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**Vliv nedávné potravní zkušenosti ptačího predátora na
fungování obranné signalizace hmyzí kořisti**

Diplomová práce

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Úvod

Aposematismus a mimikry

Pojem mimikry (mimeze pro ty co rádi skloňují) byl původně používán pro napodobování rostlinných částí živočichy za účelem zneviditelnění se pro predátora (Kirby a Spence 1815, 1817 ex. Komárek 2001). Postupně se tento pojem rozšířil na napodobování obecně, ať už organismy napodobují rostliny, živočichy, či neživou přírodu. Důležité je, že podobnost mezi organismy není zapříčiněna jejich příbuzností, ani náhodnou konvergencí, ale adaptací proti predaci (Komárek 2001). Z toho důvodu by se mimik měl odlišovat od svých blízkých příbuzných. Nejčastější případy mimikry jsou právě ty, při nichž se organismy snaží být pro predátora neviditelnými – tzv. krypse. Cott (1940) popsal nejrůznější aspekty napodobování a jiných antipredačních strategií. Kromě klasické krypse, kdy zvíře připodobňuje svou barvu a případně i tvar svému habitatu, rozlišil tzv. disruptivní zbarvení, které znemožňuje predátorovi přesné rozpoznání kořisti rozbitím plochy těla různobarevnými ornamenty (viz. též např. Endler 1978 nebo Silberglied *et al.* 1980). V každém případě jakékoliv zbarvení je závislé na okolí a proto se krypticky zbarvené druhy snaží buď barevně přizpůsobit pozadí, nebo vyhledat vhodný substrát na němž bude jejich zbarvení nejlépe fungovat (Merilaita *et al.* 2001). Poměrně časté je, že stejné zbarvení může být za určitých podmínek predátorem považováno za výstražné a v jiných podmínkách může působit krypticky (Järvi *et al.* 1981 a,b; Harvey 1983; Veselý *et al.* 2006 in press).

Aposematismus je pojem popisující zvláštní případ antipredační strategie, při níž organismus využívá výstražné zbarvení, aby signalizoval predátorovi svou nevýhodnost (Järvi *et al.* 1981a). Nevýhodnost je nejčastěji realizována chemicky (nepoživatelné látky) nebo mechanicky (trny, chlupy, žihadla). Proklamace těchto kvalit je nejčastěji optická, a to nejen výstražnými barvami, ale i nejrůznějšími vzory (nejznámější je kresba připomínající oko – Cott 1940), anebo morfologickými strukturami samotnými. Další způsob jak signalizovat predátorovi svou nepoživatelnost je používání chemické obrany na větší vzdálenost (tedy vnímatelné čichem nikoliv chutí). Méně časté způsoby jsou zvuková signalizace (Hristov a Conner 2005) nebo specifické chování (např. Srygley 1999). Není vždy úplně zřejmé, je-li daná kvalita způsob proklamace nevhodnosti nebo nevhodnost sama, dost často plní obě tyto funkce najednou (viz již zmiňované ostny nebo chlupy). Nejčastější (nejčastěji studovaný) případ aposematismu

je ten, při kterém chemicky chráněný druh signalizuje svou nevýhodnost optickými signály. I tehdy ovšem mohou chemické vlastnosti mít i signalizační funkci.

Učebnicové příklady mimikry jsou právě ty, při nichž různé druhy kořisti napodobují navzájem své výstražné zbarvení (Remington 1963; Allen a Cooper 1994). První případ, kdy chutný druh napodobuje zbarvením nechutného (nebo nebezpečného) aposematika popsal jako první H. W. Bates v roce 1862. Nutným následkem této interakce je snížená chráněnost modelového druhu. Proto tento stav funguje jen v případě, že mimetik je vzácnější než model (naivní predátor se pravděpodobně setká dříve s modelem než s mimikem) a když je model dostatečně nevýhodný (Edmunds 1974). Druhý případ, tzv. Müllerovské mimikry, popisují stav, kdy se stejně nechutné nebo nebezpečné druhy snaží sobě podobat, a tím se navzájem chránit. K jejich vzniku vedla snaha, aby se predátor musel učit co nejméně výstražných vzorů a tím dělal co nejméně chyb. Všechny zúčastněné druhy profitují tím, že se na naučení naivního predátora podílí dohromady a tím jsou ztráty jednotlivých druhů rozmělněny (viz. např. Wickler 1968; Edmunds 1974 nebo Speed 1993). Ve skutečnosti zřejmě existují komplexy druhů, které se navzájem více či méně podobají, a zároveň mají různou míru nevýhodnosti pro predátora. Méně nevýhodné druhy jsou v tomto uspořádání chráněny více nevýhodnými. O takovém uspořádání se hovoří jako o tzv. quasi-Batesovských mimikry, či o Batesovsko-Mülleriánském kontinuu (MacDougall a Dawkins 1998; Speed 1999; Speed *et al.* 2000).

Evoluce aposematismu

Množství prací se zabývá problémem vzniku výstražného zbarvení a jeho udržení se v populaci kořisti (např. Harvey a Paxton 1981; Harvey *et al.* 1982; Guilford a Dawkins 1991 nebo Rowe a Guilford 1999). Podle Guilforda (1988) je možné rozlišit tři základní cesty vzniku aposematismu: i) nejdříve vzniká výstražné zbarvení, které je následně využito k proklamaci nechutnosti, ii) nechutnost a výstražné zbarvení vzniká zároveň, iii) výstražnost vzniká v populaci již nechutné kořisti. Každá z těchto cest má ovšem svá úskalí. Představa, že výstražnost vzniká v populaci nechutné kryptické kořisti naráží na fakt, že výstražná mutace je nejsnáze odhalitelná predátorem, a proto je nejrychleji eliminována z populace (pravděpodobně dříve než se její nositel rozmnoží – Guilford a Dawkins 1993). Jistou cestou jak tento paradox vysvětlit je pomocí kin selekce. Základní hypotézu nastínil Fischer v roce 1930. Předpokládal, že aposematismus je často asociován s gregaričností, a že jedinci ve skupině si jsou navzájem příbuzní.

Potom může fungovat kin selekce, protože poté co je obětováno několik sourozenců, se predátor naučí vyhýbat jedincům s výstražnou signalizací a přeživší sourozenci s výstražným zbarvením umožní další šíření genu pro něj v populaci (které je už jednoduché, neb přináší velké zisky). Wiklund a Järvi (1982) navrhli jinou alternativu. V jejich experimentech nedocházelo při ochutnání aposematické kořisti predátorem k jejímu zabití, a proto navrhli, že by se znak pro výstražnost mohl v populaci zachovat a rozšířit individuální selekcí. Není tedy nutná podmínka příbuzenských agregací pro rozšíření výstražné signalizace. Byly ovšem i práce, které kin selekci podporovali a její význam dokládali častou existencí agregací aposematického hmyzu v přírodě (např. Benson 1971 nebo Harvey 1983). Navíc Guilford (1985) poukázal na to, že v případě výstražného zbarvení se nemusí jednat o selekci příbuzných, ale pouze o selekci podobných fenotypů, které si nemusí být příbuzné (na tomto principu fungují mimikry – viz. výše). Zřejmé je, že se uplatňují oba typy selekcí při udržení a rozšíření výstražného zbarvení v populaci nechutné kořisti (a proto se mohou aposematici vyskytovat gregaricky i soliterně).

Určité rozřešení problematiky kolem evoluce výstražnosti přinesli až Alatalo a Mappes (1996) a následně Mappes a Alatalo (1997), kteří prováděli pokusy s kořistí, která nemohla být predátorem známa ani z evolučního hlediska (tzv. novel world). Všechny dosavadní experimentální studie evoluce aposematismu byly zatíženy možnou dědičnou averzí k určité kořisti i u naivních predátorů. Tento nový postoj umožnil vytvořit si predátora, o kterém přesně víme co zná. Z výsledků této a mnohých jiných studií (cf. Lindström 1999) vyplývá, že gregaričnost je výhodná při vzniku výstražného zbarvení v populaci chutné kořisti. Pokud je predátor již obeznámen s nechutností kořisti je zvýhodněna i soliterní kořist a kin selekce tudíž není nezbytným předpokladem.

