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Proměnlivost tělesné velikosti hmyzu v prostoru a čase

Disertační práce

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Prohlášení

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Eliška Baranovská

Poděkování

Nejprve bych velmi ráda poděkovala mému konzultantovi Michalu Knappovi, který mi byl nápomocen během celého mého studia na České zemědělské univerzitě. Vždy byl ochotný mi s čímkoliv poradit i pomoci. Velké díky patří také mému školiteli Pavlu Saskovi, který mi dal vždy cenné rady a velmi užitečné komentáře k mým výzkumům. Dále děkuji celému vedení Katedry ekologie, snažili se vždy vyjít vstříc a být nápomocni při provádění experimentů v laboratořích.

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Obsah

Abstrakt

Velikost těla je nápadným znakem všech organismů, který má významný vliv na další vlastnosti daného organismu, a proto může zásadně ovlivňovat jeho život. Díky úzkému vztahu mezi velikostí těla a zdatností jedinců se velikost těla těší velké pozornosti přírodovědců. Už v druhé polovině 19. století si Carl Bergmann všiml, že teplokrevní živočichové v chladnějších oblastech jsou větší než jejich příbuzní z oblastí teplejších. Avšak až v průběhu 20. století se vědci začali zaměřovat i na výzkum vnitrodruhové variability ve velikosti těla u studenokrevných organismů. V rámci mé disertační práce jsem si položila dvě hlavní otázky: Liší se tělesná velikost a kondice u hmyzu na malém prostorovém měřítku a v čase? Mění se velikost těla hmyzu v různých nadmořských výškách a je tento rozdíl dán lokální adaptací nebo fenotypovou plasticitou? Mými modelovými organismy jsou brouci z čeledí střevlíkovitých a mrchožroutovitých, kteří zastávají významnou pozici v mnoha suchozemských potravních řetězcích. Střevlíci jsou významní predátoři škůdců a mrchožrouti se podílejí na rozkladu živočišné organické hmoty. Časová a prostorová proměnlivost na malém prostorovém měřítku a v čase byla zjišťována porovnáním jedinců *Anchomenus dorsalis* (Pontoppidan, 1763) a *Poecilus cupreus* (Linnaeus, 1758) žijících na 4 polích během 3 po sobě jdoucích let. Tělesná velikost *P. cupreus* se lišila pouze mezi pohlavími. Oproti tomu u druhu *A. dorsalis* byla proměnlivost v tělesné velikosti a v tělesné kondici zjištěna mezi lokalitami v průběhu sezóny i mezi roky. Proměnlivost tělesné velikosti na větší prostorové škále jsem zkoumala u mrchožrouta *Silpha carinata* (Herbst, 1783). Jedinci *S. carinata* následovali konverzní Bergmannovo pravidlo, tj. brouci dorůstali do větších velikostí v nižších nadmořských výškách. Laboratorní experiment, kdy jsem chovala jedince *S. carinata* za standardizovaných podmínek v klimatických boxech, naznačil, že rozdíly ve velikosti jedinců mezi populacemi z různých nadmořských výšek je dán především lokální adaptací na konkrétní podmínky. Důvod, proč *S. carinata* dorůstá menších velikostí ve vyšších nadmořských výškách, není přesně znám. Nicméně je zřejmé, že všeobecně na hmyz působí jiné vlivy, než na teplokrevné organismy. Například teplota nebo počet generací za rok se považují za hlavní faktory, které mohou generovat vzory ve velikosti těla hmyzu podél geografických gradientů. Další studie by se proto měly hlavně zaměřit na zjištění mechanismů, které generují vnitrodruhovou proměnlivost ve velikosti těla. Vnitrodruhová variabilita ve velikosti těla se projevuje nejen na velké prostorové škále. Proměnlivost ve velikosti těla na malém prostorovém měřítku může reflektovat kvalitu místních podmínek. Proto je vhodné jí zjišťovat v delším časovém horizontu, abychom mohli studovat i náhlé změny prostředí. Vedlejším produktem mého rozpracovaného

experimentu zkoumajícího vliv nadmořské výšky na několik dalších druhů střevlíků a mrchožroutů je metodologický článek porovnávající vliv návnady a konzervační kapaliny na účinnost zemních pastí pro střevlíky a mrchožrouty. Tento metodologický článek je taktéž zahrnut jako součást této disertační práce.

Abstract

Body size is the most prominent trait of all organisms, and influences other characters of a given organism, including its behaviour and physiology. The body size enjoys a close attention of scientists due to tight relationship between body size and fitness of a particular individual. Carl Bergmann noticed in the second half of the 19th century that endotherm organisms are bigger in colder climate conditions than their relatives from a warmer conditions. However, scientists began to focus on research of intraspecific variation in body size in ectotherm organisms and insects a little bit later, during 20th century. I formulated two main questions in my Ph.D. thesis: Do the body size and condition vary on a small spatial scale and over time in insects? Does the body size differ in different altitudes and is this difference due to phenotypic plasticity or local adaptation? My model organisms were beetles from the families Carabidae and Silphidae that stand in an important position in most terrestrial food chains. Carabids are among important predators of various pests and silphids are significantly involved in decomposition of animal organic matter. Small-scale spatial and temporal variation was investigated in *Anchomenus dorsalis* (Pontoppidan, 1763) and *Poecilus cupreus* (Linnaeus, 1758) in four arable fields over three consecutive years. The body size of *P. cupreus* differed only between sexes. On the contrary, I found significant variation in the body size and the body condition in *A. dorsalis* among sites during season and among years. I also investigated the body size variation along a geographic gradient in a carrion beetle *Silpha carinata* (Herbst, 1783). The carrion beetle *S. carinata* followed the converse Bergmann's rule, i.e. individuals grew bigger at a lower altitude. Common garden experiment, where individuals of *S. carinata* were held in climatic chamber under standardized condition, inferred that the differences in body size among altitudes were given mainly by an adaptation to a particular environmental conditions. The reason why *S. carinata* is smaller at higher altitudes is not completely understood. Nevertheless, it is obvious that in general, effects acting on insect are different than factors acting on endotherm animals. The main factors generating geographic cline of body size in insect are considered to be a temperature and a voltinism. However, there is need for further studies that should be focused on research of other possible proximate mechanisms. Intraspecific body size variations could be recorded even on a small spatial scale. Small-scale body size variation could reflect the quality of local environment conditions. Therefore, it is important to examine small-scale body size variation for a longer time horizon because of record of sudden environmental change. The methodological article, comparing the effect of presence of bait and type of preservative fluid on efficiency of pitfall traps for carabid and carrion beetle, is a by-

product of my unfinished experiment investigating the effect of altitude on several other species of carabid and silphid beetles. This methodological article is also included as a part of this Ph.D. thesis.

Úvod

Velikost těla patří mezi nejpozoruhodnější vlastnosti všech organismů, protože ovlivňuje mnoho znaků, které formují život daného organismu. Velikost těla má například zásadní vliv na chování a fyziologii všech živých tvorů (Chown & Gaston, 2010; Yom-Tov & Geffen, 2011). Jednou z nedůležitějších vlastností velikosti těla je přímá souvislost se zdatností daného jedince. Větší samice mají obvykle vyšší plodnost (Sota, 1985; Honěk, 1993; Mccabe & Partridge, 1997; Moya-Laraño, 2002; Teuschl, Reim & Blanckenhorn, 2007; Marshall *et al.*, 2013) a větší samci se úspěšněji páří (Juliano, 1985; Savalli & Fox, 1998). Schopnost vyrovnat se se stresovými podmínkami (například nedostatkem potravy nebo vody nebo vyrovnání se s teplotním stresem během přezimování) je také vyšší u jedinců s větší velikostí těla (Zhou *et al.*, 1995; Blanckenhorn, Fanti & Reim, 2007; Kingsolver & Huey, 2008; Kovacs & Goodisman, 2010). Proč tedy hmyz běžně nedorůstá do maximální možné velikosti? Možným vysvětlením jsou proti sobě jdoucí selekční tlaky během larválního stádia a stádia dospělce. Aby jedinec dorostl do větší velikosti těla, potřebuje více potravy během larválního vývoje a typicky i delší čas (Blanckenhorn, 2000). Z tohoto důvodu je vystaven vyššímu riziku predace a neočekávaného zhoršení podmínek prostředí během dlouhého larválního vývoje (Nylin & Gotthard, 1998; Blanckenhorn, 2000; Teuschl *et al.*, 2007). Při zhoršených podmínkách prostředí navíc nemusí platit obecné pravidlo o vyšší produkci potomstva většími samičkami. Konkrétní příklad najdeme u motýla *Pararge aegeria* (Linnaeus, 1758). Samice motýla všeobecně kladly méně vajíček při nižších teplotách, ale větší samice měly výrazněji sníženou plodnost ve srovnání se samicemi menšími a tudíž velikost těla při nízké teplotě nevedla k reprodukční výhodě (Gotthard, Berger & Walters, 2007).

Již v 19. století si všiml významný biolog Carl Bergmann prostorových vzorů v biologických znacích u příbuzných teplokrevných organismů. Na základě tohoto pozorování vzniklo nejslavnější biogeografické pravidlo tzv. Bergmannovo pravidlo (Bergmann, 1847). Bergmannovo pravidlo říká, že zvířata z chladnějších oblastí jsou větší než jejich příbuzní z teplejších lokalit. Vysvětlení je nasnadě, jelikož větší zvířata, díky relativně menšímu povrchu k objemu těla, mají nižší tepelné ztráty při nízkých teplotách prostředí. Později začaly vznikat studie, které se zaměřovaly na platnost pravidla uvnitř jednoho druhu (Blackburn, Gaston & Loder, 1999) a u studenokrevných organismů včetně hmyzu (např. Park, 1949; Ray, 1960; Blanckenhorn & Demont, 2004; Stillwell, Morse & Fox, 2007; Cvetković *et al.*, 2009; Shelomi, 2012; Boaratti & Da Silva, 2015). U hmyzu bývá jako možný mechanismus generující větší tělesnou velikost jedinců z chladnějšího prostředí označováno ekofyziologické pravidlo

„Temperature-size rule", ukazující, že larvální růstová křivka pro nižší teploty prostředí vede k větší finální velikosti dospělců (Atkinson & Sibly, 1997; Kingsolver & Huey, 2008). Jedná se tedy o důsledek fenotypové plasticity. Avšak v přírodě můžeme pro jedince v rámci daného druhu hmyzu často pozorovat opak Bergmannova pravidla, tzv. konverzní Bergmannovo pravidlo. Známy jsou i případy, kdy daný druh hmyzu neprojevuje žádnou systematickou změnu velikosti těla podél geografických gradientů (Blanckenhorn & Demont, 2004). Tedy proximátní mechanismy, které působí na studenokrevné organismy, jsou odlišné, než procesy ovlivňující teplokrevné živočichy. Přímý vliv teploty během juvenilního vývoje, délka sezóny nebo dostupnost potravy jsou označovány za hlavní předpokládané příčiny, které způsobují vnitrodruhové geografické gradienty ve velikosti těla (Chown & Klok, 2003; Stillwell *et al.*, 2007; Kingsolver & Huey, 2008; Okuzaki & Sota, 2017). Nicméně délka larválního vývoje ve spojení s délkou sezóny se v současnosti jeví jako nejpravděpodobnější faktor geografických gradientů ve velikosti těla u ektotermních terestrických organismů (Horne, Hirst & Atkinson, 2015; Zeuss, Brunzel & Brandl, 2017).

Vnitrodruhová proměnlivost ve velikosti těla se může značně lišit jak v prostoru, tak i čase. Změny ve velikosti těla v průběhu času lze snadno pozorovat u invazních druhů, jelikož často můžeme zachytit jejich vývoj v novém prostředí od začátku invaze (Huey *et al.*, 2000; Laparie *et al.*, 2010). Ve střednědobém horizontu lze také variabilitu ve velikosti těla pozorovat i jako důsledek probíhajících klimatických změn (Bowden *et al.*, 2015; Fenberg *et al.*, 2016). Například velikost motýlů, žijící v arktických podmínkách, se snížila během 18 let s rostoucí teplotou (viz Obr.: 1; Bowden *et al.*, 2015). Ale i v relativně krátkém časovém úseku se dají zaznamenat změny ve velikosti těla. V dynamických prostředích, například v agroekosystémech, kde se z roku na rok i během sezóny mohou měnit přírodní podmínky stanoviště, organismy mohou odpovídat skrze změny ve velikost těla (Östman, 2005). V rámci prostoru se nejvíce proměnlivost ve velikosti těla u jednotlivých druhů projevuje na velkém prostorovém měřítku, jelikož zde může působit více faktorů i jejich interakce (Chown & Klok, 2003; Stillwell *et al.*, 2007; Teuschl *et al.*, 2007; Kingsolver & Huey, 2008; Ikeda *et al.*, 2012; Okuzaki & Sota, 2017). Ovšem změny ve velikosti těla mohou být patrné i na menší prostorové škále, kvůli působení biotických faktorů či náhodných změn prostředí (Östman *et al.*, 2001; Haschek *et al.*, 2012; Warzecha *et al.*, 2016). Samozřejmě neexistuje jasná hranice mezi časovými a prostorovými změnami ve velikosti těla a často mohou tyto faktory působit současně (Yom-Tov & Geffen, 2011).
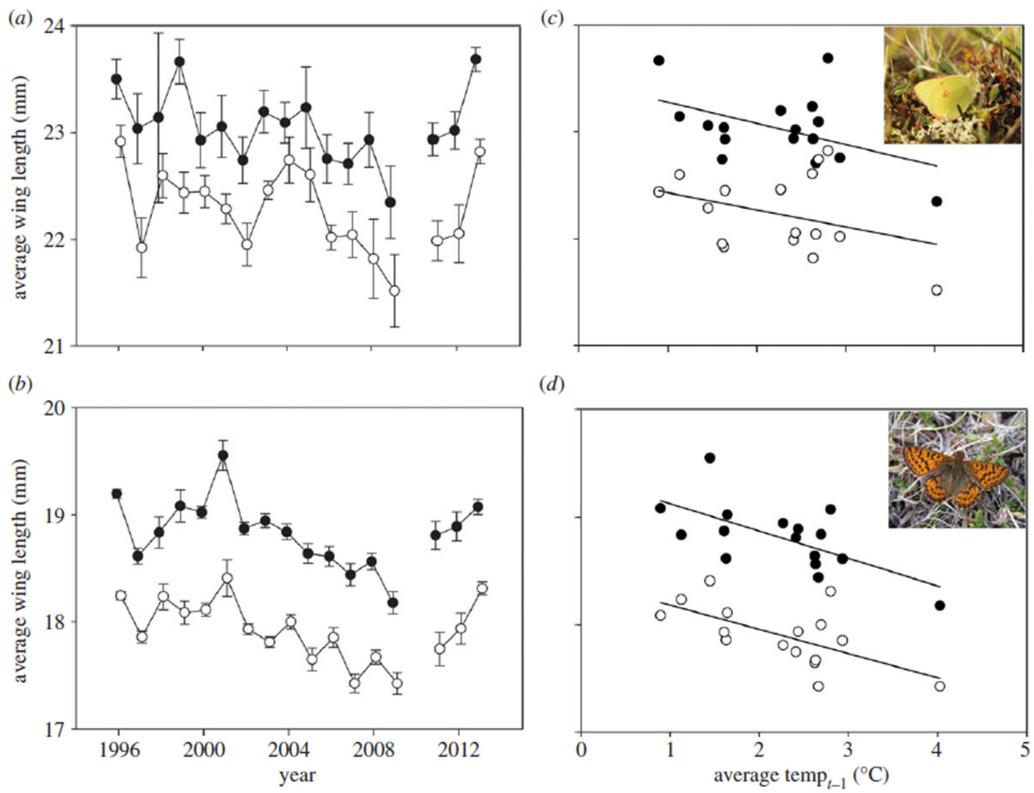
Obr.: 1: Meziroční změny v průměrné délce křídel samců (bílé kruhy) a samic (černé kruhy) pro a) Colias hecla (Lefèbvre, 1836) a b) Boloria chariclea (Schneider, 1794) a jejich odpovědi k průměrné teplotě mezi květnem a srpnem (Bowden *et al.*, 2015).

Vnitrodruhová proměnlivost velikosti těla podél zeměpisných gradientů se zdá být výsledkem odezvy k abiotickému prostředí nebo vzájemnému působení organismů na dané lokalitě (Angilletta & Dunham, 2003; Yom-Tov & Geffen, 2011). V mnoha případech je často problematické odhalit, jaké mechanismy určují geografické rozdíly ve velikosti těla v rámci druhu. Hlavními mechanismy zodpovědnými za vnitrodruhovou geografickou proměnlivost ve velikosti těla u hmyzu jsou lokální adaptace populací na podmínky lokality a fenotypová plasticita. Důkaz, že proměnlivost ve velikosti těla s měnící se zeměpisnou šířkou a nadmořskou výškou má genetický základ, poskytují tzv. „standardizované zahradní experimenty" (Common garden experiments; (Karl, Janowitz & Fischer, 2008; Stillwell & Fox, 2009; Stillwell, 2010; Tsuchiya *et al.*, 2012; Purcell *et al.*, 2016; Sniegula, Golab & Johansson, 2016). Také existují studie, které se snaží odhalit geny zodpovědné za prostorovou variabilitu ve velikosti těla (Weeks, McKechnie & Hoffmann, 2002; Levy *et al.*, 2015).

 Velikost těla u hmyzu se často liší mezi pohlavím (tzv. pohlavní dimorfismus ve velikosti těla). U většiny druhů hmyzu bývají samice větším pohlavím než samci (Teder & Tammaru, 2005; Blanckenhorn *et al.*, 2007; Chown & Gaston, 2010; Stillwell *et al.*,

7

2010). Pohlavní dimorfismus ve velikosti těla je pravděpodobně způsoben selekčním tlakem rozdílně působícím na samce a samice. Avšak směr a rozsah pohlavního dimorfismu ve velikosti těla se značně liší jak mezi druhy, tak i mezi populacemi stejného druhu (Teder & Tammaru, 2005; Stillwell *et al.*, 2010). Fenotypová plasticita, která je způsobena rozdílnými podmínkami prostředí, je patrně viníkem rozdílného pohlavního dimorfismus ve velikosti těla mezi populacemi stejného druhu (Stillwell *et al.*, 2010). Proto mohou být zaznamenány i změny v pohlavním dimorfismu ve velikosti těla podél zeměpisných gradientů (Stillwell & Fox, 2009; Tsuchiya *et al.*, 2012; Laiolo, Illera & Obeso, 2013). Zdá se, že velikost těla samců je geograficky variabilnější než velikost těla samic, protože více reaguje na změny prostředí (Stillwell *et al.*, 2010). Možným vysvětlením je vyšší selekční tlak na samičí tělesnou velikost než samčí, a proto velikost těla samic může být vystavena tzv. „kanalizaci znaků", tedy evolučnímu zakonzervování hodnoty daného znaku (Fairbairn, 2005).

Renchovo pravidlo je další významný biogeografický zákon, které vysvětluje vztah velikosti těla živočichů spojený s pohlavním dimorfismem. Pravidlo bylo odvozeno v mezidruhovém kontextu u teplokrevných organismů, ale aplikuje se i na variabilitu ve velikosti těla jedinců v rámci druhu i u studenokrevných organismů (Frynta *et al.*, 2012; Matějů & Kratochvíl, 2013; Eweleit & Reinhold, 2014; Liao, Liu & Merilä, 2015). Pravidlo konkrétně říká, zvýšením velikosti těla se zvýší i pohlavní dimorfismus ve velikosti těla pokud samci jsou větším pohlavím a pohlavní dimorfismus ve velikosti těla se sníží, pokud samice jsou větším pohlavím. Jinými slovy velikost těla samců je variabilnější (Blanckenhorn *et al.*, 2006). Nicméně použití tohoto pravidla uvnitř druhu může být sporné, jelikož mechanismy, které generují rozdíly v pohlavním dimorfismu, se zřejmě liší uvnitř druhu a mezi druhy (Teder & Tammaru, 2005). Blanckenhorn *et al.* (2006) a Hirst, Horne & Atkinson (2015) říkají, že rozdíly v pohlavním dimorfismu mezi populacemi nepotvrzují Renchovo pravidlo a environmentální podmínky ovlivňují spíše míru než směr pohlavního dimorfismu ve velikosti těla. Pokus o aplikaci Renschova pravidla v geografickém měřítku učinili Blanckenhorn *et al.* (2006). Zdá se, že velikost těla samců se mění podél geografických gradientů strměji než velikost těla samic. Na druhou stranu Teder & Tammaru (2005) naopak ve své meta-analýze prokázali, že samice jsou variabilnějším pohlavním, co se týče fenotypové plasticity, tedy přímé odpovědi jedinců na zažívané podmínky prostředí. Z toho vyplývá, že je potřeba dalších výzkumů, které by integrovaly tyto kusé znalosti do jednotné teorie.

Velikost těla hmyzu může být v různých studiích vyjádřena pomocí různých veličin. Strukturální velikost těla (např. délka krovky) je určena během juvenilního vývoje a je ovlivněna jak genetickými predispozicemi, tak i vlastnostmi prostředí – například

teplotou či kvalitou a kvantitou potravy (Ernsting & Isaaks, 1997; Nylin & Gotthard, 1998). Velikost těla lze také vyjádřit pomocí tělesné hmotnosti (z tělesné hmotnosti a strukturální velikosti těla pak lze zjistit tělesnou kondici jedinců (Knapp & Knappová, 2013). Na rozdíl od strukturální velikosti těla je tělesná hmotnost dospělého hmyzu často ovlivňována podmínkami prostředí, které zažívají během života dospělců (platí pro druhy, které přijímají potravu jako dospělci, tzv. „income breeders"; (Bommarco, 1998b; Östman, 2005).

