds (Jones 1986; Meehan and Mench 2002; Fox and Millam 2004, 2007). Studies of domestic chicks (*Gallus gallus*) and orange-winged Amazon parrots (*Amazona amazonica*) demonstrated the importance of enrichment of the home environment. Enriched environment significantly reduced the fear of novel objects in orange-winged Amazon parrots (Meehan and Mench 2002; Fox and Millam 2004, 2007), and in chicks, the home environment with assorted novel objects even increased the acceptance of novel food into their diet (Jones 1986).

The experience with variety of food reduced neophobia and increased acceptance of novel food in chicks (Jones 1986; Marples et al. 2007) and turkeys (Lecuelle et al. 2011, but see Mastrota and Mench 1995). Because the majority of birds in our study did not explore the firebugs by tactile contact, we can not decide, whether their avoidance of novel prey was a result of neophobia or dietary conservatism (Marples and Kelly 1999). However, food neophobia is described as a short-time response lasting usually only a few minutes and followed by a tactile contact with the novel food (Marples and Kelly 1999), whereas the birds in our study showed a relatively long-lasting refusal of novel prey. The rearing conditions with a limited variety of food types and an unlimited supply of them could make the birds unwilling to explore a novel prey. This would correspond with the relationship between generalist-specialist behaviour and exploration, where the food or habitat specialists exhibit greater neophobia than the species that exploit broader variety of foods and habitats (Greenberg 1984, 1990; Mettke-Hofmann et al. 2002; Webster and Lefebvre 2001; Tebbich et al. 2009).

(b) *Effect of personality*

Although the birds of both personality types showed similar aversion towards the aposematic firebugs on the first day, their behaviour on the second day considerably differed. On the second day, the "fast" birds approached the firebugs significantly earlier and more often than the "slow" ones. Whether the birds attacked the firebugs was also dependent on personality types. On the second day, half of the "fast" birds not only approached and inspected the firebugs from a close distance, but also seized and pecked it. Nevertheless, only two fast birds consumed several firebugs during the experiment. Long-lasting process of refusing to consume novel food was termed dietary conservatism (Marples et al. 1998; Marples and Kelly 1999). In the wild-caught great tits, the dietary conservatism has been shown to be independent on personality, because the dietary conservative birds were not found to be slower explorers (Marples and Mappes 2011). However, in Japanese quails (*Coturnix coturnix japonicus*), selected accordingly to their speed of recruitment of aposematic non-toxic two-spot ladybirds (*Adalia bipunctata*) into their diet, the variation in acceptance of novel prey had a genetic basis, suggesting an effect of dietary conservatism (Marples and Brakefield 1995). Whether the dietary conservatism is connected with personality traits is still a question for future research.

The relationship between exploration/object neophobia and neophobia towards novel food is also still ambiguous. Only a few studies dealt with the correlation between food neophobia and other personality traits (Exnerová et al. 2010, 2015; An et al. 2011; Bókony et al. 2012; Liebl and Martin 2014). Exnerová et al. (2010) showed a positive correlation between exploration and reaction towards novel aposematic prey in juvenile great tits. Similarly, An et al. (2011) have found positive correlation between latency to approach and feeding in the presence of novel object and approach and pecking novel type of food in wild caught blacked-capped chickadees (*Poecile atricapillus*). However, food neophobia correlated with object neophobia only in one of the four Hungarian populations of house sparrows (*Passer* *domesticus*), and in none of the eight populations studied in Kenya (Bókony et al. 2012; Liebl and Martin 2014). The correlations seem to be population-specific, not only in house sparrows, but also in great tits. While Dutch population showed strong correlation between personality traits and behaviour to novel food, no such correlation was found in Finnish and Bohemian populations of great tits (Exnerová et al. 2010, 2015).

In great tits, the slow explorers tend to be more successful in avoidance learning than the fast explorers, presumably because they are more neophobic and sensitive to negative stimuli (Exnerová et al. 2010). In our study, all birds that have overcome their neophobia and attacked the aposematic firebugs learned to avoid them very quickly, attacking mostly only one firebug during the learning session. The firebugs were mostly only pecked or seized and dropped, and only occasionally consumed. Such a behavioural response might indicate an effect of dietary conservatism, but considering the unpalatability of the firebug, it also may be a result of a rapid avoidance learning. Similarly, wild-caught adult great tits from Finnish population that were inexperienced with the aposematic firebugs, learned to avoid them considerably faster than their naive hand-reared conspecifics (Svádová et al. 2009; Exnerová et al. 2010). Interestingly, the Finnish wild-caught adults were much less cautious in attacking aposematic prey (Exnerová et al. 2015) than the hand-reared captive adults in our study.

CONCLUSIONS

On the first day of the experiment, the fast birds hesitated as long as the slow birds in approaching the aposematic firebugs. However, the type of personality influenced the approach latencies on the second day, when the slow birds hesitated longer than the fast birds. The slow birds were also approaching the firebugs less frequently than the fast birds. While the slow birds showed the same level of innate wariness towards aposematic prey during the whole experiment, the level of innate wariness of the fast birds significantly decreased. We suggest that the laboratory rearing conditions with unlimited food supply and restricted variety of food types might have enhanced the innate wariness in slow birds, whereas in fast birds the same conditions might have lead to developing a routine behaviour of feeding exclusively on a familiar food . Even though hand-reared juveniles from the same selection lines (Exnerová et al. 2010) reacted differently towards aposematic firebugs than hand-reared adults tested in this study, we were still able to observe significant differences in reactions of birds of different personality types. We can therefore conclude that the individual differences in reactions of great tits towards aposematic prey are consistent across time, but the particular way of reactions and their intensity changes during their life.

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FIGURES AND LEGENDS

Figure 1. Approach latencies of naive adult great tits of the two personality types (S-slow, F-fast) measured from the beginning of the first firebug trial to first approach to the firebug in the first (DAY 1) and second day (DAY 2) of firebug presentation. Square, mean; box, mean ± s.e.; whiskers, confidence intervals (0.95); circles, outliers; stars, extremes.

Figure 2. Number of approaches to firebugs per trial by naive adult great tits of two personality types (S-slow, F-fast) in the first (DAY 1) and second day (DAY 2) of firebug presentation. Square, mean; box, mean ± s.e.; whiskers, confidence intervals (0.95); circles, outliers; stars, extremes.

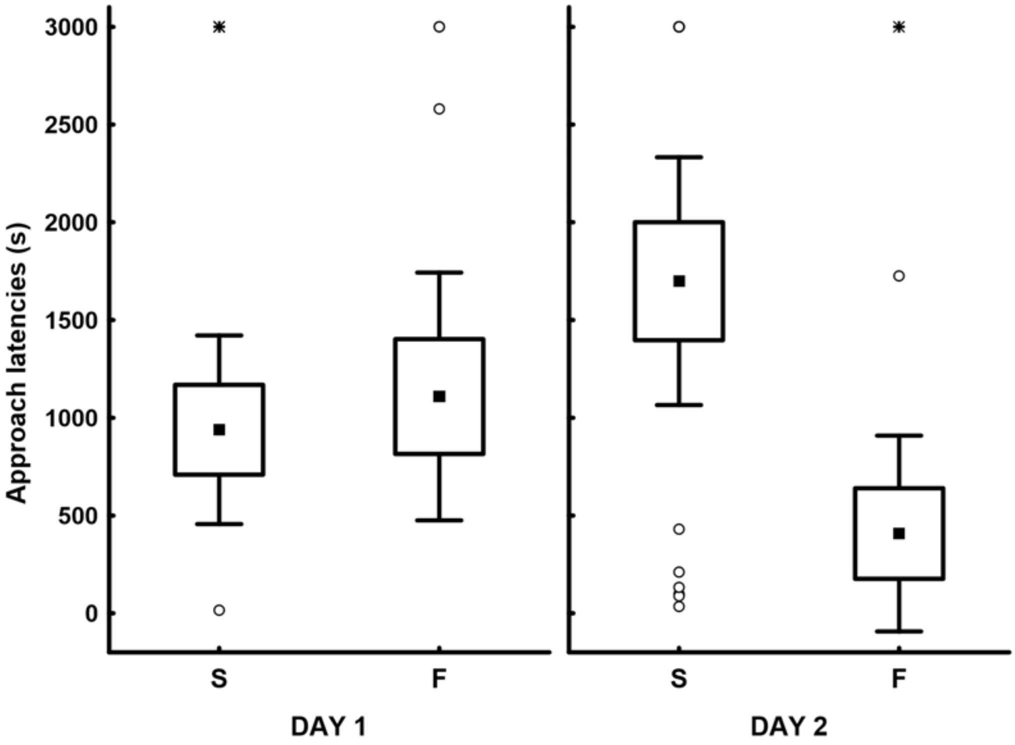

Figure 1.

