

Antipredatory function of *Graphosoma lineatum* (Heteroptera, Pentatomidae) coloration

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ÚVOD

Aposematismus a mimikry

Pojem mimikry (mimeze) byl původně používán pro napodobování rostlinných částí živočichy za účelem zneviditelnění pro predátory (Kirby and Spence 1815, 1817 ex Komárek 2001). Postupně bylo odhaleno mnoho vztahů organismů, v nichž je užíváno napodobování, ať už s funkcí antipredační či jinak signalizační. Aposematismus je jev zahrnující určité případy antipredační signalizace mezi organismy, při nichž nepoživatelná kořist zpravidla dává najevo svou nevýhodnost pro predátora nápadným zbarvením. Podíl na optické části aposematického signálu má také uspořádání barev do různých vzorů (Alatalo and Mappes 1996; Lindström 1999; Lindström, Alatalo et al. 2001). Další části optického signálu podpořující výstražnost mohou být tvar (Alatalo and Mappes 1996) a velikost těla (Gamberale and Sillén-Tullberg 1996a), které ovlivňují množství barvy, případně vzor daného aposematika. Častým jevem je kombinace optických signálů se signály čichovými (Marples, Van Veelen et al. 1994) a chuťovými (Sword 1999). Tyto jevy byly během posledních let intenzívně studovány a byly vyvinuty nejrůznější experimentální postupy odhalující různé aspekty komunikace mezi zúčastněnými organismy (Silberglied 1979; Guilford and Dawkins 1991; Guilford and Dawkins 1993; Marples, Van Veelen et al. 1994; Rowe and Guilford 1996; Speed 2000).

Experimentální výzkum aposematismu

V dosavadním výzkumu aposematismu coby antipredační strategie byly užívány čtyři hlavní přístupy: 1) observační metody: rozборы potravy (Exnerová, Štys et al. 2003), pozorování predátorů a jejich kořisti v přirozených podmínkách (Collins and Watson 1983; Peres and v. Roosmalen 1996), měření přírodního materiálu (Srygley 1994; Ohsaki 1995), 2) polní experimenty (Morrell and Turner 1970; Waldbauer and Sternburg 1987; Lyytinen, Alatalo et al. 1999) 3) laboratorní experimenty (viz. dále) a 4) uměle simulované modely (Speed 1999). Nejhojnější jsou přirozeně experimenty laboratorní při nichž je možno minimalizovat množství vnějších vlivů na průběh pokusu, ale zároveň se některé nové faktory související se zajištěním objevují (Roper 1990).

Jako predátoři byli používáni opticky se orientující živočichové, nejčastěji ptáci (viz dále), plazi (Krall, Bartelt et al. 1999; Sword 1999), ale i ryby (D'Heursel and Haddad 1999) nebo hmyz (Kauppinen and Mappes 2003). Použité predátory lze rozdělit na naivní, bez jakékoliv možné předchozí zkušenosti s experimentální kořistí a na predátory, u nichž lze zkušenost s kořistí očekávat (v přírodě odchycení či pozorování). Nejčastěji používanými v přírodě odchycenými ptačími predátory jsou na Americkém kontinentě vlhovec druhu *Agelaius*

phoeniceus (Avery and Nelms 1990), sýkory černohlavé *Parus atricapillus* (Alcock 1970), sojky chocholátá *Cyanocitta cristata* (Coppinger 1969; Bowers 1983), ale i strnadovití (Cardoso 1997) a v Evropě lejsci (*Ficedula hypoleuca*) (Lyytinen, Alatalo et al. 1999), sýkory (hlavně *Parus major*) (Järvi, Sillén-Tullberg et al. 1981; Sillén-Tullberg 1990), kosi (*Turdus merula*) (Marples, Roper et al. 1998) nebo červenky (*Erithacus rubecula*) (Gibson 1980; Marples, Roper et al. 1998). Jako naivní predátory lze použít ručně dochovaná mláďata v přírodě běžně se vyskytujících druhů, která neměla možnost setkat se s pokusnou kořistí, ačkoliv patří k druhu, který se s ní v přírodě může běžně potkávat: sýkora koňadra *Parus major* (Wiklund and Järvi 1982; Sillén-Tullberg 1985b; Sillén-Tullberg 1990; Alatalo and Mappes 1996; Lindström, Alatalo et al. 1999b), sýkora modřinka *Parus caeruleus* (Wiklund and Järvi 1982), špaček obecný *Sturnus vulgaris* (Wiklund and Järvi 1982), křepelka polní *Coturnix coturnix* (Wiklund and Järvi 1982; Evans, Castoriades et al. 1987), sojka chocholátá *Cyanocitta cristata* (Coppinger 1970), vlhovec nachový *Quiscalus quiscula* (Coppinger 1970), vlhovec červenokřídlý *Agelaius phoeniceus* (Coppinger 1970) nebo tyran žlutobřichý *Pitangus sulphuratus* (Smith 1977). Druhý typ naivních predátorů jsou druhy, které se s předkládanou kořistí nikdy setkat nemohou (ať už z geografických, ekologických či behaviorálních důvodů): zebříčky *Taeniopygia guttata* (Sillén-Tullberg 1985a), komerčně chovaná kuřata *Gallus gallus domesticus* (Gittleman, Harvey et al. 1980; Gamberale and Sillén-Tullberg 1996a; Roper and Marples 1997; Gamberale-Stille and Sillén-Tullberg 1999; Sillén-Tullberg, Gamberale-Stille et al. 2000), křepelky *Coturnix coturnix japonica* (Marples, Van Veelen et al. 1994). Predátoři se stávají naivními i tehdy je-li jim předkládána uměle vytvořená potrava (Alatalo and Mappes 1996; Lindström, Alatalo et al. 1999a; Lindström, Alatalo et al. 2001).

Pokusnou kořist lze rozdělit do dvou hlavních kategorií, na kořist umělou a na kořist přirozenou. Umělá kořist je užívána především v experimentech, které se snaží odhalit principy evoluce aposematismu (Alatalo and Mappes 1996; Lindström, Alatalo et al. 1999a; Lindström, Alatalo et al. 2001), protože predátoři mohou být považováni za naivní (nejen vzhledem k jedinci, ale i k celému druhu). Navíc umělá kořist skýtá nepřehledné množství variability jak v optických, tak v olfaktorických signálech. Poslední dobou se využívá tohoto designu stále častěji i v řešení jiných otázek – např. vliv kontrastu kořisti k pozadí (Gamberale-Stille and Guilford 2003). Jiným případem umělé kořisti je víceméně přirozená potrava, ovšem úmyslně modifikovaná experimentátorem, např. obarvené pečivo (Speed, Alderson et al. 2000), obarvený hmyz (Lindström, Alatalo et al. 1999b), nebo se jedná o mutace v přírodě se vyskytujících druhů (Sillén-Tullberg 1985b).