Tělesná kondice představuje tukové zásoby jednotlivých organismů a je dalším znakem odrážejícím zdatnost jedinců (Knapp & Knappová, 2013). Pokud, jsou samice v dobré kondici, mají vyšší plodnost, úspěšněji se páří a lépe přežívají nepříznivé podmínky (Juliano, 1986; Zhou *et al.*, 1995; Barone & Frank, 2003). Tělesná kondice jedinců je především ovlivněna kvalitou a kvantitou potravy, kterou přijme dospělý jedinec. Z toho vyplývá, že se může zásadně lišit v průběhu života jedince. Faktory, které způsobují změny v tělesné kondici hmyzu lze shrnout do dvou základních kategorií. Jsou to strategické faktory (Elkin & Reid, 2005) a faktory prostředí (van Dijk, 1994). Strategické faktory jsou začleněny do životního cyklu jedinců a druhů a ty jsou schopni na ně adaptivně reagovat. Je to vlastně odpověď (adaptivní reakce) organismů na předpovídatelné změny podmínek prostředí. Strategický faktor může být například zvýšení příjmu potravy před přezimováním (Lövei & Sunderland, 1996) nebo rozdíly v tělesné kondici mezi samci a samicemi stejného druhu (Zangger, Lys & Nentwig, 1994). Na rozdíl od strategických faktorů jsou faktory prostředí nepředvídatelné a vyskytují se náhodně v prostoru a čase. Mohou to být například náhlé změny teplot (van Dijk, 1994) nebo disturbance v prostředí, jako je zemědělský management nebo používání pesticidů (Bommarco, 1998a; Östman *et al.*, 2001). Organismy sami o sobě se na vliv faktorů prostředím nemohou dopředu připravit.

Proměnlivost tělesné velikosti u střevlíkovitých a mrchožroutovitých brouků na velkém prostorovém měřítku

Vnitrodruhová variabilita ve velikosti těla na velkém prostorovém měřítku je především posuzována podél gradientů nadmořských výšek nebo zeměpisných šířek. Jak bylo zmíněno výše, tak studií zkoumajících proměnlivosti velikosti těla hmyzu je poměrně velké množství. Je však nutné si uvědomit, že i druhů hmyzu je velké množství (jedná se o druhově nejbohatší třídu organismů; Grimaldi & Engel, 2004) a jednotlivé taxonomické skupiny se mohou ve svých odpovědích vůči geografickým gradientům podstatně lišit. Mými modelovými organismy jsou brouci z čeledí Carabidae a Silphidae a studií zkoumajících vnitrodruhovou variabilitu velikosti těla podél geografických gradientů u těchto dvou čeledí již není tolik (viz níže), a proto doplnění stávajících znalostí o nové je jistě smysluplné.

Jednou z klíčových studií je výzkum variability ve velikosti těla podél gradientu nadmořské výšky u dvou druhů asijských střevlíků *Carabus japonicus* (Motschulsky, 1857) a *Carabus tosanus* (Nakane, Iga & Ueno, 1953). Proměnlivost ve velikosti těla nebyla nalezena pro druh *C. japonicus*, zatímco pro druh *C. tosanus* ano. Délka těla střevlíka *C. tosanus* dosahovala nižší velikosti ve vyšší nadmořské výšce (Ikeda *et al.*, 2012). Ikeda *et al.*, (2012) uvádějí, že snížení tělesné velikosti *C. tosanus* mohlo způsobit nedostatek potravních zdrojů. Ve vyšší nadmořské výšce je menší početnost členovců, kterými se *C. tosanus* živí, než v nižší nadmořské výšce. Také kratší sezóna ve vyšší nadmořské výšce omezuje dobu, po kterou jedinci mohou získávat potravu, což ve výsledku snižuje velikost těla dospělců. Tento model odpovídá konverznímu Bergmannovu pravidlu (Blanckenhorn & Demont, 2004). Nicméně je zajímavé, že v jiné studii se velikost těla *C. japonicus* spolu s *Carabus dehaanii* (Chaudoir, 1848) zmenšovala s klesající průměrnou teplotou lokality (Okuzaki, Sugawara & Sota, 2015; Okuzaki & Sota, 2017). Tudíž ještě jedna věc by mohla stát za prostorovou variabilitou ve velikosti těla. Okuzaki & Sota (2017) našli u *C. japonicus* vztah mezi velikostí larvy a velikostí kořisti. Největší jedinci se nacházeli v nejnižší nadmořské výšce, kde ale jejich kořist - žížaly, dorůstaly také do největších velikostí. Menší larvy střevlíků se těžko vypořádají s větší kořistí, a proto z dalších možných vysvětlení se jeví adaptace na větší velikost těla z důvodu predace větší kořisti. Délka sezóny může mít také vliv na množství vyprodukovaných generací (Gullan & Cranston, 2005). Velikost těla střevlíka *Pterostichus melanarius* (Illiger, 1798) se měnila nepravidelně podél zeměpisné šířky v Rusku. *P. melanarius* je schopen mít i dvě generace do roka ve středních zeměpisných šířkách, kde je délka sezóny minimálně 6 měsíců. Aby jedinci stihli dvě generace za rok, dorůstají do menších velikostí těla. Pokud jedinec žije více

na sever, má pouze jednu generaci za rok, ale má dostatek času na vývoj, takže dorůstá do větších velikostí. Pokud se postupuje více k severu, doba sezóny se ještě více zkracuje a střevlíci zase dorůstají do menších velikostí těla (Sukhodolskaya & Saveliev, 2016). Ale zdá se, že střevlíci převážně následují konverzní Bergmannovo pravidlo (Park, 1949; Tsuchiya *et al.*, 2012; Sukhodolskaya & Saveliev, 2016; Okuzaki & Sota, 2017).

Rovněž velikost těla mrchožrouta *S. carinata* s rostoucí nadmořskou výškou klesá (Růžička, 2002). Pravděpodobně opět za tím stojí podmínky prostředí. Šustek (1983) uvádí, že velikost těla *S. carinata* úzce souvisí s délkou vegetační doby. Jedinci z vyšších nadmořských výšek, kde vegetační doba je 140 – 180 dní, dosahují menších velikostí. Pokud je vegetační doba dlouhá 240 – 280 dní, jedinci dorůstají obřích velikostí (podobný vzorec vykazuje například i velikost těla u druh *Silpha longicornis* (Portevin, 1926; Nishikawa, 2010). Naproti tomu proměnlivost velikosti těla u hrobaříka *Nicrophorus investigator* (Zetterstedt, 1824) ukazuje opačný model. S rostoucí nadmořskou výškou se prodlužuje délka vývoje a velikost těla roste, tento jev odpovídá Bergmannovu pravidlu. Ale zde mohl ovlivnit změnu velikosti těla mezi vyšší a nižší nadmořskou výškou i jiný faktor. U hrobaříka se v rámci druhu vyvinuly odlišné reprodukční strategie. Samice kladly přibližně stejně velkou snůšku bez ohledu na velikost mršiny ve vyšší nadmořské výšce. Ve výsledku larvy z větších mršin dorůstaly do větších velikostí. Naopak v nižší nadmořské výšce s rostoucí váhou mršiny rostla i velikost snůšky a larvy byly stejně velké bez rozdílu hmotnosti mršiny. Tudíž je možné, že ve vyšší nadmořské výšce brouci vybírají větší mršiny, do kterých kladou vajíčka (Smith *et al.*, 2000).

Jestli jsou změny ve velikosti těla podél geografických gradientů dány lokální adaptací na místní podmínky nebo fenotypovou plasticitou, lze zjistit provedením tzv. standardizovaného zahradního experimentu (Stillwell & Fox, 2009; Tsuchiya *et al.*, 2012). Taková studie byla provedena v Japonsku pro střevlíka *C. tosanus*. Jedinci z různých populací byli chováni v laboratoři při teplotách 15 °C a 20 °C a i když jedinci dosahovali větších velikostí při vyšších teplotách, tak rozdíly ve strukturální velikosti těla způsobené teplotou prostředí byly výrazně menší než rozdíly ve velikosti těla způsobenými geografickým původem (vyšší či nižší nadmořskou výškou zdrojové populace). Z toho plyne, že fenotypová plasticita přispívá zřejmě jen relativně málo k rozdílům ve strukturální velikosti těla mezi populacemi z nižší a vyšší nadmořské výšky. Tyto rozdíly jsou hlavně dány geneticky, adaptací populací k lokálním podmínkám prostředí (Tsuchiya *et al.*, 2012).

Proměnlivost velikosti těla a tělesné kondice u střevlíkovitých a mrchožroutovitých brouků na malém prostorovém měřítku

Jak už jsem zmínila výše, prostorová proměnlivost velikosti těla u střevlíků a mrchožroutů je studována převážně na velkém prostorovém měřítku (např. Park, 1949; Smith *et al.*, 2000; Ikeda *et al.*, 2012; Tsuchiya *et al.*, 2012; Sukhodolskaya & Saveliev, 2016; Okuzaki & Sota, 2017). Studií pro malé prostorové měřítko je minimum (u mrchožroutů zcela chybí), jelikož na první pohled nemusejí být vidět výrazné rozdíly ve velikosti těla. Nicméně, studie druhů s obrovským geografickým rozsahem se vyznačují vysokým množstvím různorodých míst, které zahrnují různé environmentální a historické proměnné ovlivňující velikost těla jedince. To může produkovat falešné výsledky oproti studiím provedeným na malém geografickém měřítku (Shelomi, 2012).

Existuje mnoho faktorů, které ovlivňují velikost těla jedince. Tyto faktory se liší od jednoho stanoviště k druhému a mohou způsobit variabilitu ve velikosti těla na malém prostorovém měřítku (Desender, 1989; Chown & Gaston, 2010). Obecně se uvádí, že teplota, která působí během vývoje jedince, významně ovlivňuje velikost těla imaga. Vlivem nízké teploty se jedinci mohou vyvíjet déle, ale přitom dorůstat do větších velikostí („Temperature-size rule"; Ernsting & Isaaks, 1997; Kingsolver & Huey, 2008). Na druhou stranu při prodlužujícím se vývoji jsou jedinci vystaveni vyššímu riziku mortality (Teuschl *et al.*, 2007). Dalším významným faktorem ovlivňujícím velikost těla je množství a kvalita potravy, kterou má k dispozici larva. Větší množství potravy v době larválního vývoje způsobuje dorůstání do větší velikosti těla (Lenski, 1984; van Dijk, 1994). Velikost těla může být ovlivněna i hostitelem, na kterém se některé druhy střevlíků vyvíjejí. Velikost těla střevlíka *Brachinus explodens* (Duftschmid, 1812) se zvýšila s rostoucí hmotností kukel hostitelů (Saska & Honěk, 2012).

Proměnlivost ve velikosti těla hmyzu na malém prostorovém měřítku může být výrazná obzvláště v intenzivně obhospodařované zemědělské krajině, jelikož je to velice dynamické prostředí s častými disturbancemi (Bommarco, 1998a). Zemědělský management pravděpodobně způsobuje podstatné změny ve strukturální velikosti těla brouků tím, že ovlivňuje potravní zásobu pro larvy (Bommarco, 1998b). Používání pesticidů, podíl jednoletých plodin, přítomnost mezí a dalších ostrůvků neobhospodařovaných lokalit i parametr poměru obvodu pole k jeho ploše, může mít zásadní vliv na množství i složení dostupné potravy (Zangger, 1994; Bommarco, 1998a; Holland & Luff, 2000). Proto se velikost těla může významně lišit mezi jednotlivými poli. Například strukturální velikost těla střevlíka *P. cupreus* byla ovlivněna zemědělským managementem, jelikož jedinci z farem s ekologickým managementem dorůstali do větší velikosti než střevlíci z konvenčních farem (Bommarco, 1998a).

Důležitou složkou, která zlepšuje potravní podmínky pro střevlíky, ale i jiný hmyz, žijící v zemědělské krajině, jsou meze. Bylo prokázáno, že strukturální velikost těla druhu *P. cupreus* se významně liší mezi polem a přilehlou mezí. Samice dosahovaly větších velikostí na mezích než v polích (Zangger *et al.*, 1994). Vliv na strukturální velikost těla může mít i přítomnost městské krajiny. U střevlíka *Carabus nemoralis* (Müller, 1764) se snižovala strukturální velikost těla jedinců směrem od okrajových částí do centra města (Weller & Ganzhorn, 2004). Podobný vzor byl pozorován i u samic druhu *Carabus aeruginosus* (Fischer, 1822; Sukhodolskaya & Eremeeva, 2013). Tedy zvyšující se znečištění prostředí může také způsobit změny ve strukturální velikosti těla (Lagisz, 2008).

Krajinná struktura vedle strukturální velikosti významně ovlivňuje i aktuální hmotnost dospělců a tím i jejich tělesnou kondici. Tělesná kondice je u střevlíků úzce spojena s reprodukčním úspěchem jedinců (Zangger *et al.*, 1994; Bommarco, 1998a; Östman *et al.*, 2001; Haschek *et al.*, 2012). Proměnlivost tělesné kondice jedinců je především způsobena diverzitou plodin, množstvím neobhospodařovaných remízků nebo polních mezí či konvenčním versus ekologickým zemědělství (Bommarco, 1998a; Östman *et al.*, 2001; Haschek *et al.*, 2012). Například střevlíci z ekologických farem jsou v lepší tělesné kondici než ti z konvenčních farem. Krajina ekologických farem je více heterogenní s větším množství remízků a mezí a vyšší rozmanitostí pěstovaných plodin, proto může obsahovat více zdrojů nutných pro život brouků (Bommarco, 1998a; Östman *et al.*, 2001). Na druhou stranu menší diverzita plodin může vézt k přemnožení některých škůdců (Östman, 2005). Zvýšením počtu škůdců se zvýší množství dostupné potravy pro některé druhy střevlíků a to vede i k růstu jejich tělesné kondice (viz obr. 2; Haschek *et al.*, 2012). Další důležitou složkou zemědělské krajiny, která má pozitivní vliv na tělesnou kondici, jsou meze. Zvyšují množství a rozmanitost potravy (Zangger, 1994; Zangger *et al.*, 1994) a jsou vhodným úkrytem i stanovištěm pro přezimování (Lys, 1994; Andersen, 1997). Na mezích se navíc v menší míře uplatňují negativní vlivy zemědělské činnosti, jako je užívání pesticidů nebo časté disturbance (Barone & Frank, 2003). Věk mezí je také důležitým faktorem, který ovlivňuje velikost i kondici střevlíků. Starší meze poskytují lepší podmínky pro výživu střevlíků a ve výsledku, zde jedinci dosahují lepší tělesné kondice (Barone & Frank, 2003; Frank, Kehrli & Germann, 2007).
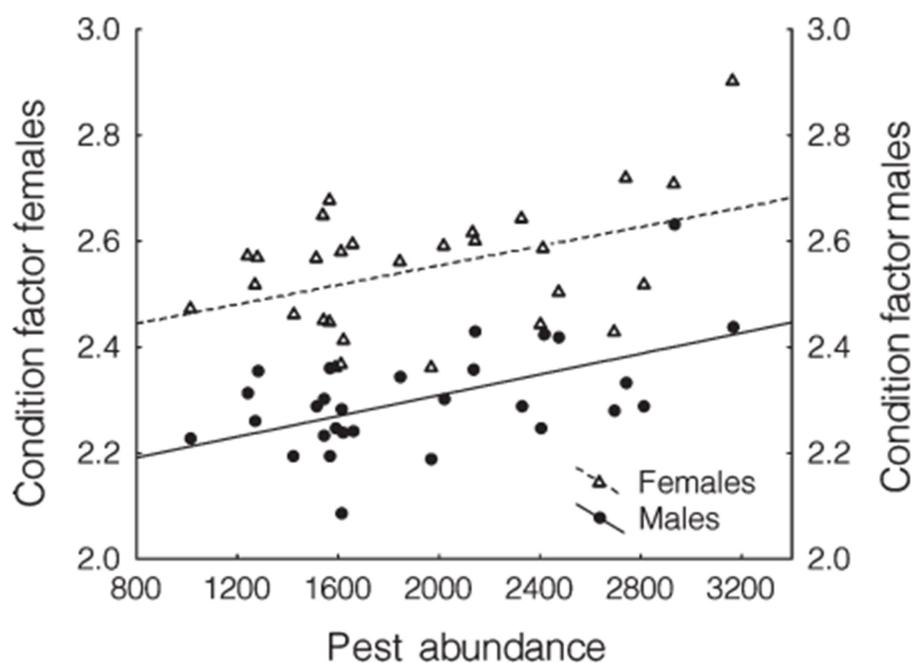
Obr. 2: Regresní analýza pro *P. cupreus*. Vztah mezi faktorem kondice a hustotou škůdců.

Proměnlivost velikosti těla a tělesné kondice u střevlíkovitých a mrchožroutovitých brouků v čase

Vnitrodruhovou proměnlivost velikosti těla lze sledovat ve střednědobém horizontu v řádu jednotek až desítek let i v krátkodobém horizontu během jediné sezóny. Časové změny ve vnitrodruhové proměnlivosti ve velikosti těla jsou pravděpodobně způsobeny podobnými abiotickými a biotickými vlivy jako prostorové změny ve velikosti těla. Například teplota, potravní nabídka, náhodné změny prostředí nebo kvalita prostředí se může měnit v průběhu roku i let a způsobovat proměnlivost ve velikosti těla hmyzu obývajícího danou lokalitu (Bilde & Toft, 1998; Bommarco, 1998b; Östman, 2005; Teder, Tammaru & Esperk, 2008; Chown & Gaston, 2010).

Teplota se jeví jako významný faktor, který způsobuje změny ve strukturální velikosti těla brouků v průběhu času (Ernsting & Isaaks, 1997). U střevlíka *Notiophilus biguttatus* (Fabricius, 1779) samice kladly větší vajíčka, ze kterých se líhly větší larvy, na začátku sezóny, kdy teploty dosahovaly nižších hodnot. Naopak samice, které kladly později v sezóně, produkovaly menší vajíčka (Ernsting & Isaaks, 1997). Sezónní změny ve strukturální velikost těla u jedinců mohou být způsobeny kvůli přezimování. Vyšší míra přežívání větších jedinců z jedné sezóny do druhé byla potvrzena pro mrchožrouta *N. investigator* (Smith, 2002). Na druhou stranu u mrchožrouta *Nicrophorus americanus* (Olivier, 1790) se nepotvrdila výhoda větší velikosti těla při přezimování (Schnell *et al.*, 2008).

V delším časovém horizontu, lze snadno pozorovat změny ve strukturální velikosti těla například u invazních druhů. U invazního střevlíka *Merizodus soledadinus* (Guerin-Meneville, 1832) souvisí změny ve strukturální velikosti těla s dobou, kdy střevlík kolonizoval určité území souostroví Kergueleny. Jedinci z lokality, která byla kolonizována nejdříve (1913), dosahovali menších velikostí než jedinci z lokality, která byla kolonizována nejpozději (2003). Autoři studie uvádějí několik důvodů, proč by to tak mohlo být: 1) Střevlíci mají silný vliv na diverzitu a početnost členovců a proto jsou menší na lokalitě, odkud již zmizela jejich oblíbená potrava (bezkřídlé mouchy) a byli nuceni přejít na mnohem menší potravu (např. chvostoskoky); 2) Teplotní podmínky, které se mohou lišit na lokalitách, mají vliv na vývoj larvy a na následnou velikost těla dospělého jedince; 3) Když jsou potravní zdroje omezené, tak se u střevlíka může projevit kanibalismus - dospělí střevlíci predují larvy svého druhu a riziko takové predace se zvyšuje s prodloužením doby vývoje larvy, což by mohlo vézt k selekčnímu tlaku na zkrácení doby vývoje larvy a tím i strukturální velikosti těla dospělců. Avšak studie se zabývá spíše geografickou proměnlivostí velikosti těla, jelikož porovnává různé lokality kolonizované po různě dlouhou dobu (Laparie *et al.*, 2010). Změny ve

strukturální velikosti těla v průběhu několika let se zabývala studie Smith (2002). Ve dvouleté studii hrobaříka *N. investigator* se meziroční změny ve velikosti těla nepotvrdily (Smith, 2002). Nýbrž prodloužením doby výzkumu na 6 let se již objevila průkazná meziroční proměnlivost v průměrné délce krovky. Jelikož velikost těla mrchožrouta může odpovídat změnám v populační dynamice malých hlodavců, tak v delším časovém horizontu se může lépe odrážet proměnlivost ve strukturální velikosti těla (Smith *et al.*, 2000).