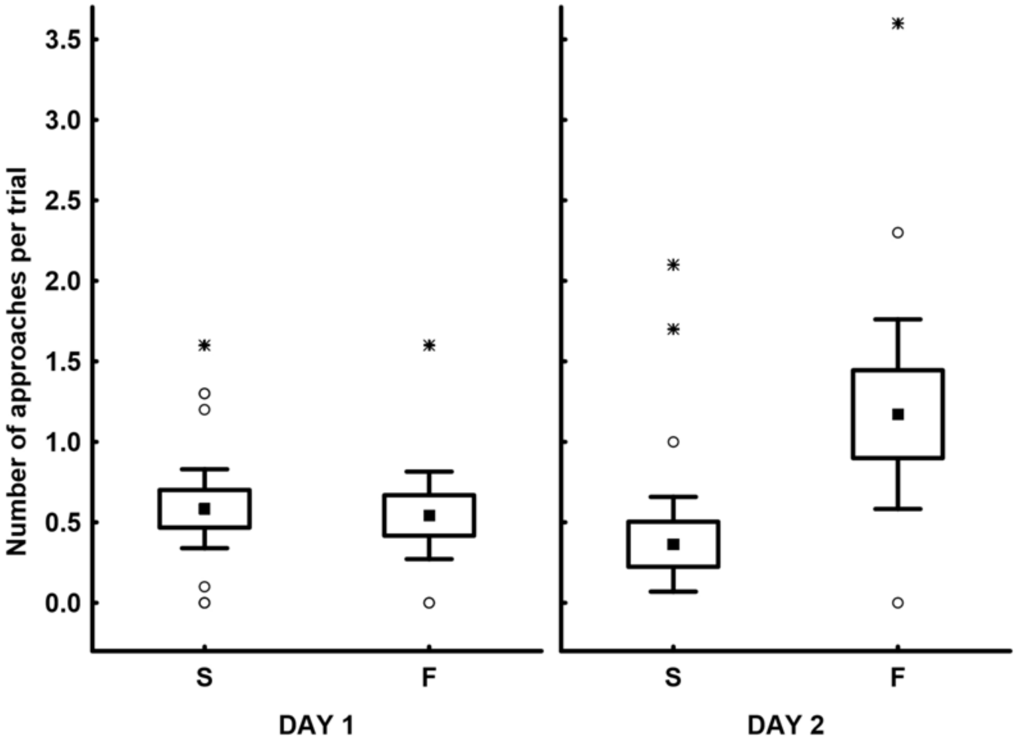

Figure 2.

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Scared by shiny? The value of iridescence in aposematic signalling of the hibiscus harlequin bug

Fabricant Scott, Exnerová Alice, Ježová Dana & Štys Pavel

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I, Scott Fabricant, declare the following:

The student, Dana Adamová (Ježová) participated in the design and execution of the experiments, data analyses and preparation of the manuscript.

I agree with including the outputs of this publication in her Ph.D thesis.

Scott Fabricant, Ph.D

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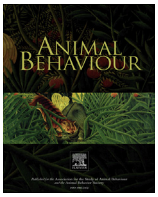

Scared by shiny? The value of iridescence in aposematic signalling of the hibiscus harlequin bug

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Studies on aposematism have generally focused on the benefits of red or yellow coloration, occasionally in contrast with green or brown, but rarely blue or orange. Furthermore, almost no studies have explicitly studied the utility of iridescent coloration in aposematism. To evaluate the survival benefit of iridescent coloration, we tested the ability of the natural colour extremes of *Tectocoris diophthalmus* jewelbugs to induce initial avoidance, learned avoidance, discrimination from palatable alternatives and broad generalization against avian predators: naïve hand-reared and experienced wild-caught great tits, *Parus major*. Artificial baits were created by hollowing out bugs and inserting pieces of mealworm. Preference tests presented iridescent and orange baits simultaneously, then birds were divided into training groups and sequentially exposed to palatable black baits alternated with iridescent or orange baits made unpalatable by soaking mealworms in quinine solution. This was followed by simultaneous black/coloured discrimination tests, then a generalization test with both previously experienced and novel baits (all palatable). All groups showed a preference for orange baits over ones with iridescent patches. For wild-caught birds, attack latencies of iridescent and orange training groups were statistically indistinguishable, although only orange caused increased attack latency over the sequence. Hand-reared birds showed no change in attack rate/latency towards iridescent bugs over the sequence. In postlearning discrimination tests, all groups showed equally strong preference for palatable black baits and their unpalatable training baits. In generalization tests, hand-reared birds were most averse towards trained baits, but increased avoidance of iridescent-and-black baits suggests iridescence alone can contribute to aposematism. Wild-caught birds showed strong aversion to iridescent and novel orange-and-black baits regardless of training, suggesting birds may be broadly generalizing experience from local red-and-black aposematic bugs. Results suggest iridescent coloration and patterning can be an effective aposematic signal, especially in the presence of alternative palatable prey and/or other aposematic species.

Aposematism is the phenomenon of prey advertising their unprofitability to potential predators through conspicuous and memorable signals that are often visual in nature (reviewed in Ruxton, Sherratt, & Speed, 2004). There is a rich literature on how predators, particularly birds, respond to visual aposematic signals and their individual components. Several studies have demonstrated that birds attend mostly to colour when learning to avoid aposematic prey (Exnerová et al., 2006; Ham, Ihalainen, Lindström, & Mappes, 2006; Svádová et al., 2009). Other studies have demonstrated the relative value of background contrast (Gamberale-Stille, 2001; Gamberale-Stille & Guilford, 2003), the relative value of internal contrast and patterning (Aronsson & Gamberale-Stille, 2008, 2009; Hegna, Saporito, Gerow, & Donnelly, 2011), the importance of pattern regularity/symmetry (Forsman & Merilaita, 1999; Stevens, Castor-Perry, & Price, 2009) and the breadth/direction of generalization (Gamberale-Stille & Tullberg, 1999; Svádová et al., 2009) for aposematic signals. Theory and empirical studies also suggest that aposematic patterns tend towards signal uniformity (Greenwood, Cotton, & Wilson, 1989; Lindström, Alatalo, Lyytinen, & Mappes, 2001), and maximal conspicuousness or distinctiveness (Roper & Redston, 1987; Sherratt & Beatty, 2003), but instances of intraspecific variation and/or low conspicuousness are common (Stevens & Ruxton, 2012). Understanding the selection pressures upon these 'non-textbook' aposematic systems is currently an active area of research (Blount, Speed, Ruxton, & Stephens, 2009; Endler & Mappes, 2004; Lindstedt, Talsma, Ihalainen, Lindström, & Mappes, 2010; Speed & Ruxton, 2007).

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However, despite the breadth of the literature, some areas have received considerably less attention. For example, many authors cite the prevalence of red and yellow in aposematic signals (Endler & Mappes, 2004; Ruxton et al., 2004; Théry & Gomez, 2010), but no research has quantified this prevalence. Few authors have experimented with other colours. One paper showed ultraviolet cues are not effective in avoidance learning of great tits, *Parus major* (Lyytinen, Alatalo, Lindström, & Mappes, 2001). While there are few potentially aposematic species with blue components in their signals, seemingly none have been tested empirically (reviewed in Umbers, 2013), although artificial blue/cyan prey have been used successfully by Gamberale-Stille and Guilford (2003) and Aronsson and Gamberale-Stille (2008). The efficacy of green as a colour in avoidance learning is difficult to establish, as green or brown is often used as an 'inconspicuous' control in experiments. However, exposure to pyrazine odours (Rowe & Guilford, 1996) or the bitter taste of quinine (Rowe & Skelhorn, 2005) can cause unlearned biases against yellow or red but not green. However, there is at least one study showing green food as being more resistant to overcoming dietary conservatism than yellow or red (Marples, Roper, & Harper, 1998). Better understanding of the learning value of less common 'warning' colours such as green or blue requires more study.

Similarly to short wavelengths, iridescent colours in aposematism are an understudied phenomenon. By definition, the hue of iridescent colours changes with viewing angle, owing to the arrangement of multiple reflecting layers creating a coherent Bragg mirror (Seago, Brady, Vigneron, & Schultz, 2009). Additionally, small variations in the layer spacing can result in a large change in hue or saturation (Kurachi, Takaku, Komiya, & Hariyama, 2002). These aspects of iridescent coloration may affect their efficacy in providing a reliable aposematic signal. However, iridescent and other structural colours can be very bright (Seago et al., 2009), and are capable of creating short-wavelength hues such as blues and ultraviolets uncommon in pigments (Doucet & Meadows, 2009; Umbers, 2013). Paired with pigmentary colours, this ability to create chromatic and/or luminance contrast may enhance the conspicuous and distinctiveness of the aposematic signals (Doucet & Meadows, 2009; Endler, 1992). Therefore, there is great value in studying the role of iridescence in aposematic learning.

There are scattered studies documenting a role for iridescent colours in aposematic signals in beetles (Schultz, 2001) and butterflies (Bowers & Larin, 1989; Pegram, Lillo, & Rutowski, 2013; Rutowski, Nahm, & Macedonia, 2010), but none for true bugs. Iridescence is relatively common in some families of true bugs (Heteroptera), particularly in the assassin bugs (Reduviidae), plant bugs (Miridae), leaf-footed bugs (Coreidae), burrower bugs (Cydnidae), shield bugs (Pentatomidae and Scutelleridae) and some minor families (P. Štys, unpublished data). In many of them, iridescence is combined with yellow, orange or red pigment coloration producing a potentially aposematic effect. This type of coloration is sometimes limited only to larvae for poorly understood reasons (P. Štys, unpublished data).