K problému způsobu selekce umožňujícímu výstražnému zbarvení uchytit se v populaci se přidávají ještě problémy spojené se schopnostmi predátora. Maria Servedio (2000) prokázala, že na prosazení výstražného zbarvení mají výrazný vliv predátorovy schopnosti učit se a pamatovat si. Pokud se predátor setká s aposematickou kořistí po delším čase není již schopen ji poznat a proto na ní může opět zaútočit. Pravděpodobnost, že si predátor kořist zapamatuje, je závislá na míře špatné zkušenosti s první kořistí. Proto je podle této práce evoluce výstražného zbarvení v populaci nechutné kořisti možná za předpokladu, že je kořist opravdu hodně nevýhodná a nutí predátora, aby si ji dobře pamatoval. Stejně tak jako vše v přírodě, ani otázka evoluce aposematického zbarvení nenajde nikdy jedno uspokojivé vysvětlení. Na tomto procesu

se podílí mnoho parametrů a proto se může prosadit mnoha cestami, které musí ovšem být vždy evolučně stabilní.

Pokud se týče druhé cesty evoluce výstražného zbarvení, při kterém se objevuje najednou zbarvení i nechutnost, hlavní problém tkví v malé pravděpodobnosti této události. Pokud by mělo jít o simultánní vznik obou mutací najednou v jedné generaci potomků, i když selekce na takovou schopnost je jistě velká, je z molekulárně genetického hlediska málo pravděpodobná (viz. např. Miura a Sonigo 2001). Je zřejmé, že jeden znak (nevhodnost nebo výstražná signalizace) musí vzniknout jako první následován druhým (Guilford 1988), i když třeba ve velmi krátkém časovém horizontu. První z námi presentovaných prací se zabývá otázkou vzniku výstražného zbarvení v populaci chutné kořisti. Již Guilford (1988) považoval tuto alternativu vzniku aposematismu za nejméně pravděpodobnou. Tato teorie předpokládá vznik nápadného zbarvení za jiným, než antipredačním účelem (např. vnitrodruhová komunikace). Nápadnost s sebou přináší zvýšené náklady a rizika, která jsou následně kompenzována vznikem chemické ochrany (Ruxton *et al.* 2004). Tato teorie je často považována za nepravděpodobnou až nemožnou (Yachi a Higashi 1998; Riipi *et al.* 2001; Stuart-Fox *et al.* 2003), nicméně jsou důkazy z přírody, že je tento postup možný (Acarina, Hydrachnida – Proctor a Garga 2004). Navíc jak dokázali Marples *et al.* (1998) do hry ještě vstupuje potravní konservatismus predátora, který způsobí, že predátor se určitý čas vyhýbá i chutné výstražné kořisti a po tuto dobu se může gen pro výstražnost rozšířit (viz. níže).

Nicméně úplně jiná situace nastává, je-li nově vznikající výstražná mutace mimikem jiného predátorovi dobře známého nechutného druhu. Podle pravidel Batesiánské mimeze je tato mutace velmi rychle fixována a šíří se v populaci chutné kořisti. V tomto případě ovšem nastává problém v okamžiku, kdy je výstražnost v populaci chutné kořisti příliš hojná a mimici jsou častější než model. V takovém případě se naivní predátor může naučit, že výstražnost je korelována s chutností a tím ohrozit populace aposematického modelu (Lindström *et al.* 1997). Tím se sníží populace mimika a ve finále se ustaví rovnováha mezi populacemi mimiků a modelů, která přináší všem zúčastněným největší prospěch.

Neofobie a potravní konservatismus

Neofobie i potravní konservatismus jsou procesy ovlivňující vztah predátora ke kořisti. V obou případech se jedná o odmítání neznámé kořisti (viz. Marples a Kelly 1999).

Oba jevy byly původně slučovány pod pojmem neofobie, ale v posledních letech byly odlišeny. Neofobie je okamžitý odpor k nové kořisti (a nejen kořisti) spuštěný v okamžiku setkání s novostí, není příliš silně zafixován a je-li nová kořist vhodná jako potrava, během relativně krátké doby mizí (často během několika minut). Důvod vyprchání neofobického odmítání je nejčastěji generalizace nové kořisti s jinou, již známou (Schlenoff 1984). Samozřejmě čím je nová kořist odlišnější od všeho co predátor doposud poznal, tím je generalizace obtížnější a neofobie vytrvalejší. Smyslem neofobie je ochránit predátora před potencionálně nebezpečnou potravou, dává predátorovi čas důkladně potravu ohledat - chrání ho před zbrklostí (známé jsou pokusy s potkany a otrávenou kořistí – Domjan 1980 ex Schlenoff 1984), predátor musí kořist podrobněji prozkoumat než se rozhodne zda ji akceptovat či odmítnout.

Potravní konservatismus je naproti tomu dlouhodobý proces, který se vytváří během predátorovi života, je silně fixován a obtížně zrušitelný. Experimentálně bylo prokázáno (Kelly a Marples 2004), že se jedná o až několik měsíců trvající odpor a byla popsána 4 stadia vyhasínání: i) optické prozkoumávání, ii) občasné ochutnání až pozření nové kořisti, iii) pravidelná konzumace nové kořisti, iv) přijmutí kořisti mezi běžnou potravu. Byly popsány tři možné způsoby jak zvíře může získat odpor k novému: i) averze spuštěná v okamžiku setkání s novou kořistí (Schuler a Roper 1992) – neofobie, ii) vrozená averze (Lindström *et al.* 1999), iii) averze naučená během predátorova života (Tinbergen 1960, Shettleworth 1972). Poslední dva případy se týkají jak potravního konservatismu tak nastavení neofobie. A právě v tomto smyslu je často obtížné přesně definovat oba pojmy (viz. Marples a Kelly 1999). Potravní konservatismus je nastavení parametrů vhodné potravy. Toto nastavení může být vrozené nebo naučené, pravděpodobně existuje určitý vrozený mustr vhodné potravy, který je během predátorova mládí ještě dopilován. Naproti tomu neofobie je z definice spuštěna až v okamžiku střetu s novou potravou. V tom ovšem vězí problém. Nastavení citlivosti spuštění neofobické reakce může být u různých druhů nebo i jedinců různé (viz. níže). Stejná kořist může být některými predátory vnímána jako nová, zatímco jinými může být generalizována s jinou již známou kořistí okamžitě a proto se neofobická reakce vůbec nedostaví. Nastavení citlivosti spuštění neofobické reakce proto může být považováno za jeden z aspektů potravního konservatismu. Samozřejmě může tento parametr být vrozený nebo naučený, i když v tomto případě je snazší si představit vrozené nastavení citlivosti spuštění neofobické reakce, které je jen lehce korigováno životními zkušenostmi.

Jak bylo řečeno výše, lze rozpoznat zřetelné mezi i vnitrodruhové rozdíly v míře neofobie a potravního konservatismu (např. Coppinger 1970; Schuler a Hesse 1985; Marples *et al.* 1998). Rozdíly jsou dány především potravními strategiemi a strukturou využívaného habitatu. Obecně lze říci, že potravní generalisté mají menší sklony k neofobii a potravnímu konservatismu než druhy specializovanější (Greenberg 1983; Webster a Lefebvre 2000; Mettke-Hofmann *et al.* 2002). Lze to ovšem definovat i obráceně, že vyšší míra potravního konservatismu vede ke specializaci. Je to poměrně logické, protože zvýšený potravní konservatismus zužuje model vhodné potravy a způsobuje specializovanost na určitou, tomuto modelu vyhovující, kořist. Bylo ovšem prokázáno, že toto spojení je mnohem obecnější, a zvýšená míra potravního konservatismu se vyskytuje u druhů specializovaných nejen potravně, ale i ekologicky a habitatově (Mettke-Hofmann *et al.* 2002). Dále bylo prokázáno, že určité rozdíly v míře neofobie a potravního konservatismu se projevují i na vnitrodruhové úrovni (Verbeek *et al.* 1994; Dingemans *et al.* 2003), nicméně tyto rozdíly nejsou tak markantní jako rozdíly mezi druhy a jsou spíše dány historií a zkušenostmi každého jednotlivce.