Předpokládá se, že počasí a především teplota, ale také rozdílná kvalita a kvantita dostupné potravy má vliv na variabilitu tělesné hmotnosti a kondice dospělých brouků v průběhu času (Bilde & Toft, 1998; Bommarco, 1998b). Tělesná hmotnost střevlíka *Carabus interruptus* (Dejean, 1831) obývajícího Kanárské ostrovy kolísala mezi jednotlivými měsíci a nárůst hmotnosti byl zaznamenán od podzimu do zimy s vrcholem v lednu. Střevlík se rozmnožuje v zimních měsících, a proto je pravděpodobné, že před rozmnožováním zvyšuje přísun potravy a tedy i stoupá jeho hmotnost (de los Santos Gómez, 2009). Podobný trend ve variabilitě tělesné hmotnosti a s ní úzce související tělesné kondice ukázal i Zangger *et al.*, (1994) pro druh *P. cupreus*. Tělesná kondice i hmotnost se významně lišila mezi měsíci a nárůst hmotnosti a kondice opět probíhal hlavně před dobou reprodukce (Zangger *et al.*, 1994). Vyšší tělesná kondice samicím umožňuje zvýšení počtu nakladených vajíček (Bommarco, 1998a; Haschek *et al.*, 2012) nebo prodloužení doby, kdy samice je schopna klást vajíčka (Sota, 1985). Tudíž tělesná hmotnost i tělesná kondice mají významný vliv na fitness jedince. Tělesná hmotnost či kondice se neliší pouze v průběhu sezóny, ale může odrážet i změny v kvalitě prostředí z roku na rok. Například střevlíci *P. cupreus* a *P. melanarius* se lišili jak v hmotnosti, tak i v obsahu tuku na téže lokalitě v průběhu dvou let (Östman, 2005). Také stádium sukcese může ovlivnit kondici střevlíků na dané lokalitě. Na nově vytvořených mezích v zemědělské krajině, střevlíci obývající mez měli v prvním roce nižší tělesnou kondici než během následujících tří let. Po řádném zapojení meze v druhém roce existence se zvýšilo i množství dostupné potravy a tělesná kondice střevlíků již dále nerostla (Barone & Frank, 2003; Frank *et al.*, 2007).

Také přezimování má významný vliv na tělesnou kondici střevlíků. Střevlík *Calathus melanocephalus* (Linnaeus, 1758) úspěšněji přezimoval, pokud před přezimováním měl dostatečný příjem potravy. Avšak musím poznamenat, že průkazný rozdíl v přežití zimní hibernace mezi jedinci, kteří byli dostatečně krmeni a jedinci, kteří hladověli před přezimováním, byl zjištěn pouze pro jednu sezónu v rámci dvouleté studie (van Dijk, 1994). Vliv na změny tělesné hmotnosti během přezimování má také teplota. Například

u druhu *Bembidion lampros* (Herbst, 1784) dlouhodobé denní kolísání teplot způsobilo větší úbytek tělesné hmotnosti než dlouhodobé teploty pod bodem mrazu (Petersen et al., 1996).

Vliv biotických, ale i abiotických faktorů může způsobit, že variabilita ve velikosti těla a tělesné kondici brouků v čase není synchronní mezi lokalitami. Tedy lokalita, kde střevlíci měli nejnižší tělesnou kondici v porovnání s jinými lokalitami jeden rok, tak další rok už může být naopak vnímána jako kvalitní – brouci zde mají nadprůměrnou tělesnou kondici (Östman, 2005). Na lokalitě se mohly změnit podmínky prostředí, například z důvodu nedostatečného střídání plodin se přemnožili škůdci a zvýšením příjmu potravy se i zvyšuje tělesná kondice střevlíků (Östman, 2005). Samozřejmě na hmotnost či kondici nepůsobí pouze jeden faktor, ale i interakce mezi nimi. I když množství potravy má pozitivní vliv na hmotnost jedinců, tak při nízkých teplotách mohou být jedinci lehčí. Samice střevlíka *Poecilus versicolor* (Sturm, 1824) měly signifikantně nižší hmotnost, když byly chovány při 12 °C než při 19 °C, bez ohledu na množství potravy (van Dijk, 1994).

Pohlavní dimorfismus ve velikosti těla u střevlíků a mrchožroutů

U střevlíků a mrchožroutů je literatura o pohlavním dimorfismu ve velikosti těla velice omezená. Alespoň existuje několik studií, které zkoumají vlivy působící na velikost těla brouků zvlášť pro samce a samice. I když u některých druhů střevlíků samci bojují o samice a jsou větší, například u některých druhů tribů Scaritini, Cicindelini nebo Harpalini (Oberprieler & Arndt, 2000). Dokonce u *Carabus uenoi* (Ishikawa, 1960) a u rodu *Brachinus* nebyly zaznamenány rozdíly ve velikosti těla mezi samci a samicemi (Juliano, 1983; Sota *et al.*, 2000). Tak střevlíci zpravidla vykazují stejný model jako většina druhů hmyzu, tedy samice jsou zpravidla větším pohlavím (Sota *et al.*, 2000; Lagisz, 2008; Henríquez, Donoso & Grez, 2009; Laparie *et al.*, 2010; Tsuchiya *et al.*, 2012). Díky velice omezené literatuře není zcela jasné, jestli u mrchožroutů převažuje nějaký model v pohlavním dimorfismu ve velikosti těla. Například u *N. investigator* se velikost těla mezi samci a samicemi neliší. Tento méně častý vzorec u hmyzu je pravděpodobně způsoben tím, že zde existuje vnitrodruhový souboj pohlaví o mršinu a proto zde mohou působit podobné selekční tlaky na velikost těla jak pro samce, tak i pro samice (Smith *et al.*, 2000). Na druhou stranu u *S. carinata* a *S. longicornis* samice jsou větším pohlavím (Růžička, 2005; Nishikawa, 2010).

Míra pohlavního dimorfismu může být ovlivněna několika faktory a ne všechny souvisí s evoluční historií druhu či populace. Například na přírodních, člověkem neovlivněných lokalitách jsou samice druhu *C. aeruginosus* větším pohlavím, ale pokud byli brouci sbíráni z luk obklopených městskou zástavbou, pak nebyly zaznamenány žádné rozdíly ve velikosti těla mezi pohlavími (Sukhodolskaya & Eremeeva, 2013). Podobný trend byl pozorován i u střevlíka *Pterostichus melas* (Creutzer, 1799), kde Giglio *et al.* (2011) našli rozdíly v míře pohlavního dimorfismu mezi jedinci, kteří byli ošetřeni organofosfátovým pesticidem a bez ošetření. Velikost těla samic, které byly ošetřeny pesticidem, byla stejná jako velikost těla samců. Navíc se ve studii objevil ještě jeden překvapivý vzorec, průměrná délka krovky samců, kteří byli ošetřeni pesticidem, byla delší než u samců neošetřených. Tedy i pesticidy mohou vyvolat změny v pohlavním dimorfismu ve velikosti těla.

Není zcela jasné, zda samice nebo samci střevlíků jsou citlivější k environmentálním faktorům. Například velikost těla samic *P. versicolor* se výrazněji snížila ve srovnání se samci, pokud byly chovány při 12 °C než při 19 °C (van Dijk, 1994). Také v agroekosystémech samice střevlíka *P. cupreus* reagovaly více na podmínky prostředí než samci. Samice z mezí měly delší krovku ve srovnání s polními samicemi (Zangger *et al.*, 1994), ale u samců se žádný rozdíl nenašel. U střevlíka *C. tosanus*, kde samice jsou větším pohlavím, pohlavní rozdíly ve velikosti těla se snižovaly s rostoucí

nadmořskou výškou a tedy klesající teplotou. Ve výsledku to způsobilo snížení pohlavního dimorfismu ve velikosti těla (Tsuchiya *et al.*, 2012). Na druhou stranu u jiných druhů hmyzu se uvádí, že samci jsou více variabilní ve velikosti těla než samice (Blanckenhorn *et al.*, 2006; Stillwell *et al.*, 2010).

Cíle disertační práce

Hlavním cílem mé disertační práce je studium proměnlivosti strukturální velikosti těla a tělesné kondice hmyzu a faktorů, které tyto změny způsobují. Tento cíl byl sledován prostřednictvím několika dílčích úkolů:

1. Jak se mění tělesná kondice a strukturální velikost těla hmyzu na malém prostorovém měřítku a jaké faktory ovlivňují tyto změny?

Existuje mnoho faktorů, které mohou ovlivňovat strukturální velikost těla a tělesnou kondici jedinců. Snažím se určit, které z nich působí na strukturální velikost těla a tělesnou kondici a také se snažím zodpovědět, které faktory na ně mají větší vliv.

2. Mění se proměnlivost strukturální velikosti těla a tělesné kondice hmyzu v čase?

Faktory, které působí na strukturální velikost těla a na tělesnou kondici jedince, mohou působit rozdílně v průběhu času. Například Östman (2005) uvádí, že při přemnožení škůdců na polích se může zvýšit tělesná kondice i strukturální velikost těla z roku na rok, jelikož je aktuálně dostatek potravy pro střevlíky. Avšak následující rok, pokud bude potravy méně, se tělesná kondice a i strukturální velikost těla mohou opět snížit. Studie zkoumající strukturální velikost těla nebo tělesnou kondici jsou zpravidla prováděny pouze jeden rok a nemusí tak odhalit synchronní či asynchronní proměnlivost strukturální velikosti těla a tělesné kondice v průběhu času. Z tohoto důvodu jsem se rozhodla zjistit, zda rozdíly ve strukturální velikosti těla a tělesné kondice se na jednotlivých lokalitách opravdu liší v průběhu let.

Pro zjištění těchto dvou cílů jsem si jako modelový druh vybrala brouky z čeledi střevlíkovitých, konkrétně druhy *Poecilus cupreus* (Linnaeus, 1758) a *Anchomenus dorsalis* (Pontoppidan, 1763). Tyto druhy jsou významnými predátory škůdců v zemědělské krajině, která je vhodná pro studium těchto změn, jelikož se vyznačuje vyšší dynamikou biotických i abiotických činitelů.

3. Existuje proměnlivost strukturální velikosti těla hmyzu podél gradientu nadmořské výšky?

Například u některých teplokrevných organismů se mění velikost těla s měnícím se gradientem nadmořské výšky nebo zeměpisné šířky. Toto pravidlo bylo popsáno již na konci 19. století Carlem Bergmannem. Tzv. Bergmannovo pravidlo nám říká, že

s rostoucí nadmořskou výškou eventuálně zeměpisnou šířkou se velikost těla zvětšuje. Avšak hmyz a jiní studenokrevní živočichové vykazují i jiné vzorce v proměnlivosti velikosti těla vlivem geografických gradientů. *Silpha carinata* (Herbst, 1783) je vhodný druh pro studium proměnlivosti ve velikosti těla, jelikož je schopna vyprodukovat pouze jednu generaci za rok, má značně proměnlivou velikost těla a omezené disperzní schopnosti, což jsou dobré předpoklady pro tvorbu gradientu ve velikosti těla podél gradientu nadmořské výšky.

4. Jaké mechanismy generují geografickou proměnlivost ve strukturální velikosti těla hmyzu?

Uvádí se, že proměnlivost ve velikosti těla může být způsobena fenotypovou plasticitou či lokálními adaptacemi. Ale do jaké míry za proměnlivost ve velikosti těla mohou adaptace a do jaké míry fenotypová plasticita není zcela jasné, a proto je to předmětem mého bádání. Mým modelovým druhem pro zjištění mechanismů generující geografickou proměnlivost ve velikosti těla je opět *S. carinata*.

Samozřejmě nezůstala jsem pouze u těchto otázek, ale další dílčí cíle jsou popsány v přiložených manuskriptech.

Výstupy disertační práce

Konkrétní výsledky této doktorské disertační práce najdete v následujících článcích

1. Baranovská E & Knapp M (2014): Small-scale spatiotemporal variability in body size of two common carabid beetles. Central European Journal of Biology 9: 476–494.

2. Baranovská E, Knapp M & Saska P (2014): The effects of overwintering, sex, year, field identity and vegetation at the boundary of fields on the body condition of *Anchomenus dorsalis* (Coleoptera: Carabidae). European Journal of Entomology 111: 1–7.

3. Baranovská E, Knapp M & Jakubec P (2016): Effects of Bait Presence and Type of Preservative Fluid on Ground and Carrion Beetle Samples Collected by Pitfall Trapping. Environmental Entomology 45: 1022–1028.

4. Baranovská E & Knapp M: Steep converse Bergmann's cline in a carrion beetle: between and within population variation in body size along an elevational gradient. Manuskript v recenzním řízení v Journal of Zoology.

1. Článek

Small-scale spatiotemporal variability in body size of two common carabid beetles

Baranovská E & Knapp M

Central European Journal of Biology

VERSITA

Small-scale spatiotemporal variability in body size of two common carabid beetles

Research Article

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Abstract: Adult body size is one of the most ecologically relevant quantitative traits that underlies many other life-history traits of particular organism. In insects, there is positive intraspecific relationship between body size and female fecundity. In this study small scale temporal and spatial and space variability in structural body size of *Poecilus cupreus* and *Anchomenus dorsalis* was investigated. The beetles were collected in four fields near Prague-Suchdol in autumn 2009 and 2010, and in spring 2010, 2011 and 2012. In both species structural body size was significantly affected by sex (females were the larger sex). In *A. dorsalis* structural body size was also significantly affected by arable field identity, overwintering (post-overwintering individuals collected in spring were larger in comparison to pre-overwintering individuals collected in autumn), sampling year, overwintering by year and arable field by year interactions. Our results suggest that spatiotemporal variation in environmental conditions experienced by *A. dorsalis* during larval growth resulted in differences in adult structural body size among particular fields and particular sampling years. In addition, mean structural body size in *A. dorsalis* was affected by overwintering, which was probably caused by size-specific winter mortality. Moreover, effect of overwintering varied among years, probably according to the specific weather conditions during a particular winter.

Keywords: Agricultural landscape • Anchomenus dorsalis • Arable field • Overwintering • Poecilus cupreus • Seasonal variation • Structural body size

1. Introduction

Adult body size is a noticeable feature of each organism and one of the most ecologically relevant quantitative individual characteristics [1,2]. Individual body size determines many other life-history traits of particular organism, including ecological, physiological and ethological traits [2,3]. In insects, there is quite close intraspecific relationship between body size and fecundity in females, where fecundity increases with increasing body size [4]. In addition to higher fecundity, bigger individuals commonly have higher mating success, enhanced longevity and winter survival in comparison to smaller ones [5-7].

Intraspecific variation in body size is frequently studied at large spatial scale, *e.g.* variation along latitudinal or altitudinal gradients. Such variation in body size at large scale stimulated searching for zoogeographical rules, for example Bergmann's rule or converse Bergmann's rule [3,8]. In insects, continuum of latitude and altitude effects on body size was reported by Blanckenhorn and Demont [1], who suggested that with increasing latitude or altitude and thus decreasing temperature, specimens of large species with typically longer developmental time become smaller (converse Bergmann's rule), whereas specimens of smaller species with typically shorter developmental time become bigger (Bergmann's rule). Changes in body size with altitude or latitude could be caused either by adaptation of local populations or by phenotypic plasticity [8,9]. For example, increase in rearing temperature generally leads to decrease in body size in insects, a phenomenon known as temperature-size rule (TSR) [10].

Interestingly, studies focused on small–scale spatial and temporal variation in body size are lacking. To our knowledge, the only exception for carabids is the study performed by Östman [11], who studied variation in structural body size and body condition of beetles

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on 10 farms around Uppsala in Sweden in two subsequent years. It is important to note that two different measures of body size could be measured: structural body size (*e.g.* elytron length) and body mass (sometimes corrected for structural body size and then called "body condition") [12]. Structural body size (SBS) is determined during juvenile development and is affected by genetic predispositions as well as by temperature (see TSR above), food quantity and quality [13,14]. In contrast, adult body mass (and body condition derived from it) in income breeding insects is determined by environment condition, *e.g.* food availability, experienced by adults [14-16]. Carabids inhabiting arable fields are known to be food limited as adults, which could have substantial effects on their reproductive success [17,18]. Thus variation in body condition of carabids is frequently investigated in agricultural landscape [13,19,20]. Carabids are holometabolous insects in which different life stages (*e.g.* larvae and imagos) frequently occupy various niches and experience diverse environmental conditions, *e.g.* food availability, at the same locality [15,18]. Thus, adult body condition (computed from body mass measurement) may not to be tightly correlated to structural body size. However, studies investigating variability in SBS of carabids are rare [11].

Agroecosystems are suitable habitats to study small-scale spatial and temporal variation in body size of predatory insects as food availability could vary between particular fields based on their properties, such as field area, crop identity or agricultural management [13,19]. Moreover, agroecosystems host several ubiquitous and abundant carabid species, which makes them suitable for performance of a study investigating spatio-temporal variation in SBS. These species typically overwinter in field boundaries neighbouring the arable land [21]. Therefore beetles are aggregated in field margins in autumn and in early spring, enabling collection of large numbers of specimens. In addition, autumn and spring sampling enable to investigate effect of overwintering on SBS, *i.e.* size-specific winter survival. Winter is thought to be a period with substantial mortality risk for temperate insects, reaching up to 90% in some species [22].

In present study, we investigate small-scale spatial and temporal variation in SBS of two carabid beetles: *Anchomenus dorsalis* and *Poecilus cupreus* in agricultural landscape. We hypothesize that: SBS in both species is affected by gender as a result of selection pressure on large body size in females; SBS is affected by arable field identity as a result of different food availability among particular fields; SBS is affected by sampling year as a result of different food availability among particular seasons (due to variation in the course

of weather conditions). In order to test these hypotheses we address the following questions: 1) Does SBS vary among particular fields?; 2) Does SBS vary among years?; 3) Does SBS differ between autumn and subsequent spring?; 4) Does SBS vary between males and females?; 5) Is there any other complex effect of above mentioned variables on SBS represented by their interactions?

2. Experimental Procedures

2.1 Study species
Poecilus cupreus (Lineaus, 1758) (Coleoptera: Carabidae) and *Anchomenus dorsalis* (Pontoppidan, 1763) (Coleoptera: Carabidae) are open habitat generalist species differing in body size, *A. dorsalis* is 5.6 – 7.7 mm long and *P. cupreus* is 9.6 – 14.0 mm long [23]. Both are common in agricultural landscapes and frequently occur also in arable fields [24]. However, non-crop habitat plays crucial role for their overwintering and reproduction [13,24]. *A. dorsalis* and *P. cupreus* are typical spring breeders, *i.e.* species reproducing in spring and early summer, larval growth takes place during summer and overwintering stage are adult beetles. Both species are polyphageous predators of diverse arthropods including these considered as serious pests, *e.g.* aphids, thus *A. dorsalis* and *P. cupreus* are classified as beneficial organisms with biocontrol potential [13,15,19,24].

2.2 Experimental design
Carabid beetles were collected repeatedly in four arable fields situated few kilometers north-west of Prague, the Czech Republic (Figure 1). Fields were distanced few kilometers from each other, differed in size (area), but were similar in structure of field boundaries. All fields were conventionaly managed during sampling period (2009-2012), however crops planted in particular fields differed (for details see Supplementary material Table S1). Within each field two field margin sites, one neighbouring forest boundary and second neighboring grassy boundary, were sampled using pitfall traps. Traps were made of plastic gutters 80 cm long, 15 cm wide and 15 cm deep, which were buried in the ground. The rim of the traps was precisely flushed with the soil level to enhance trap efficiency for smaller specimens. So-called "live traps" were employed, *i.e.* traps were not filled with any conservation fluid. Each trap was covered by metal plate (90 x 30 cm) made of aluminium, fixed by large (15 cm long) nails. At each site (neighbouring forest or grassy boundary) three pitfall traps were operated. Traps were emptied every other day.
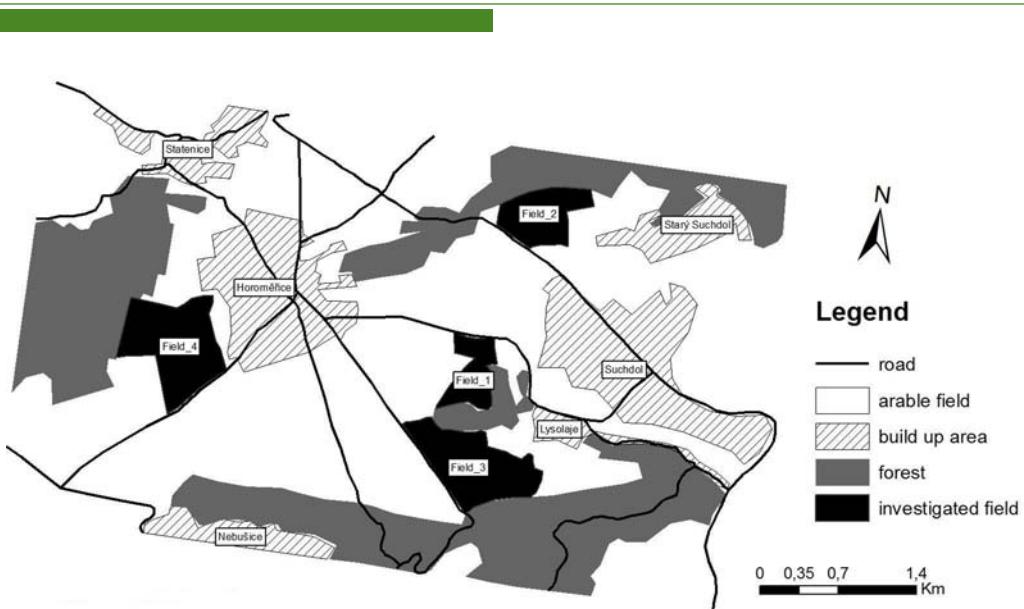
Figure 1. Map of localities investigated in this study.Investigated arable fields were situated near Prague-Suchdol, the Czech Republic (GPS coordinates of field centres: field 1 – 50°7'46.942''N 14°21'37.574''E, field 2 50°8'28.480''N 14°21'54.754''E, field 3 – 50°7'16.933''N 14°21'44.419''E, field 4 – 50°7'38.451''N 14°19'30.361''E).