Přirozená kořist může být předkládána v různých formách: živá nebo znehybněná či umrtvená (Coppinger 1969; Coppinger 1970), soliterní nebo gregarická (Sillén-Tullberg 1990; Gamberale and Sillén-Tullberg 1996b). Mohou to být semena rostlin (Gittleman,

Harvey et al. 1980; Peres and v. Roosmalen 1996), ale častější je testování aposematismu u živočichů. Jako přirozenou živou kořist lze použít měkkýše (Allen, Raymond et al. 1988) nebo obojživelníky (Brodie 1980; D'Heursel and Haddad 1999), ale bezkonkurenčně nejčastější jsou pokusy s hmyzem.

Ty ovšem mohou mít také různý design podle cíle výzkumu: byla používána různá vývojová stadia téhož druhu hmyzu (Sillén-Tullberg, Wiklund et al. 1982), barevně polymorfní druhy (Sillén-Tullberg 1985a; Sillén-Tullberg 1985b) nebo více různých hmyzích druhů (Wiklund and Järvi 1982). První experimenty a stále jedny z nejrozšířenějších jsou pokusy v nichž jsou jako kořist předkládáni motýli a jejich larvy. Bylo popsáno několik mimetických komplexů motýlů na nichž byly prováděny experimentální studie (*Chlosyne harrisii* – *Euphydryas phaeton* – (Bowers 1983), *Limenitis archippus* – *Danaus plexippus* – *Danaus gilippus* – (Ritland 1991), nebo studie pouze testují aposematicnost signálů vybraného druhu (druhů) – např. podčel. Heliconiinae (Benson 1971), rod *Papilio* (Järvi, Sillén-Tullberg et al. 1981; Minno and Emmel 1992), čel. Pieridae (Lyytinen, Alatalo et al. 1999). Jako další hmyzí kořist byla použita sarančata (Sword 2001), slunéčka (Marples, Van Veelen et al. 1994) a jiní brouci (Hetz and Slobodchikoff 1988; De Cock and Matthysen 2001), časté jsou studie zabývající se blanokřídlým a dvoukřídlým hmyzem (Evans and Waldbauer 1982; Howarth and Edmunds 2000; Kauppinen and Mappes 2003).

Experimentální výzkum na ploštících

Můžeme říci, že druhou nejhojnější skupinou hmyzu, na níž je prováděn výzkum v oblasti aposematismu jsou ploštice (Heteroptera). Velká část zástupců tohoto řádu je vybavena určitým zdrojem chemické obrany, jehož chemické složení a účinky (včetně změn během ontogenetického vývoje jedince) byly podrobovány mnohým studiím – např. Pentatomidae (Akpana and Olagbemiro 1982; Aldrich, Avery et al. 1996; Krall, Bartelt et al. 1999), Pyrrhocoridae (Farine, Bonnard et al. 1992; Farine 1992), Coreidae (Gunawardena and Bandumathie 1993), Miridae (Wardle, Borden et al. 2003). Obranné látky vznikají obdobně jako u většiny hmyzích aposematiků sekvestrací z potravy (Sillén-Tullberg, Gamberale-Stille et al. 2000; Aliabadi, Renwick et al. 2002), vzácněji syntézou z vlastních látek (Cardoso 1997). Méně časté jsou práce pojednávající o morfologii žláz produkující obranné látky (Cassier, Nagnan et al. 1994). Také druhá podmínka aposematismu - výstražnost zbarvení - byla prokázána v mnoha čeledích heteropter – Coreidae (Schlee 1986), Pentatomidae (Schlee 1986), Pyrrhocoridae (Wiklund and Järvi 1982; Schlee 1986; Exnerová, Landová et al. 2003), Lygaeidae (Sillén-Tullberg 1985a).

Konkrétní výzkumy se pokoušely zodpovědět mnohé otázky týkající se aposematismu. Základní otázkou je do jaké míry se ploštice vyskytují v potravě ve volné přírodě žijících druhů (Creutz 1953; Exnerová, Štys et al. 2003). První experimenty zkoušely požitelnost

ploštičích druhů pro různé predátory (Ullrich 1953; Schlee 1986). Krall a Barlet et al.(1999) provedli chemickou analýzu obranných látek plošnice druhu *Cosmopepla bimaculata* (Pentatomidae) a zároveň otestovali jejich antipredační funkci. Jako predátory použili naivní ptáky (*Charadrius vociferus*, *Sturnus vulgaris*, *Turdus migratorius*) i plazy (*Anolis carolinensis*). Po opakovaném předložení ploštic schopných sekrece dráždivých látek se predátoři naučili této kořisti vyhýbat. U jedinců, u nichž byla sekrece repelentních látek znemožněna, byla míra predace vyšší.

Reakce predátora na aposematickou kořist však skrývá mnohé další aspekty. Problém generalizace mezi jednotlivými různě intenzivně výstražně zbarvenými a různě chemicky chráněnými zástupci čeledi Lygaeidae se snažily řešit Gamberale-Stille a Sillén-Tullberg (1999). Použití naivní predátoři (*Gallus gallus domesticus*) byli schopni generalizovat mezi jednotlivými druhy a odmítali i plošnice jen podobné těm, jež se naučili odmítat. Obdobnou studii provedl Evans et al. (1987) na zástupcích tří čeledí ploštic (Cercopidae, Lygaeidae, Pentatomidae – všichni vybraní zástupci měli černo-červenou kresbu). Predátoři (křepelky) se naučili vyhýbat pestře zbarvené kořisti bez ohledu na druhovou příslušnost. Zkoumán byl také vliv barevné variability uvnitř jednoho ploštičského druhu. Sillén-Tullberg (1985b) nabízela ručně odchovaným koňadrám (*Parus major*) dvě barevné (aposematická a kryptická) formy druhu *Lygaeus equestris*. Aposematická forma přežívala v daleko větší míře než kryptická

Gamberale-Stille a Sillén-Tullberg (1996a) dále prokázaly, že tělesně větší instary aposematické plošnice (*Tropidothorax leucopterus*, Lygaeidae) jsou silněji chráněny před útokem predátora (kuřata) než menší. Na stejném druhu plošnice byl také testován vliv gregariozности kořisti (Gamberale and Sillén-Tullberg 1998). Byla prokázána větší averze predátora (kuřata) vůči kořisti shromážděné ve větší skupině. U neaposematické plošnice (*Graptosthetus servus*) se stejný efekt nedostavil. Sillén-Tullberg (2000) prováděla u *Lygaeus equestris* a *Tropidothorax leucopterus* pokusy se schopností sekvestrovat obranné látky z potravy. Testovaná kuřata odmítala více plošnice jejichž živná rostlina mohla poskytovat obranné látky