Potravní zkušenost

Jak bylo popsáno v předchozí kapitole, reakci predátora na nabídnutou kořist neoddiskutovatelně ovlivňuje jeho předchozí zkušenost (Coppinger 1970). Obecně lze předpokládat, že odmítána bude kořist výrazně se lišící od všech dosavadních zkušeností predátora (viz teorie neofobie a potravního konservatismu – Marples a Kelly 1999; Lindström *et al.* 2001). Naopak kořist na níž je predátor zvyklý by neměla být odmítnuta nikdy. Otázka ovšem je, co to znamená, že predátor je na kořist zvyklý. Poté co se predátor setká s novou a chutnou kořistí a zmizí neofobická averze, je kořist přijata jako vhodná. To, zda stačí jen jedno setkání k definitivnímu začlenění kořisti do svého jídelníčku závisí na kognitivních a paměťových schopnostech predátora, ale je zřejmé, že při druhém setkání se stejnou kořistí by se již neměla neofobická reakce dostavit. Úplně jiná situace nastává, je-li odmítání nové kořisti způsobeno potravním konservatismem predátora. Jak bylo popsáno výše, potravní konservatismus je velmi pevně zakořeněn v predátorově mysli a pozměnit představu vhodné potravy je velmi nesnadné (Kelly a Marples 2004), tedy u dospělého predátora. Pokud se tedy setká s kořistí, která je pro něj nevyhovující bude ji odmítat po delší dobu než ji akceptuje, případně ji nemusí akceptovat nikdy. Neboli pozměnit predátorovi jeho představu vhodné kořisti nemusí být vždy lehké. Přesto některé práce experimentující s potravní

zkušeností predátora (Coppinger 1969, 1970 nebo Lindström *et al.* 2001) prokázali, že poměrně nedávná rozličná potravní zkušenost muže výrazně ovlivnit predátorovu reakci vůči stejné kořisti.

Jak již bylo řečeno další aspekt problému vlivu potravní zkušenosti je predátorova schopnost učit se a pamatovat si (Speed 2000). Je evidentní, že predátor neschopný pamatovat si parametry vhodné kořisti může dělat při útocích na rozličnou potravu chyby, které mohou být osudové. Proto je schopnost bezpečně rozpoznat vhodnou kořist jedna z nejdůležitějších vlastností každého organismu (viz. Optimal foraging theory - Stephens and Krebs 1986). Bylo prokázáno, že co se učení a pamatování u ptáků týče, jsou na prvním místě krkavcovití a sýkorovití. Tyto skupiny prokazují v divokých populacích jak schopnost učit se novým potravním zdrojům a experimentovat s potravou (Sherry a Galef 1984, 1990; Kothbauer-Hellmann 1990), tak vynalézat nové přístupy k získávání potravy a dokonce používat nástroje (např. Chappell a Kacelnik 2002). Co se paměti týče jedná se o druhy, u kterých bylo často pozorováno schovávání potravy na horší období, což vyžaduje poměrně rozsáhlou prostorovou paměť (např. Clayton 1998 nebo Pravosudov *et al.* 2003).

Schopnost učit se a pamatovat si je zároveň silně ovlivněna motivací (Pravosudov a Clayton 2001). Aplikováno na nevhodnou kořist, čím horší zážitek je pro predátora napadení kořisti, tím déle si ho bude pamatovat a tím déle na ni (a jí podobné) bude reagovat odmítavě (Edmunds 1974). Navíc Gamberale-Stille (2000) dokázala, že čím delší je příležitost predátora vtisknout si templát nevhodné potravy, tím je jeho odmítavost vůči ní silnější. Bylo prokázáno, že potravní zkušenost může změnit predátorovy preference natolik, že začne útočit na nevhodnou kořist. Má-li predátor předchozí dobrou zkušenost s kořistí podobnou nevhodné kořisti s kterou je právě konfrontován, mohl by tím být zmaten a napadnout ji (Lindström *et al.* 1997). Pokud je obraz vhodné kořisti vtištěn dostatečně silně může změnit reakci i na velmi nevhodnou podobnou kořist. Zároveň je z předchozího zřejmé, že čím kratší časový úsek je mezi dobrou zkušeností s kořistí a konfrontací s podobnou nevhodnou kořistí, tím je větší šance omylu a napadení nevhodné kořisti. Lze tedy předpokládat, že neofobie, ale dokonce i potravní konservatismus by se měl dát odstranit pomocí specifické nedávné zkušenosti a vytvořením určitého obrazu jedlé kořisti. O této problematice pojednává druhá prezentovaná práce.

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Evolution of warning colouration in palatable prey. An experimental approach

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ABSTRACT

An evolution of Batesian mimics was experimentally tested with wild caught avian predators (*Parus major*). The Guyana spotted cockroach (*Blaptica dubia*) was used as a palatable prey in which the mimic evolves, and the red firebug (*Pyrrhocoris apterus*) was used as a model. The optical signalization of insect prey was modified using paper sticker shield placed on its back. The effect of predator familiarity with the mimic prey was tested. The cockroaches carrying the shield of the firebug pattern were significantly better protected than those with the cockroach shields. The protectivity of the firebug shield was of the same effectivity on the cockroach as on the firebug, but the firebug was protected regardless of the shield pattern – the warning shield pattern provided much better protection to the edible prey. Great tits familiar with the cockroach as the prey attacked cockroaches regardless of their shield pattern. The cockroaches with firebug shields were no longer protected against predator attack. This result suggests the need of the predator low familiarity with the prey in which the Batesian mimics evolve.

Keywords: mimicry, aposematism, palatability, food experience.

INTRODUCTION

The evolution of warning colouration has been very tricky problem for several last decades. Fisher (1930) proposed the first theory explaining the problem with origin of conspicuous individuals in population of cryptic and unpalatable animals, using the kin selection. According to this theory, gregariousness and kinship of the basal population was postulated. Under these terms, several warningly coloured individuals removed from the population by naïve predator attack, leave their brothers which carry genes for warning colouration. At the same time their deaths are useful in teaching the predator

the connection between the colouration and the unpalatability. Subsequently, the warning colouration is very profitable and spreads in the population. Nevertheless, there are several aposematic insect species which do not live in aggregations (e.g. larvae of swallow tail – *Papilio machaon*). This problem was particularly solved in experiments conducted by Wiklund and Järvi (1982). In these experiments with *P. machaon* the naïve predators did not kill offered aposematic prey during handling them, so that individual selection of the warning colouration was considered possible.

Guilford (1988) proposed three possible situation in which warning colouration evolves: i) the colouration evolves first and then is followed by evolution of chemical defence, ii) colouration and unpalatability evolves at the same time (in one individual), iii) the warning colouration evolves in unpalatable prey. First two processes were supposed to be very rare, but there were some works supporting the theory of an evolution of warning colouration in palatable prey. Ruxton *et al.* (2004) suggested evolving of unpalatability in warningly coloured prey as a compensation of costs and risk connected with conspicuous colouration. Moreover, Thomas *et al.* (2003 and 2004) proved the dietary conservatism as an important factor increasing predators' avoidance to conspicuous, edible prey and subsequent spreading of the colouration in population. See Marples *et al.* (2005) for review.

Our experiments should simulate evolution of warning colouration in population of edible prey, but in this case the colouration mimicked an aposematic model familiar to the predator. This situation suits the definition of the Batesian mimicry. Batesian mimic is edible, profitable animal that simulates the signalization of another unpalatable or dangerous one (the model) (Bates 1862 *ex.* Komárek 2003). The mimic is protected by the model as far as there are more models than mimics. The Batesian mimicry is recognizable in case, when the model and mimic are not closely relative, so that we can say that their similar colouration truly is an adaptation to predation and not the result of heritability (Komárek 2003). Another basic form of mimicry is the situation when one unpalatable or dangerous prey mimics the colouration of another, similarly dangerous (Müllerian mimicry). The aim of this system is to disburden the predator the learning of unprofitability, so that it needs not to learn lots of different signals connected with some danger (Edmunds 1974). Recent studies (MacDougall and Dawkins 1998; Speed 1999, Speed *et al.* 2000) showed that in fact there is no sharp border between Batesian and Müllerian mimicry in nature, as less protected prey is mimicking more protected one

and prey without protection can mimic both of them and so called quasi-Batesian complexes evolve.