At the same sites, beetles were collected in autumn (October) 2009 and 2010, and in spring (April) 2010, 2011 and 2012. At each site in each sampling period we tried to collect 40 specimens per particular species (*A. dorsalis* or *P. cupreus*), *i.e.* 80 specimens per particular species per field. To prevent depletion of local populations, sampling of beetles at particular site was terminated immediately after sufficient number of specimens was collected. Live beetles were transported to the laboratory, killed by freezing and stored in a freezer at -20°C until sample processing. Before size measurements, specimens of particular species from particular site and particular sampling period were sexed and 15 males and 15 females were selected at random. If there were fewer than 15 males or females per site, then all available specimens were used (see Supplementary material Table S1). Elytron length, hind femur length and pronotum width were subsequently measured for all selected specimens using digital calliper with a precision to 0.01 mm. Although it is a common practice to measure only one size, it was shown that measurement of more sizes is meaningful in carabids, because individuals of particular length could be either wide or thin [12].

In *A. dorsalis* we analyzed two datasets: overwintering dataset (A1) consisted of two autumn sampling events (2009 and 2010) and two spring sampling events (2010 and 2011); spring dataset (A2) consisted of three spring sampling events (2010, 2011 and 2012). Low abundances or activity of *P. cupreus* in some sampling events resulted in insufficient numbers of collected specimens from some sites. Therefore, we had to analyze only limited datasets for this species.

Overwintering dataset for *P. cupreus* (P1) consisted of one autumn sampling event (2009) and one spring sampling event (2010). Spring dataset for *P. cupreus* (P2) consisted of two spring sampling events (2010 and 2011). Complete dataset including morphometrical measurements for all specimens analyzed in this study is attached (see Supplementary material Table S2).

2.3 Statistical analyses

To analyze effects of sex, field, overwintering (autumn or spring), sampling year and their interactions on body size, we employed direct multivariate ordination techniques called Redundancy analysis (RDA). All three measured sizes, *i.e.* elytron length, hind femur length and pronotum width, were used as dependent variables. Data were analysed separately for particular datasets (A1 and A2 for *A. dorsalis* and P1 and P2 for *P. cupreus*; see above), whereas all possible explanatory variables (*e.g.*, sex, field, year for A2 dataset) and their interactions were included for each particular dataset.

Significant terms were identified using forward selection procedure and significance of the final model (all canonical axes together) was tested using randomisation test with 999 permutations. Subsequently, net effects of particular significant terms were analysed and tested. For such purposes all other significant terms in particular model were used as covariates and randomisation tests with 999 permutations were performed under restricted permutation scenarios (permutations were made only within blocks defined by main effect covariates, *i.e.* non-interaction covariates). All analyses were performed in Canoco for Windows 4.5 software [25].

3. Results

In total, we collected and measured 1133 individuals of *A. dorsalis* (934 were analyzed in overwintering dataset A1 and 677 were analyzed in spring dataset A2) and 683 individuals of *P. cupreus* (468 were analyzed in overwintering dataset P1 and 455 were analyzed in spring dataset P2; for details see Supplementary material Table S1).

Structural body size of *A. dorsalis* was significantly affected by sex (females are larger in comparison to males), field of origin, overwintering (post-overwintering individuals collected in spring were larger in comparison to pre-overwintering individuals collected in autumn) and overwintering×year interaction (Table 1; Figure 2). All other investigated interactions were insignificant (P> 0.05). Effect of sampling year was not significant in overwintering dataset (2 years sampling; RDA: F= 2.77, P= 0.085), but it became significant when spring data were analyzed (3 years sampling; RDA: F= 4.02, P= 0.013). In spring dataset, there was also significant field×year interaction (Table 2; Figure 3). Other interactions between investigated terms were insignificant (P> 0.05).

In *P. cupreus*, body size was significantly affected by sex (females are larger than males; RDA: overwintering 1 year dataset: F= 5.31, P= 0.009; spring 2 years dataset: F= 9.66, P= 0.002). There was no significant effect of field of origin (RDA; overwintering 1 year
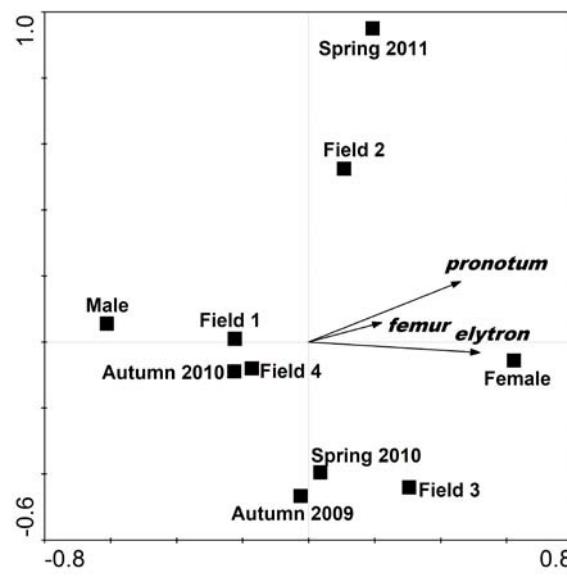
Figure 2. The effects of sex, field and interaction between overwintering and year on structural body size in *Anchomenus dorsalis*. Ordination diagram displays results of redundancy analysis (RDA) performed on „overwintering dataset" (A1 – see Experimental Procedures). Total variability in data explained by environmental variables included in the final model was 22.9% (the first canonical axis explained 22.1% of variability in data, the second canonical axis explained 0.5% of variability in data; permutation test for all canonical axes: F= 39.235; P= 0.001). The first canonical axis is strongly correlated with structural body size of *A. dorsalis*.

	Term	F-value	P-value	R^2
Forward selection	Sex	216.51	0.001	
	Field	19.43	0.001	
	Overwintering	8.06	0.002	
	Year #	2.77	0.069	
	Overwintering×Year	4.78	0.018	
Net effects	Sex	225.78	0.001	0.189
	Field	10.60	0.001	0.027
	Overwintering	7.98	0.004	0.007
	Year #	2.77	0.085	0.002
	Overwintering×Year	4.78	0.027	0.004
	all together †	39.24	0.001	0.229

Table 1. The effects of sampling site,, sampling year and overwintering on structural body size in *Anchomenus dorsalis*. Presented results are outcome of direct linear multivariate ordination analyses (RDA) performed on dataset consisting of beetles sampled repeatedly at four field sites in autumn and spring during two subsequent years. Structural body size is represented by elytron length, hind femur length and pronotum width. Final model presented in the table was selected using forward selection procedure, where selection was made among following terms: sex, field, overwintering, year and all their possible interactions.

 # there was no significant (at P= 0.05) main effect of year, however the term was included in the final model because of significant overwintering×year interaction
† represents the final model (shown in Figure 2) including all above mentioned terms

	Term	F-value	P-value	R²
Forward selection	Sex	188.91	0.001	
	Year	6.29	0.013	
	Field	6.03	0.010	
	Field×Year	15.85	0.001	
Net effects	Sex	193.62	0.001	0.219
	Year	4.02	0.013	0.009
	Field	3.83	0.009	0.013
	Field×Year	4.24	0.001	0.028
	all together †	20.33	0.001	0.269

Table 2. The effects of sampling site, sampling year on structural body size in *Anchomenus dorsalis*. Presented results are outcome of direct linear multivariate ordination analyses (RDA) performed on dataset consisting of beetles sampled repeatedly at four field sites in spring during three subsequent years. Structural body size is represented by elytron length, hind femur length and pronotum width. Final model presented in the table was selected using forward selection procedure, where selection was made among following terms: sex, field, year and all their possible interactions.

† represents final model (shown in Figure 3) including all above mentioned terms

dataset: F= 3.11, P= 0.059; spring 2 years dataset: F= 2.84, P= 0.069) neither sampling year (RDA; spring 2 years dataset: F= 1.48, P= 0.217). There were no significant interactions between investigated terms (all P> 0.05).

4. Discussion

There are only few studies investigating small-scale spatial and temporal variation in SBS in insects. The only study on carabid beetles was published by Östman [11], who studied variation in SBS and body condition of carabid beetles in two subsequent years at several farms in Sweden. To our knowledge, this is the first study investigating effect of winter period on shift in SBS of carabid beetle within populations at several sites in two subsequent years. Our results indicate that SBS in *Anchomenus dorsalis* differed between autumn and subsequent spring, varied among sites and this spatial variation was asynchronous through time. Interestingly, for the second studied species (*Poecilus cupreus*) there were no significant effects of site, overwintering or sampling year on SBS. Possible cause could be slightly higher within sample variability (measured as coefficient of variation for particular sample) in body size in *P. cupreus* in comparison to *A. dorsalis* or lower number of collected specimens in *P. cupreus*. SBS in both species was significantly influenced by sex, where females were larger than males. Female biased sexual size dimorphism in investigated species is in agreement with general trends observed in insects [26]. Bigger females are able to reach greater fecundity [4], thus
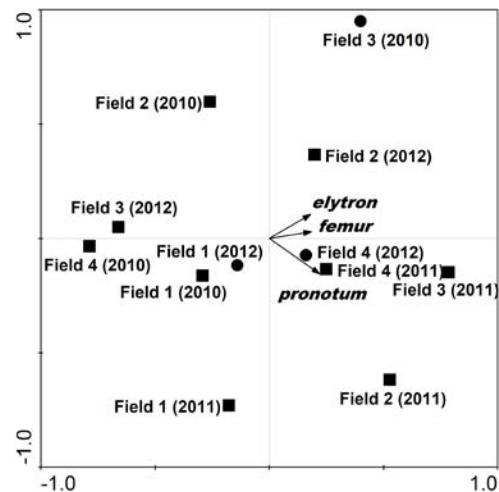
Figure 3. The effects of field identity and year on structural body size in *Anchomenus dorsalis*. Ordination diagram displays results of redundancy analysis (RDA) performed on „spring dataset" (A2 – see Experimental Procedures). Samples from particular fields in particular years were classified according to crop type grown there in preceding year (*i.e.* time of larval growth of collected specimens): squares represent field with cereal crop, circles represent field with other crop than cereal (sugar beet, rape or poppy). Total variability in data explained by field, year and their interaction was 5.0% (the first canonical axis explained 4.6%, the second canonical axis explained 0.4%; permutation test for all canonical axes: F=4.127; P=0.001; sex was used as covariable in the analysis). The first canonical axis is strongly correlated with structural body size of *A. dorsalis*.

body size in females is under strong selection favoring larger size [27].

SBS in *A. dorsalis* was significantly affected by overwintering. Specimens of species collected in autumn

were bigger than these collected in subsequent spring. Possible explanation for this pattern is size-dependent winter mortality (*i.e.* large individuals have higher probability of surviving). As adult SBS is determined during preimaginal development [18,28], it is possible that survival of beetles during winter is indirectly influenced by food and temperature experienced during preimaginal stages. Interestingly, SBS differed between beetles collected in autumn and in subsequent spring mainly during winter in 2010-2011 (there was significant interaction between overwintering and year). Season-specific effect of physiological condition on winter survival in carabids has been previously reported by van Dijk [28]. Winter mortality in insects is substantially affected by temperature and other environmental conditions [22,29]. Carabids could suffer from extremely low winter temperatures causing chill injuries as well as from mild winters causing depletion of energy reserves, whereas probably the most unfavorable conditions correspond to temperature fluctuations around zero point (which was the case of the winter 2010-2011) [29]. The course of winter temperatures differ strongly among particular years in Central Europe, thus varying effects of overwintering on SBS is not surprising.

Variation in SBS among particular years is probably caused by year to year variation in environmental conditions. The weather, mainly temperature and moisture, could substantially affect abundance of prey and thus determine feeding conditions of carabid larvae [18]. Moreover, preimaginal growth and final adult SBS are also affected by experienced temperature *per se.* In general, body size of individuals experienced lower temperatures during preimaginal development is larger than those experienced higher temperatures (so-called temperature-size rule) [10].

Variation in SBS of *A. dorsalis* among fields could be a result of difference in local feeding conditions, which could be affected for example by landscape parameters (structure) or by agricultural management taking place at a particular field [19]. Bommarco [13] reported that body size of beetles increases as area of arable field and perimeter-to-area of a particular field decreases. However, it is difficult to identify particular causes of variation in SBS of beetles among fields in this study as only four fields were investigated. It is important to note that just developmental plasticity (proximate

causes) is discussed above. However, variation in SBS could be also caused by local adaptation of particular populations (ultimate causes). But at a small spatial scale investigated in this study ultimate causes are unlikely. We assume that beetles have been able to move among the particular investigated fields. The majority of ground beetles, including both species investigated in this study, have quite high dispersal ability as they are able to fly [24]. Thus, high gene flow probably leads to low level of genetic differentiation in our study system [30].

Inconsistency of variation in SBS of *A. dorsalis* among fields in time (significant field and year interaction) could be caused by rotation of annual crops grown. Crop identity and connected specific agricultural operations and their timing may substantially alter environmental conditions within fields [13,24]. Unfortunately, limited extent of our study (just four fields investigated) do not allow us to investigate the effect of crop identity rigorously.

In conclusion, SBS in both investigated species was determined mainly by gender. However, smaller portion of variation in body size of *A. dorsalis* was also explained by field identity, sampling year, overwintering, interaction of overwintering and year and interaction of field identity and year. This small-scale spatial and temporal variation in SBS was probably caused by differences among particular fields in larval food supply and differences in weather conditions of particular years. This study shows that it is important to perform long-term research (spanning more than one year) in order to record not only spatial variation, but also temporal variation in body size.

Acknowledgments

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Supplementary material

	Crop			Number of specimens measured							
	2009	2010	2011	Autumn 2009		Spring 2010		Autumn 2010	Spring 2011		Spring 2012
				Ad*	Pc*	Ad	Pc	Ad	Ad	Pc	Ad
Field 1	winter wheat	winter wheat	poppy	60	60	60	59	60	60	58	47
Field 2	winter wheat	spring barley	spring barley	60	49	60	60	46	60	52	60
Field 3	winter rape	winter wheat	winter wheat	60	60	60	60	51	60	52	57
Field 4	spring barley	winter wheat	sugar beet	60	60	59	60	60	58	54	36

Table S1. List of crops planted and numbers of specimens measured in particular fields.

Ad = Anchomenus dorsalis; Pc = Poecilus cupreus

Sampling period	Site	Field	Sex	Elytron	Pronotum	Femur	Y10	Y11	M	F	F1	F2	F3	F4
s10	F1 - 1	F1	M	7.30	3.61	2.94	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	7.43	3.66	3.16	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	7.63	3.74	3.22	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	7.47	3.42	3.04	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	8.17	3.83	3.15	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	8.02	3.66	3.04	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	7.69	3.43	3.12	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	7.58	3.60	2.90	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	8.10	3.47	3.17	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	8.32	3.83	2.89	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	8.02	3.78	3.23	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	8.40	3.87	3.12	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	8.08	3.82	3.18	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	8.06	3.69	3.43	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	M	8.10	3.73	3.24	1	0	1	0	1	0	0	0
s10	F1 - 1	F1	F	8.44	3.68	3.29	1	0	0	1	1	0	0	0
s10	F1 - 1	F1	F	7.85	3.75	3.87	1	0	0	1	1	0	0	0
s10	F1 - 1	F1	F	8.21	3.71	2.90	1	0	0	1	1	0	0	0
s10	F1 - 1	F1	F	8.17	3.93	3.08	1	0	0	1	1	0	0	0
s10	F1 - 1	F1	F	7.80	3.61	2.77	1	0	0	1	1	0	0	0
s10	F1 - 1	F1	F	8.04	3.88	3.24	1	0	0	1	1	0	0	0
s10	F1 - 1	F1	F	7.44	3.21	3.17	1	0	0	1	1	0	0	0
s10	F1 - 1	F1	F	7.80	3.64	3.21	1	0	0	1	1	0	0	0
s10	F1 - 1	F1	F	7.20	3.67	3.09	1	0	0	1	1	0	0	0
s10	F1 - 1	F1	F	8.11	3.90	3.42	1	0	0	1	1	0	0	0
s10	F1 - 1	F1	F	7.95	3.75	3.15	1	0	0	1	1	0	0	0

Table S2. Raw morphometrical data for each particular specimen analyzed in this study.

Sampling period	Site	Field	Sex	Elytron	Pronotum	Femur	Y10	Y11	M	F	F1	F2	F3	F4
s10	F1 - 1	F1	F	8.10	3.73	3.32	1	0	0	1	1	0	0	0
s10	F1 - 1	F1	F	8.10	3.69	3.17	1	0	0	1	1	0	0	0
s10	F1 - 1	F1	F	7.99	3.78	3.19	1	0	0	1	1	0	0	0
s10	F1 - 1	F1	F	7.85	3.99	3.04	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	M	7.82	3.28	3.19	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	8.22	3.95	3.30	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	8.32	3.86	3.35	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	7.60	3.43	3.21	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	8.03	3.85	2.99	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	8.06	3.73	3.17	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	7.94	3.34	3.19	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	8.26	3.94	3.34	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	7.70	3.55	3.00	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	8.12	3.59	3.29	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	7.72	3.60	3.22	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	7.89	3.60	3.10	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	8.49	3.94	3.31	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	8.78	3.97	3.30	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	M	8.45	3.88	3.24	1	0	1	0	1	0	0	0
s10	F1 - 2	F1	F	7.87	3.56	2.77	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	F	7.64	3.34	2.98	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	F	8.33	3.96	3.40	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	F	8.09	3.68	3.16	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	F	8.35	3.87	3.24	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	F	8.83	4.01	3.37	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	F	7.62	3.63	3.11	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	F	8.54	3.71	3.45	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	F	8.06	3.56	3.12	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	F	8.07	3.70	3.23	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	F	7.61	3.64	3.15	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	F	8.06	3.90	3.22	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	F	8.28	3.96	2.98	1	0	0	1	1	0	0	0
s10	F1 - 2	F1	F	8.14	3.65	3.22	1	0	0	1	1	0	0	0
s10	F2 - 1	F2	M	8.40	3.79	2.83	1	0	1	0	0	1	0	0
s10	F2 - 1	F2	M	8.26	3.63	3.17	1	0	1	0	0	1	0	0
s10	F2 - 1	F2	M	8.19	3.85	3.22	1	0	1	0	0	1	0	0
s10	F2 - 1	F2	M	8.42	3.83	3.35	1	0	1	0	0	1	0	0
s10	F2 - 1	F2	M	8.21	3.68	3.26	1	0	1	0	0	1	0	0
s10	F2 - 1	F2	M	7.94	3.63	3.27	1	0	1	0	0	1	0	0
s10	F2 - 1	F2	M	7.64	3.67	3.08	1	0	1	0	0	1	0	0
s10	F2 - 1	F2	M	8.68	4.03	3.30	1	0	1	0	0	1	0	0

continued **Table S2.** Raw morphometrical data for each particular specimen analyzed in this study.

Sampling period	Site	Field	Sex	Elytron	Pronotum	Femur	Y10	Y11	M	F	F1	F2	F3	F4
s10	F2 - 1	F2	M	8.30	3.88	3.25	1	0	1	0	0	1	0	0
s10	F2 - 1	F2	M	8.10	3.84	3.28	1	0	1	0	0	1	0	0
s10	F2 - 1	F2	M	8.33	3.80	3.39	1	0	1	0	0	1	0	0
s10	F2 - 1	F2	F	8.58	4.16	3.22	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	8.03	3.80	3.13	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	7.20	3.59	2.78	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	7.72	3.63	3.15	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	8.44	3.90	3.22	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	7.70	3.79	2.95	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	8.18	3.81	2.79	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	7.90	3.57	3.21	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	7.64	3.55	2.41	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	8.40	4.05	3.04	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	8.04	3.80	3.15	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	8.53	4.01	3.46	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	8.27	3.86	3.33	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	7.99	3.64	3.04	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	8.45	3.95	3.30	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	8.60	3.97	3.33	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	8.11	3.53	3.03	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	8.03	3.91	2.96	1	0	0	1	0	1	0	0
s10	F2 - 1	F2	F	8.08	3.78	3.15	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	M	8.00	3.59	3.11	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	7.60	3.70	2.95	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	7.75	3.90	3.25	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	7.49	3.47	2.96	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	8.23	3.74	3.30	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	7.35	3.48	2.99	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	7.60	3.66	3.07	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	7.69	3.54	3.12	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	7.84	3.88	3.15	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	8.12	3.70	2.94	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	8.19	3.97	3.37	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	7.67	3.50	3.14	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	8.13	3.78	3.54	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	7.20	3.48	2.90	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	M	7.96	3.78	2.92	1	0	1	0	0	1	0	0
s10	F2 - 2	F2	F	8.27	4.03	3.27	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	F	7.80	3.67	3.07	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	F	8.28	3.81	3.21	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	F	8.42	3.98	3.32	1	0	0	1	0	1	0	0

continued **Table S2.** Raw morphometrical data for each particular specimen analyzed in this study.