Tectocoris diophthalmus (Heteroptera: Scutelleridae), the hibiscus harlequin bug, is an ideal study system to engage with questions of intraspecific variation and learning value of iridescent coloration. Rather than employing the more 'typical' aposematic colour scheme of red or yellow with black markings, *T. diophthalmus* bugs display a matte orange background overlaid with bright metallic blue-green iridescent patches. These patches are highly variable between individuals, ranging in size from almost covering the dorsal surface to being entirely absent (Fabricant, Kemp, Krajíček, Bosáková, & Herberstein, 2013). Like other true bugs, *T. diophthalmus* produces defensive secretions (Staddon, Thorne, & Knight, 1987), and has been demonstrated to be capable of inducing avoidance learning in chickens (Fabricant & Smith, 2014). The species lives in Australia and Malesian and Melanesian islands, and is arboreal and mainly phytophagous, feeding on *Hibiscus*, *Gossypium* and *Lagunaria* species (Malvaceae; Cassis & Gross, 2002).

The aim of this study was to test the efficacy of the iridescent coloration of *T. diophthalmus* in inducing initial aversion, avoidance learning and generalization. We tested this bug, a native to Australia, against European great tits, an allopatric species which has no experience with *T. diophthalmus* or any other iridescent species of true bug. We tested both wild-caught and hand-reared birds; hand-reared great tits are completely naïve to noxious prey, and have been shown in experiments to lack innate bias against red-and-black coloured bugs (Exnerová et al., 2007; Svádová et al., 2009), but wild-caught birds are likely to have experience with noniridescent true bugs, including local red-and-black aposematic species (Hotová Svádová, Exnerová, Kopečková, & Štys, 2010). We compared wild-caught and hand-reared birds with respect to their initial wariness towards palatable iridescent and noniridescent (orange) bugs, and in their behaviour towards unpalatable iridescent bugs across a learning sequence, discrimination tests and generalization tests using novel bugs sharing features of conditioned bugs. Because we could not control the previous experience of wild-caught birds, we also compared the behaviour of wild-caught birds exposed to unpalatable iridescent bugs with that of birds exposed to unpalatable orange bugs that lacked iridescent patches.

Our specific questions were as follows. (1) Do the iridescent patches of *T. diophthalmus* increase initial avoidance? (2) Are iridescent patches efficient in inducing avoidance learning and discrimination from palatable alternatives? (3) Do birds generalize broadly among different colour phenotypes of the bugs, including to more 'typical' orange-and-black bugs? (4) Do experienced and naïve birds differ in their patterns of initial avoidance, learning to avoid unpalatable iridescent prey, and generalization of novel bug phenotypes? By comparing the results of these experiments to previous studies, we aimed to elucidate what components of the visual warning signals of *T. diophthalmus* are most salient to birds, and how variation may affect their survival.

METHODS

Avian Predators

Great tits are small, predominantly insectivorous passerine birds inhabiting mostly woodlands, parks and gardens (Cramp & Perrins, 1993). They are frequently used as model predators in studies of warning signals and mimicry (Lindström, Alatalo, & Mappes, 1997; Sillén-Tullberg, Wiklund, & Järvi, 1982; Svádová et al., 2009) and are suitable for studies involving novel prey, because they are less neophobic than other European species of Paridae, and their avoidance of aposematic insects is mostly based on individual learning (Exnerová et al., 2007).

Hand-reared Birds

We used 20 hand-reared great tits as naïve predators, and all were trained only with iridescent bugs. Juvenile birds were taken from their nestboxes in spring 2012 when 12–16 days old. At this age the nestlings have no visual experience with their food. The nestboxes were placed in parks and orchards in the outskirts of Prague, Czech Republic. No more than two nestlings were taken from the same brood. The nestlings were transported to the laboratory in the same artificial nests they were housed in (plastic boxes for keeping small animals with textile and tissue-paper lining for insulation and comfort). The length of transport did not exceed 30 min. Nestlings were

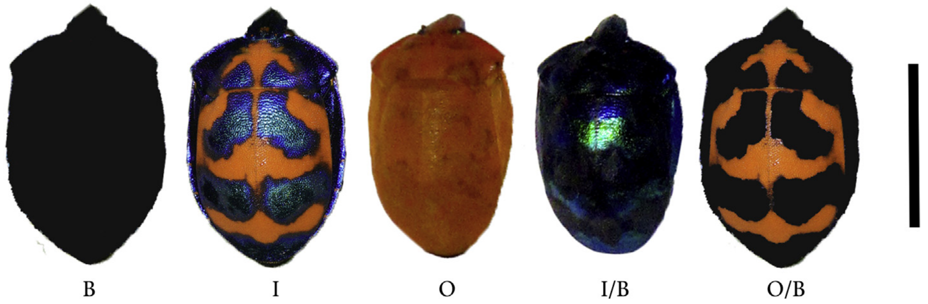

Figure 1. Examples of bug baits: black (B), iridescent (I), orange (O), iridescent-and-black (I/B) and orange-and-black (O/B). Black bar is 1 cm.

kept in artificial nests until fledging, and then housed individually in plastic cages (40 × 50 cm and ×40 cm high) with a wire-mesh front wall. The home cages were equipped with three wooden perches, two water bowls with drinking and bathing water provided ad libitum and with two feeders situated on the bottom of the cage. The birds were housed at 22–25 °C. The photoperiod was set the same as outdoors (16:8 h light/dark). The birds were hand-reared with a standard food for passerines (Handmix, Orlux) along with mealworms. When they started to feed independently, they received food mixtures for insectivorous passerines (egg mixtures Oké-bird and Nutribird, Versele-Laga; Uni patee and Insect patee, Orlux). They were tested when they were fully independent at the age of 50–60 days. Individuals were released after experiments in the third week of July, on days without rain or wind, in the same locality they were taken, as specified by our permits. They were first given a veterinary check-up to ensure they were at normal weight and in good condition, their wing and tarsus lengths were measured, and they were individually banded. They were provided with supplementary food (mealworms, sunflower seeds and the commercial mixture for insectivorous birds) in the feeders placed at the locality for several days.

Wild-caught Birds

Forty adult great tits (20 males and 20 females; 18 yearlings and 22 older birds) were caught in mist nets in September 2012 in the Botanical Garden of Charles University in Prague. The birds were housed individually in the same cages, at the same temperature and photoperiod, and fed with the same food as hand-reared juveniles. After the experiments, they were released after testing in the third week of September, in the same location they were caught as specified by our permits, on days without rain or wind. As for the hand-reared birds, the wild-caught birds were given a veterinary check-up, measured and banded and provided with supplementary food on release. While no detailed monitoring took place afterwards, regular catching and banding of birds at this location suggests a number of the birds survived and bred in the same locale for multiple years.

Prior to the experiment, the wild-caught birds were allocated to two experimental groups, one assigned for the avoidance learning with iridescent bugs and the other with orange bugs. The composition of both groups was balanced with respect to sex (iridescent group: 10 males/ 10 females; orange group: 11 males/ 9 females) and age (iridescent group: 10 yearlings/ 10 adults; orange group: 10 yearlings/ 10 adults) to maximize validity of between-group comparisons.

Tectocoris *Baits*

Adult *T. diophthalmus* were hand picked off *Lagunaria patersonia* trees in a suburban population in Narrabeen, New South Wales, Australia. Bugs were killed by freezing, and then dried completely in a fume cupboard. Their ventral abdominal cuticle, remnants of internal organs, antennae and legs were then removed, creating a hollow dome consisting of the insect's head, thorax, wings and body length scutellum (abdominal shield). The bug retained its natural shape and colour patterns when viewed from above (see Johansen et al., 2010 for details). Inside the hollow body cavity we placed half a mealworm, *Tenebrio molitor* (henceforth referred to as 'mealworm'), serving as an unconditioned stimulus. The mealworms were attached to the ventral side of bugs and were not visible until the birds seized or turned over the baits.

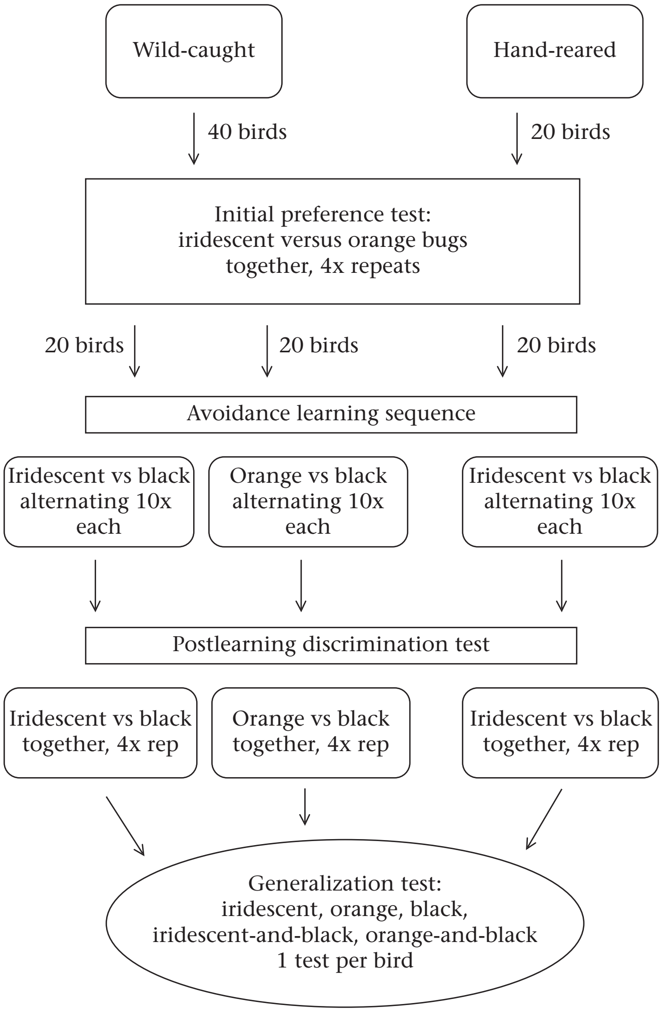

Figure 2. Flowchart of the experimental design. Following the arrows down shows the series of experiments that each training group underwent, as well as their sample size and the number of repetitions of each experiment. Pretraining regimes, identical for all groups, are not included in this flowchart.