Lastly, our experiments tested the effect of former experience of the predator with the palatable prey on potential evolution of warning signalization. Coppinger (1970) as one of the first pointed out the importance of predators experience on its reaction toward any prey. Two principles were supposed to affect the avoidance of the predator toward its prey: the neophobia and the dietary conservatism (see Marples & Kelly 1999, for review). The neophobia rises in case when predator meets new prey. It rises immediately at the moment of encounter and usually takes only short time. After this time the prey is generalized with some familiar one and subsequently accepted. The dietary conservatism is supposed to be rather long term event. There are two possible ways of the origin of it: innate (e.g. Lindström *et al.* 1999) or learned (e.g. Shettleworth 1972) avoidance to a certain prey. This avoidance is realized by making some number of images of potential prey in predators' mind. Any prey different from these images is avoided (Kelly and Marples 2004). This effect is strongly rooted in predator brain and can be suppressed only very hardly. Certain variability in extend of dietary conservatism was proved in the interspecific (e. g. Coppinger 1970; Marples *et al.* 1998) as in the intraspecific level (e. g. Schuler and Hesse 1985; Verbeek *et al.* 1994; Dingemanse *et al.* 2003). Generally, more specialized species (or individuals) show higher extend of dietary conservatism than the generalists.

In the present study, we studied the efficiency of warning colouration of palatable prey experimentally; using great tits as predators. The palatable prey was mimicking another one earlier proved as unpalatable - the red firebug (see e.g. Exnerová *et al.* 2003, 2006). Wild caught adult birds, supposed to be familiar with the model from the nature, were used as predators. We tested, whether the warning colouration of an edible prey (a cockroach) is protecting the insect from the bird attack, by installing a paper sticker shield with a demonstrably warning colour pattern (the pattern of the firebug). Further, we tested whether the edible prey is protected equally as the unpalatable red firebug (a comparison with reaction of great tits to the red firebug with the firebug shield). Subsequently, we tested if there is the same protective effect of the firebug pattern on palatable and unpalatable prey, by comparing the difference between firebug-shielded and cockroach-shielded firebugs and cockroaches. Lastly, we tested the effect of predator experience on their reaction to warningly coloured edible prey. Higher described experiments were carried out using great tits used to the mealworm as

predators. We repeated experiments with cockroaches with cockroach vs. firebug shields with great tits used to the experimental species of cockroach in purpose to test the effect of familiarity of the edible prey on evolution of warning colouration.

MATERIAL AND METHODS

Experimental prey

The red firebug, *Pyrrhocoris apterus* (L., 1758) has a red and black colouration proved as warning (Wiklund & Järvi 1982, Schlee 1986, Exnerová *et al.* 2003, 2006) together with chemical defence based on short-chained alkanes and their derivatives, produced by repugnatory metathoracic glands (Farine *et al.* 1992). The experimental firebug individuals were collected in the surrounding of České Budějovice (South Bohemia, Czech Republic) during years 2004 and 2005. Groups of circa 50 individuals were kept in the laboratory in glass jars (15 cm in diameter). Dry linden tree seeds (*Tilia cordata*) and water were supplied *ad libitum*.

The Guyana spotted roach, *Blaptica dubia* (Audinet-Serville, 1838) is used as common feed for insectivorous animals. In our experiments the second and third larval instar were used (8-14 mm in length). It is of brownish colours with dark and pale spots and has no chemical defence. The experimental individuals were kept in glass terrarium (40 x 30 x 20 cm) in high densities. Fresh vegetable (carrot, beet root and potatoes), dry cat and dog foods and water were supplied *ad libitum*.

Both insect species were reared at 25°C and long-day (18 h light, 6 h dark) conditions.

The natural colouration of experimental prey was modified using paper sticker shields (see Appendix – Figures 1, 2, 3 and 4). There were two possible patterns on the shields. The first pattern represented the cockroach and the other the firebug as seen from above. Both patterns were obtained by taking photographs of both insect species.

Experimental predators

Adult great tits (*Parus major* L) caught with the mist net in the vicinity of České Budějovice (South Bohemia, Czech Republic), were used as experimental predators. Captures were conducted during years 2004 to 2006 except for the breeding seasons (May to July). We are provided with licenses permitting catching and ringing birds (Bird Ringing Centre Praha No. 1004) and experimentation with animals (Czech Animal Welfare Commission No. 489/01). Birds were kept in standard birdcages at lowered indoor temperature and under outdoor photoperiod conditions. Birds were

acclimated to the laboratory conditions and food (sunflower seeds and mealworms or cockroaches – see chapter trials) for 1–2 days prior to experiments. They were ringed and released immediately after trials finished.

Experimental equipment

The experimental cages were made from wooden cubic frames (0.7 m × 0.7 m × 0.7 m) covered with wire mesh (2 x 2 mm) with the front wall made of one-way mirror (see Exnerová *et al.* 2003, for details). The cages were equipped with one perch, a bowl with water, and a rotating circular feeding tray, containing six small cups (a single cup contained a prey item during a trial). The distance between the perch and the tray was approximately 25 cm. The bottom of cups was of white colour. Standard illumination was generated by light source (LUMILUX COMBI 18W, OSRAM) that simulates the full daylight spectrum.

Trials

The 120 tested individuals of great tits were divided in two groups. The first one of 80 individuals was used to mealworms as a prey during the acclimatization to the laboratory. The other of 40 individuals was used to cockroaches. Subsequently the birds from the first group were divided into four groups (20 individuals each). The first group was offered the cockroach with the shield with pattern of the cockroach. The second group was offered the cockroach with the shield with pattern of the firebug. The third group was offered the firebug with the cockroach shield and the fourth group was offered the firebug with the firebug shield. The birds used to the cockroaches were divided into two groups of 20 individuals. The first one was offered the cockroach with the cockroach shield and the other the cockroach with the firebug shield. To avoid pseudo-replication, each individual bird was used for a single series of trials only.

Each bird was placed into the experimental cage before the experiment in order to adapt to the new conditions, and was provided with food (mealworms or cockroach) and water. The bird was deprived of food for 1.5–2.5 hours before the experiment to increase its motivation. The bird was assumed to be ready for experiment as soon as it attacked offered prey (mealworm or cockroach) immediately after offering. Each experiment with an individual bird consisted of a series of 4 immediately succeeding trials, in which 2 control prey (mealworm or cockroach) and 2 experimental prey (shielded cockroach or firebug) were offered alternately, starting with the control prey. Repetition of several trials within a single experiment was used to eliminate effects of

the individual differences in predators' neophobia, which was proved as a very short-term event (see Marples & Kelly 1999, for review). The control prey was used to check bird's motivation to forage, and the trial ended after the prey had been eaten. The trials with experimental prey always lasted 5 minutes.

A continuous description of the bird behaviour was recorded in the program Observer ver. 3 (1989–1992, ©Noldus) and simultaneously the experiment was recorded with the camera. We distinguished three possible results of each trial: (1) the experimental prey was neither handled nor killed during the 5 minutes lasting trial, (2) the prey was handled (touched, pecked or taken by the bird's bill) but not killed, (3) the prey was killed.

Statistical analysis

We used the numbers of birds in each experimental group that handled/killed at least one of the two offered experimental prey for the statistical analyses (all tests were performed in Statistica 5.5, 1984-1999, © StatSoft, Inc.). The Fisher's exact test was used for their analysis.

RESULTS

See Appendix for numbers of great tits handling or killing offered prey (Fig. 5) and for results of statistical tests (Tab. 1).

The warning function of the firebug pattern on palatable prey (experiments with predators used to the mealworm)

The great tits avoided to attack the cockroach carrying the shield with the firebug pattern more than cockroach carrying shield with the cockroach pattern. Significantly smaller number of great tits killed offered cockroach with the firebug shield than those with the cockroach shield. Cockroach carrying the firebug shield was protected equally (against the attack as well as against the death) as a firebug with the firebug shield. Nevertheless, the firebug with the cockroach shield was protected as strongly as the firebug with the firebug shield.

The warning function of the firebug pattern on palatable prey (experiments with predators used to the cockroach)

Great tits handled and killed cockroaches with the cockroach shield regardless of their experience with the cockroach. Predators used to the cockroach were not discouraged

from the attack by the firebug shield, and handled and killed cockroaches with the firebug shield more often than predators used to mealworms.