Sampling period	Site	Field	Sex	Elytron	Pronotum	Femur	Y10	Y11	M	F	F1	F2	F3	F4
s10	F2 - 2	F2	F	8.60	4.04	3.34	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	F	8.20	4.04	3.70	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	F	7.92	3.72	3.01	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	F	7.98	3.54	3.07	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	F	8.46	3.89	3.24	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	F	8.27	3.67	3.31	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	F	8.07	3.72	3.02	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	F	8.46	4.03	3.49	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	F	8.20	3.96	3.29	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	F	7.96	3.91	3.21	1	0	0	1	0	1	0	0
s10	F2 - 2	F2	F	8.10	3.75	3.09	1	0	0	1	0	1	0	0
s10	F3 - 1	F3	M	8.31	3.96	3.28	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	7.64	3.60	3.06	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	7.80	3.46	2.76	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	8.24	3.91	3.12	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	8.06	3.80	3.20	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	8.17	3.92	3.23	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	7.94	3.64	3.19	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	7.85	3.54	3.06	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	8.42	3.89	3.28	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	7.98	3.79	3.22	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	8.22	3.83	3.12	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	8.46	4.00	3.16	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	8.26	3.82	3.08	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	7.86	3.57	3.02	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	M	7.77	3.82	3.10	1	0	1	0	0	0	1	0
s10	F3 - 1	F3	F	8.32	4.00	2.89	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	8.23	3.77	3.16	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	8.61	3.96	3.38	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	8.26	3.73	3.40	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	8.28	3.83	3.19	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	8.27	3.72	3.02	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	8.01	3.81	3.17	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	8.27	3.80	3.26	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	8.09	3.82	3.13	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	8.40	4.01	3.22	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	7.53	3.53	2.98	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	8.45	3.75	3.22	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	8.05	3.75	3.10	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	7.99	3.83	2.68	1	0	0	1	0	0	1	0
s10	F3 - 1	F3	F	8.21	3.95	3.26	1	0	0	1	0	0	1	0

continued Table S2. Raw morphometrical data for each particular specimen analyzed in this study.

Sampling period	Site	Field	Sex	Elytron	Pronotum	Femur	Y10	Y11	M	F	F1	F2	F3	F4
s10	F3 - 2	F3	M	7.85	3.70	3.15	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	7.83	3.84	3.21	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	8.01	3.69	3.09	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	7.34	3.61	3.32	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	7.99	3.53	3.18	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	7.40	3.88	3.05	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	8.04	3.74	3.28	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	8.12	3.94	2.87	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	8.35	3.62	3.17	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	7.81	3.44	3.06	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	7.59	3.66	3.20	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	7.72	3.58	3.00	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	7.95	3.53	3.16	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	8.19	3.85	3.01	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	M	8.05	3.67	3.10	1	0	1	0	0	0	1	0
s10	F3 - 2	F3	F	7.56	3.65	2.98	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	8.46	3.93	2.88	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	8.34	3.70	3.34	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	8.39	3.83	3.19	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	7.70	3.58	3.32	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	7.93	3.92	3.25	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	7.90	3.84	3.20	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	8.73	4.12	3.34	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	8.70	3.93	3.22	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	8.15	3.78	2.86	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	7.76	3.60	2.93	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	8.68	4.16	3.36	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	7.66	3.62	3.01	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	7.72	3.80	3.16	1	0	0	1	0	0	1	0
s10	F3 - 2	F3	F	8.35	4.01	3.37	1	0	0	1	0	0	1	0
s10	F4 - 1	F4	M	7.78	3.64	3.00	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	M	7.98	3.61	3.17	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	M	7.65	3.47	2.86	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	M	7.90	3.76	3.15	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	M	8.11	3.64	3.24	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	M	8.50	3.99	3.29	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	M	7.83	3.60	3.12	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	M	7.70	3.53	3.08	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	M	7.92	3.74	3.20	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	M	7.72	3.68	2.98	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	M	7.58	3.50	2.90	1	0	1	0	0	0	0	1

continued Table S2. Raw morphometrical data for each particular specimen analyzed in this study.

Sampling period	Site	Field	Sex	Elytron	Pronotum	Femur	Y10	Y11	M	F	F1	F2	F3	F4
s10	F4 - 1	F4	M	7.94	3.91	3.14	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	M	8.13	3.80	3.06	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	M	7.82	3.42	3.07	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	M	7.80	3.70	2.95	1	0	1	0	0	0	0	1
s10	F4 - 1	F4	F	8.40	4.06	2.97	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	8.16	3.70	3.12	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	8.23	3.58	3.24	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	8.00	3.64	3.06	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	7.91	3.62	3.00	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	8.37	4.09	3.34	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	7.94	3.66	2.96	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	8.40	3.88	3.27	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	7.49	3.43	2.89	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	8.31	3.94	3.23	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	8.42	3.79	3.26	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	7.98	3.72	3.04	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	8.23	3.77	3.11	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	8.30	3.90	3.27	1	0	0	1	0	0	0	1
s10	F4 - 1	F4	F	7.68	3.52	2.28	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	M	7.54	3.62	3.09	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	7.97	3.71	3.24	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	8.16	3.62	3.23	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	7.88	3.82	3.12	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	8.11	3.82	3.03	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	7.85	3.51	2.88	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	8.18	3.84	3.26	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	7.98	3.71	3.12	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	7.75	3.63	2.86	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	7.85	3.65	3.06	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	7.93	3.53	2.82	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	7.64	3.57	2.87	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	8.26	3.81	3.16	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	8.14	3.75	3.20	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	M	7.70	3.49	3.01	1	0	1	0	0	0	0	1
s10	F4 - 2	F4	F	8.00	3.97	3.29	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	F	8.02	3.90	3.22	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	F	8.04	3.82	3.23	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	F	8.19	3.92	3.28	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	F	8.12	3.82	2.98	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	F	7.30	3.54	2.99	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	F	8.06	3.54	3.09	1	0	0	1	0	0	0	1

continued **Table S2.** Raw morphometrical data for each particular specimen analyzed in this study.

Sampling period	Site	Field	Sex	Elytron	Pronotum	Femur	Y10	Y11	M	F	F1	F2	F3	F4
s10	F4 - 2	F4	F	8.39	3.93	3.38	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	F	7.82	3.71	2.92	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	F	7.88	3.74	3.13	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	F	8.48	3.96	3.27	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	F	8.02	3.70	2.79	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	F	8.00	3.70	3.15	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	F	7.62	3.47	2.97	1	0	0	1	0	0	0	1
s10	F4 - 2	F4	F	7.90	3.78	2.96	1	0	0	1	0	0	0	1
s11	F1-1	F1	M	7.78	3.55	3.15	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	8.25	4.02	3.28	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	8.11	3.59	2.96	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	7.75	3.62	3.11	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	8.25	3.93	3.27	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	8.19	3.85	3.18	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	8.44	3.68	3.14	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	7.56	3.56	3.10	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	7.19	3.62	2.85	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	7.59	3.59	3.03	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	8.18	3.83	3.13	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	7.71	3.72	3.12	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	7.35	3.84	3.23	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	7.67	3.51	3.01	0	1	1	0	1	0	0	0
s11	F1-1	F1	M	7.54	3.40	3.02	0	1	1	0	1	0	0	0
s11	F1-1	F1	F	8.31	3.85	3.34	0	1	0	1	1	0	0	0
s11	F1-1	F1	F	8.08	3.73	3.22	0	1	0	1	1	0	0	0
s11	F1-1	F1	F	8.14	3.79	3.30	0	1	0	1	1	0	0	0
s11	F1-1	F1	F	7.48	3.60	3.05	0	1	0	1	1	0	0	0
s11	F1-1	F1	F	8.26	3.73	3.17	0	1	0	1	1	0	0	0
s11	F1-1	F1	F	7.94	3.62	3.13	0	1	0	1	1	0	0	0
s11	F1-1	F1	F	8.38	4.13	3.12	0	1	0	1	1	0	0	0
s11	F1-1	F1	F	7.87	3.88	3.22	0	1	0	1	1	0	0	0
s11	F1-1	F1	F	8.00	3.86	3.14	0	1	0	1	1	0	0	0
s11	F1-1	F1	F	7.50	3.62	2.92	0	1	0	1	1	0	0	0
s11	F1-1	F1	F	8.46	3.98	3.42	0	1	0	1	1	0	0	0
s11	F1-1	F1	F	7.76	3.52	3.09	0	1	0	1	1	0	0	0
s11	F1-1	F1	F	8.15	3.89	3.27	0	1	0	1	1	0	0	0
s11	F1-2	F1	M	7.78	3.67	3.16	0	1	1	0	1	0	0	0
s11	F1-2	F1	M	8.14	3.87	3.14	0	1	1	0	1	0	0	0
s11	F1-2	F1	M	8.02	3.70	3.21	0	1	1	0	1	0	0	0
s11	F1-2	F1	M	7.98	3.62	3.28	0	1	1	0	1	0	0	0
s11	F1-2	F1	M	7.89	3.69	3.08	0	1	1	0	1	0	0	0

continued Table S2. Raw morphometrical data for each particular specimen analyzed in this study.

Sampling period	Site	Field	Sex	Elytron	Pronotum	Femur	Y10	Y11	M	F	F1	F2	F3	F4
s11	F1-2	F1	M	7.70	3.88	3.16	0	1	1	0	1	0	0	0
s11	F1-2	F1	M	7.92	3.83	3.11	0	1	1	0	1	0	0	0
s11	F1-2	F1	M	8.25	3.76	3.14	0	1	1	0	1	0	0	0
s11	F1-2	F1	M	7.99	3.71	3.13	0	1	1	0	1	0	0	0
s11	F1-2	F1	M	8.23	3.87	3.27	0	1	1	0	1	0	0	0
s11	F1-2	F1	M	7.48	3.65	3.14	0	1	1	0	1	0	0	0
s11	F1-2	F1	M	7.73	3.63	3.05	0	1	1	0	1	0	0	0
s11	F1-2	F1	M	8.25	3.95	3.40	0	1	1	0	1	0	0	0
s11	F1-2	F1	M	8.24	3.96	3.24	0	1	1	0	1	0	0	0
s11	F1-2	F1	M	8.34	3.85	3.27	0	1	1	0	1	0	0	0
s11	F1-2	F1	F	8.20	3.74	3.20	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	8.53	3.99	3.34	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	8.00	3.86	3.23	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	7.98	3.89	3.20	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	7.20	3.56	3.10	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	7.82	3.84	3.30	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	7.74	3.54	3.03	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	8.28	3.82	3.25	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	8.16	4.12	3.36	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	7.97	3.78	3.09	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	7.25	3.60	3.04	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	7.97	3.65	3.07	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	7.76	3.71	3.01	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	7.96	3.18	3.01	0	1	0	1	1	0	0	0
s11	F1-2	F1	F	7.70	3.74	3.14	0	1	0	1	1	0	0	0
s11	F2-1	F2	M	7.95	3.77	3.22	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	7.97	3.72	3.27	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	7.75	3.62	3.22	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	7.98	3.53	3.22	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	8.24	3.91	3.02	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	7.74	3.80	3.20	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	7.62	3.47	2.92	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	7.99	3.68	2.80	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	8.04	3.84	3.27	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	7.72	3.60	2.99	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	8.01	3.68	3.31	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	7.78	3.59	2.93	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	7.91	3.90	3.18	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	8.04	3.83	2.75	0	1	1	0	0	1	0	0
s11	F2-1	F2	M	8.23	3.92	3.33	0	1	1	0	0	1	0	0
s11	F2-1	F2	F	8.22	3.96	3.20	0	1	0	1	0	1	0	0

continued Table S2. Raw morphometrical data for each particular specimen analyzed in this study.

Sampling period	Site	Field	Sex	Elytron	Pronotum	Femur	Y10	Y11	M	F	F1	F2	F3	F4
s11	F2-1	F2	F	8.27	3.86	3.07	0	1	0	1	0	1	0	0
s11	F2-1	F2	F	8.05	3.77	3.25	0	1	0	1	0	1	0	0
s11	F2-1	F2	F	8.02	3.85	3.14	0	1	0	1	0	1	0	0
s11	F2-1	F2	F	8.13	3.90	3.22	0	1	0	1	0	1	0	0
s11	F2-1	F2	F	8.09	3.82	3.24	0	1	0	1	0	1	0	0
s11	F2-1	F2	F	7.73	3.84	2.93	0	1	0	1	0	1	0	0
s11	F2-1	F2	F	7.98	3.67	3.10	0	1	0	1	0	1	0	0
s11	F2-1	F2	F	8.16	3.85	3.22	0	1	0	1	0	1	0	0
s11	F2-1	F2	F	7.52	3.52	2.80	0	1	0	1	0	1	0	0
s11	F2-1	F2	F	8.09	3.81	3.12	0	1	0	1	0	1	0	0
s11	F2-1	F2	F	8.23	3.83	3.27	0	1	0	1	0	1	0	0
s11	F2-1	F2	F	8.16	3.97	3.40	0	1	0	1	0	1	0	0
s11	F2-1	F2	F	8.45	4.10	3.42	0	1	0	1	0	1	0	0
s11	F2-1	F2	F	7.45	3.75	3.03	0	1	0	1	0	1	0	0
s11	F2-2	F2	M	7.62	3.61	3.05	0	1	1	0	0	1	0	0
s11	F2-2	F2	M	8.02	3.80	3.36	0	1	1	0	0	1	0	0
s11	F2-2	F2	M	8.37	3.86	3.06	0	1	1	0	0	1	0	0
s11	F2-2	F2	M	8.20	3.85	3.31	0	1	1	0	0	1	0	0
s11	F2-2	F2	M	7.22	3.41	2.97	0	1	1	0	0	1	0	0
s11	F2-2	F2	M	7.55	3.64	3.28	0	1	1	0	0	1	0	0
s11	F2-2	F2	M	8.15	3.91	3.30	0	1	1	0	0	1	0	0
s11	F2-2	F2	F	8.45	3.98	3.37	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	7.73	3.83	3.24	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	8.55	4.12	3.37	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	7.74	3.67	3.10	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	8.29	3.84	3.29	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	7.65	3.74	2.95	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	8.17	4.02	3.01	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	8.05	3.99	3.25	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	7.63	3.72	3.15	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	7.82	3.66	3.08	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	7.50	3.63	2.82	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	8.35	3.97	3.24	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	7.78	3.69	3.06	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	8.03	3.92	3.18	0	1	0	1	0	1	0	0
s11	F2-2	F2	F	7.92	3.59	3.06	0	1	0	1	0	1	0	0
s11	F3-1	F3	M	8.14	3.97	3.22	0	1	1	0	0	0	1	0
s11	F3-1	F3	M	7.80	3.72	3.28	0	1	1	0	0	0	1	0
s11	F3-1	F3	M	7.98	3.72	3.18	0	1	1	0	0	0	1	0
s11	F3-1	F3	M	7.81	3.65	3.16	0	1	1	0	0	0	1	0
s11	F3-1	F3	M	7.52	3.47	3.06	0	1	1	0	0	0	1	0

continued Table S2. Raw morphometrical data for each particular specimen analyzed in this study.

Sampling period	Site	Field	Sex	Elytron	Pronotum	Femur	Y10	Y11	M	F	F1	F2	F3	F4
s11	F3-1	F3	M	7.74	3.68	3.04	0	1	1	0	0	0	1	0
s11	F3-1	F3	M	7.84	3.82	3.28	0	1	1	0	0	0	1	0
s11	F3-1	F3	M	8.17	3.71	3.10	0	1	1	0	0	0	1	0
s11	F3-1	F3	M	7.38	3.79	2.84	0	1	1	0	0	0	1	0
s11	F3-1	F3	M	7.70	3.62	3.07	0	1	1	0	0	0	1	0
s11	F3-1	F3	M	8.12	3.80	3.14	0	1	1	0	0	0	1	0
s11	F3-1	F3	M	8.14	3.63	3.27	0	1	1	0	0	0	1	0
s11	F3-1	F3	M	8.00	3.81	3.20	0	1	1	0	0	0	1	0
s11	F3-1	F3	M	7.63	3.56	3.12	0	1	1	0	0	0	1	0
s11	F3-1	F3	M	7.82	3.51	3.08	0	1	1	0	0	0	1	0
s11	F3-1	F3	F	7.66	3.71	3.01	0	1	0	1	0	0	1	0
s11	F3-1	F3	F	7.80	3.65	3.10	0	1	0	1	0	0	1	0
s11	F3-1	F3	F	7.73	3.77	3.00	0	1	0	1	0	0	1	0
s11	F3-1	F3	F	8.43	3.74	3.23	0	1	0	1	0	0	1	0
s11	F3-1	F3	F	8.13	3.62	3.13	0	1	0	1	0	0	1	0
s11	F3-1	F3	F	8.10	3.78	3.24	0	1	0	1	0	0	1	0
s11	F3-1	F3	F	7.86	3.70	3.06	0	1	0	1	0	0	1	0
s11	F3-2	F3	M	7.98	3.76	3.22	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	7.92	3.64	3.07	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	7.89	3.71	3.12	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	7.89	3.62	3.04	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	8.16	3.63	3.26	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	8.23	3.74	3.19	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	8.23	3.87	3.22	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	7.95	3.95	3.18	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	7.71	3.62	2.92	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	8.30	4.00	3.20	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	7.74	3.62	3.09	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	8.00	3.68	3.18	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	8.24	3.79	3.25	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	7.93	3.78	3.27	0	1	1	0	0	0	1	0
s11	F3-2	F3	M	8.19	3.99	3.25	0	1	1	0	0	0	1	0
s11	F3-2	F3	F	7.81	3.63	3.10	0	1	0	1	0	0	1	0
s11	F3-2	F3	F	8.19	3.61	3.00	0	1	0	1	0	0	1	0
s11	F3-2	F3	F	8.02	3.62	3.20	0	1	0	1	0	0	1	0
s11	F3-2	F3	F	8.29	3.98	3.20	0	1	0	1	0	0	1	0
s11	F3-2	F3	F	7.89	3.95	3.26	0	1	0	1	0	0	1	0
s11	F3-2	F3	F	7.90	3.62	2.77	0	1	0	1	0	0	1	0
s11	F3-2	F3	F	7.59	3.46	2.92	0	1	0	1	0	0	1	0
s11	F3-2	F3	F	8.28	3.89	3.21	0	1	0	1	0	0	1	0
s11	F3-2	F3	F	7.67	3.49	3.04	0	1	0	1	0	0	1	0

continuedTable S2. Raw morphometrical data for each particular specimen analyzed in this study.

Sampling period	Site	Field	Sex	Elytron	Pronotum	Femur	Y10	Y11	M	F	F1	F2	F3	F4
s11	F3-2	F3	F	8.09	3.70	3.26	0	1	0	1	0	0	1	0
s11	F3-2	F3	F	8.20	3.85	3.06	0	1	0	1	0	0	1	0
s11	F3-2	F3	F	8.66	4.05	3.33	0	1	0	1	0	0	1	0
s11	F3-2	F3	F	8.23	3.66	3.10	0	1	0	1	0	0	1	0
s11	F3-2	F3	F	8.25	3.91	3.24	0	1	0	1	0	0	1	0
s11	F3-2	F3	F	8.10	3.83	3.06	0	1	0	1	0	0	1	0
s11	F4-1	F4	M	8.05	3.80	3.15	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	7.75	3.62	3.10	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	8.51	3.78	3.20	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	8.43	4.01	3.42	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	7.98	3.61	3.13	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	8.22	3.75	3.05	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	8.31	3.84	3.25	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	8.26	3.83	3.27	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	7.91	3.67	3.07	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	8.57	3.85	3.28	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	8.16	3.84	3.30	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	8.04	3.73	3.18	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	8.46	3.91	3.30	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	8.14	3.63	3.15	0	1	1	0	0	0	0	1
s11	F4-1	F4	M	8.33	3.95	3.22	0	1	1	0	0	0	0	1
s11	F4-1	F4	F	8.37	3.84	3.09	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	7.98	3.67	3.00	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	8.20	3.80	3.16	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	8.57	3.94	3.14	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	8.66	4.20	3.37	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	7.95	3.71	3.10	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	8.62	4.02	3.30	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	7.73	3.74	2.76	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	7.94	3.73	3.15	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	7.37	3.52	2.99	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	7.97	3.69	3.04	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	7.99	3.77	3.01	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	8.06	3.94	3.29	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	7.82	3.62	2.96	0	1	0	1	0	0	0	1
s11	F4-1	F4	F	8.17	3.84	3.16	0	1	0	1	0	0	0	1
s11	F4-2	F4	M	7.99	3.70	3.10	0	1	1	0	0	0	0	1
s11	F4-2	F4	M	8.03	3.84	3.21	0	1	1	0	0	0	0	1
s11	F4-2	F4	M	7.74	3.70	3.02	0	1	1	0	0	0	0	1
s11	F4-2	F4	M	8.02	3.77	3.06	0	1	1	0	0	0	0	1
s11	F4-2	F4	M	7.67	3.52	2.97	0	1	1	0	0	0	0	1

continued Table S2. Raw morphometrical data for each particular specimen analyzed in this study.