Disruptivní zbarvení

Za disruptivní je považováno takové zbarvení, které rozkládá obrys nebo plochu jedince na menší části (somatolytické zbarvení) (Cott 1940; Komárek 2001). Následkem toho není predátor schopen zaměřit svou pozornost na kořist (nebo se zaměřuje na životně méně důležité partie) a ta má větší pravděpodobnost přežití. Tato strategie je hojně užívána v kombinaci s nenápadnými barvami a vzniká tím kryptické zbarvení nepostřehnutelné na vhodném pozadí (můry, hlavonožci, platýsi, mláďata na zemi hnízdících ptáků, lelci, pruhování a skvrnění kočkovitých šelem a mnohé jiné – Cott l.c.) . Někdy je však somatolytické zbarvení užíváno i v kombinaci s výraznými barvami (např. oční pruh ryb,

křídla motýlů – Cott l.c.). Často je také využívána gregaričnost k vytvoření souvislého prostoru s daným vzorem, v němž jednotlivec zaniká – např. u zeber (Cloudsley-Thompson 1984). U hmyzu se somatolýza nejčastěji vyskytuje u motýlů jak v kryptické podobě (noční motýli) tak v kombinaci s barevným vzorem (Silberglied, Aiello et al. 1980).

Experimenty testující účinnost disruptivního zbarvení nejsou příliš časté, neboť je třeba dokonale napodobit přírodní podmínky. Jedna z prací testující nenápadnost motýlů druhů *Catocala ilia* (tmavý) a *Catocala relictata* (světlý) (Kono, Reid et al. 1998) byla provedena tak, že kořist byla predátorovi (*Cyanocitta cristata*) pouze promítána na monitoru na určitém pozadí (kůra břízy nebo dubu) a pták byl při útoku odměněn potravou.

Častější jsou pokusy při nichž se testuje celkový vliv pozadí na optický signál kořisti (častěji zaměřené na aposematickou kořist). Experiment s kuřaty a larvami hmyzu (bělásek zelný *Pieris rapae* – zelené, pilatka *Athalia rosae* – černé) (Ohara, Nagasaka et al. 1993) testoval míru predace na různě barevných pozadích. Černé larvy se na přírodním zeleném pozadí jevily výstražně, kdežto zelené krypticky. Volně žijícím zahradním ptákům byla předkládána černě pruhovaná kořist (těsto) na stejně pruhovaném pozadí (Sellers and Allen 1991). Byl prokázán vliv orientace pruhů kořisti vůči pozadí na schopnost predátora nalézt kořist. Schopnost krypticky zbarvené kořisti (larva jepice *Baetis rhodani*) aktivně nalézt vhodné pozadí byla prokázána v další studii (Tikkanen, Huhta et al. 2000). Sillén-Tullberg (1985b) zkoumala reakce naivních koňader na červenočernou a šedočernou morfu nymf plošnice *Lygeus equestris* prezentované na šedém pozadí. Tento design pokusu však nemůže prokázat kryptické vlastnosti zbarvení šedočerné morfy, protože predátor ví, kde se má kořist nacházet a vyhne se jí jen pokud mu přijde nevhodná. Pokus Lindstrom, Alatalo et al (1999b) s výstražně a krypticky zbarvenými moučnými červy, podávanými naivním koňadram, nepotvrzuje, že by nápadnost vůči pozadí vyvolávala averzi predátora, pouze urychluje jeho učení se nové kořisti (Gamberale-Stille 2001).

Při studiu kryptického zbarvení je stále častější využití počítačových simulací, modelující koevoluci kořisti a predátora s definovanými parametry (úspěšnost útoku/přežívání, nechutnost kořisti, populační charakteristiky apod.). Příkladem tohoto typu studia je práce (Merilaita 2003), která se snažila vysvětlit podmínky, za nichž je využití kryptického zbarvení v různorodém prostředí nejoptimálnější. Tyto experimentální metody bývají užívány hojně i při studiu mimize (vznik, udržení, fungování mimetických vztahů) (Turner, Kearney et al. 1984; Speed 1999; Speed and Turner 1999). Výhodou tohoto postupu je eliminace nepredikovatelných vnějších vlivů, nevýhodou mohou být nesprávné závěry v případě nevhodně zadaných parametrů.

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RUKOPIS ČLÁNKU

Antipredatory function of *Graphosoma lineatum* (Heteroptera, Pentatomidae) coloration

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**Heteroptera, Pentatomidae, *Graphosoma lineatum*, aposematic coloration. background,
color pattern, *Parus major*, *Parus caeruleus***

Abstract

The reaction of two tit species to the shieldbug (*Graphosoma lineatum*) was observed. The coloration of the shieldbug (red and black longitudinally striped pattern) could have two basic antipredatory functions. Red and black color in combination with chemical defense of this species supports the theory that *G. lineatum* is aposematic. The second hypothesis is based on presence of the striped pattern in this bug species. This type of coloration can be present in cryptic species, and has somatolytic (or disruptive) function. These two hypotheses were tested in experiments with avian predators (*Parus major* and *Parus caeruleus*). The first hypothesis was tested by making artificially non-aposematic form of the bug and by comparing results of these experiments to the results of similar experiments with red firebug (*Pyrrhocoris apterus*). The second hypothesis was tested in experiments with modified background that should imitate 1) the natural surroundings of the shieldbug (the umbel of carrot plant) and 2) the color pattern present in *G. lineatum*. The blue tit avoided both wild and non-aposematic forms of shieldbug. The great tit avoided wild form more often than the non-aposematic one. The reaction of both tested predators to the shieldbug was equal to the reaction to the red firebug. The wild shieldbug presented on modified background is attacked by great tit equally often as the nonaposematic form on the white background. The coloration of the shieldbug on contrasting background has a warning function. The power of this signal is lowered on specific background.

Introduction

The pentatomid true bugs are generally considered to dispose of repellent defensive stuff. Numerous chemical analysis observed the composition of the contents of their defensive glands (Akpata and Olagbemi 1982; Aldrich, Lusby et al. 1984; Gunawardena and Herath 1991; Borges and Aldrich 1992; Gunawardena and Bandumathie 1993; Cassier, Nagnan et al. 1994; Pavis, Malosse et al. 1994; Aldrich, Avery et al. 1996; Ho and Millar 2001; Ho, Kou et al. 2003). Some experiments showed the effect of these chemicals on predators (Krall, Bartelt et al. 1999). But the pentatomid bugs usually do not use this chemical defense in combination with warning coloration. There are few warningly colored species in this family for example in genus *Murgantia* or in genus *Eurydema* (Aldrich, Avery et al. 1996; Aliabadi, Renwick et al. 2002). The antipredatory function of these species coloration has been tested rarely (Aliabadi, Renwick et al. 2002).