We used five types of bait (Fig. 1) differing in their coloration: (1) iridescent bugs with approximately 50% of their dorsal surface covered by iridescent blue-green patches and the rest remaining orange; (2) naturally orange bugs with only orange coloration; (3) black bugs, individuals painted all black with nontoxic Tim & Tess poster colour (shade 105 – carbon black); (4) iridescent-and-black bugs with their orange parts painted black; and (5) orange-and-black bugs with their iridescent parts painted black. Both iridescent-and-black and orange-and-black bugs had approximately half of their dorsal surface painted black and the other half orange or iridescent, respectively.

Experimental Set-up

Experiments were carried out in wooden cages (70 × 70 × 70 cm) with wire-mesh walls and the front wall made of one-way glass. Each cage was equipped with a wooden perch, water bowl and rotating circular feeding tray with six cups. The perch was 20 cm from the feeding tray. The cups had a white lining on which all types of baits appeared highly conspicuous. The cage was illuminated by daylight-simulating bulbs (Biolux Combi 18 W, Osram). A continuous record of the bird's behaviour in the experiment was made using the Observer XT 8.0, and the behaviour was also videorecorded. Experiments were spread across 3 days and were divided into several successive steps (Fig. 2). Before the experiment, the birds were caught by hand in their home cages, put into a textile bag and transported to the experimental cages; they were transported to their home cages in the same manner after the experiments. The birds were allowed to acclimate to the experimental cage and to search for food in one of the cups of the feeding tray before the experiment. Each day before starting the respective part of the experiment, the birds were deprived of food for 2 h. They showed no behavioural markers of stress during or after the experiment.

Pretraining (Days 1 and 2)

To minimize the number of bugs destroyed during the experiment we used black *Tectocoris*-shaped silhouettes cut from cardboard with a mealworm glued to their lower side by nontoxic glue (Kores glue stick) to train the birds to handle the baits and extract the edible piece of mealworm from the inedible shell. The birds had to pass three successive stages of the pretraining: (1) handling the upside-down bait with the mealworm on the top; (2) handling the bait with the mealworm beneath the paper silhouette, but with part sticking out; (3) handling the bait with the mealworm hidden completely beneath the paper silhouette. The birds had to handle successfully two baits with a hidden mealworm in a row to participate in the experiment. At the beginning of the second-day session, the pretraining was repeated with black-painted *Tectocoris* baits to check whether the birds remembered their experience and generalized it to the real *Tectocoris* baits. Identical pretraining was performed for both hand-reared and wild-caught birds.

Initial Preference Test (Day 2)

To determine whether iridescent coloration increases initial avoidance, we tested whether the birds had any preference for either of the two natural forms of *Tectocoris* bugs used in the experiment. The birds were offered two baits, one iridescent and one orange, both with a palatable piece of mealworm hidden inside, together in the same dish. This process was repeated a total of four times. We recorded the order in which baits were chosen, and measured the latency to attack each bait. The latency to attack the second bait was adjusted to account for the handling and feeding time of the first bait. After the bird finished handling the first bait, it had 3 min (180 s) to handle the other bait; otherwise the trial was stopped and a nonattack was recorded. The initial preference testing procedure was identical for both hand-reared and wild-caught birds.

Avoidance Learning Sequence (Day 3)

The next phase involved training birds against a specific colour morph of bug, to determine the efficiency of avoidance learning. Wild-caught birds were divided into two experimental groups of 20 birds: one group were trained to avoid iridescent bugs, the other to avoid orange bugs. All 20 hand-reared birds were trained to avoid iridescent bugs. Aposematic baits were made unpalatable by soaking the mealworm in 6% quinine (chloroquine phosphate) solution, while the mealworms for palatable baits were soaked in water. Baits were presented in alternating sequence; in each trial only one bait (either black/palatable or coloured/unpalatable) was offered. Although this sequential discrimination task is likely to be more difficult than simultaneous presentation (Beatty & Franks, 2012), we argue that it reflects better a natural situation. Sequences always started with black/palatable, and baits were reused in a sequence unless the birds inflicted damage on them. A trial was terminated after 3 min (180 s) if no attack had occurred, and a nonattack was recorded. Birds were exposed to 10 trials of presentation of each alternating bait type, for a total of 20 trials.

Postlearning Discrimination Test (Day 3)

To assess whether there was a difference in how well the birds learned discrimination between palatable and unpalatable baits, we offered birds a simultaneous presentation of the two bait types used in the preceding learning sequences. Those birds trained with iridescent baits were now offered black and iridescent baits side by side in one dish, and likewise with birds trained on orange baits. This test was repeated a total of four times, and unpalatability of the aposematic baits was maintained during tests. We recorded the order of baits attacked as a binary choice (yes/no palatable first).

Generalization Test (Day 3)

Finally, trained birds were given a generalization test to determine whether training stimulus colour or previous experience in the wild influenced patterns of generalization. In a single trial, birds were offered five baits, each of a different 'phenotype': iridescent (I), orange (O), black (B), iridescent-and-black (I/B) and orange-and-black (O/B) (Fig. 1). The test was carried out under extinction conditions (all baits palatable) to prevent further learning from interfering with the birds' previous experience. The baits were presented in a rectangular dish (17 × 8 cm), aligned in a row parallel to the enclosure's perch. The order of baits in the row was randomly generated for each bird. We recorded the order of attack and the latency to attack for each bird. The latency was adjusted for the handling time of the preceding baits. There was no time limit; the trial was stopped after the bird had attacked all five baits, although no trial lasted longer than 1000 s. The generalization test procedure was identical for all birds.

Statistical Analyses

Differences in the total number of each bait type attacked by each bird in the initial preference test were compared using a Wilcoxon signed-ranks test. The repeated binary choices of the initial preference tests and postlearning discrimination tests, and attack decisions of the initial preference tests and learning sequences, were analysed using generalized linear mixed models, GLMMs (logistic regression with random intercepts for individual birds). Attack latencies in the initial avoidance tests and the learning sequences were analysed using Cox regression frailty models with random intercept and slopes for birds, a form of survival analysis. The models were built and tested in R3.0.2 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org), with packages 'lme4' (for GLMM) and 'coxme' (for survival analysis). Models were fitted by maximum likelihood, with Laplace approximations in GLMMs. Coefficients are given as estimates plus 95% confidence interval, and their significance is tested using Wald's Z test, as recommended in Bolker et al. (2009). However, log-likelihood tests result in qualitatively identical results. The generalization tests were analysed using Friedman's tests with post hoc analysis as implemented in SPSS v20 (SPSS Inc., Chicago, IL, U.S.A.). The training groups were analysed separately. The post hoc test P values were adjusted using the false discovery rate (Benjamini & Hochberg, 1995), a stepwise procedure wherein P values are ordered lowest to highest ($P_{(1)} \ldots P_{(i)} \ldots P_{(m)}$), and all P values that satisfy the inequality $P_i \leq (i/m)\alpha$ have their null hypotheses rejected. As this correction was performed on a per experiment basis, the post hoc correction was $P_i \leq (i/10)0.05$.

Ethical Note

We obtained Czech government permission for catching the adult birds (MHMP-154521/04/OOP-V-25/R-40/09/Pra), for taking juvenile great tits from nestboxes placed in parks at the outskirts of Prague (S-MHMP-043585/2009/OOP-V-26/R-8/Pra), and for laboratory experimentation with birds (No. 150/99 and 29532/2006-30). The collection of *T. diophthalmus* from council land in Narrabeen, New South Wales is permitted under Australian law, and transfer of dried *Tectocoris* between two CITES-listed institutions for research purposes is also permitted under CITES rules.

RESULTS

Initial Preference Test

In simultaneous presentations, birds showed a preference to attack the orange baits before those with iridescent patches (Fig. 3). Despite repeated exposure, there was no effect of trial number (Wald test: $Z = -0.648$, $P = 0.49$), and there was no difference in the bias pattern between wild-caught and hand-reared birds (Wald test: $Z = 0.095$, $P = 0.92$). Within wild-caught birds, there was no statistically significant difference between the initial preferences for the iridescent and orange training groups (Wald test: $Z = 1.365$, $P = 0.17$). However, the 95% confidence interval for the orange training group includes parity (50/50 odds), so this group did not share the bias of the other training groups despite not being statistically different from them (Fig. 3). When we pooled all groups, the odds of choosing an orange bait was 1.75 (95% CI 1.31–2.32; Wald test: $Z = 3.791$, $P = 0.0002$), or an average probability of 64%.