DISCUSSION

Our experiments proved the protective function of the firebug pattern even if placed on edible and unpalatable prey. The edible prey was protected against the predator attack equally as the unpalatable one. These results support the theory of evolution of Batesian mimicry. We have proved that prey protected by no chemical defence or other trait making it unprofitable gains big profit from obtaining signalization mimicking another protected prey species as described in the theory of mimicry formulated in 19th century (Bates 1862 and Poulton 1890 ex. Komárek 2003). Nevertheless, it is possible that evolution of signalization of unprofitability may evolve in population of not protected prey even when mimicking no model. This theory supposes some natural degree of neophobia or dietary conservatism of the predator to conspicuous prey. The predator avoids conspicuous prey *a priori*, regardless of its palatability (see Marples *et al.* 2005 for review). Nevertheless, the aversion to conspicuous prey may be caused by generalisation to other, to predator familiar, aposematic prey. We do not know exactly the generalisation abilities of avian predators, so it is possible, that avoidance to novel warningly coloured prey may be caused by generalisation of it to another familiar aposematic species (even if there is no similarity in pattern, just because of conspicuousness). Moreover, warning colouration is realized usually in several few colours – red, yellow in combination with white and black (see Rowe and Guilford 1999) and predators may generalize these colours. It is possible that neophobia to conspicuousness as described higher may be caused by generalisation of new conspicuous prey to another unprofitable, familiar one.

Our experiments showed that the unpalatable prey was protected regardless of its colouration, which suggests that another parameter may influence the firebug signalization. It is possible that birds could smell the scent of firebugs when approaching it, but part of birds avoided attacking the cockroach shielded firebug without approaching it to proximity. Moreover, the firebug releases the solution actively when disturbed (Socha 1993), so that most of tested firebugs did not smell during the trial at all. We can guess other optical signals sent by the firebug – the body posture, the shape of antennae and legs or the whole body shape; which could help the predator to

recognize the firebug. These results suggest more future exploration of the optical signalization of the firebug.

Experiments with predators used to cockroach brought quite surprising results. Firstly we proved equal attack rate of birds of both food experience to cockroach-shielded cockroach. Nevertheless, in subsequent trials the birds used to the cockroach were not repelled by the warning colouration from attacking the firebug-shielded cockroaches. Bird used to the mealworm were more ready to attack prey of worm-like appearance, showed lower degree of curiosity to cockroach shaped prey and were not able to find the edible prey under the warningly coloured shield. Birds used to the cockroach were more ready to attack prey of cockroach-like appearance, so that they examined the cockroach more and were able to find the prey under the shield. The cause of this observation can be different prey image in predators' mind formed during conditioning to experimental prey (see Kelly and Marples 2004). Lindström *et al.* (2001) proved positive effect of familiarity of predator to cryptic prey on avoiding the aposematic prey. These results demonstrated possible evolution of warning colouration as the warningly coloured prey was avoided more by birds used to the cryptic prey. The evidence for formation of a certain prey image can be deduced from these results. We can suppose that formation of certain food image increases the avoidance to something different.

Both groups of experimental birds had roughly the same experience from the field and in the laboratory there were only few days to learn the prey (cockroach or mealworm). This suggests that the formation and fixation of the prey image is quite fast. Gamberale-Stille (2000) proved the positive effect of time given to the predator to watch potential prey on formation of the prey image, but in these experiments she was interested in the minute scale. Several days surely are time enough to memorize and fixate potential prey. Nevertheless, length of this period is dependent on degree of dietary conservatism of the predator (Marples and Kelly 1999). The great tit can be supposed as quite foraging generalist species (see Kothbauer-Hellmann 1990); so that it has relatively low degree of dietary conservatism. So it is possible for these predators to change their food preferences during few hours or days.

Our results suggest possible evolution of Batesian mimicry in population of edible prey, but only under condition, that the avian predator has low experience with the edible prey in which the mimics evolve. If the prey is too abundant so that predator is very familiar with it, the predator is able to recognize the edible prey even when mimicking

an aposematic species and attacks it. If the prey is relatively rare, the effect of prey image supports the mimicry and warningly coloured edible mimic is avoided. Marples *et al.* (1998) proved the importance of predator's dietary conservatism in evolution of warning colouration not only in unpalatable prey, but also in the edible one. Moreover, the necessity of low density of model species is one of the conditions of mimicry, so that it enables the predator to have larger experience with the model, and the mimic can be protected (Komárek 2003).

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Effect of recent food experience on the reaction to aposematic prey

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ABSTRACT

Experiments with wild caught great and blue tits (*Parus major*, *P. caeruleus*) testing the effect of their recent food experience on their reaction to new or familiar, palatable or unpalatable, cryptic or warningly coloured prey was carried out. Two species of prey was used – the palatable Guyana spotted cockroach (*Blaptica dubia*) and unpalatable red firebug (*Pyrrhocoris apterus*). To modify the insect prey, paper sticker shield placed on its back was used. The shield was of two possible patterns – the cryptic cockroach pattern or the warning firebug one. Predators used to the cockroach or to the mealworm were used. Firstly, the effect of neophobia of predators used to the mealworm toward the shielded cockroach was tested. Later the effect of predator experience with the cockroach to i) the neophobia to the shield, ii) the aversion to the unpalatable but cryptic prey, iii) the aversion to unpalatable, warningly coloured prey was tested. The great tit did not show any degree of neophobia to the paper shield, so that it could not be affected by its cockroach experience. Contrary to this, there was a significant degree of neophobia in blue tits. This neophobia was eliminated by familiarization of blue tits with the cockroach. There was a significantly higher proportion of attack of great tits as well as blue tits familiarized with the cockroach, to the firebug with a cockroach shield than those used to the mealworm. This effect was not proved in case of warningly coloured firebugs (in both predator species) – the aposematic signalization is very well rooted in predator mind and cannot be influenced by its food experience.

Keywords: neophobia, palatability, food experience, warning signalization.

INTRODUCTION

Several decades ago, the importance of predators experience on its reaction toward prey was experimentally examined (Coppinger 1969, 1970). Especially important were parameters affecting the reaction to novel prey (predator was assumed to have no experience). Since these times two basic processes affecting reaction of a predator to novel prey were described (see Marples and Kelly 1999 for review). Both of them may cause avoidance but both of them work completely different. Nevertheless, it always is not easy to distinguish between them. The first one is called neophobia and was described as an immediate aversion to novel prey. But basically, this term had broader meaning describing any aversion to novelty regardless of its cause (e.g. Greenberg 1983 or Schlenoff 1984). In 90^{ths} Marples and Roper (1996) and Marples *et al.* (1998) showed the difference between immediate aversion (neophobia) and aversion caused by life experience of predator – so called dietary conservatism. This other process basically has the same effect on the result of encounter of predator and prey (the avoidance), but it has been proved that dietary conservatism is more stable state and it usually causes stronger avoidance than neophobia. Generally, the dietary conservatism forms strict model of potential prey and we can say it affects the degree of specialization of the predator (Greenberg 1983; Webster and Lefebvre 2000; Mettke-Hofmann *et al.* 2002). The difference between dietary conservatism and neophobia can be distinguished according to the diminishing of the aversion to novel but profitable prey (Marples and Kelly 1999). Aversion caused by the neophobia usually is of quite short duration and in a few minutes is the novel prey accepted as generalized to another familiar one (Schlenoff 1984). The dietary conservatism usually does not allow the predator to generalize the novel prey to another one; the aversion last longer and sometimes may not disappear at all. Kelly and Marples (2004) described the process of accepting novel prey which is refused because of the dietary conservatism. There are two possible ways of obtaining the dietary conservatism (as well as the neophobia) – learned and innate avoidance. There were several studies proving the innateness of the avoidance (Smith 1975; Lindström *et al.* 1999; Rowe and Guilford 1999; Jetz *et al.* 2001), nevertheless it is not always evident if this innate avoidance is caused by the neophobia or dietary conservatism. In the other case, the avoidance is learned during predators live as it meets unprofitable prey, tries to attack and finds out the unprofitability. It is a probability, that both processes may play its role. The predator is born with some

picture of an acceptable prey and this picture is formed (widened or narrowed) during predators' lives.

As mentioned above, dietary conservatism may cause different degrees of food specialization of a predator. It has been proved that different predator species show different levels of dietary conservatism toward the same novel prey (Coppinger 1970; Marples *et al.* 1998). Greenberg (1983) showed different access to novel prey in two closely related but differently specialized warbler species. The species differed not only in their diet, but also in their habitat and microhabitat specialization. Similar results described a quite comprehensive study with 61 parrot species (Mettke-Hofmann *et al.* 2002). Webster and Lefebvre (2000, 2001) examined the neophobia and foraging skills in neotropical passerine and columbiform species and proved high interspecific differences. It is evident that species with a higher degree of specialization (foraging specialization may be in connection to habitat and other forms of specialization) show higher aversion to novelty. At the same time, more generalist species show higher readiness to experiment and are considered to be of higher intelligence (e.g. Kothbauer-Hellmann 1990; Chappell and Kacelnik 2002). In last years, the effect of intraspecific variation on the novelty avoidance had been examined (e.g. Verbeek *et al.* 1994; Dingemanse *et al.* 2003). These results show that we can measure the degree of specialization even within one species; nevertheless, these results are not as considerable as between species.