The object of this study, the shieldbug (*Graphosoma lineatum* L.), is red and black longitudinally striped insect. This pattern could be considered to have warning function (Wallace 1867 ex Komárek 2001), but such hypothesis has never been tested before among insects. We can find longitudinal stripes in insect species with epidermal derivate covering their abdomen (elytra in Coleoptera, scutellum in Heteroptera). The striped coloration present in genus *Graphosoma* has no parallel among european Heteroptera. The most famous example of longitudinally striped beetle is colorado potato beetle (*Leptinotarsa decemlineata*). Also coloration of this species in combination with its chemical defense (Hough-Goldstein, Geiger et al. 1993) could be thought to be aposematic. We can find the transversal organisation of stripes in Insecta much more often, because it is in accordance with the metameric morfological organisation of the insect body (Zrzavý, Nedvěd et al. 1993). The warning function of this pattern has been proved in many species of orders Hymenoptera and Lepidoptera (Järvi, Sillén-Tullberg et al. 1981; Evans and Waldbauer 1982; Wiklund and Järvi 1982; Howarth and Edmunds 2000; Kauppinen and Mappes 2003).

On the other hand, the striped pattern present in genus *Graphosoma* could have completely other antipredatory function. Stripes are sometimes used by animals for disruption of their body outline e.g. in zebras (Cloudsley-Thompson 1984) or for making important parts of body invisible (eye stripe of fish) (Cott 1940). Shieldbug lives and feeds mostly on carrot plants (Apiaceae, Rosopsida). The inflorescence of carrot plants is usually formed into an umbel that consists of many short twigs. On the background made of these twigs can the coloration of the shieldbug have disruptive function (the surface of the bug is fallen apart and the outline of its body is not apparent).

We studied both functions of the shieldbug coloration in experiments with wild caught avian predators. We could not test the disruptive (cryptic) function of the coloration of the shieldbug in our experimental design (the predator expects the prey and attacks every suitable prey offered). We had assumed that prey considered by predator as suitable is not aposematic and could be thought as disruptively colored (in this case).

We tested these null hypotheses: 1) The coloration of the shieldbug presented on the white background is not warning (attack rate of bird predator to the wild form of the shieldbug presented on white background does not differ from the attack rate to non-aposematic form of shieldbug presented on the white background). 2) The coloration of the shieldbug presented on modified background is not warning (attack rate of the bird predator to the wild form of shieldbug presented on modified background does not differ from the attack rate to nonaposematic form of shieldbug presented on white background). 3) The coloration of the shieldbug has lower defence effect than the coloration of the red firebug (attack rate of the bird predator to the wild form of shieldbug presented on white background differs from the attack rate to the wild form of red firebug presented on white background).

Methods and material

Prey

The shieldbug (*Graphosoma lineatum* L., 1758) is a member of family Pentatomidae (Heteroptera, Pentatomorpha), that are considered to be distasteful for predators (Krall, Bartelt et al. 1999). It disposes of active secretion of irritating stuff, when touched. Bugs are considered to be able to acquire repellent secretions from their nutrient (Sillén-Tullberg, Gamberale-Stille et al. 2000). It is predominantly produced in metathoracical scent glands and consists of short chained alkanes and its derivatives (Stransky, Valterova et al. 1998). The shieldbug is widespread species that inhabits most of the Palearctic. Both larvae and adults feed on seeds and vegetative parts of plants of family *Apiaceae* (Popov 1971; Musolin and Saulich 1995). The experimental individuals were collected in the surrounding of České Budějovice (South Bohemia) in the spring 2002 and 2003. Groups of bugs (cca 50 individuals) were kept in laboratory in transparent plastic boxes (16 × 13 × 7 cm). Dry seeds of carrot (*Daucus carota sativa*), cow parsley (*Anthriscus sylvestris*), wild angelica (*Angelica sylvestris*) and water were supplied *ad libitum*. The insects were reared at 25°C and long day conditions (18 h light, 6 h dark).

The second type of the prey was the red firebug (*Pyrrhocoris apterus* L., 1758). This species was used to compare the reaction of tested birds. Previous study proved that the red firebug was considered aposematic by tested tit species (Exnerová, Landová et al. 2003). The

experimental individuals of the firebug were collected during years 1995 – 2003. They were kept in glass jars (15 cm in diameter) at 25°C and long day conditions (18 h light, 6 h dark). Dry seeds of linden tree (*Tilia cordata*, *Tilia platyphyllos*) and water were supplied to them.

Predators

Adult birds of two tit species (*Parus caeruleus* L., 1758, *Parus major* L., 1758 see Fig 11. and Fig. 12) caught to the mist net in the surrounding of České Budějovice were used as predators. Captures were conducted during years 1995 to 2003 except for the breeding seasons (May to July). We are provided with license permitting catching and ringing birds by Bird Ringing Centre Praha (No. 1004) and experimentation with animals issued by Czech Animal Welfare Commission (No. 150/99).

Birds were kept in standard birdcages to get acclimatized to laboratory conditions one to three days prior the experiments. Their sex and age was determined according to the handbook (Hromádka, Horáček et al. 2002). The light conditions corresponded to outdoor fotoperiod. The birds were offered a varied diet (sunflower seeds, mealworms (larvae of *Tenebrio molitor* L., 1758), crickets (*Acheta domestica* (L., 1758), insects swept in the field) not to be fixed on one type of prey. They were ringed and released immediately after trials.

Experimental equipment

The experimental cages were 71 cm wooden cubic frames covered with wire mesh (2 x 2 mm) with the front wall of one-way mirror (see Fig. 13). The cages were equipped with perch, bowl with water and rotating circular feeding tray containing 6 small cups. Only one of the cups contained the prey during the individual trial. The distance between the perch and the tray was approximately 25 cm. The colour of the bottom of cups was white or modified (see chapter Trials). Standard illumination was obtained by light source (LUMILUX COMBI 18W, OSRAM) simulating the full daylight spectrum.

Trials

Tested sample of blue tits (80 individuals) was divided into four groups of 20 individuals. The first group was offered wild (Fig. 4) and the second one artificially non-aposematic form (Fig. 5) of the shieldbug. The third and fourth group was offered wild (Fig. 9) and non-aposematic form (Fig. 10) the red firebug. We eliminated the aposematic coloration of bugs using brown watercolour (burned sienna) spread all over the pronotum, scutellum, hemielytra and abdomen. This dye is odourless and non-toxic, and the treatment did not influenced the bugs as to their locomotion and secretion.

Great tits (120 individuals) were divided into six groups of 20 individuals. The first one was offered wild and the second one non-aposematic form of the shieldbug. The third and

fourth group was offered wild and non-aposematic form of the red firebug. The fifth and the sixth group was offered the shieldbug presented on different background to test the disruptive function of its colouration. There was a brown (burned sienna) pattern made of crossed lines in the first type of experiment (form UMBEL). The lines were organised to the shape of a schematised umbel of a carrot plant as seen from above (Fig. 6 and Fig. 8). This pattern was used to disrupt the uniform white bottom of the cup. The “shieldbug” pattern was used in the second type of background experiment (form STRIPES) (Fig. 7). Colours and span of this pattern was obtained by scanning the shieldbug by scanner (HP scanjet 5470c).