The hand-reared birds did not show a significant difference in latency in attacking orange versus iridescent baits (Wald test: $Z = -1.34$, $P = 0.18$). Hand-reared birds also attacked virtually all baits offered, with only two nonattacks recorded, in different birds and against different bait types. Wild-caught birds showed a bias in attack, as their preference for orange baits was accompanied by a small but statistically significant reluctance to attack iridescent baits during the observation period (mean baits attacked ± SD: iridescent = 3.38 ± 1.03, orange = 3.78 ± 0.53; Wilcoxon signed-ranks test: $V = 19$, $P = 0.034$). Overall, 33 of 40 birds sampled three or more iridescent baits, whereas 38 of 40 birds sampled three or more orange baits. This attack bias was also accompanied by a significantly greater latency to attack iridescent baits (hazard ratio = 0.65, 95% CI 0.51–0.83; Wald test: $Z = -3.43$, $P = 0.0006$). Neither attack rate (Wald test: $Z = 0.684$, $P = 0.494$) nor latency (Wald test: $Z = 1.56$, $P = 0.12$) changed between trials.

Avoidance Learning Sequence

To test the efficiency of avoidance learning, latency to attack was measured over a series of alternating presentations of palatable black and unpalatable coloured baits. There was no effect of bait type on latency for wild-caught birds (Wald test: $Z = 1.35$, $P = 0.18$). With bait types pooled, there was an increase in attack latencies over the course of the learning sequence (hazard ratio = 0.928, 95% CI 0.872–0.989; Wald test: $Z = -2.34$, $P = 0.019$), translating into a 7.7% reduced instantaneous risk of attack in each subsequent trial (Fig. 4). When analysed within groups, a significant increase in attack latencies over the course of the training sequence was observed in the orange bait training group (hazard ratio = 0.916, 95% CI 0.286–0.967; Wald test: Z = −3.17, $P = 0.002$), but not the

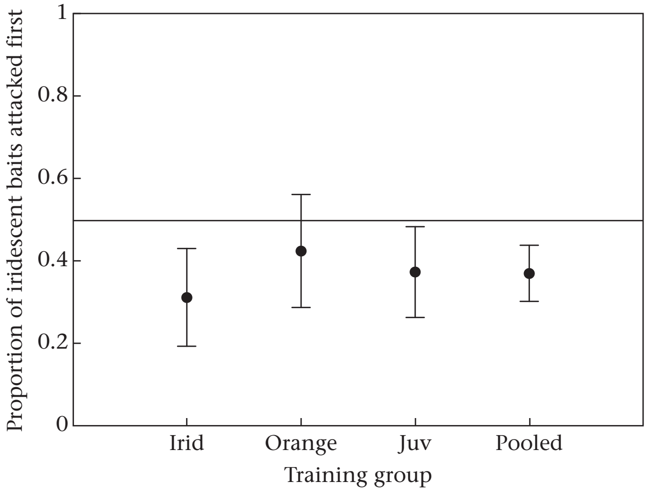

Figure 3. Mean proportion (and 95% CI) of times a bird attacked an iridescent bait first for training groups in the preference test. 'Irid' is wild-caught iridescent-trained birds, 'Orange' is wild-caught orange-trained birds, 'Juv' is hand-reared iridescent-trained birds and 'Pooled' is all birds combined. Data from each bird were summed across the four trials and normalized to account for repetition.

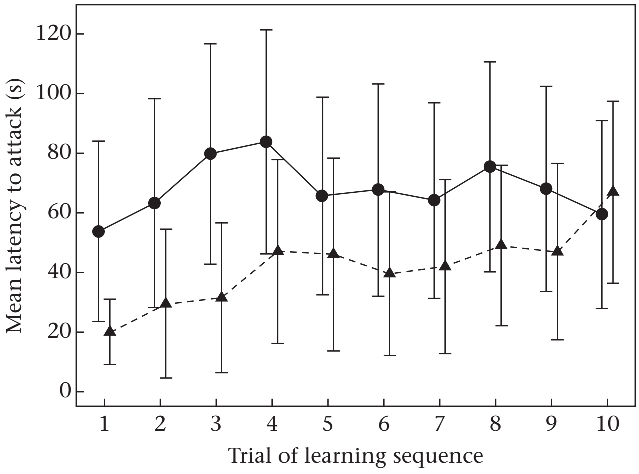

Figure 4. Mean attack latency (and 95% CI) for unpalatable coloured baits at each trial of the learning sequence of wild-caught birds. Each point is the group mean for that trial of the learning sequence. Circles connected by solid lines are iridescent baits; triangles connected by dashed lines are orange baits.

iridescent bait training group (Wald test: $Z = -1.31$, $P = 0.19$) or hand-reared birds trained to iridescent baits (Wald test: $Z = 1.67$, $P = 0.094$; Fig. 5). Overall, wild-caught birds trained to iridescent baits had significantly higher latencies than their hand-reared counterparts, with a 3.2 times lower instantaneous risk of attack across the sequence (hazard ratio = 0.301, 95% CI 0.120–0.804; Wald test: $Z = -2.41$, $P = 0.016$). Prior to first exposure to quinine, the wild-caught birds displayed a nonsignificant trend towards greater latency to attack iridescent bugs (hazard ratio = 0.518, 95% CI 0.259–1.039; Wald test: $Z = -1.85$, $P = 0.06$). This trend disappeared by the end of the learning sequence as the hazard ratio approached unity (Wald test: $Z = 0.066$, $P = 0.95$; Fig. 5).

Wild-caught birds also showed differences between training colours in how they handled the palatable control black baits (Fig. 6). Both groups showed a sharp increase in latency for the first one or two trials after initial exposure to baits containing quinine-laced mealworm pieces. Despite this, there was an overall small but significant reduction in latency to attack black baits over the learning sequence (hazard ratio = 1.099, 95% CI 1.034–1.167, Wald test: $Z = 3.03$, $P = 0.002$). Over the course of the trials there was a comparatively greater latency to attack black bugs for wild-caught birds trained to iridescent bugs, compared to wild-caught birds trained to orange bugs (hazard ratio = 0.421, 95% CI 0.205–0.862; Wald test: $Z = -2.37$, $P = 0.018$). However, this difference was not present on first presentation of black baits, before any exposure to quinine (Wald test: $Z = -0.728$, $P = 0.47$). Despite not showing a significant increase in latency to attack iridescent bugs over the course of the trials, hand-reared birds showed significantly higher latency to attack iridescent bugs compared to black bugs over the entire sequence (hazard ratio = 0.621, 95% CI 0.501–0.768; Wald test: $Z = -4.37$, $P < 0.001$; Fig. 7).

Despite a longer latency towards attacking unpalatable baits compared to black baits, few birds learned to abstain from attacking the unpalatable baits. In the wild-caught birds, four of 20 birds trained to iridescent baits abstained from attacking on at least half of the presentations, whereas only two of 20 birds trained to orange and only one of 20 hand-reared birds did. Within wild-caught birds, there was no difference between patterns of nonattack between iridescent- and orange-trained birds (Wald test: $Z = 1.315$, $P = 0.19$), nor any significant change over the course of the learning sequence (Wald test: $Z = -1.333$, $P = 0.18$). There was a nonsignificant trend towards wild-caught birds trained against iridescent abstaining from attack more often than their hand-reared

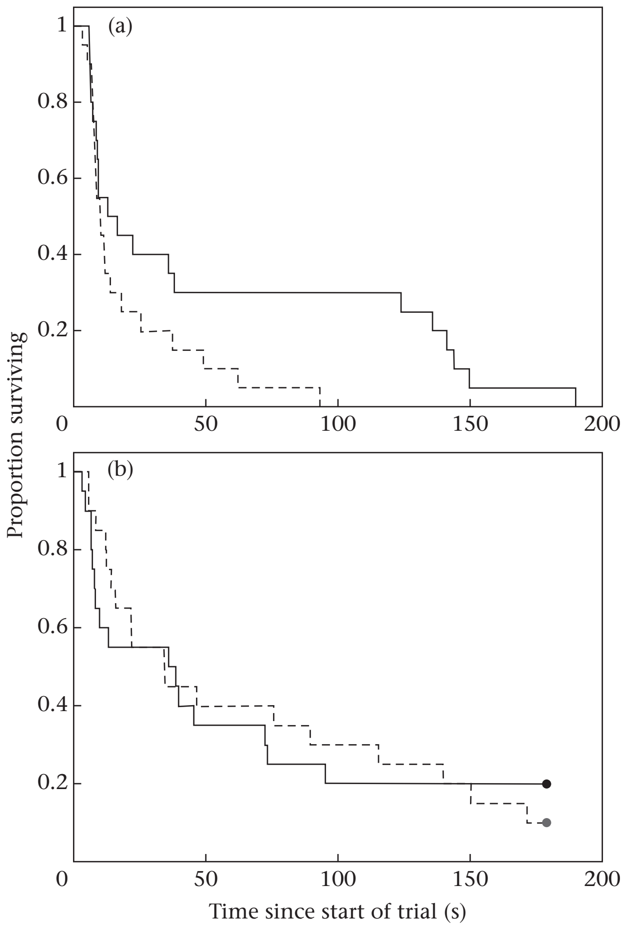

Figure 5. Survival curves (proportion surviving) for unpalatable baits as attacked by wild-caught birds. (a) The first trial of the learning sequence (before exposure to quinine). (b) The last trial of the learning sequence. Solid lines are iridescent baits; dashed lines are orange baits. Trials were terminated after 180 s, so any baits not attacked are considered having 'survived'.