We have already said that dietary conservatism may be innate, and may be formed during predators' lives. But it is not clear yet, how much experience does the predator need to accept or refuse the prey in different stages of its life. We can guess that the degree of dietary conservatism is constituted during several first months of predators' lives and after this period it is quite hard for it to accept something new. Length of this sensitive period may differ between species but generally it should correspond to sensitive period of learning of other important life abilities like mate resolution (e.g. Bischof and Clayton 1991) or singing (e.g. Marler 1991). According to this assumption we can suppose that adult predators do not change their dietary preferences much easily, and any short food experience would not change their reaction to their prey. The effect of immediate food experience on the reaction of predator to prey has rarely been examined before. Several studies tested the effect of experience on the reaction to aposematic prey (Coppinger 1969, 1970). Results of Lindström *et al.* (2001) proved lowered number of attacks of predators familiar with cryptic prey to conspicuous prey.

The birds familiar with the cryptic one avoided the aposematic prey more than those without any experience. This suggests that bird evolved during familiarization with the cryptic prey a certain picture of potential prey and anything different from this picture was avoided (especially when conspicuous). According to higher mentioned, we can assume that different picture of preferred prey made in predators mind might change its reaction to any other prey.

In the present study, we studied the importance of effect of recent experience on neophobia and prey image of individuals of two tit species (*Parus major*, *Parus caeruleus*). Our experiments were carried out using paper sticker shields with different patterns placed on insect prey, modifying prey colouration. Firstly, we tested the neophobic aversion of tested birds toward edible prey carrying the paper shield with non-warning pattern (a cockroach with shield with cockroach pattern). Later the effect of food experience on this neophobia was tested. First experiment compared reaction of birds used to the mealworm and a cockroach as a prey to cockroaches with cockroach shields. Second experiments tested the effect of predator experience on its reaction to an unpalatable but cryptic prey (a red firebug with a cockroach shield). Lastly we tested the effect of recent experience of the bird predator on its reaction to an aposematic unpalatable prey (a firebug with a firebug shield). The difference between both tested predator species was described.

MATERIAL AND METHODS

Experimental prey

The red firebug, *Pyrrhocoris apterus* (L., 1758) has a red and black colouration proved as warning (Wiklund & Järvi 1982, Schlee 1986, Exnerová *et al.* 2003, 2006) together with chemical defence based on short-chained alkanes and their derivatives, produced by repugnatory metathoracic glands (Farine *et al.* 1992). The experimental firebug individuals were collected in the surrounding of České Budějovice (South Bohemia, Czech Republic) during years 2004 and 2005. Groups of circa 50 individuals were kept in the laboratory in glass jars (15 cm in diameter). Dry linden tree seeds (*Tilia cordata*) and water were supplied *ad libitum*.

The Guyana spotted roach, *Blaptica dubia* (Audinet-Serville, 1838) is used as common feed for insectivorous animals. In our experiments the second and third larval instars were used (8-14 mm in length). It is of brownish colours with dark and pale spots and

has no chemical defence. The experimental individuals were kept in glass terrarium (40 x 30 x 20 cm) in dens population. Fresh vegetable (carrot, beet root and potatoes), dry cat and dog foods and water were supplied *ad libitum*.

Both insect species were reared at 25°C and long-day (18 h light, 6 h dark) conditions. The natural colouration of experimental prey was modified using paper sticker shields (see Appendix Figures 1, 2, 3, 4). There were two possible patterns on these shields. The first pattern represented the cockroach and the other the firebug as seen from above. Both patterns were obtained by taking photographs of both insect species.

Experimental predators

Adults of two tit species (*Parus major* L. 1758 and *Parus caeruleus* L. 1758) caught with the mist net in the vicinity of České Budějovice (South Bohemia, Czech republic), were used as experimental predators. Captures were conducted during years 2004 to 2006 except for the breeding seasons (May to July). We are provided with licenses permitting catching and ringing birds (Bird Ringing Centre Praha No. 1004) and experimentation with animals (Czech Animal Welfare Commission No. 489/01). Birds were kept in standard birdcages at lowered indoor temperature and under outdoor photoperiod conditions. Birds were acclimated to the laboratory conditions and food (sunflower seeds and mealworms or roaches – see chapter trials) for 1–2 days prior to experiments. They were ringed and released immediately after trials finished.

Experimental equipment

The experimental cages were made from wooden cubic frames (0.7 m × 0.7 m × 0.7 m) covered with wire mesh (2 x 2 mm) with the front wall made of one-way mirror (see Exnerová *et al.* 2003, for details). The cages were equipped with one perch, a bowl with water, and a rotating circular feeding tray, containing six small cups (a single cup contained a prey item during a trial). The distance between the perch and the tray was approximately 25 cm. The bottom of cups was of white colour. Standard illumination was generated by light source (LUMILUX COMBI 18W, OSRAM) that simulates the full daylight spectrum.

Trials

There were 140 tested individuals of each tit species. The birds of each species were divided into two groups. The first one of 80 individuals was used to mealworms as a prey during the acclimatization to the laboratory. The other of 60 individuals was used

to cockroaches. Subsequently the birds from the first group were divided into four groups (20 individuals each). The first group was offered the cockroach without any shield. The second group was offered the cockroach with the shield with pattern of the cockroach. The third group was offered the firebug with the cockroach shield and the fourth group was offered the firebug with the firebug shield. The birds used to the cockroaches were divided into three groups of 20 individuals. The first one was offered the cockroach with the cockroach shield, the second the firebug with the cockroach shield and the third the firebug with the firebug shield. To avoid pseudo-replication, each individual bird was used for a single series of trials only.

Each bird was placed into the experimental cage before the experiment in order to adapt to the new conditions, and was provided with food (mealworms or cockroach) and water. The bird was deprived of food for 1.5–2.5 hours before the experiment to increase its motivation. The bird was assumed to be ready for experiment as soon as it attacked offered prey (mealworm or cockroach) immediately after offering. Each experiment with an individual bird consisted of a series of 4 immediately succeeding trials, in which 2 control preys (mealworm or cockroach) and 2 experimental prey (cockroach or firebug) were offered alternately, starting with the control prey. The control prey was used to check bird's motivation to forage, and the trial ended after the prey had been eaten. The trials with experimental prey always lasted 5 minutes.

A continuous description of the bird behaviour was recorded in the program Observer ver. 3 (1989–1992, ©Noldus) and simultaneously the experiment was recorded with the camera. We distinguished three possible results of each trial: (1) the experimental prey was neither handled nor killed during the 5 minutes lasting trial; (2) the prey was handled (touched, pecked or taken by the bird's bill) but not killed; (3) the prey was killed.

Statistical analysis

We used the numbers of birds in each experimental group that handled/killed at least one of the two offered experimental prey for the statistical analyses (all tests were performed in Statistica 5.5, 1984-1999, © StatSoft, Inc.). The Fisher's exact test was used for their analysis.

RESULTS

See Appendix for numbers of great and blue tits handling or killing offered prey (Figures 6 and 7) and for results of statistical tests (Tab. 2).

The effect of neophobia to the paper shield (experiments with predators used to the mealworm)

Great tits handled and killed the shielded cockroaches equally as the non-shielded ones. Blue tits showed some degree of neophobia as they handled and killed the cockroaches with cockroach shields significantly fewer than the cockroaches without any shields.

The effect of recent experience on the presence of neophobia

Great tits handled and killed cockroaches with cockroach shields regardless of their experience with the cockroach. Blue tits used to the cockroach showed no degree of neophobia as they handled and killed shielded cockroaches more often than birds used to the mealworm.

The effect of recent experience on the reaction to unpalatable prey

Great tits as well as blue tits used to the cockroach attacked firebugs with cockroach shields more often than those used to the mealworm. Both tit species killed the firebug with the cockroach shield minimally regardless of their experience with the cockroach.

The effect of recent experience on the reaction to aposematic prey

Great tits as well as blue tits used to the cockroach handled and killed firebugs with firebug shields as rarely as those used to the mealworm.