To avoid pseudoreplication, each bird was used for one series of trials only.

The bird was placed into the experimental cage before the experiment to get used to the conditions and it was provided with food (mealworms) and water. The bird was deprived from food for 1,5 to 2,5 hours before the experiment. It consisted of 10 trials. The first trial started at the moment when the bird attacked the offered mealworm immediately after offering. The bird was successively offered one specimen of the mealworm and one of the tested bug in the individual trials. Series of 10 trials (sequence: mealworm, bug, mealworm, bug...) was used in order to minimise the effect of neophobia, which was supposed to be rather short-term event (Marples and Kelly 1999). Each trial was 5 minutes in length.

Continuous description of bird's behaviour was recorded in program Observer ver. 3 (1989–1992, ©Noldus). We distinguished 12 elements of bird's behaviour but only two were used in subsequent analyses: whether the bird handled (touched or pecked the prey by the bill) or killed some of the offered bugs.

Statistical analysis

We used the data in two forms for statistical analysis.

First one were numbers of birds in each experimental group that handled or killed at least one of five offered bugs. Fisher exact test was used for their comparison.

Second one were numbers of bugs handled or killed by individual birds in each experimental group. Distribution of data in this form was proved as Poisson by Kolmogorov – Smirnov test. We have used square root transformation for normalisation of this type of data. Multiple comparisons of data were made by Tukey HSD test in two – way ANOVA (Statistica 5.5, 1984-1999, © StatSoft, Inc).

Generalised linear model (S-Plus 4.0, 1988-1997, © MathSoft, Inc.) was used for selection of significantly important parameters. We used data in both forms in this case.

Results

Numbers of observed reactions

Numbers of birds that handled or killed at least one offered shieldbug (*G. lineatum*) and red firebug (*P. apterus*) and numbers of bugs handled and killed by individual birds are summarized in Tab. 2 and Tab. 3, Fig. 1, Fig. 2 and Fig. 3.

Wild vs. brown non-aposematic form of *Graphosoma lineatum* (GL)

- *Parus caeruleus*

The blue tit avoided both forms of GL extremely. There was no difference found between numbers of birds that handled (Fisher exact test, $p=0,605$) or killed (Fisher exact test, $p=1$) both forms of shieldbug.

- *Parus major*

Tested birds tended to avoid the wild form of GL more than the brown one. Although the difference between numbers of great tits that handled both forms of shieldbug was not strongly significant (Fisher exact test, $p=0,111$), the difference in killing rate was more important (Fisher exact test, $p=0,0915$). Nevertheless, the comparison of numbers of both handled and killed bugs was proved as highly significant (Tukey HSD test: handling – $p=0,0079$; killing – $p=0,0056$).

Brown GL on white background vs. wild GL on modified background (*P. major*)

- *UMBEL*

There was no difference found in numbers of birds that handled, resp. killed at least one brown bug presented on white background and wild bug presented on UMBEL background (Fisher exact test: handling – $p=0,3431$; killing – $p=0,2351$). Nevertheless, the number of handled resp. killed wild shieldbugs presented on UMBEL background was higher than the number of handled or killed brown bugs on white background (Tukey HSD test: handling – $p=0,0868$, killing – $p=0,0256$).

- *STRIPES*

There was no difference found in numbers of birds that handled, resp. killed at least one brown bug presented on white background and wild bug presented on STRIPES background (Fisher exact test: handling – $p=0,3431$; killing – $p=0,7164$). There was no difference in

numbers of handled and killed brown shieldbugs on white background and wild shieldbugs presented on STRIPES background (Tukey HSD test: handling – $p=0,5519$, killing – $p=0,8181$).

Wild form of *Graphosoma lineatum* vs. wild form of *Pyrrhocoris apterus*

- *Parus caeruleus*

Blue tit avoided wild forms of both bug species equally intensely (Fisher exact test: handling – $p=1$, killing – $p=1$).

- *Parus major*

Birds tended to handle wild form of the shieldbug more often than the wild form of firebug (Fisher exact test, $p=0,0915$) but they killed them equally (Fisher exact test, $p=1$).

Generalized linear models

The most important parameters explaining data variability were bug coloration (wild red-black or artificially non-aposematic brown) and bird species (*P. caeruleus* or *P. major*). For details see Tab. 1.

Tab. 1 – Results of GLM.

| activity | data distribution | factor | F | p | % v.e. |
|----------|-------------------|--------------|-------|---------|--------|
| handling | binomial | color of bug | 20,11 | <<0,001 | 8 |
| | | bird species | 15,46 | <0,001 | 7 |
| | poisson | bird species | 22,69 | <<0,001 | 13 |
| | | color of bug | 14,37 | <0,001 | 9 |
| killing | binomial | background | 6,23 | <0,01 | 7 |
| | | color of bug | 14,71 | <0,001 | 10 |
| | poisson | bird species | 13,73 | <0,001 | 9 |
| | | color of bug | 17,37 | <<0,001 | 15 |
| | | bird species | 16,83 | <<0,001 | 15 |
| | | background | 6,5 | <0,01 | 10 |

v.e. – variability of data explained by selected factor

Discussion

The blue tit avoided both wild and non-aposematic forms of the shieldbug equally. There was no difference in the reactions to wild and brown painted shieldbug, because only few blue tits decided to attack (and much fewer to kill) presented bug. These results show that there is another effect influencing their reaction than the color of the bug (body size or body shape of the shieldbug). The blue tit did not find the shieldbug as suitable prey. The results of experiments with great tit as predator show a difference in reactions to wild and brown form of the bug. There were more brown bugs handled by great tit. On the other hand, number of birds that handled the wild form of shieldbug did not significantly differ from number of birds that handled brown form. Birds that manipulated the brown form of bug manipulated usually more than one offered bug whereas the wild form of bug was manipulated repetitively very rarely. This finding was the reason for using the numbers of handled or killed bugs also in subsequent comparisons.

The rate of brown bugs handling presented on white background is higher than the rate of wild bugs handling presented on UMBEL background. But this difference was not proved as significant. This result indicates that the warning function of the shieldbug coloration is on this type of background still present but its power is weakened. Bugs presented on background imitating the shieldbug pattern were handled equally as brown bugs on white background. This result indicates that the warning function of the shieldbug coloration is lowered on this type of background.

The blue tit avoided the wild shieldbug equally as the red firebug. Both bug species were strongly refused. (see above). The great tit handled and killed both tested bug species similarly. Nevertheless, the wild shieldbug was handled little bit often than the firebug.

On the other hand, many tested birds refused the shieldbug regardless of the color signal (the blue tit absolutely). The warning coloration is more important in cases of repeated encounter of predator and prey. If the warning signal is received the predator will usually not attack the prey repetitively.