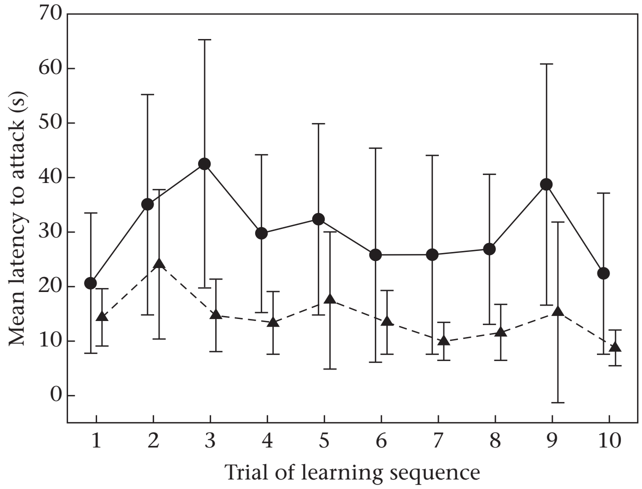

Figure 6. Mean attack latency (and 95% CI) for palatable black bugs at each trial in the wild-caught birds' learning sequences. Circles connected by solid lines are the black bait attack latencies of birds in the iridescent training group. Triangles connected by dashed lines are the black bait attack latencies of birds in the orange training group.

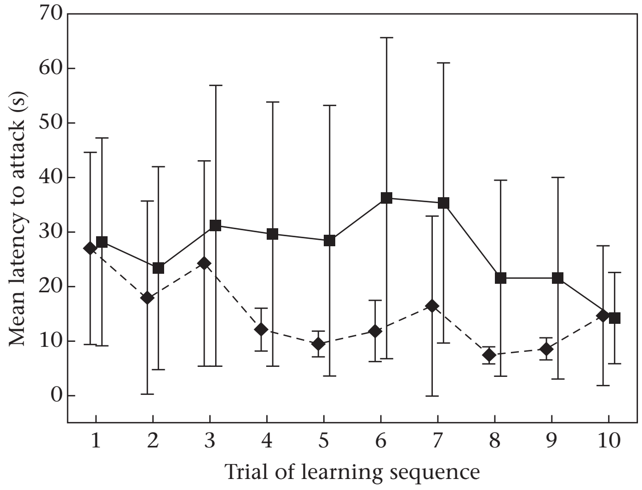

Figure 7. Mean attack latency (and 95% CI) for hand-reared birds at each trial in the learning sequence. Squares connected by solid lines are mean attack latencies at each trial for the unpalatable iridescent baits. Diamonds connected by dashed lines are the mean attack latencies at each trial for the palatable black baits.

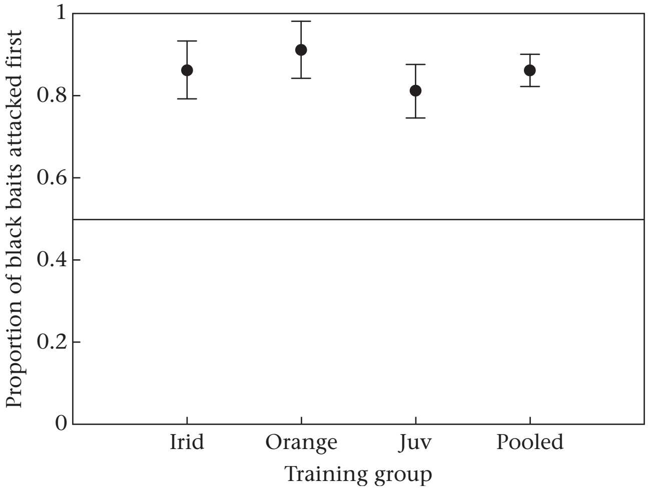

Figure 8. Mean proportion (and 95% CI) of times a bird attacked a black (palatable) bait first for training groups in the postlearning discrimination tests. 'Irid' is wild-caught iridescent-trained birds, 'Orange' is wild-caught orange-trained birds, 'Juv' is hand-reared iridescent-trained birds and 'Pooled' is all birds combined. Each bird was summed across the four trials and normalized to account for repetition.

counterparts (odds ratio = 8.78, 95% CI 0.84–81.26; Wald test: $Z = -1.87$, $P = 0.07$). All black baits were attacked by all birds on all presentations.

Postlearning Discrimination Test

For all training groups, there was a strong preference for the palatable black baits over their respective unpalatable coloured baits in side-by-side preference tests, suggesting clear discrimination learning for all training groups (Fig. 8). Over a series of four trials, there were no effects of trial order (Wald test: $Z = -1.251$, $P = 0.21$), or between wild-caught and hand-reared birds (Wald test: $Z = -1.581$, $P = 0.11$). Within the wild-caught birds, there was no difference between being trained against orange or iridescent baits (Wald test: $Z = 0.993$, $P = 0.32$). The pooled odds ratio of attacking a black bait first is 6.27 (95% CI 4.35–9.05; Wald test: $Z = 9.796$, $P < 0.0001$). Therefore the mean probability of attacking a palatable black bait over the trained unpalatable stimulus is 86%, regardless of the prior experience of birds or bug colour morph.

Generalization Test

All three training groups of birds had distinctly different results in the generalization tests, suggesting effects of training colour and between wild-caught and hand-reared birds (Table 1, Fig. 9). Within-group Friedman tests comparing the five stimuli (three familiar, two novel) were all significant (wild-caught orange-trained: $Q_4 = 13.56$, $P = 0.009$; wild-caught iridescent-trained: $Q_4 = 11.48$, $P = 0.02$; hand-reared iridescent-trained: $Q_4 = 41.28$, $P < 0.001$). For wild-caught birds trained to orange baits, there were significant pairwise differences between black baits and orange baits, but also between black baits and iridescent baits, and between black baits and orange-and-black baits. For wild-caught birds trained to iridescent, there were significant pairwise differences between orange baits and iridescent baits, and between orange baits and orange-and-black baits. Neither wild-caught

Table 1
Mean rank of each bait type in the generalization tests

Group	Black	Iridescent	Orange	Iridescent/Black	Orange/Black
Orange	1.90	3.15	3.55	3.00	3.40
Iridescent	2.58	3.50	2.22	3.08	3.62
Juvenile (Iridescent)	1.50	4.65	2.95	3.25	2.65

Scores denote the average rank (out of 5) that each bait type was chosen, so a bait type with a lower mean rank is chosen on average before one with a higher rank. Mean rank values are the result of a within-group Friedman test, so each mean rank is relative to the other values in its respective row.

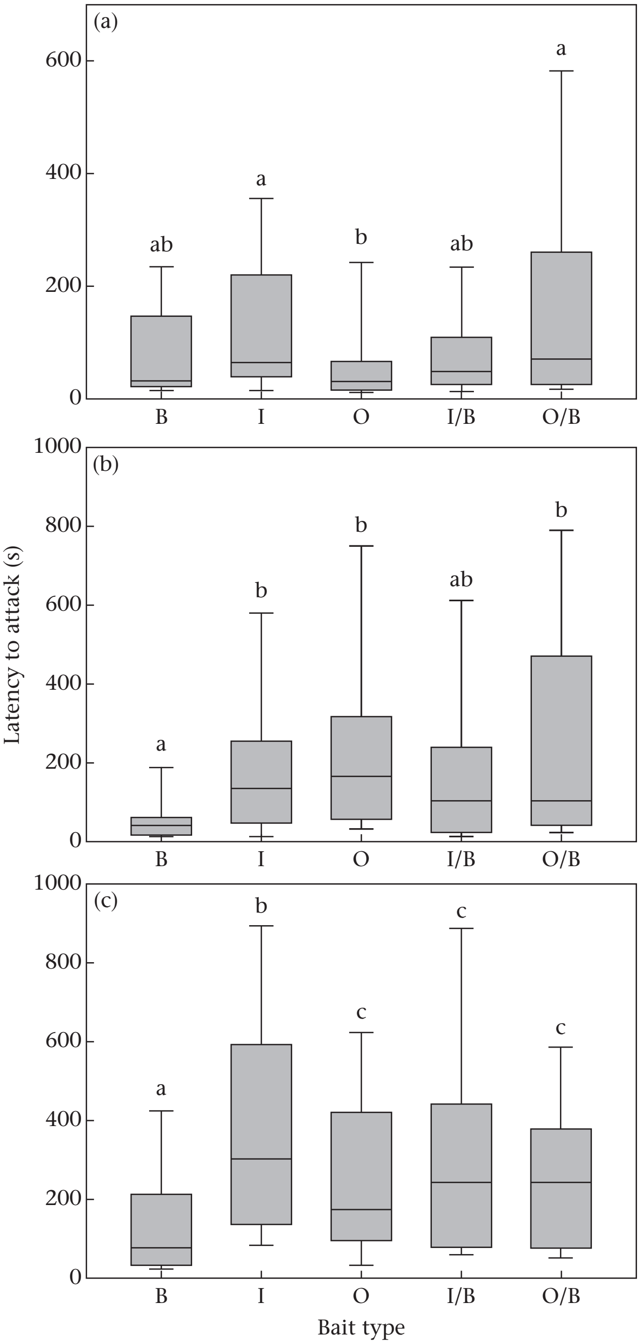

Figure 9. Latency (s) for birds to attack each bait, with handling times of previous baits subtracted, in the generalization tests. (a) Wild-caught birds trained against iridescent baits, (b) wild-caught birds trained against orange baits, and (c) hand-reared birds trained against iridescent baits. Bait types are black (B), iridescent (I), orange (O), iridescent-and-black (I/B) and orange-and-black (O/B). Box plots include median, interquartile range and range. Within each plot, significantly different groups (as judged by a Friedman test post hoc analysis with P values adjusted for the false discovery rate) are marked with different letters. Boxes marked 'ab' are therefore not significantly different from 'a' or 'b'.

training group showed a significant difference between iridescent baits and orange-and-black baits with the shared pattern.