DISCUSSION

Our experiments proved difference in neophobic reaction between both predator species. The great tits showed lower degree of aversion to shielded cockroaches than the blue tits did. This result can be explained by quite different foraging ecology and behaviour of these two species as the great tit is able to exploit broader supply of niches (see Harrap and Quinn 1996 for details). Contrary to these differences, results of studies describing the learning and memory abilities (Clayton 1998) and willingness to experimentation (Kothbauer-Hellmann 1990) proved the blue tit practically as gifted as the great tit. Nevertheless the works mentioned in previous sentence described the abilities of wild populations of birds; we can suppose that in laboratory conditions the blue tit is not as adaptable as the great tit is.

The effect of different food experience on predator reaction to novel or unpalatable prey was surprisingly weighty. As the great tits showed no neophobia, there was no effect of food experience on it. Nevertheless, the blue tits familiar with the cockroach, showed high increase of willingness to attack shielded cockroach and there no longer was any noticeable mark of neophobia. We can suppose that birds familiar with the cockroach had changed picture of potential prey and were not neophobic toward shielded prey.

The aversion of both tested species (with mealworm experience) to non-warningly coloured unpalatable prey was enormous. We can suppose that birds were able to recognize the firebug even when covered with the cockroach shield. We cannot suppose that birds were able to smell its repugnant scent, because many of experimental birds refused to attack the shielded firebug without approaching it to proximity and the firebug scent can not be smelled on several decimetres. Moreover, the firebug releases the solution actively when disturbed (Socha 1993), so that most of tested firebugs did not smell during the trial at all. The firebug has to send another optical signal than the colouration to the predator. We can guess several optical characterisations according which is the firebug quite recognizable, like its body posture or leg and antenna shape.

Overwhelming majority of birds of both species used to the cockroach attacked the cockroach-shielded firebug. We can suppose that the experience with the cockroach or the mealworm changed their picture of potential prey in two different ways. Birds used to the mealworm were ready to attack a worm-like prey and any prey different from this picture was *a priori* avoided. Contrary to this, birds used to cockroach were ready to attack a beetle-shaped prey and this beclouded their ability to recognize the firebug hidden under brownish paper shield. Nevertheless, we can guess that there was the effect of colouration, as the firebug was mimicking the cockroach with shield pattern, and this enhanced the probability of mistake in predator attack. Birds used to the cockroach might attack the firebug because they thought it is the cockroach.

Reaction of both tested predator species to warningly coloured unpalatable prey was not affected by their food experience. Birds used to the cockroach avoided the firebug-shielded firebug equally as birds used to the mealworm. That suggests that the effect of food experience is not as important in encounter of a predator and an aposematic prey. The birds did not have to show much effort to recognize the firebug, so that there were much fewer mistakes caused by their experience with the cockroach. The avoidance to aposematic prey is strongly rooted in birds mind and is not so easily changeable. Nevertheless there was some insignificant increase in attacking the red firebug in birds

of both species which were used to the cockroach. That supports the theory that the birds, which decided to attack the firebug with the firebug shield, were more ready to attack some beetle-like insect rather than some insect of cockroach colouration like in previous experiments. They did not think they are attacking the cockroach because the firebug was completely differently coloured. This suggests that their prey picture was changed. This is quite serious result as many of experimental works testing the aposematic signalization of insect prey (usually butterflies or bugs) use as a control prey some worm-like prey (e.g. Järvi *et al.* 1981; Gamberale and Sillén-Tullberg 1998; Exnerová *et al.* 2003, 2006). Because of the control prey, the picture of potential prey in predators mind may be changed and predators show avoidance caused not only by the warning signalization but also by the food experience. That suggests the necessity of diversity of control prey in any experiments with avian predators, so that we can be sure about the width of their prey image.

It is evident that food experience has very significant effect on bird foraging behaviour. It is quite surprising that the experience can be changed so easily. Birds used in all of our experiments were adult wild caught, so that they could be supposed to have the same food experience from the field. After being caught, there were only one to two days given to the birds to get used to experimental prey (mealworm or cockroach). This quite short time sufficed to them for changing their food preference. These preferences were changed so strongly that they made the predators to attack unpalatable and potentially dangerous prey. In chapter introduction a theory, that predators form their dietary habits during some quite short time after being fledged, was mentioned. This theory assumes that older predators are not able to change their food spectrum easily and this effect was described as dietary conservatism (see Marples and Kelly 1999). Our results show that food preferences of adult (at least four months old) birds can be changed quite easily. The reasons for this discrepancy may be the species of tested predators. Regardless of foraging differences between great and blue tit mentioned above, both tit species can be doubtless assumed as quite generalist species of birds. Generalists are supposed to be more ready to experiment and change their food preferences (Greenberg 1983), so that practically no dietary conservatism is present in these species. We can guess if the effect of food experience would have the same importance in some more specialist species.

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Appendix

Table1 – Results of statistical analyses (Fisher exact p-values)

First prey in analysis			Second prey in analysis			Activity	
Predator used to	Prey species	Shield type	Predator used to	Prey species	Shield type	Handling	Killing
Mealworm	Cockroach	Cockroach	Mealworm	Cockroach	Firebug	0.0003	0.0012
Mealworm	Cockroach	Firebug	Mealworm	Firebug	Firebug	0.4075	0.3416
Mealworm	Firebug	Firebug	Mealworm	Firebug	Cockroach	1	1
Mealworm	Cockroach	Cockroach	Cockroach	Cockroach	Cockroach	0.2308	0.0471
Mealworm	Cockroach	Firebug	Cockroach	Cockroach	Firebug	0	0

Table 2 - Results of statistical analyses (Fisher exact p-values)

Predator species	First prey in analysis			Second prey in analysis			Activity	
	Predator used to	Prey species	Shield type	Predator used to	Prey species	Shield type	Handling	Killing
<i>Parus major</i>	Mealworm	Cockroach	None	Mealworm	Cockroach	Cockroach	1	0.6948
	Mealworm	Cockroach	Cockroach	Cockroach	Cockroach	Cockroach	0.2308	0.0471
	Mealworm	Firebug	Cockroach	Cockroach	Firebug	Cockroach	<<0.001	0.0471
	Mealworm	Firebug	Firebug	Cockroach	Firebug	Firebug	0.4075	1
<i>Parus caeruleus</i>	Mealworm	Cockroach	None	Mealworm	Cockroach	Cockroach	0.0004	0.0004
	Mealworm	Cockroach	Cockroach	Cockroach	Cockroach	Cockroach	0.0033	0.0004
	Mealworm	Firebug	Cockroach	Cockroach	Firebug	Cockroach	0.0012	1
	Mealworm	Firebug	Firebug	Cockroach	Firebug	Firebug	0.3416	1