Sampling period	Site	Field	Sex	Elytron	Pronotum	Femur	Y10	Y11	M	F	F1	F2	F3	F4
s11	F4-2	F4	M	7.65	3.52	2.95	0	1	1	0	0	0	0	1
s11	F4-2	F4	M	8.00	3.75	3.18	0	1	1	0	0	0	0	1
s11	F4-2	F4	M	8.00	3.70	3.10	0	1	1	0	0	0	0	1
s11	F4-2	F4	M	8.52	3.89	3.32	0	1	1	0	0	0	0	1
s11	F4-2	F4	F	8.32	3.80	3.30	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	8.19	3.78	3.11	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	7.53	3.43	3.00	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	7.67	3.51	3.01	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	7.39	3.40	2.94	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	8.34	3.83	3.32	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	7.75	3.60	2.82	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	8.46	3.98	3.21	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	8.47	3.82	3.25	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	8.37	3.96	3.26	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	8.06	3.97	3.35	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	7.76	3.56	3.07	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	7.80	3.60	2.84	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	8.05	3.82	3.22	0	1	0	1	0	0	0	1
s11	F4-2	F4	F	7.72	3.70	3.02	0	1	0	1	0	0	0	1

continued**Table S2.** Raw morphometrical data for each particular specimen analyzed in this study.

2. Článek

The effects of overwintering, sex, year, field identity and vegetation at the boundary of fields on the body condition of *Anchomenus dorsalis* (Coleoptera: Carabidae)

Baranovská E, Knapp M & Saska P

The effects of overwintering, sex, year, field identity and vegetation at the boundary of fields on the body condition of *Anchomenus dorsalis* (Coleoptera: Carabidae)

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Abstract. Body condition, in terms of body mass corrected for structural body size, represents an animal's energetic reserves and is an indicator of the health and fitness of an organism. This study investigates the variability in the body condition of the carabid beetle, *Anchomenus dorsalis*, in time and space at a small scale. The beetles were collected in four fields with two types of boundary (grassy and forest) near Prague-Suchdol in autumn 2009 and 2010 and in spring 2010 and 2011. Body condition was significantly affected by overwintering (the body condition of post-overwintering individuals collected in spring was worse than that of pre-overwintering individuals collected in autumn), sex (females were in better condition than males) and to a lesser extent by sampling year, field identity and several interactions, e.g., overwintering : sex (greater decrease in body condition during overwintering in males than in females), overwintering : year (difference in body condition between pre-overwintering and post-overwintering individuals was greater in 2009/2010 than 2010/2011). Type of boundary had no substantial effect on the body condition of *A. dorsalis*. This study shows the importance of sampling body condition several times a year for more than one season, which has rarely been performed to date.

INTRODUCTION

Body condition indicates the energy (fat) reserves of an animal (Peig & Green, 2009; Knapp & Knappová, 2013) and is presumed to substantially influence the fitness of particular organisms. In general, females in good condition mate more frequently with high-quality males (Cotton et al., 2006), have a higher fecundity (Bommarco, 1998b; Moya-Laraño, 2002) and survive better in unfavourable environments (Petersen, 1999) than females in poor condition.

Variation in body condition can be caused either by "strategic" factors (Elkin & Reid, 2005) or "environmental" factors (van Dijk, 1994). Strategic factors are incorporated in the life cycle of a species and individuals are able to adaptively shape them. Variation in body condition due to strategic factors represents an animal's response to predictable conditions. An example of such a strategic factor is maximisation of food intake before hibernation (van Dijk, 1994; Lövei & Sunderland, 1996) or sex-specific body condition. Female carabids have higher food demands than males, at least before reproduction (Bilde & Toft, 1998), because egg production required more energy than sperm production. On the other hand, environmental factors are unpredictable and occur at random in time and space. The course of the weather or disruptive changes in the environment, such as soil operations or pesticide treatments (Östman et al., 2001; Östman, 2005), are environmental factors that can affect the body condition of insects.

Environmental factors are assumed to be responsible for a substantial proportion of the spatial and temporal variation in body condition, of which the uneven distribution of prey in time and space may be the most important (Bommarco, 1998a; Östman et al., 2001). At the farm scale, food availability for carabid beetles can be affected by extreme weather conditions or drastic agronomical practice (Huusela-Veistola, 1996; Bilde & Toft, 1998). For example, carabid beetles starve less in June than July, most likely a result of the hot and dry weather in the latter month, which may reduce the amount of available prey (Bilde & Toft, 1998). Increased prey abundance not only positively affects the body condition of males and females of carabid beetles but also increases oocyte numbers (Haschek et al., 2012) or extends the oviposition period (Sota, 1985). Sufficient fat reserves may also be important for prolonged survival after hibernation in early spring, when prey is temporarily scarce (Petersen, 1999).

As predatory carabid beetles often starve in arable fields (Bilde & Toft, 1998; Bommarco, 1998b), their body condition provides information about the quality of this habitat for particular species (Barone & Frank, 2003). In addition to local environmental conditions, the body condition of carabid beetles seems to be affected by the surrounding landscape. Crop diversity and high perimeter-to-area ratio (i.e., landscapes with more abundant field boundaries; Östman et al., 2001) both affect the availability of prey for carabid beetles (Haschek et al., 2012). Thus, a heterogeneous landscape may provide a better environment with more
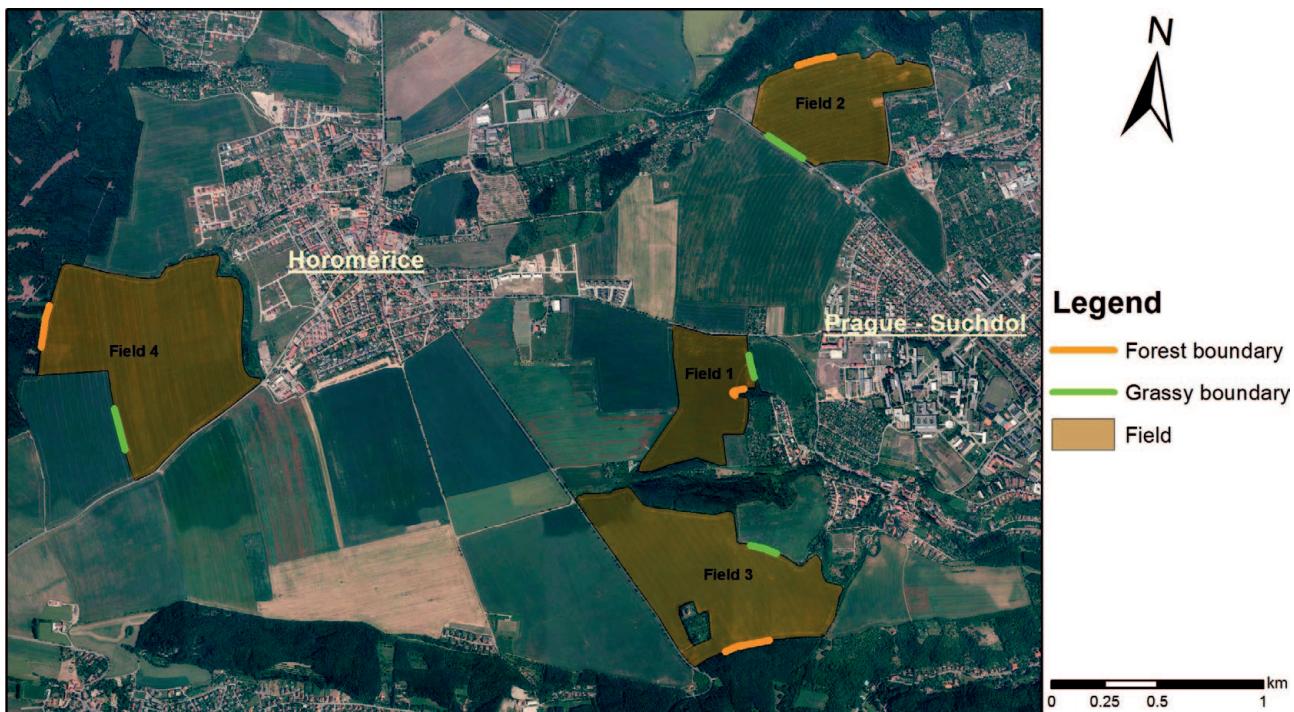
Fig. 1. Map of localities investigated in this study. The arable fields investigated were situated near Prague-Suchdol, in the Czech Republic (GPS coordinates of field centres: field 1 – 50°7´46.942″N 14°21´37.574″E, field 2 – 50°8´28.480″N 14°21´54.754″″, field 3 – 50°7´16.933″N 14°21´44.419″E, field 4 – 50°7´38.451″N 14°19´30.361″E). Orange and green lines mark the sites where pitfall traps were set. The map background was taken from an aerial photograph provided by the Czech Office for Surveying, Mapping and Cadastre (ČÚZK, 2014).

food, resulting in increased fat reserves of carabids and higher population densities (Holland, 2002). Field boundaries are also important, especially for carabid reproduction (Zangger et al., 1994) and overwintering (Andersen, 1997).

Most studies concerning carabid body condition investigate spatial variation or temporal variation separately. To our knowledge, the exception for carabids is the study performed by Östman (2005), who investigated variation in structural body size and body condition on 10 farms around Uppsala, Sweden in two subsequent years. He found asynchronous temporal variation in body condition among sites, suggesting that body condition is affected also by other factors than those related to site characteristics. Recently, Baranovská & Knapp (2014) investigated the variation in structural body size of two species of carabids in arable fields. They found that structural body size, which is determined during pre-imaginal development and remains stable during adult life, is mainly affected by gender and to a lesser extent also by field identity, year and overwintering. Both studies showed that it is important to study spatial and temporal variation together because there may be an interaction between spatial and temporal variation that affects body condition (Östman, 2005) or structural body size (Östman, 2005; Baranovská & Knapp, 2014). Moreover, there is no information on the effect of spatial differences in intra-seasonal temporal variation (pre- vs. post-winter) in determining carabid body condition.

In this study, we examined the temporal and spatial variation in the body condition of the polyphagous predatory carabid A. dorsalis at eight different sites (four arable

fields and two types of boundary within each field) over two seasons (autumn 2009 and spring 2010, autumn 2010 and spring 2011). The same study design was used as in our previous study (Baranovská & Knapp, 2014), in which we only investigated structural body size. In this paper, we study the variation in body condition of A. dorsalis. Unlike structural body size, body condition (here, the dry body mass corrected for structural body size) is substantially affected by the environmental conditions experienced by the adults. First, we investigated how body condition varied among fields, which differed in their properties (area, shape etc.) and in surrounding landscape parameters, which can affect the food supply for the beetles. We also expected that the body condition of A. dorsalis would be better in autumn than in spring, as energy reserves will be spent during winter, and females would have better body condition than males because of sex-specific selection pressure on accumulating energy reserves (females have to be prepared for reproduction, which has a high energy cost). Finally, we investigated whether there was any variation in the body condition of A. dorsalis between years associated with the variation in environmental conditions in particular seasons.

MATERIAL AND METHODS

Study species

Anchomenus dorsalis (Pontoppidan, 1763) is a medium-sized ground beetle (Coleoptera: Carabidae), ca. 5.6–7.7 mm long (Hůrka, 1996). In the Czech Republic, this species occurs in open habitats and is common even in intensively managed agricultural landscapes. Although A. dorsalis occurs within arable fields, surrounding field boundaries are very important for its re-

production and overwintering (Holland et al., 2009; Roume et al., 2011). Beetles mate and lay eggs in spring and early summer, larval growth takes place during summer, and the new adults that emerge in late summer or early autumn need to feed intensively before overwintering. *A. dorsalis* is an important generalist predator feeding on aphids and other arthropod crop pests (Wratten & Vickerman, 1985; Bilde & Toft, 1997) and is thus considered a beneficial organism with biocontrol potential (Zaller et al., 2009).

Experimental design

Beetles were collected repeatedly in four arable fields situated a few kilometres northwest of Prague, the Czech Republic (Fig. 1). The fields were conventionally managed during the sampling period (autumn 2009 – spring 2011). The fields investigated were located 1–3 km from each other and were surrounded mainly by fragments of forest and other arable fields (separated by grassy boundaries or roads with a grassy ditch). Within each field, six gutter pitfall traps were set (for details see Baranovská & Knapp, 2014), three at the edge adjacent to a fragment of forest and three at the edge adjacent to a grassy boundary. The traps lacked preservative because it can alter body mass (Knapp, 2012), which would have biased our data. Samples were collected every other day to minimise predation within the pitfall traps. Beetles were collected in autumn (October) 2009 and 2010 and in spring (April) 2010 and 2011. In each field and sampling period, we tried to collect 80 specimens of *A. dorsalis* (40 from the grassy boundary and 40 from the forest boundary). Live beetles were transported to the laboratory, frozen and stored at –20°C until processed. The specimens were sexed and 30 males and 30 females (15 per each type of boundary) were selected at random for each field and sampling period. If there were fewer than 15 males or 15 females per particular sampling site (type of boundary within a particular field), then all specimens collected were used. The beetles were dried for 48 h at 50°C and were then weighed using a Sartorius analytical laboratory balance with a precision to 10^{-5} g. Elytron length, hind femur length and pronotum width were measured using a digital calliper with a precision to 0.01 mm. Although it is common practice to take only one measurement (e.g., Bommarco, 1998a; Östman, 2005), it has recently been shown that measuring of more than one dimension is meaningful for carabids due to violated structural isomorphy among the specimens (Knapp & Knappová, 2013). In total, 936 individuals of *Anchomenus dorsalis* were weighed, measured and analyzed.

To compare the course of the temperature experienced during the two subsequent winters (2009/2010 and 2010/2011), temperature data were obtained from the weather station of the Crop Research Institute (Prague-Ruzyně; http://www.vurv.cz/meteo/). Temperature measurements were made at the surface of the soil and recorded at hourly intervals.

Statistical analyses

Several methods of estimating body condition are used in ecological studies, e.g., Fulton's index, Quételet's index, Relative condition, Relative mass, Residual index, Scaled mass index and ANCOVA (Peig & Green, 2010). In this study, body condition was computed as body mass (dry mass) corrected for structural body size as suggested by García-Berthou (2001). Principal component analysis (PCA) was used to integrate the three measures of structural size (elytron length, pronotum width, hind femur length) into one variable. All specimens were pooled across all sampling sites and all sampling periods and analyzed using a single analysis. Sample scores on the first axis (corresponding to the structural body size of a particular specimen) were then used as co-variables in subsequent univariate analyses of body condition.

The PCA was performed in Canoco for Windows 4.5 software (ter Braak & Šmilauer, 2002).

To analyse the effects of sex, field, year, overwintering, and boundary type and their interactions on the body condition of *A. dorsalis*, a generalised linear model with gamma distribution of errors (GLM-g) was employed. Gamma distribution of errors was used because our data were not normally distributed and this distribution is generally appropriate for data based on size measurements (e.g., length, weight). Structural body size (scores on the first axis resulting from PCA) was used as the covariable in the model. Dry body mass of particular specimen was used as the response variable in the model. To identify significant terms, a manual backward selection procedure based on deletion tests (F-tests) was employed. At first, a full model containing all main effects and all their possible interactions was constructed. Subsequently, the full model was simplified by omitting non-significant terms (deletion test; P > 0.05). Terms were tested (and omitted) in the following order: four-way interactions, three-way interactions, two-way interactions and main terms. The analysis was performed using R 3.0.1 software (R Core Team, 2013).

RESULTS

Strategic factors had substantial effects on the body condition of *Anchomenus dorsalis* in this study. Overwintering was the most important factor determining body condition in *A. dorsalis* (Table 1). Pre-overwintering individuals collected in autumn were in significantly better body condi-

TABLE 1. Effects of site, year, overwintering, boundary and sex on body condition in *Anchomenus dorsalis*. Final generalised linear model with gamma distribution of errors is presented. The final model was selected based on a backward selection procedure applied to the full model consisting of the following explanatory variables (terms): sex, field, overwintering, year, boundary and all possible interactions. The score on the first axis of the PCA based on elytron length, pronotum width and hind femur length measurements of particular specimens was used as a covariable (size) in the model to correct dry body mass for structural body size.

Term	Df	F-value	P-value	R^2
size	1	1201.53	<0.001	0.382
overwintering	1	765.50	<0.001	0.244
sex	1	45.90	<0.001	0.015
field	3	3.46	0.016	0.003
year	1	4.31	0.038	0.001
boundary[#]	1	0.74	0.390	0.000
overwintering : sex	1	16.38	<0.001	0.005
overwintering : field	3	8.64	<0.001	0.008
overwintering : year	1	18.11	<0.001	0.006
field : year	3	6.55	0.000	0.006
field : boundary	4	4.14	0.003	0.005
overwintering : sex : year	2	3.66	0.026	0.002
overwintering : field : year	3	6.12	0.000	0.006
overwintering : field : boundary	4	11.44	<0.001	0.015
field : year : boundary	4	5.60	0.000	0.007
overwintering : field : year : boundary	4	2.81	0.025	0.004

[#]There was no significant main effect of boundary type (P > 0.05); however, the term had to be included in the final model because of significant field : boundary, overwintering : field : boundary, field : year : boundary and overwintering : field : year : boundary interactions.
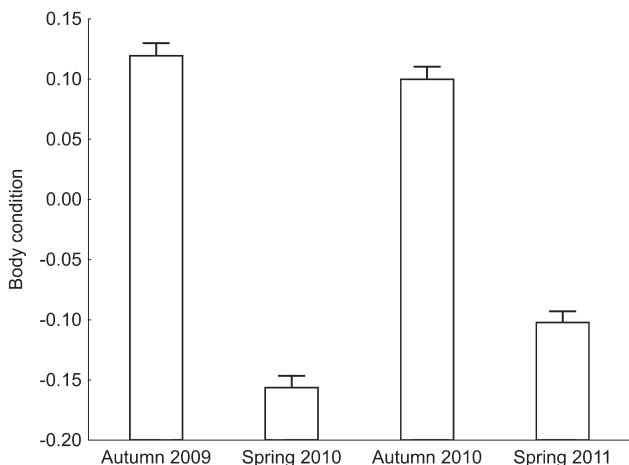
Fig. 2. The effect of overwintering on the body condition of *A. dorsalis* recorded over two winters. To visualise differences in body condition within a season (pre- and post-overwintering) and between seasons (2009/2010 and 2010/2011), residuals from the dry mass-structural body size relationship based on generalised linear model with gamma distribution of errors were used. Mean residuals + SE are shown.

Fig. 3. The effect of sex on the pre- and post-winter body condition of *Anchomenus dorsalis*. To visualise differences in the pre- and post-winter body condition between sexes, residuals from dry mass-structural body size relationship based on a generalised linear model with gamma distribution of errors was used. Mean residuals + SE are shown. M Autumn = males collected before overwintering; M Spring = males collected after overwintering; F Autumn = females collected before overwintering; F Spring = females collected after overwintering.

tion than the post-overwintering individuals collected in spring (Fig. 2). In addition, body condition was significantly affected by sex, as females were in better body condition than males (Fig. 3). The overwintering : sex interaction was also significant (in males the decrease in body condition during overwintering was more substantial than in females; Fig. 3).

The second group of factors affecting body condition are those linked to environmental variability. There was significant variation in the body condition of *A. dorsalis* among particular fields. Body condition also differed slightly between the years (beetles were in better condition in 2010/2011 than in 2009/2010; for differences in the course of temperature between seasons see Appendix A1. In 2009/2010 temperatures below freezing occurred 6 weeks earlier than in 2010/2011 and there were also more frost days in 2009/2010 than in 2010/2011). There were also some significant interactions: overwintering : year (the difference in body condition between pre-overwintering and post-overwintering individuals was greater in 2009/2010 than 2010/2011; Fig. 2), overwintering : field, field : year, field : boundary and some significant higher interactions (see Table 1). It is important to note that the variation explained by the factors linked to environmental variability was rather low (see R^2-values reported in Table 1).

DISCUSSION

Our results indicate that body condition in *Anchomenus dorsalis* differs significantly between autumn and the subsequent spring, i.e., there is a substantial effect of overwintering. Pre-overwintering specimens were in better body condition than post-overwintering individuals. It is believed that adult carabids preparing for overwintering increase their fat reserves in order to enhance winter survival. However, there is little evidence in the literature in support of this. Individuals of *Calathus melanocephalus*

that fed before overwintering, survived better than those that did not, but this was recorded in only one out of two experimental seasons (Van Dijk, 1994). Unfortunately, studies investigating variation in carabid body condition within a season are limited mainly to spring and summer months (den Nijs et al., 1996; Bilde & Toft, 1998). However, a decrease in body condition during hibernation similar to that recorded in this study is reported by Chaabane et al. (1996) for *Abax ater*. That *A. dorsalis* feeds intensively for a few days following emergence from winter diapause, which results in an increase in body mass and lipid content, is recorded (Raubenheimer et al., 2007); however, pre-overwintering body mass and lipid content have not been reported. Small changes in body mass during winter are reported by Petersen at al. (1996) for *Bembidion lampros*. Interestingly, the decrease in body condition of *A. dorsalis* during overwintering was more pronounced for beetles collected in 2009/2010 than in 2010/2011. Such inter-annual changes in the decrease in body condition during overwintering could be caused by weather, mainly differences in temperature. Long-term daily temperature fluctuations around freezing (0°C) causes body mass reduction in *Bembidion lampros*, whereas constant low temperature does not (Petersen et al., 1996). Thus, the greater reduction in the body condition of overwintering individuals in 2009/2010 may be due to differences in temperature in the two winters (for details see Appendix A1).