Most pairwise differences in hand-reared birds were significant (Fig. 9). Of particular note is that orange and orange-and-black baits were not significantly different. Additionally, iridescent-and-black baits were significantly higher ranked than black baits, but significantly lower ranked than iridescent baits; these distinctions are not made by either training group in the wild-caught birds (Table 1).

DISCUSSION

These results permitted an evaluation of the question of whether iridescent coloration is effective in inducing initial avoidance, efficient avoidance learning and discrimination, and broad generalization. Iridescent patches (when paired with quinine defences) were effective in inducing greater initial avoidance and broad generalization. For wild-caught birds, attack latencies and rates of nonattack were not statistically distinguishable between birds trained to avoid iridescent or orange baits. All training groups showed equivalent strong preference for baits associated with palatability after their respective training sequences. Hand-reared and wild-caught birds trained against iridescent baits showed group differences in generalization behaviour that were probably related to previous experience, but iridescence was an effective discrimination cue for both groups.

Initial Preference Tests

There was a significant initial bias against the iridescent baits, and this preference was the same for wild-caught and hand-reared birds. In hand-reared birds this bias was likely to be innate, since they had no previous experience with any aposematic prey. This result is surprising, since in previous studies naïve hand-reared great tits did not show any innate avoidance of aposematic prey (Exnerová et al., 2007; Hotová Svádová, Exnerová, Kopečková, & Štys, 2013; Svádová et al., 2009; but see Lindström, Alatalo, & Mappes, 1999). It is possible that the innate bias in great tits is more a question of preference than a strong avoidance, and that it can be observed only when the birds are given a choice between the alternative prey types (Lindström et al., 1999) and not when they encounter only a single prey item at a time (Exnerová et al., 2007; Hotová Svádová et al., 2013; Svádová et al., 2009).

The question arises: what specific stimulus is responsible for the preference for orange baits over the iridescent ones? Several studies (Hauglund, Hagen, & Lampe, 2006; Mastrota & Mench, 1995; Schuler & Hesse, 1985) have demonstrated that blue and green are chosen by birds preferentially over yellow and red, so it is unlikely that in this experiment, the great tits were being driven off by blue or green coloration per se. It is possible that bias against iridescent bugs was caused by higher luminance contrast of the iridescence (Sandre, Stevens, & Mappes, 2010), or by the presence of a contrasting pattern itself (Hauglund et al., 2006; Roper & Cook, 1989), both enhancing the prey's conspicuousness, although for hand-reared naïve birds, the bias is unlikely to be caused by aversion to patterning per se (Aronsson & Gamberale-Stille, 2008, 2009; Osorio, Jones, & Vorobyev, 1999). Conversely, the birds may have had an innate preference for the orange stimulus and preferred the prey with more of an orange colour, but preference for red over green has only been found when the birds recognize the food as berries and not insects (Schmidt & Schaefer, 2004). In other studies, birds did not show any preference for red berries over green ones, but always preferred green insects to red ones (Gamberale-Stille, Hall, & Tullberg, 2007; Gamberale-Stille & Tullberg, 2001). Future research should perform preference tests using the range of baits used in the generalization tests on naïve hand-reared birds, to verify that iridescence per se is the aversive trait.

The wild-caught birds appeared more cautious, with greater latencies to attack overall in the preference tests. They also showed a significant difference in attack latencies between orange and iridescent bugs, something not seen with the hand-reared juveniles. Since both colour morphs were novel to the great tits, this could be due to increased neophobia or dietary conservatism (Marples et al., 1998; Marples & Kelly, 1999) of older birds. However, in other experiments, adult wild-caught great tits frequently attacked novel colour morphs of prey (Exnerová et al., 2006), and adult individuals were even less cautious than yearlings (Lindström et al., 1999). It is therefore more likely that wild-caught birds partly generalized their previous experience with local red-and-black aposematic true bugs (Exnerová et al., 2006; Hotová Svádová et al., 2010) to the *Tectocoris* baits, especially as the great tits generalize easily from red to orange prey (Exnerová et al., 2006; Ham et al., 2006).

Avoidance Learning Sequence

The results suggest that over a learning sequence of this duration, iridescent and orange baits were equally effective for wild-caught birds. There was no statistically significant difference between the two groups, although this may be an artefact of the high variance in latency. When analysed separately, birds trained on iridescent baits did not show an increase in latency over the learning sequence, whereas those trained to orange baits did show an increase. This, however, is likely to be due to the demonstrated initial aversion towards iridescence (see above), and may potentially be influenced by birds choosing to sample fewer iridescent baits during the preference tests. It is worth noting that while there was a nonsignificant trend for higher latency against iridescent baits at the beginning of the learning sequence, this difference evaporated by the last trial as latency against orange baits increased. It is unclear whether latency against orange would have continued to increase to greater levels or whether the two groups would have remained equally aversive over a long period of exposure. It is also unclear whether more birds would have abstained from attack in a longer learning sequence or with stronger chemical defences, although the natural chemical defences of *T. diophthalmus* are rather weak (Staddon et al., 1987).

Hand-reared juveniles, like their wild-caught counterparts, did not show an increase in attack latency or change in attack rate over the course of the learning sequence. There was, however, a large difference in latency between hand-reared and wild-caught birds in how they treated iridescent bugs. Therefore, previous experience with more noxious prey may be essential to increase attack latency. Odorous secretions, which live *T. diophthalmus* produces but quinine baits do not, may also be important in promoting avoidance (Jetz, Rowe, & Guilford, 2001; Rowe & Guilford, 1996; Siddall & Marples, 2008). Conversely, juvenile birds may simply be more hungry and/or more tolerant of weak defences, which may influence their eagerness to attack (Alcock, 1973; Barnett, Bateson, & Rowe, 2007; Sandre et al., 2010). Despite a lack of observed increase in attack latency, hand-reared birds had significantly higher attack latencies overall against iridescent unpalatable baits than palatable black baits, suggesting discrimination was occurring.

One surprising result from the learning trials was the increased latency for attacking the black control baits in the iridescent-trained group, compared to the orange-trained group. Perhaps from an oblique viewing angle the iridescent patches appeared dark to the birds. It has been suggested that luminance contrast is not as relevant as colour in bird learning (Osorio et al., 1999), which may explain this unusual finding. Sandre et al. (2010) suggested that luminance contrast influences initial wariness, but this may not extend to a prolonged learning sequence. Studies of the aposematic effect of luminance contrast alone are rare in bird research, although it is discussed by Stevens (2007) and luminance contrast is effective in teaching mantids to avoid emetic seed bugs (Prudic, Skemp, & Papaj, 2007). The importance of chromatic versus achromatic contrast in aposematism is a ripe area for further research.

Postlearning Discrimination Test

Both colour morphs were shown to be equally effective cues in a postlearning discrimination test with simultaneous presentation of palatable alternative prey. Age and experience of birds did not affect this outcome, as wild-caught adults and hand-reared juveniles were equally keen to pick the black control baits over their respective unpalatable baits. The presence of alternative prey may therefore be very important to the harlequin bug's survival, especially if this weakly defended stinkbug (Staddon et al., 1987) is capitalizing on imperfect Müllerian or quasi-Batesian mimicry to other sympatric noniridescent noxious bugs (Kokko, Mappes, & Lindström, 2003; Lindström, Alatalo, Lyytinen, & Mappes, 2004). The different performances of birds at the end of the learning sequence and in the postlearning discrimination test also support the idea that the sequential discrimination task may be more difficult than the simultaneous one (Beatty & Franks, 2012), or at least that sequential discrimination learning can in some cases be almost behaviourally silent and become evident only when the two stimuli are presented simultaneously.

Generalization Test

The generalization trials exposed the major differences between the wild-caught birds and the hand-reared birds, distinguished patterns of generalization between iridescent- and orange-trained wild-caught birds, and allowed predictions of how aposematic signals utilizing iridescence may fare in the wild. Regardless of training colour morph, the orange-and-black baits were highly aversive to wild-caught birds. Furthermore, the iridescent baits remained repellent to the birds trained to avoid orange baits, despite these birds only experiencing the iridescent colour morph as a palatable food item. The reverse was not true for birds trained to avoid iridescent baits: orange baits remained the most preferred food choice, even more so than the black baits. Despite its pattern being fairly distinct from local red and black Heteroptera (Exnerová et al., 2008), the harlequin bugs appear to be benefiting from experience with the local Müllerian/quasi-Batesian mimetic complex (Hotová Svádová et al., 2010; Hotová Svádová et al., 2013; but see Veselý, Veselá, & Fuchs, 2013). Although bird species do vary in how they respond to aposematic prey (Exnerová et al., 2003; Exnerová et al., 2007), these findings are likely to be relevant to the harlequin bug's survival against avian predators in its home range.