Fig. 1 – The Guyana spotted roach (*Blaptica dubia*) with the cockroach shield



Fig. 2 – The Guyana spotted roach (*Blaptica dubia*) with the firebug shield



Fig. 3 – The red firebug (*Pyrrhocoris apterus*) with the cockroach shield



Fig. 4 – The red firebug (*Pyrrhocoris apterus*) with the firebug shield

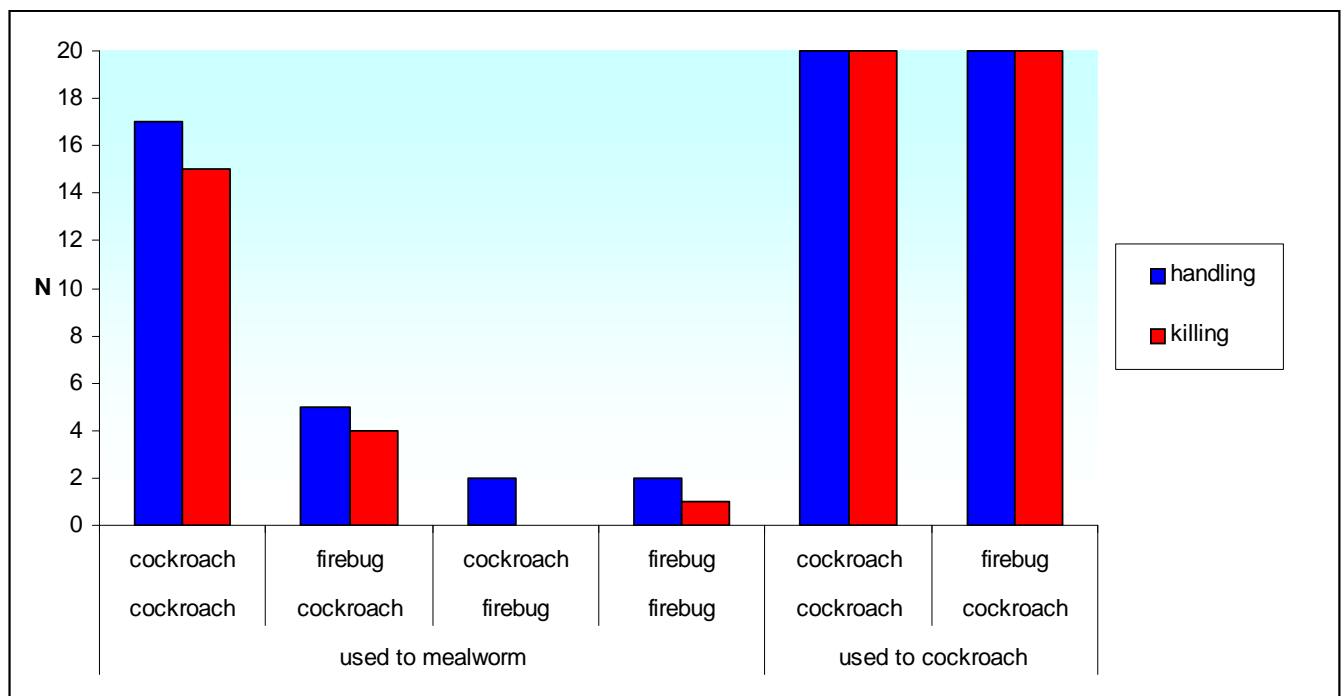


Fig. 5 – Numbers of great tits that handled or killed at least one of two offered prey.
 Under each column there is: i) Shield type
 ii) Prey species
 iii) Experience of bird predator

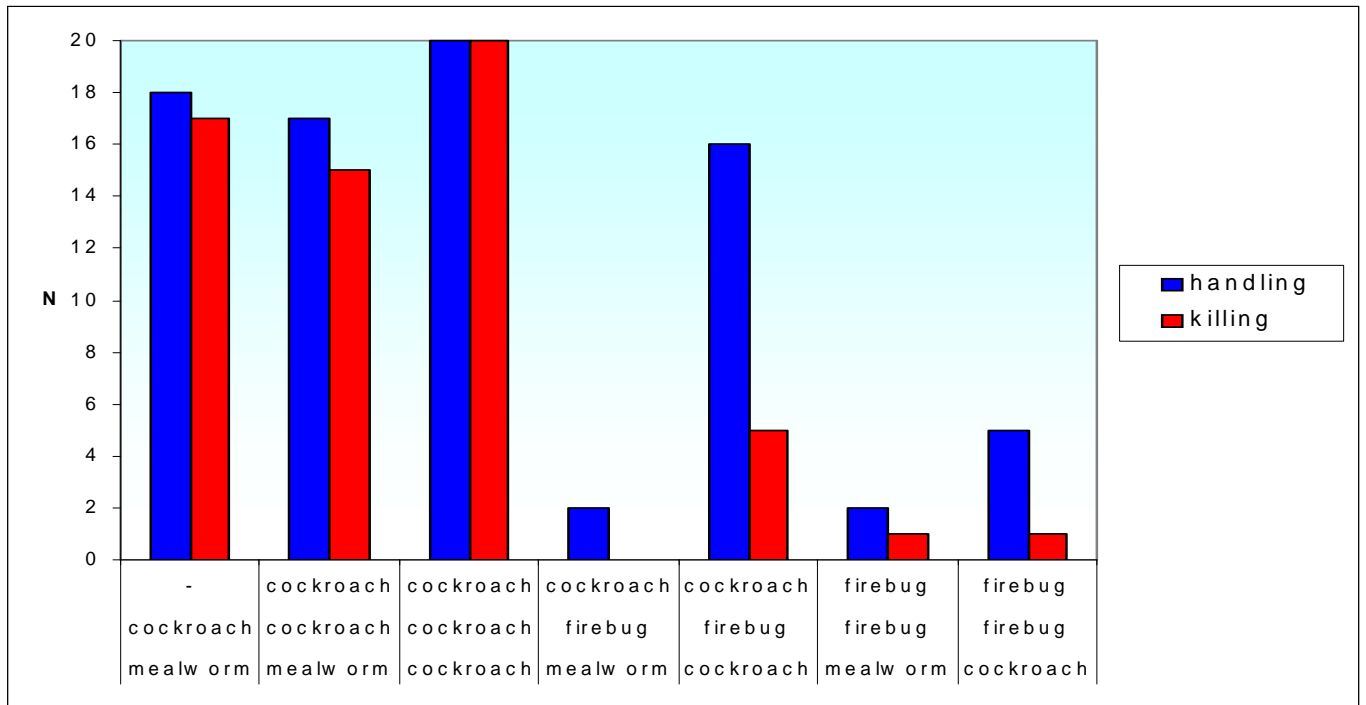


Fig. 6 – Numbers of great tits (*Parus major*) that handled or killed at least one of two offered prey. Under each column there is: i) Shield type
ii) Prey species
iii) Experience of bird predator

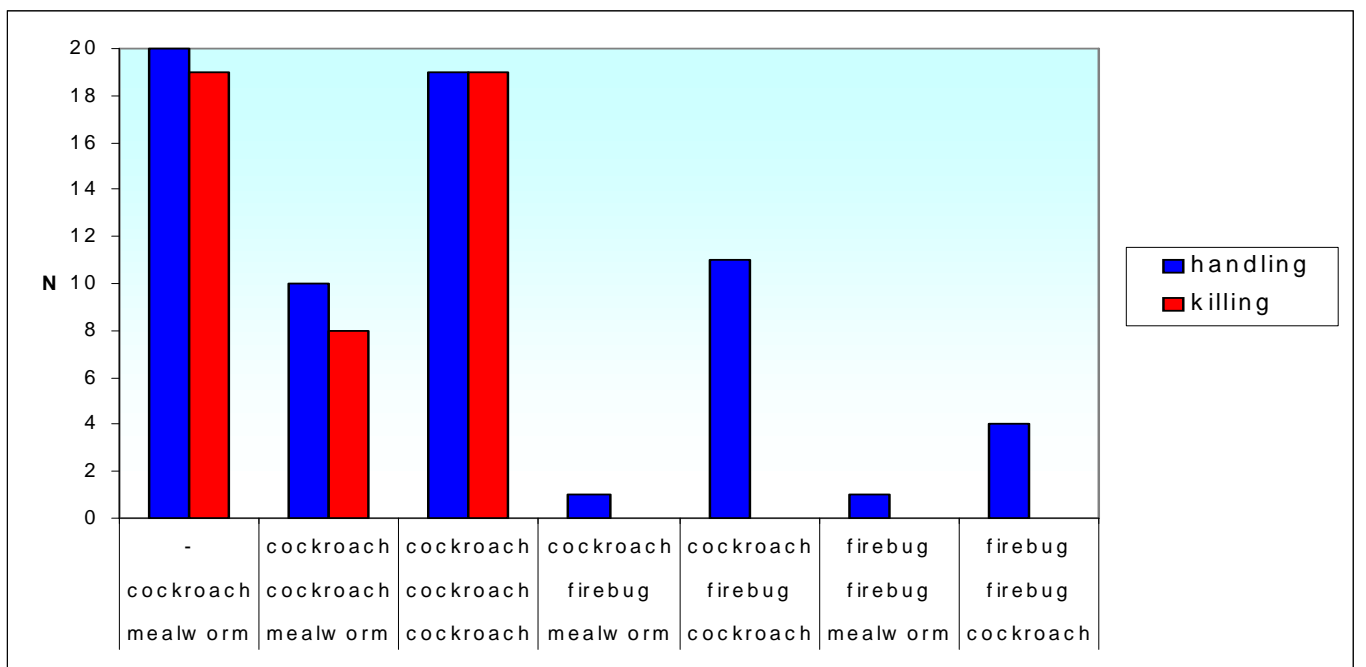


Fig. 7 – Numbers of blue tits (*Parus caeruleus*) that handled or killed at least one of two offered prey. Under each column there is: i) Shield type
ii) Prey species
iii) Experience of bird predator