Our results also indicate that body condition in *A. dorsalis* differed between sexes. The better body condition of females reported in this study is not surprising as females generally invest much more energy in reproduction than males, and thus selection pressure on female body condition is expected to be higher. Enhanced body condition in post-overwintering females compared to males is also re-

ported in *Bembidion lampros* by Knapp & Saska (2012). Interestingly, post-overwintering females were in a relatively better body condition than males (there was significant interaction between sex and overwintering). A possible explanation of the better ability of females to maintain their body condition over winter lies in their larger body size, as large individuals have proportionally lower metabolism rates than small ones (Glazier, 2005). An alternative explanation is the existence of sex-specific selection pressures on physiological processes or behavioural mechanisms linked to managing energy reserves during winter. Unfortunately, studies investigating sexual dimorphism in the body condition of carabid beetles are lacking, and the link between post-overwintering body condition and subsequent egg production in females needs to be investigated. Currently, high food quality and quantity, resulting in good body condition, are considered to be the main factors affecting egg production in carabid beetles (Sota, 1985; Bilde & Toft, 1994; van Dijk, 1994; Bommarco, 1998b). The importance of food quality, especially lipid and protein content, for egg production is also recorded specifically for *A. dorsalis* (Jensen et al., 2012). Although *A. dorsalis* females have better body condition in early spring than males, they still have larger requirements for food before reproduction than males (May to June; Bilde & Toft, 1998).

In previous studies, the spatially based variation in body condition of carabid beetles within arable fields is reported to be affected by the structure of the surrounding landscape, perimeter-to-area ratio (i.e., shape), farming operations, farming practice (organic vs. conventional) or crop diversity at the landscape level (e.g., Bommarco, 1998b; Östman, 2001; Barone & Frank, 2003; Frank et al., 2007). Interestingly, in this study, features of the fields and boundary type explained only a very small portion of total variability recorded in the body condition of *A. dorsalis*. This is surprising, as different boundary types are thought to contain different sources of food. For example, body condition in *A. dorsalis* (species inhabiting open habitats) should be positively affected by a neighbouring grassy boundary in comparison to a forest boundary. There are three possible explanations: (1) our experimental sites are too close to each other, and, thus, the surrounding landscape is similar (shared); (2) our experimental fields did not differ enough in terms of area and shape; (3) all our experimental fields were conventionally managed, with 3 out of 4 fields being managed by the same agricultural company.

Although spatial variation in carabid body condition has been investigated quite intensively, only the study by Östman (2005) investigated spatial variation in carabid body condition over several (two) years. Östman (2005) found that in addition to spatial variation in body condition, there is also substantial temporal variation. Thus, individuals need not have a better body condition in particular fields as the suitability of particular fields for carabids may change from year to year. Such a finding was also confirmed by this study. Asynchronous temporal variation in carabid body condition is most likely caused by variation in environmental conditions e.g., crop rotation or a local pest

outbreak as argued by Östman (2005). Unfortunately, the limited scope of our study (just four fields investigated in two subsequent years) does not allow to us to investigate rigorously the effect of the crop and we have no direct information on prey availability at the sites sampled.

In conclusion, the body condition of *A. dorsalis* seems to be affected mainly by strategic factors, which are integrated into the life cycle of this species and thus under the animal's control (e.g., variation in body condition within a season, sex-specific body condition). Environmental factors investigated in this study (year to year variation, field identity and boundary type) were of limited importance in determining its body condition.

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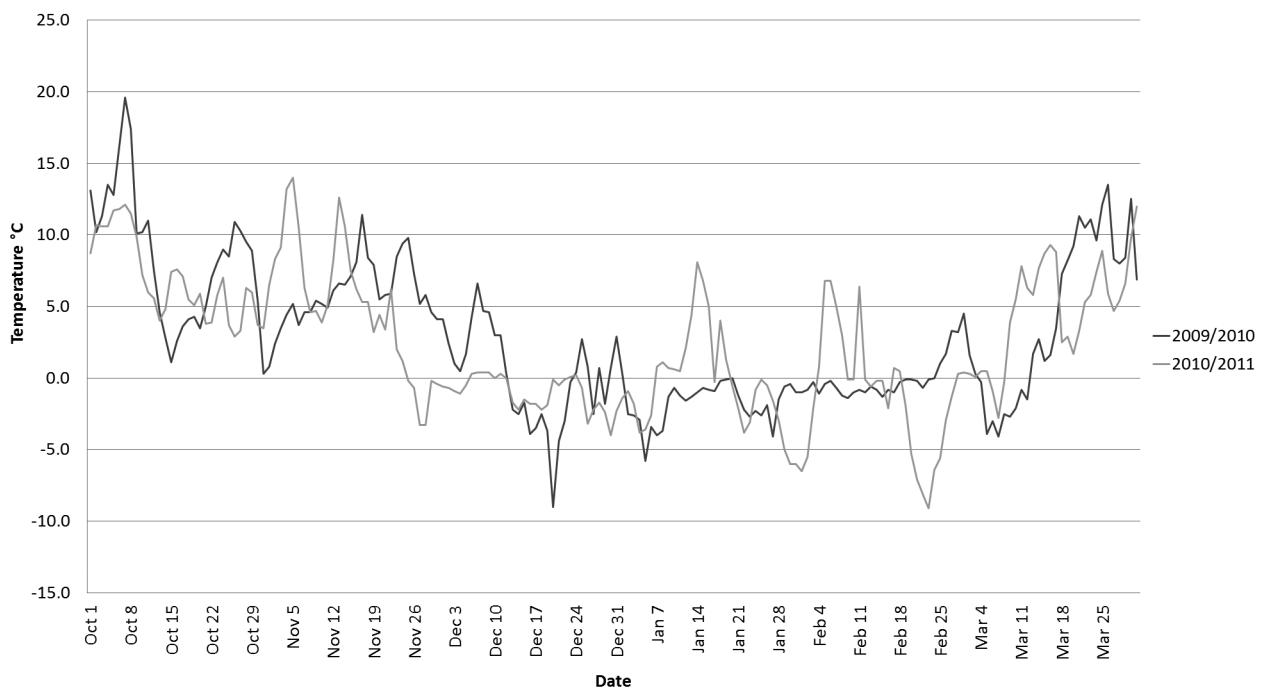
APPENDIX 1. Fluctuations in the average daily temperature from October to March recorded in two consecutive seasons (2009/2010 and 2010/2011). Temperature data were obtained from the weather station of the Crop Research Institute (Prague-Ruzyně). Visualised temperatures represent the temperature measured at the surface of the soil. The black line indicates the course of the average daily temperature recorded in winter 2009/2010 and the grey line that recorded in winter 2010/2011. In winter 2009/2010, there were 59 fluctuations around freezing (0°C), and in winter 2010/2011, there were 142 fluctuations around freezing. The number of fluctuations around freezing was counted based on hourly measurements of temperature at the surface of the soil.

3. Článek

Effects of Bait Presence and Type of Preservative Fluid on Ground and Carrion Beetle Samples Collected by Pitfall Trapping

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Environmental Entomology

OXFORD

Sampling

Effects of Bait Presence and Type of Preservative Fluid on Ground and Carrion Beetle Samples Collected by Pitfall Trapping

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Abstract

Pitfall trapping is a sampling technique frequently used by entomologists around the world. However, there exist sampling biases linked to particular trapping designs, which require investigation. In this study, we compared the effects of the type of preservative fluid (propylene glycol or formaldehyde) and the presence of fish bait in pitfall traps on the number of specimens (individuals) collected, the species richness, and the species composition of carabid (Coleoptera: Carabidae) and silphid (Coleoptera: Silphidae) beetle assemblages. Traps containing propylene glycol collected a substantially higher number of individuals of both taxa and a higher number of silphid species compared with traps containing formaldehyde. The use of fish bait in the traps increased the number of individuals collected and the number of species collected for silphid beetles but had no effect on the collection parameters for carabids. The species composition of the carabid assemblages was minimally affected by the presence of fish bait or the type of preservative fluid, whereas the fish bait had a substantial effect on the species composition of silphids. The silphid species that feed directly on vertebrate carcasses were almost completely absent in the nonbaited traps. The results suggest that pitfall traps baited with fish and containing propylene glycol as a preservative fluid are optimal for the simultaneous sampling of carabid and silphid beetles, which both provide important ecosystem services (e.g., predation of pests and decomposition of vertebrate carcasses) and are therefore interesting for ecological research.

Key words: activity-density, baited trap, catch size, sampling bias, sampling technique

Pitfall trapping is a traditional sampling method that has been used by entomologists for almost a century. One of the first studies describing the pitfall trapping technique was published by Barber (1931), which explains why the pitfall trap is called the "Barber trap" in some countries. Today, pitfall traps are commonly used in a range of studies of ground-dwelling arthropods (e.g., spiders, carabid, and staphylinid beetles), covering, among others, the areas of applied agriculture, biodiversity, autecology, and evolutionary biology (Drees et al. 2008, Kotze et al. 2011, Knapp et al. 2013, Butler et al. 2013, Baranovska et al. 2014, Knapp and Rezac 2015). On the bases of a literature survey we conducted using the Web of Science database, pitfall traps were used in more than 200 studies published in 2014. The pitfall trapping technique is also used for investigation of smaller vertebrates, e.g., mammals (dos Santos-Filho et al. 2015), lizards (Rotem et al. 2013), and amphibians (Gibbs 1998). The widespread popularity of pitfall trapping as a research technique is mainly based on its low cost and the relatively large samples that can be obtained. Moreover, pitfall trapping allows for systematic research in which the sampling effort is standardized. However, this

sampling technique has also some shortcomings that have been recognized for a long time and have been investigated in the past decades (Greenslade 1964, Adis 1979, Spence and Niemela 1994).

The majority of entomologists acknowledge that the number of animal specimens collected by pitfall traps does not only reflect the abundance (i.e., population density) of the animals in the vicinity of a particular trap but also reflects the movement of those animals and their probability of being captured by the pitfall trap itself. Arthropod activity may be significantly affected, for example, by the ambient temperature (Saska et al. 2013), or it may vary between the sexes during mating because of sex-specific behaviors (Topping and Sunderland 1992). Thus, pitfall trap users became familiar with the term "activity-density", which better describes the variable measured than the term "abundance" (Spence and Niemela 1994); nevertheless, interspecific differences in capture probabilities are still largely overlooked. For example, the evidence indicates that pitfall trap samples are biased toward larger species, which are overrepresented because of their higher probability of capture, whereas smaller species are relatively more numerous in absolute density

samples (Hancock and Legg 2012). Some species are able to hang on to the pitfall trap edge, which results in a lower capture rate, and species also differ in their ability to escape from a trap after falling into it (Halsall and Wratten 1988).

The differences observed between pitfall trap samples (e.g., catch size) are also attributable to the trap design; this complicates the comparison of data between pitfall trap studies because the design has to be taken into account to ensure correct interpretation. For example, higher catches were obtained with open traps than with traps covered by a roof (Spence and Niemela 1994), and traps made of a smoother material have been found to be more efficient (Luff 1975, Waage 1985). Arthropod samples have also been shown to be affected by trap size (Brennan et al. 1999, Luff 1975), trap color (Buchholz et al. 2010), and the preservative fluid used in the traps (Knapp and Ruzicka 2012). Many different types of preservative fluids have been used in pitfall traps for ecological research in the past decades, and the efficiency of several of them has been investigated in methodological studies (Knapp and Ruzicka 2012, Koivula et al. 2003, Lemieux and Lindgren 1999, Pekar 2002, Schmidt et al. 2006, Waage 1985, Weeks and McIntyre 1997). However, when choosing a particular preservative fluid, an entomologist must consider also its efficiency in preservation of different taxa and other issues such as environmental risks (Restani and Galli 1991, Sasakawa 2007, Jud and Schmidt-Entling 2008, Thomas 2008, Aristophanous 2010). Thus, at present, there is no widely preferred preservative fluid for use in pitfall traps in ecological research.

The trap efficiency may be affected not only by the trap design but also by the experimental design, e.g., the frequency of sample collection or the spatial distribution of traps. Sampling for short sampling intervals results in considerably higher catches than sampling for long time intervals (Schirmel et al. 2010). Higher catches found in recently installed traps could be the result of the small-scale disturbance from trap installation or the lower pool of available specimens after several days of trapping (i.e., a depletion of the local population; Digweed et al. 1995). Some authors are convinced that the effect of local depletion in combination with proper spatial distributions of traps could be used to reveal the real arthropod population densities using pitfall trapping (Perner and Schueler 2004, Shi et al. 2014). However, the densities of species obtained by the traditional pitfall trapping techniques (installation of separate traps) need to be interpreted with caution because the correlations between these densities and densities obtained by alternative sampling techniques are quite low in some cases (Hancock and Legg 2012, Spence and Niemela 1994, Topping and Sunderland 1992).

Pitfall traps are not only used in studies of ground-dwelling arthropods but also in studies of insects of decomposer communities, e.g., silphid beetles (Kocarek 2000), dung beetles (Hector Flechtmann et al. 2009), and dipterans (Goni et al. 2012). Insect decomposers are attracted to pitfall traps by carrion or dung baits that emit volatiles that the animals are able to detect (Kalinova et al. 2009, Dormont et al. 2010, Stavert et al. 2014). The efficiency of the bait may vary depending on the bait type (Rintoul et al. 2005, Marsh et al. 2013). To the best of our knowledge, there is no information on the effect of the type of preservative fluid and interaction between preservative fluid and bait on carrion beetle assemblages collected by pitfall traps. Similarly, there is only anecdotal information on the effects of bait on carabid beetle assemblages sampled using pitfall traps. Seldon and Beggs (2010) found no effect of bait in pitfall traps on the sampling efficiency of large carabid species. With sampling and analysis of several arthropod taxa in a single study becoming commonplace in ecological and conservation research, the simultaneous study of carabid and carrion beetles using

pitfall traps would seem to be an appropriate combination. The carabids are a diverse group of beetles that include many species that are beneficial for agriculture (Hůrka 1996, Kotze et al. 2011). In consideration of the existing wealth of knowledge of carabid beetle biology, the habitat specialization of some carabid species, and the high diversity of carabid species traits, these beetles are an ideal taxon for ecological studies and are frequently used as bioindicators (Rainio and Niemela 2003, Kotze et al. 2011). Carrion beetles also play an important role in ecosystem functioning, as they contribute to the decomposition of carrions (i.e., they provide a sanitary service), which is beneficial for many vertebrate populations (Matuszewski et al. 2008, Dekeirsschieter et al. 2011).

In this study, we investigated whether the pitfall trap design used for simultaneous sampling of carabid and silphid beetles affects the samples obtained. In particular, the effects of the type of preservation fluid (propylene glycol or formaldehyde) and the presence of fish bait in pitfall traps were tested in terms of the number of specimens collected, the recorded species richness, and the recorded species composition of carabid and silphid beetle assemblages across six sampling sites of two habitat types (meadow and spruce forest). We hypothesized that: 1) pitfall traps using propylene glycol will collect more specimens and more species of both investigated taxa when compared with pitfall traps using formaldehyde; 2) pitfall traps using fish bait will collect more specimens and more species of both investigated taxa when compared with traps without any bait; and 3) the difference in species composition of samples originating from baited and nonbaited traps will be driven by zoophagous carabid species and carrion beetles specialized to consume vertebrate carcasses, as these groups could be attracted by bait presence. The results of the study will be used to make recommendations on the appropriate pitfall trap design for ecological studies on carabid and silphid assemblages.

Materials and Methods

Sampling Design

Beetles were collected during June and July of 2013 in the Giant Mountains in the northeast region of the Czech Republic. Six sites were chosen to investigate the effects of the preservative fluid and fish bait on beetle assemblages collected in pitfall traps. Traps were installed in open (meadow) and forested (spruce forest) habitats, and the two habitats were replicated at three altitudes (400 m a.s.l., 700 m a.s.l., and 1,000 m a.s.l.). For details of the sampling sites, see Supp. Table 1 [online only]. Within each site, 20 pitfall traps of four trap design types were installed (five traps per trap design type). All pitfall traps comprised a 0.5-litre plastic cup with a diameter of 10 cm at the opening of the cup; cups were covered by aluminium roof held in place by three nails to prevent inundation from rainfall. The trap design types differed in the type of preservation fluid and in the presence or absence of fish bait as follows: 1) propylene glycol without bait; 2) formaldehyde without bait; 3) propylene glycol with bait; and 4) formaldehyde with bait. Propylene glycol was diluted with water (1 part propylene glycol, 3 parts water). Formaldehyde was diluted with water (1 part formaldehyde, 24 parts water). Fish bait consisted of 15 g of fresh Alaskan cod that was placed in a small plastic pot suspended beneath the roof of the trap. At each site, traps were arranged in five blocks, each consisting of four traps (one trap for each trap design type). Traps within a particular block were located ∼3 m from each other, and each block was located at least 25 m from each other. Thus, traps in a particular block shared the pool of available specimens for trapping. Traps

were operated for 6 weeks, after which samples were collected, transported to the laboratory, and stored at –20°C until processing (i.e., identification to species level). Carabid beetles were identified using the key of Hůrka (1996), and carrion beetles were identified using the key of Šustek (1981).

Statistical Analyses

Data analyses were performed only on the data originating from "complete blocks"; in other words, blocks that contained damaged or missing samples were omitted from the analyses. A portion of the traps were destroyed by large animals (e.g., deer or wild boar), whereas others were flooded by cloudbursts. The final data set comprised data from 20 complete blocks (i.e., 20 blocks × 4 trap types = 80 traps in total) distributed among the six sampling sites.

To analyze the effects of fish bait presence and the type of preservative fluid on the number of individuals and the number of species collected in the pitfall traps for each taxon, generalized linear mixed models (GLMMs) were used (Crawley 2013). A quasi-Poisson distribution of errors was applied in all models due to the overdispersion present in previously fitted models with a Poisson distribution of errors. Block identity was set as a random effect term in all the models to ensure that traps with different designs (bait presence/absence; propylene glycol/formaldehyde as preservative fluid) from a single place (i.e., traps sharing the same available specimen pool) were compared among each other. Fish bait presence, type of preservative fluid, and their interaction were set as fixed effect terms. The statistical significance of the fixed effect terms was tested using F-tests. Separate models were run for each taxon (carabid beetles and carrion beetles) and for both investigated dependent variables (number of collected individuals and recorded species richness). All univariate models were run using the software R, version 3.0.2 (R Development Core Team 2014).

Canonical correspondence analysis (CCA) was used to analyze the effects of fish bait presence and the type of preservative fluid on the species composition of carabid beetle and carrion beetle assemblages. Data were transformed logarithmically ($\log_{10}(x+1)$) prior to the analyses, and rare species were down-weighted. The forward selection procedure was applied to identify the significant terms among 1) fish bait presence, 2) type of preservative fluid, and 3) the interaction of fish bait presence and preservative fluid type. Block identity was used as a covariate to identify blocks in permutation tests, which tested for the statistical significance of the particular terms (sample exchange was only permitted within a particular block). There were 999 permutations for each randomization test. Goodness of fit of the original data matrix, i.e., the nonpermuted data matrix (species data × environmental data matrix; where environmental data are bait presence, preservative fluid, and their interaction), was compared with the fit of randomized data matrixes and tested using pseudo-F values. Pseudo-F values are analogous to F values computed in univariate regression models (for details see Smilauer and Leps 2014). Separate multivariate analyses were conducted for the carabid beetle and carrion beetle assemblages. All multivariate analyses were performed using Canoco 5.0 software (Smilauer and Leps 2014).

Results

The number of specimens collected in the pitfall traps was significantly affected by the type of preservative fluid for both taxa (carabids: $F_{1,57} = 25.00$, $P < 0.001$; silphids: $F_{1,57} = 11.02$, $P = 0.002$). Propylene glycol was more efficient in trapping than formaldehyde (Fig. 1). The presence of fish bait significantly increased the number

of carrion beetles collected ($F_{1,57} = 100.35$, $P < 0.001$; Fig. 1B); however, it had no effect on the number of carabid beetles collected ($F_{1,57} = 2.04$, $P = 0.158$). In addition, the number of carabids collected was significantly affected by the interaction of the type of preservative fluid with bait presence ($F_{1,57} = 9.56$, $P = 0.003$). The efficiency of traps containing propylene glycol was enhanced in the presence of fish meat bait for carabids (Fig. 1A). The opposite trend was observed for the number of carrion beetles collected in these traps; however, the result was not statistically significant (GLMM: $F_{1,57} = 2.75$, $P = 0.103$).