The coloration of the shieldbug on contrasting background is considered by great tit as warning. This function could be lowered when the shieldbug is presented on background with disrupted surface.

The coloration of the shieldbug has ever been thought to have warning function, but this has never been tested. This study is the first one that proves this hypothesis in experiments with wild caught avian predators.

The presumption that the shieldbug is an aposematical species was supported by many features of this species. The combination of red and black color is widely considered to have warning function (Wallace 1867). There are some defensive chemicals present in this bug. This is the other very important precondition of aposematism. The chemicals used as repellent are probably sequestered from the nutrient (carrot plants) as proved at other members of family Pentatomidae (Aliabadi, Renwick et al. 2002). Sequestration of repellent stuffs was proved at other warningly colored bugs (Sillén-Tullberg, Gamberale-Stille et al. 2000). The shieldbug mostly lives and feeds on upper parts of herbs and is very good visible for predators. It lives there with other insects that use warning coloration as antipredatory strategy - for example ladybird (Whitmore and Pruess 1982).

But there are some differences in antipredatory signalization of the shieldbug and of other true bugs. The coloration of this species is not usual among aposematical true bugs. The red and black combination is very common – *Pyrrhocoris apterus* (Exnerová, Landová et al. 2003), *Lygaeus equestris*, *Tropidothorax leucopterus* (Gamberale-Stille and Sillén-Tullberg 1999), but formation of these colors in longitudinal stripes is among true bugs unique. There are very few warningly colored species in the family Pentatomidae. The usual coloration of pentatomids could be thought as cryptic (mostly brown or green colors). The species that were proved as aposematically colored e. g. in genus *Eurydema* (Aliabadi, Renwick et al. 2002) are mostly black with red or yellow spots of different size and shape. Red and black longitudinal stripes of genus *Graphosoma* are very unique type of coloration within aposematical pentatomids.

The other factor influencing the warning signalization, the chemical defense, is present in most warningly colored bug species. The repellent stuff of the shieldbug is mostly composed of n-alkanes and 2-alkenals (Stransky, Valterova et al. 1998). Similar chemicals were found in defensive glands of other members of family Pentatomidae (Akpata and Olagbemiro 1982; Aldrich, Lusby et al. 1984; Gunawardena and Herath 1991; Borges and Aldrich 1992; Gunawardena and Bandumathie 1993; Cassier, Nagnan et al. 1994; Pavis, Malosse et al. 1994; Aldrich, Avery et al. 1996; Ho and Millar 2001; Ho, Kou et al. 2003). Other bug families use in their defense also similar stuff but their content may be different: e. g. Pyrrhocoridae (Farine, Bonnard et al. 1992; Farine 1992), Coreidae (Gunawardena and Bandumathie 1993) or Miridae (Wardle, Borden et al. 2003).

The theory of mimicry assumes that warningly colored species should compose mimetic complexes to increase their probability of survival. The shieldbug has no similarly colored parallel within relative insects. It can be perceived by predators as member of group (pentatomids or all true bugs) that is considered to be distasteful regardless of the color.

A study exploring the presence of pentatomids and other true bugs in middle european birds diet was made (Exnerová, Štys et al. 2003). This study shows that the chemical defence

does not prevent the bird attack totally. Seventeen pentatomid species were found in the diet of fourteen bird species (mostly of genera *Oenanthe*, *Saxicola*, *Lanius* and *Sitta*). There was also one aposematically colored species found (*Eurydema oleracea*) in the diet of the nuthatch and the wheatear. The shieldbug has not been found in bird diet in this study likewise in another wild bird diet analysis (Creutz 1953). On the other hand according to these results we can say anything about the aposematical signalisation to the predator only very hardly.

Bird species used in this study as predators (*Parus major* and *Parus caeruleus*) are widespread tits that can easily acclimate to the laboratory conditions. These species were proved to be relatively intelligent and ready to experiment with new food sources (Kothbauer-Hellmann 1990). But the great tit seems to be more adaptable to changed foraging conditions than the blue tit (Sasvári 1979). That is the reason why is this species used in numerous studies as predator. Reaction of wild caught specimens of this species to aposematically colored prey has been tested several times. But these studies usually did not only test the aposematical signalization of an insect species. They tested different aspects of the relationship: aposematic prey – predator. For example the ability of the great tit to generalize among different aposematic bugs (Sillén-Tullberg, Wiklund et al. 1982), the reaction of the great tit to gregaric prey (Sillén-Tullberg 1990; Lindström, Alatalo et al. 1999; Sillén-Tullberg, Leimar et al. 2000) or the function of Batesian mimicry (Lindström, Alatalo et al. 1997).

Studies that tested only aposematical function of an insect signalization with adult great tit as predator are scarce. In experiments with larvae of *Papilio machaon* (Järvi, Sillén-Tullberg et al. 1981) the great tits were able to learn to avoid this unprofitable prey. Experiments with European white butterflies (Pieridae) showed that the white coloration of this prey do not signal the unpalatability (Lyytinen, Alatalo et al. 1999).

The other hypothesis tested in this study, the influence of the background to the optical antipredatory signal of the prey, has also been tested many times. Results of several studies testing this hypothesis are various. One (Sillén-Tullberg 1985) proved that there is no effect of background to the predator reaction. Tested aposematic prey (*Lygaeus equestris*) had been refused by predators more than the nonaposematic form on every tested background. This result agrees with ours. On the other hand another study (Roper 1994) showed that prey presented on background making it more visible suffers higher predation than that one on background making it cryptic. We did not test the visibility of the prey but the effectiveness of its warning coloration.

Results of this study supports the theory that the shieldbug is warningly colored species. Experiments with modified background did not test the extent of visibility of the prey but the

extent of warningness of the shieldbug. These results show that it is possible to find background that would lower the warning signal of the prey.

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Appendix

Tab 2. – Number of birds that handled or killed at least one offered shieldbug (*G. lineatum*) and red firebug (*P. apterus*).

| tit species | bug species | bug color | background | N | handling | killing |
|-------------|-------------|-----------|------------|----|----------|---------|
| PM | GL | WILD | WHITE | 20 | 6 | 1 |
| PM | GL | BROWN | WHITE | 20 | 12 | 6 |
| PM | GL | WILD | UMBEL | 20 | 8 | 2 |
| PM | GL | WILD | STRIPES | 20 | 8 | 4 |
| PC | GL | WILD | WHITE | 20 | 1 | 0 |
| PC | GL | BROWN | WHITE | 20 | 3 | 1 |
| PM | PA | WILD | WHITE | 20 | 1 | 1 |
| PM | PA | BROWN | WHITE | 20 | 14 | 6 |
| PC | PA | WILD | WHITE | 20 | 2 | 0 |
| PC | PA | BROWN | WHITE | 20 | 12 | 1 |

PM – *Parus major*, PC – *Parus caeruleus*, GL – *Graphosoma lineatum*, PA – *Pyrrhocoris apterus*