For the wild-caught birds, the presence of a contrasting pattern was necessary for generalizing unpalatability, as birds trained to iridescent baits did not show elevated avoidance of orange baits despite iridescent colour morphs containing small orange patches. This is surprising since several studies have doubted the importance of contrasting patterns for learning to recognize aposematic prey (Aronsson & Gamberale-Stille, 2008, 2009; Exnerová et al., 2006; Svádová et al., 2009; but see Aronsson & Gamberale-Stille, 2013). However, the attention that predators give to the pattern may depend on the amount of their previous experience, which agrees with findings that experienced predators usually select for a closer mimetic resemblance than naïve ones (Ihalainen, Lindström, Mappes, & Puolakkainen, 2008; Lindström, Lyytinen, Mappes, & Ojala, 2006; Rowe, Lindström, & Lyytinen, 2004). Iridescent-and-black baits, which test iridescence as a signal without the contribution of contrasting orange, were not a strong signal to wild-caught birds. To birds trained on orange baits, they were much less aversive than the other stimuli (except black), possibly because of the colour or luminance contrast. For birds trained to iridescent baits, black and iridescent-and-black baits were statistically even, supporting the notion that the iridescent patches are harder to distinguish from black than orange is. Behaviour of wild-caught birds thus seemed to be affected by their experience with red-and-black aposematic bugs in the wild, since they attended mostly to orange colour and contrasting dark pattern (either black or iridescent) when learning to avoid unpalatable baits. This pattern of generalization may be overridden with extensive training, as Pegram et al. (2013) found no difference between how birds responded to selective blacking-out of blue or orange components of the warning signal of *Battus philenor* (Papilionidae) butterflies.

The hand-reared, formerly naïve birds, on the other hand, performed exactly as would be expected from being conditioned that iridescent-and-orange bugs are unpalatable. Their most aversive stimulus was the one to which they were trained, while the black baits were most preferred. Both novel phenotypes (iridescent-and-black and orange-and-black) were less aversive than the trained unpalatable bait, because they were imperfect matches but also possibly because they shared part of their pattern (black colour) with the positive stimulus (the palatable bait). The iridescent-and-black baits were the second most aversive stimuli, suggesting that naïve birds can associate iridescent coloration with unpalatability, and that iridescence alone, without taking advantage of a mimetic complex, can function as an aposematic signal. This result is unlikely to be due to neophobia since the other novel stimulus, the orange-and-black baits, was the second-least aversive stimuli, supporting the idea that aposematic patterns must be learned in great tits and are not innately aversive (Exnerová et al., 2007; Hotová Svádová et al., 2013; Sillén-Tullberg, 1985). The surprise here was that the orange baits, which had previously only been experienced as palatable, were significantly more aversive than black ones. This could be evidence for biased generalization or peak shift (Gamberale & Tullberg, 1996; Gamberale-Stille & Tullberg, 1999; ten Cate & Rowe, 2007), as the training stimulus had both iridescent and orange patches, so very large (in this case, whole body) orange patches could also increase avoidance (Remmel & Tammaru, 2011). It is also worth noting the greater latency against iridescent baits, even after correcting for the handling time of other baits, further supporting the possible role of alternative prey in promoting survival in *T. diophthalmus*.

Conclusion

It appears as though the iridescent patches of *T. diophthalmus* can act as an aposematic signal. This may be through either the innate avoidance of high luminance contrast (Sandre et al., 2010) or avoidance learning. The presence of iridescent patches creating a contrasting pattern was important for initial avoidance and generalization in wild-caught birds. However, this benefit appears to be greatly increased by the presence of alternative palatable prey. The large differences we observed between wild-caught and hand-reared birds may reflect behavioural differences by age (Lindström et al., 1999), or less cautiousness and discrimination of hand-reared birds (Exnerová et al., 2007), but above all else they appear to reflect experience. The wild-caught birds probably had experience with local red-and-black Heteroptera (Hotová Svádová et al., 2010), and their performance supports the idea of broad generalization in colour and pattern (Aronsson & Gamberale-Stille, 2009, 2013; Exnerová et al., 2006; Ham et al., 2006). Iridescent bugs may thus benefit from experience of bird predators with other similar red or orange prey with contrasting but noniridescent patterns.

Our results suggest that orange is not superior to iridescence (with small orange patches) for learning to avoid aposematic prey. This result is surprising, given the noted prevalence of long-wavelength colours in aposematic signalling (Ruxton et al., 2004). This result also supports previous findings that internal contrast is not more important than colour in avoidance learning (Aronsson & Gamberale-Stille, 2009; Roper & Cook, 1989), although internal contrast may be beneficial in unlearned avoidance (Sandre et al., 2010). Despite within-group differences in the learning sequence, both morphs were equally efficient discrimination cues compared with palatable alternative prey. Together, these observations support the idea that the iridescent patches may be selectively advantageous despite not being maximally aposematic. Furthermore, the equivalent survival of the extreme morphs suggests that, at least in a one-predator system, variation in aposematic signals may be evolutionarily stable.

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Závěr

1) Sýkory koňadry (*Parus major*) ze dvou vzdálených populací (populace ze středního Finska a populace ze středních Čech) se nelišily v projevech svého exploračního chování. Obě populace prozkoumávaly nový předmět umístěný v domovské kleci stejně ochotně. Populace se však vzájemně lišily neofobií vůči neznámému předmětu umístěnému v blízkosti potravy. Vyšší míra neofobie sýkor z finské populace však nijak neovlivnila jejich přístup k nové kořisti. Sýkory koňadry z obou testovaných populací napadaly modrého cvrčka stejně rychle. Přesto, že explorace nového předmětu a neofobie spolu vzájemně pozitivně korelovaly u obou studovaných populací, reakce na novou kořist dospělých sýkor koňader nesouvisela s mírou jejich explorace ani neofobie.

2) Mláďata tří středoevropských druhů sýkor z čeledi Paridae *(Parus major, Periparus ater, Cyanistes caeruleus*) se liší mírou vrozené opatrnosti vůči nové a aposematické kořisti. Zatímco u sýkor koňader a sýkor uhelníčků pozitivní zkušenost s jedlou potravou červené barvy snížila neofobii vůči nové i aposematické kořisti, sýkory modřinky vykazovaly stále stejně vysokou míru vrozené opatrnosti bez ohledu na jejich předchozí zkušenost. Mláďata sýkor koňader a sýkor uhelníčků, která měla předchozí zkušenost s jedlou potravou červené barvy, navíc také aposematickou červeno-černou ploštici *Pyrrhocoris apterus* více konzumovala. Jestli k deaktivaci neofobie a potravního konzervatizmu u sýkor koňader a sýkor uhelníčků vedla jejich pozitivní zkušenost s různou potravou nebo u nich došlo k široké generalizaci zkušenosti s jedlou potravou červené barvy na jinou červeně zbarvenou kořist, však nemůžeme prozatím rozhodnout.

3) Vnitrodruhové rozdíly v přístupu sýkor koňader (*P. major*) k aposematické kořisti mohou být dány typem jejich personality. Přesto, že způsob, jakým na aposematickou kořist reagovaly dospělé ručně odchované sýkory koňadry, se značně lišil od reakce naivních ručně odchovaných mláďat, vliv typu personality jedince se v reakci na aposematickou ploštici *P. apterus* u testovaných sýkor projevil. Zdá se tedy, že rozdíly v přístupu k aposematické kořisti u sýkor koňader zůstávají v průběhu života u jednotlivých typů personalit konzistentní. Způsob a intenzita projevované reakce vůči aposematické kořisti u sýkor koňader se však může měnit s věkem jedince. Vysoká míra počáteční opatrnosti vůči aposematické ploštici dospělých jedinců pocházejících z chovů, však může být ovlivněna jejich chybějící zkušeností s explorací různých podnětů nebo/a absencí zkušenosti s nedostatkem potravy.

4) Iridescentní zbarvení aposematické ploštice *Tectocoris diophthalmus* zvyšuje u dospělých i naivních sýkor koňader jejich počáteční opatrnost k tomuto typu kořisti. Obě věkové kategorie testovaných sýkor prokázaly schopnost naučit se rozpoznávat nejedlou iridescentní kořist od její jedlé načerno nabarvené varianty a tyto zkušenosti poté generalizovat na uměle vytvořenou iridescentně-černou ploštici se zabarvenými oranžovými částmi. Iridescentní zbarvení aposematické ploštice *T. diophthalmus* je tedy důležitou součástí její výstražné signalizace.

5) Jedním z hlavních faktorů, které ovlivňují reakci sýkor koňader (*P. major*) vůči aposematické kořisti je jejich dosavadní zkušenost. Z přírody odchycení jedinci z finské populace, která nemá s výstražně zbarvenou červeno-černou kořistí žádnou dosavadní zkušenost, napadali aposematickou červeno-černě zbarvenou ploštici *P. apterus* signifikantně více než sýkory koňadry pocházející z Čech, které se s touto kořistí v přírodě běžně setkávají. Rozdílná reakce vůči aposematické kořisti u dospělých sýkor koňader nemusí být způsobena pouze rozdílnou zkušeností s konkrétním typem kořisti, ale také jejich celkovou zkušeností s explorací různých podnětů a různých zdrojů potravy, se kterými se jedinec v průběhu svého života setká. Dospělé sýkory koňadry pocházející z laboratorních chovů vykazovaly vůči aposematické červeno-černě zbarvené ploštici vysokou míru počáteční opatrnosti, aniž by s takovým či podobným typem kořisti měly jakoukoli dosavadní zkušenost.