Tab 3. – Median, mean, minimum and maximum of bugs handled and killed by individual birds.

| tit species | bug species | bug color | background | handling | | | killing | | |
|-------------|-------------|-----------|------------|----------|--------|-------|---------|--------|-------|
| | | | | min-max | median | mean* | min-max | median | mean* |
| PM | GL | WILD | WHITE | 0-2 | 0 | 1,33 | 0-1 | 0 | 1 |
| PM | GL | BROWN | WHITE | 0-5 | 1 | 2,69 | 0-5 | 0 | 3,71 |
| PM | GL | WILD | UMBEL | 0-2 | 0 | 1,5 | 0-2 | 0 | 1,5 |
| PM | GL | WILD | STRIPES | 0-5 | 0 | 2,5 | 0-4 | 0 | 3,25 |
| PC | GL | WILD | WHITE | 0-1 | 0 | 1 | 0-0 | 0 | 0 |
| PC | GL | BROWN | WHITE | 0-2 | 0 | 1,67 | 0-1 | 0 | 1 |
| PM | PA | WILD | WHITE | 0-1 | 0 | 1 | 0-1 | 0 | 1 |
| PM | PA | BROWN | WHITE | 0-5 | 1 | 1,71 | 0-5 | 0 | 2 |
| PC | PA | WILD | WHITE | 0-1 | 0 | 1 | 0-0 | 0 | 0 |
| PC | PA | BROWN | WHITE | 0-3 | 1 | 1,25 | 0-2 | 0 | 2 |

PM – *Parus major*, PC – *Parus caeruleus*, GL – *Graphosoma lineatum*, PA – *Pyrrhocoris apterus*

* - mean computed only from data from birds that were handling or killing.

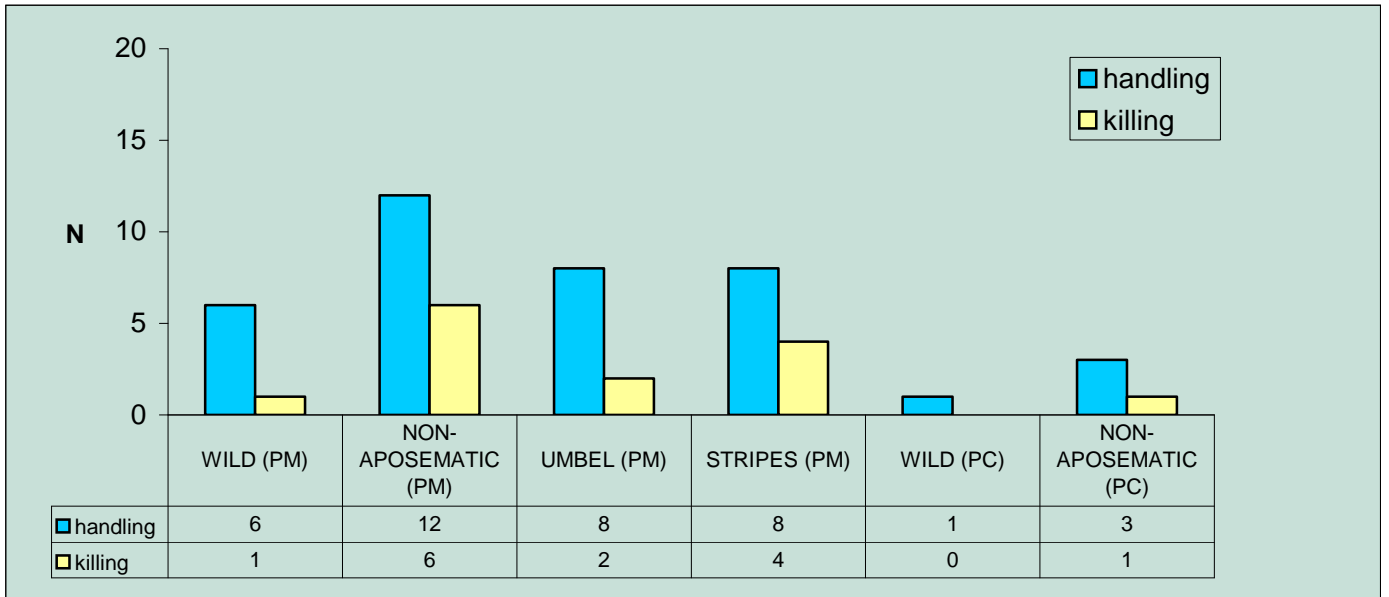


Fig. 1 – Numbers of birds that handled and killed at least one offered shieldbug (*Graphosoma lineatum*).
PM – *Parus major*, PC – *Parus caeruleus*.

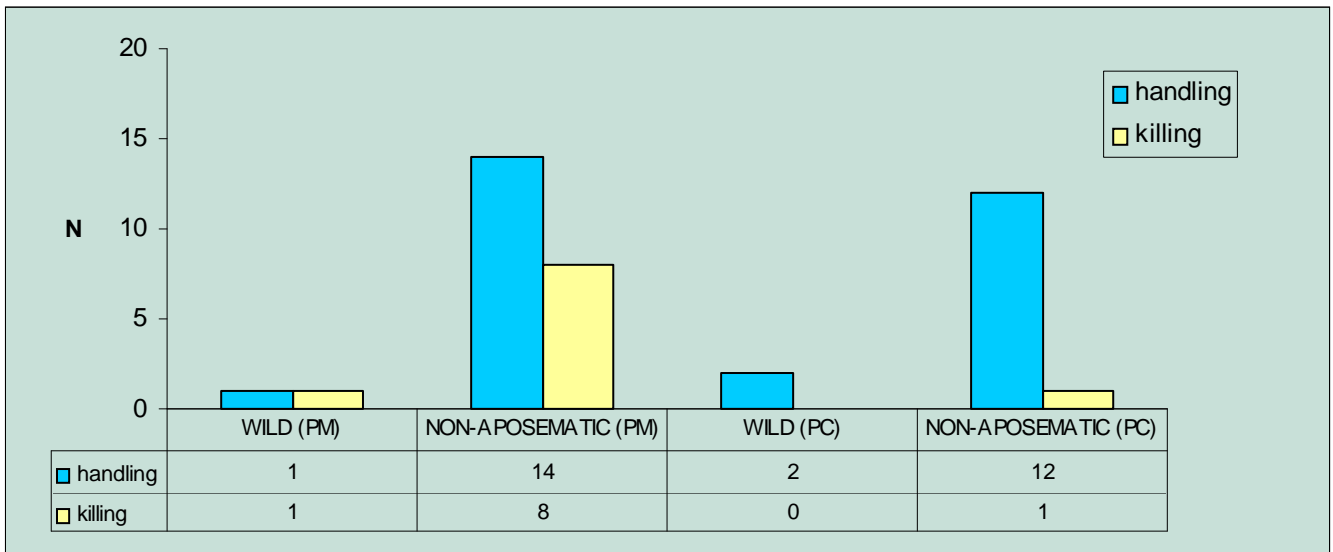


Fig. 2 – Numbers of birds that handled and killed at least one offered firebug (*Pyrrhocoris apterus*).
PM – *Parus major*, PC – *Parus caeruleus*.

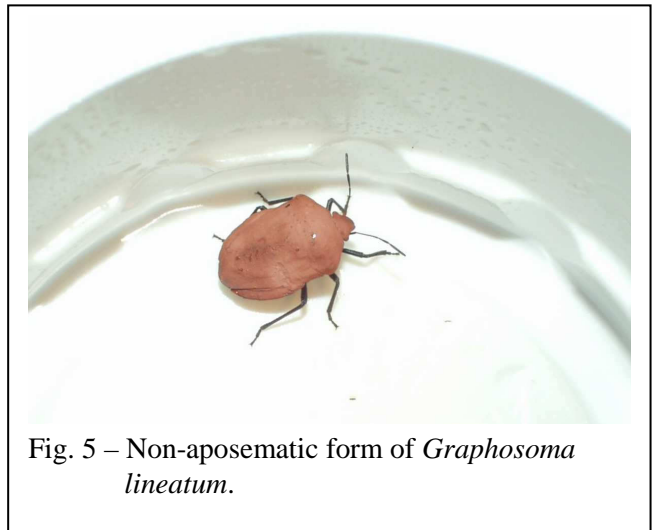
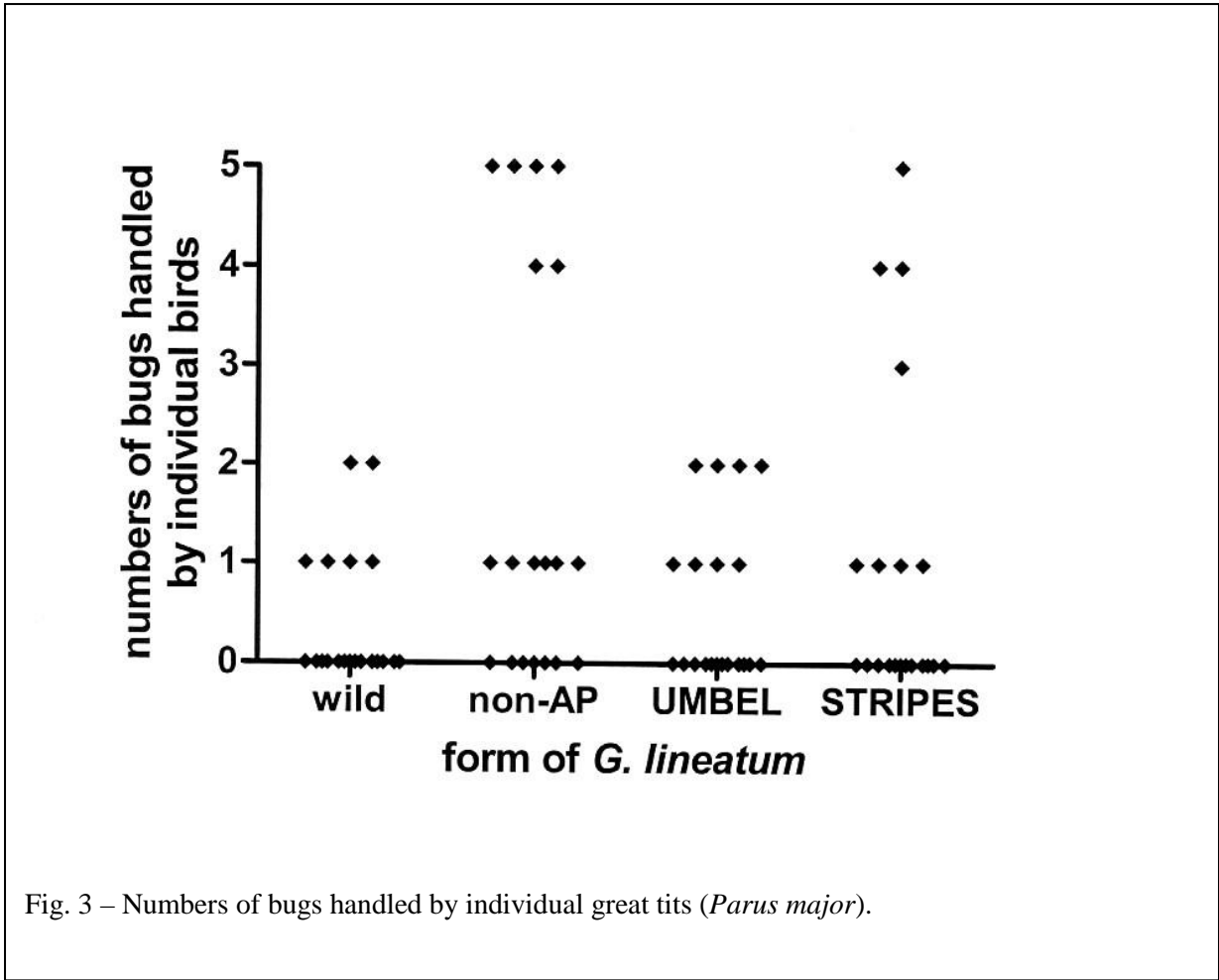




Fig. 6 – Wild form of *Graphosoma lineatum* on UMBEL background.



Fig. 7 – Wild form of *Graphosoma lineatum* on STRIPES background.

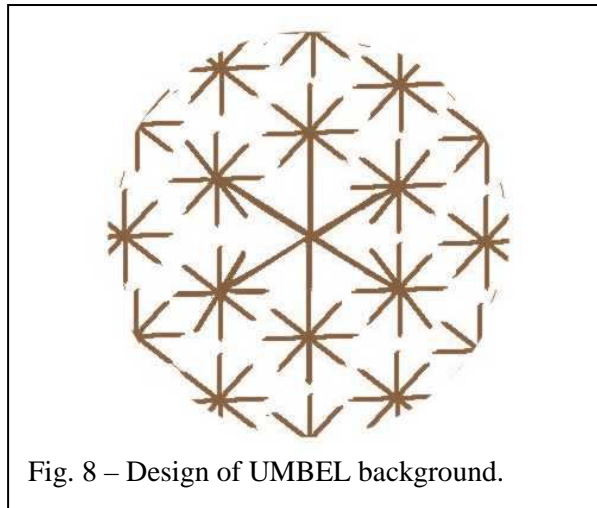


Fig. 8 – Design of UMBEL background.



Fig. 9 – Wild form of *Pyrrhocoris apterus*.



Fig. 10 – Non-aposematic form of *Pyrrhocoris apterus*.



Fig. 11 – *Parus caeruleus*.



Fig. 12 – *Parus major*.



Fig. 13 – Experimental cage.