The recorded species richness was not significantly affected by the type of preservative fluid for either taxon (carabids: $F_{1,57} = 0.22$, $P = 0.639$; silphids: $F_{1,57} = 0.28$, $P = 0.599$; Fig. 2). The presence of fish bait did not significantly affect the recorded species richness of carabids ($F_{1,57} = 2.235$, $P = 0.141$; Fig. 2A), but it did significantly increase the recorded number of species of carrion beetles ($F_{1,57} = 104.24$, $P < 0.001$; Fig. 2B). The interaction of the type of preservative fluid with the presence of fish bait on species richness was marginally significant for carrion beetles ($F_{1,57} = 4.00$, $P = 0.050$). The presence of fish bait reduced the relative efficiency of propylene glycol as a preservative for collecting species of carrion beetles (Fig. 2B). There was no significant interaction between explanatory variables in terms of the recorded species richness of carabid beetles ($F_{1,57} = 1.94$, $P = 0.169$).
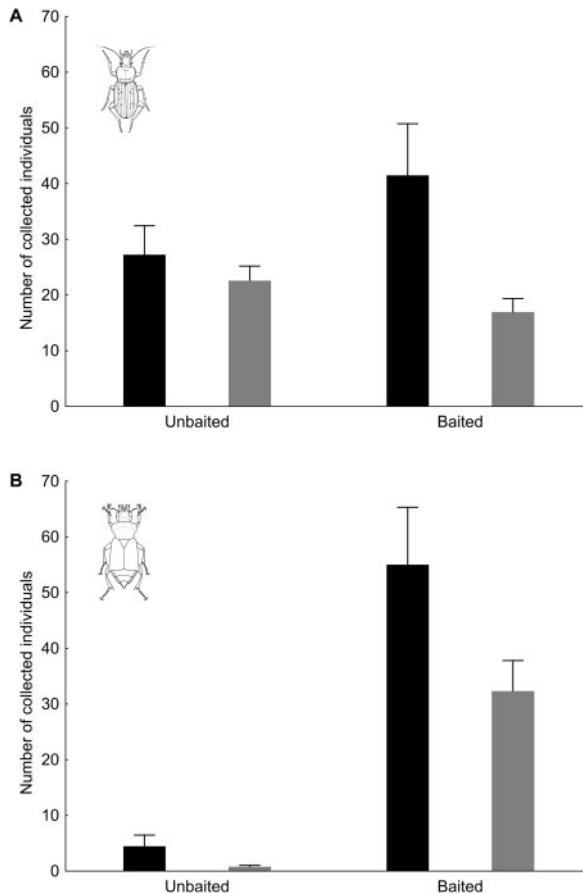
Fig. 1. Effects of bait presence and type of preservative fluid on the number of carabid and silphid beetles collected by pitfall traps. Mean values per one trap ± SEM are shown separately for (A) carabid beetles and (B) silphid beetles. Black columns represent samples from pitfall traps containing propylene glycol, and gray columns represent samples from traps containing formaldehyde.
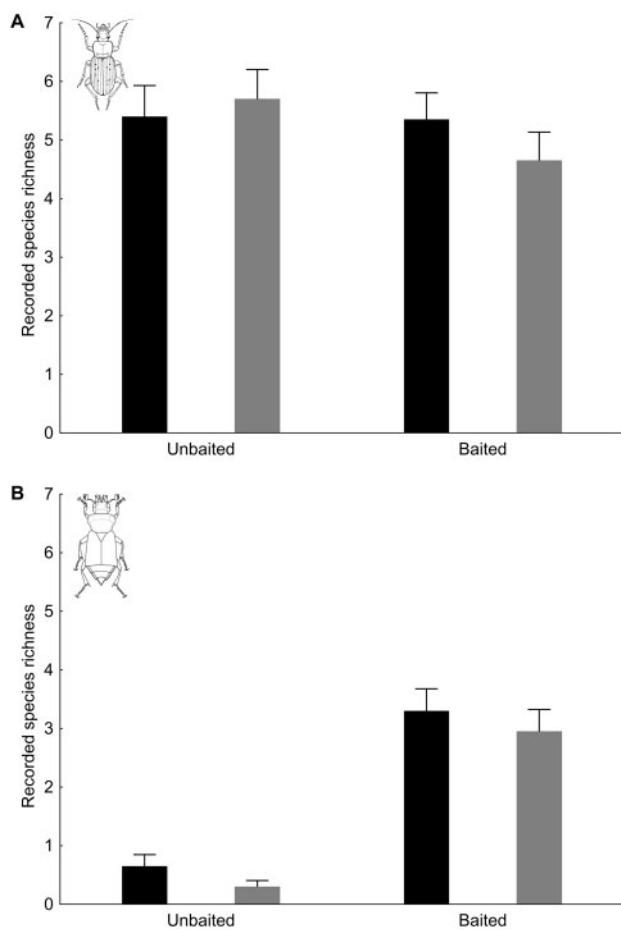
Fig. 2. Effects of bait presence and type of preservative fluid on the number of carabid and silphid species collected by pitfall traps. Mean values per one trap ± SEM are shown separately for (A) carabid beetles and (B) silphid beetles. Black columns represent samples from pitfall traps containing propylene glycol, and gray columns represent samples from traps containing formaldehyde.

The recorded species composition of carabid beetle assemblages was significantly affected by the presence of fish bait (pseudo-$F = 1.9$, $P = 0.008$) and by the type of preservative fluid (pseudo-$F = 1.9$, $P = 0.008$). However, the percentage of variance in species composition explained by trap design was low (6.3%; Fig. 3A). The interaction between the type of preservative fluid and fish bait presence did not significantly affect the species composition of carabid beetles (pseudo-$F = 1.2$, $P = 0.284$). The species composition of carrion beetles was only affected by the presence of fish bait (pseudo-$F = 13.8$, $P = 0.001$; Fig. 3B). The samples with no recorded silphid species originated always from the nonbaited traps (Fig. 3B). The species composition of carrion beetles was not significantly affected by either the type of preservative fluid (pseudo-$F = 0.9$, $P = 0.570$) or the interaction between fish bait presence and the type of preservative fluid (pseudo-$F = 1.3$, $P = 0.248$). A list of the carabid and carrion species including the numbers of individuals collected by the particular pitfall trap design type is included in the Supp. Table 2 [online only].

Discussion

The results of this study confirm previous findings that the pitfall trap design (e.g., type of preservation fluid used in the trap) can have an effect on the recorded species composition, the recorded species richness, and the number of collected specimens of ground-dwelling arthropods. Our findings suggest that propylene glycol is more efficient for collecting carabid and silphid beetles than formaldehyde. Our results suggest that the presence of fish bait has only a limited effect on the number of carabid beetles collected but has a strong effect on the number of silphid beetles collected. To the best of our knowledge, this is the first study in which an interaction between the type of preservation fluid and the presence of bait was found to have a significant effect on the number of carabid beetles collected and the number of silphid beetle species recorded. Based on published studies, significant effects of interactions between different trap properties (e.g., trap size, presence of funnel, preservative fluid) on arthropod collection parameters are uncommon and the effects of trap properties are thought to be additive (Koivula et al. 2003, Lange et al. 2011, Knapp and Ruzicka 2012). The presence of fish bait increased the number of carabids in traps with propylene glycol but decreased the number of carabids in traps with formaldehyde. A possible explanation for these trends is that the species or individuals attracted by the bait are those that substantially rely on olfactory cues for their orientation and are thus more sensitive to olfactory cues emitted by preservative fluid, i.e., propylene glycol (or may be volatiles from animals already present in traps with propylene glycol) is relatively more attractive for these beetles and formaldehyde is more deterrent for them. When compared with the nonbaited traps filled with formaldehyde, the higher species richness of silphids recorded for the nonbaited traps containing propylene glycol is probably caused by a difference between the two fluids in their ability to prevent the decomposition of organic tissue. Formaldehyde is well-known for its exceptional ability to prevent animal tissue decomposition, whereas propylene glycol (and similar substances) is less effective (Fox et al. 1985). Thus, propylene glycol probably enables the emission of some volatiles as the animal tissue decomposes (Kalinova et al. 2009). Both carabid and silphid beetles use olfactory cues (Kielty et al. 1996, Kalinova et al. 2009). The insignificant effect of the type of preservative fluid on silphid species richness in fish-baited traps may be a result of the strength of the olfactory cues generated by the bait, which may override the olfactory cues generated by the preservative fluids and varying rate of trap content decay in different preservative fluids. Bait that is not immersed in a preservative fluid decomposes at a higher rate and probably produces greater amounts of semiochemicals when compared with decaying trap content that is immersed in a preservative fluid.

The strong effect of the type of preservative fluid on the number of beetles collected, the species richness, and the species composition in terms of carabid and silphid assemblages can probably not be attributed solely to a difference in the attractiveness or repellence of the particular preservative fluid. The preservative fluids could, for example, also differ in their effectiveness in killing arthropods that have fallen into the pitfall traps (i.e., the time it takes for them to die), which may play a role in the trap escape rate (Pekar 2002). There is evidence that beetles are able to escape from pitfall traps and that the escape rate increases with the amount of time spent alive within the trap (Halsall and Wratten 1988). It is important to note that particular preservative fluids also differ in their ability to harm wildlife, e.g., nontarget animals could be exposed to the preservative fluid (LaKind et al. 1999, 1991). Thus, a decision on which preservative fluid to use should not be driven solely by its trapping efficiency. Fortunately, of the two fluids tested here, the more efficient one (propylene glycol) is also less toxic to mammals (LaKind et al. 1999, Restani and Galli 1991).
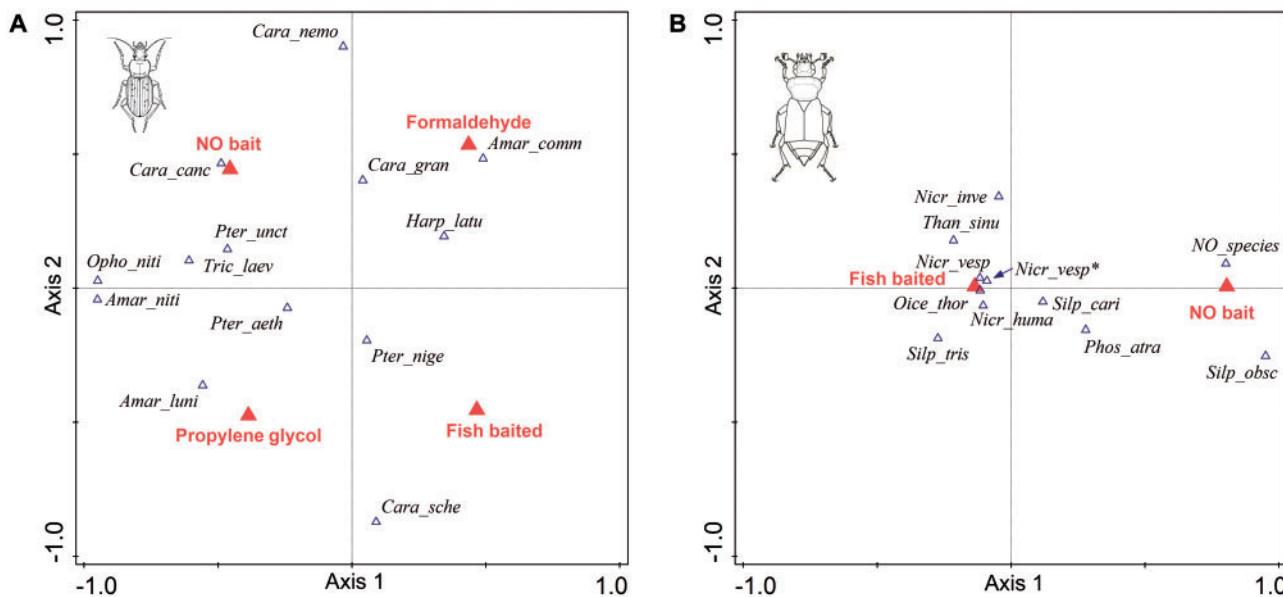
Fig. 3. Effects of bait presence and type of preservative fluid on the species composition of carabid and silphid assemblages collected by pitfall traps. The additive effects of bait presence and type of preservative fluid explained 6.3% of the variability in the species composition of carabid assemblages (A). The effect of bait presence explained 36.9% of the variability the species composition of silphid assemblages (B). The abbreviations of the species names consist of the first four letters of the genus followed by the first four letters of the species. To allow for the inclusion in the analysis of samples that did not contain any silphid species, the denomination "NO_species" was used. * indicates the species *Nicrophorus vespilloides* Herbst, 1784, which has the same abbreviation as *Nicrophorus vespillo* (Linnaeus, 1758). For the full names of the recorded species, see the Supp. Table 2 [online only].

Although fish bait attracted some species into the pitfall traps, it is uncertain whether it could also act to deter other species. We hypothesized that species composition of assemblages sampled by baited and nonbaited traps will differ in relative abundance of species belonging to particular food guilds. Zoophagous carabids should be attracted by fish bait, whereas phytophagous carabids should not (Kotze et al. 2011). Similarly, some carrion beetle species are more highly specialized to feed on vertebrate carcasses than others (Ikeda et al. 2007). On the bases of our results, it was not possible to assess the effects of the bait on food specialization of carabid beetles. There were similarly low catches of granivorous species (e.g., genera *Amara* and *Ophonus*) and of polyphagous species (e.g., genus *Harpalus*) from both baited and nonbaited traps. However, there is an effect of the level of food specialization on the silphid species composition found in both baited and nonbaited traps. Species from the genera *Silpha* and *Phosphuga* have also been recorded in nonbaited traps and they are not typical carrion feeders. *Silpha* spp. are predators of other arthropods (frequently occurring on mammal carcasses), and *Phosphuga atrata* (Linnaeus, 1758) feeds primarily on snails (Ikeda et al. 2007). The presence of silphid species that generally feed directly on vertebrate carcasses (e.g., genera *Nicrophorus* and *Thanatophilus*) was limited almost completely to traps baited with fish meal (Matuszewski et al. 2008, Dekeirsschieter et al. 2011).

In conclusion, this study confirmed that sampling both carabid and silphid beetles is affected by the trap design. Based on its greater efficiency (e.g., collection of more individuals) and a lower toxicity to wildlife, we recommend the use of propylene glycol over formaldehyde as the preservative fluid of choice in pitfall studies. We also conclude that fish-baited traps provide similar data in terms of carabid assemblages when compared with nonbaited traps. In contrast, the use of fish bait substantially enhances the sampling efficiency of the pitfall traps for silphid beetles. Thus, traps baited with fish and containing propylene glycol as a preservation fluid are ideal when

simultaneous sampling of carabid and silphid assemblages is desirable. Carabids are important predators of various arthropod species and are frequently used as bioindicators, whereas silphids play an important role in the decomposition of vertebrate carcasses. Simultaneous sampling of these two taxa that provide important ecosystem services thus seems to comprise a meaningful taxa combination for future ecological research that will rely more seriously on a multi-taxa approach.

Acknowledgments

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4. Manuskript článku

Steep converse Bergmann's cline in a carrion beetle: between and within population variation in body size along an elevational gradient

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Manuskript v recenzním řízení v Journal of Zoology

1   **Steep converse Bergmann's cline in a carrion beetle: between and within**

2   **population variation in body size along an elevational gradient**

3

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16   Short Title: Between and within population variation in body size

17   <u>Abstract</u>

18   Body size is an integrative trait with substantial fitness consequences in animals. Geographical clines

19   in body size have fascinated biologists because of their potential to provide insight into the

20   mechanisms governing local adaptation and phenotypic plasticity. In this study, we investigated

21   between- and within-population variation in body size of *Silpha carinata* (Coleoptera: Silphidae) along

22   elevation gradients in Central Europe. To distinguish between local adaptation and phenotypic

23   plasticity as potential sources of altitudinal clines in body size, a common garden experiment was

24   performed. Body size of *S. carinata* sharply decreased with increasing altitude in both genders,

25 whereas sexual size dimorphism did not change with altitude. Higher altitude females produced

26 smaller eggs than those from lower altitude. This size difference persisted to the very end of the larval

27 period. Unfortunately, only few offspring survived till adulthood and thus precise analysis of offspring

28 adult sizes was unfeasible, but between population differences in body size seems to persist to

29 adulthood. By contrast, the degree of intrapopulation variation in body size did not change with

30 altitude. The observed converse Bergmann's cline in *S. carinata* fits well with what has been predicted

31 for large, carnivorous, univoltine species. Until now, investigation of intrapopulation variation in body

32 size is rare in insects, and future complementary studies focused on this issue are highly needed.

33

Introduction

Body size is an integrative trait (Wikelski & Romero, 2003) that affects many life-history traits as well as behaviour and physiology (e.g. Chown & Gaston, 2010 for insects; Yom-Tov & Geffen, 2011 for vertebrates). Outstanding pivotal position of body size in evolutionary biology and ecology research is given in particular by close relationships between body size and fitness components. For example, larger females are more fecund (Sota, 1985; Honěk, 1993; Marshall *et al.*, 2013), whereas larger males often have a higher mating success (Savalli & Fox, 1998). In addition, larger sized organisms are frequently better able to cope with stressful environments, e.g., food and water limitation or survival at low temperatures during overwintering (Blanckenhorn *et al.*, 2007; Kingsolver & Huey, 2008; Kovacs & Goodisman, 2010; Teder *et al.*, 2010). However, insects do not always grow to a maximal possible size, likely due to counterbalancing selection favouring smaller size, e.g. due to viability costs associated with growing larger (Blanckenhorn, 2000). All else being equal, larger body size usually requires a longer development time and higher food consumption during juvenile stages, which may lead to increased risk of predation (Nylin & Gotthard, 1998). Moreover, benefits of large body size could be limited under certain environmental conditions (Gotthard, Berger & Walters, 2007; Teuschl, Reim & Blanckenhorn, 2007). Thus, variation in optimal body size patterns along environmental gradients are expected within species.

First studies about spatial patterns in biological traits (i.e. ecogeographical rules) were published in the 19th century, and investigation of geographic gradients (i.e. clines; Endler, 1977) in body size has a long conceptual (sometimes anecdotal) history (Blackburn, Gaston & Loder, 1999; Angilletta *et al.*, 2004). The most well-known ecogeographical rule is undoubtedly associated with a seminal work published by Carl Bergmann, describing interspecific variation of body size along latitudinal gradients in endotherms, known now as Bergmann's rule (Bergmann, 1847). The rule was originally defined for endotherms, with the underlying mechanism proposed to be the lower surface to volume ratio in larger animals, resulting in lowered relative heat loss at low ambient temperatures. However, the validity of this rule was later tested also at the intraspecific level (James' rule, cf.

63   Blackburn *et al.*, 1999) and for ectotherms (Ray, 1960; Blanckenhorn & Demont, 2004; Stillwell, Morse

64   & Fox, 2007; Jensen & Mayntz, 2012; Shelomi, 2012). Respective insect responses at the intraspecific

65   level are mixed, including species following Bergmann's cline, species without a significant change in

66   their body size with latitude/altitude as well as species following converse Bergmann's cline

67   (Blanckenhorn & Demont, 2004). Despite the fact that proximate mechanisms generating these

68   intraspecific clines are not completely known, several candidate explanations exist: e.g. direct effects

69   of temperature during preimaginal development (Kingsolver & Huey, 2008), local food availability

70   (Teuschl *et al.*, 2007) or season length (Chown & Klok, 2003; Stillwell *et al.*, 2007). Recently, Horne,

71   Hirst & Atkinson (2015) and Zeuss, Brunzel & Brandl (2017) emphasized that an increase in ectotherms'

72   body size at lower developmental temperatures (i.e. temperature-size rule; Atkinson & Sibly, 1997) is

73   not always the main driver of geographical size clines in terrestrial arthropods and that season length

74   - and thus voltinism - is likely a more frequent determinant.

75   Geographical variation in intraspecific body size could be a product of reaction to abiotic

76   environmental conditions or a result of biological interactions with other organisms present at a given

77   site (Angilletta & Dunham, 2003; Yom-Tov & Geffen, 2011). Exact mechanisms determining

78   intraspecific geographical variation in insect body size are rarely known and often problematic to

79   reveal in many cases. Body size of insects originating from a given population could be a result of local

80   adaptation and/or a result of developmental phenotypic plasticity (Stillwell, 2010; Purcell *et al.*, 2016).

81   Evidence for that latitudinal or altitudinal clines in body size have genetic basis can be gained from

82   common garden experiments (Stillwell, 2010; Tsuchiya *et al.*, 2012; Purcell *et al.*, 2016). For example,

83   Tsuchiya *et al.* (2012) found that body size variation in the carabid beetle *Carabus tosanus* is mainly

84   explained by local adaptations (i.e. genetic differences), whereas phenotypic plasticity contributes only

85   a little to body size variation. However, examples of insect species in which developmental plasticity

86   plays an important role in shaping geographical clines in body size are also frequent (e.g., Stillwell &

87   Fox, 2009; Purcell et al., 2016).

88   In insects, body size frequently differs between males and females (sexual size dimorphism,

89   SSD), with females being the larger sex in most species (Teder & Tammaru, 2005; Blanckenhorn *et al.*,

90   2007; Chown & Gaston, 2010). The difference in body size between sexes is likely caused by different

91   net selection pressures acting on males and females (Blanckenhorn, 2000). Thus changes in abiotic

92   conditions and biotic interactions along geographical gradients could result in different body size clines

93   in males and females (Stillwell *et al.*, 2007; Laiolo, Illera & Obeso, 2013). Species identity may influence

94   the sensitivity of females or males to the environmental conditions (Blanckenhorn *et al.*, 2006; Stillwell

95   *et al.*, 2007; Laiolo, Illera & Obeso, 2013), however males generally seem to produce steeper

96   geographical clines than females (Blanckenhorn *et al.*, 2006). A possible explanation lies in canalization

97   of female body size, which could be under higher selection pressure than male body size (Fairbairn,

98   2005). Interestingly, however, a meta-analysis of mostly laboratory case studies performed by Teder

99   & Tammaru (2005) showed an opposite pattern, with females being generally more variable in body

100   size when exposed to various environmental conditions. Responsible for above mentioned finding

101   could be sex specific phenotypic plasticity (Stillwell *et al.*, 2010), as case studies included in meta-

102   analysis by Teder & Tammaru (2005) were based on animals originating from a single population and

103   thus, solely their phenotypic plasticity was investigated.

104   Interestingly, only a few studies have investigated geographical clines in the most fine-scale

105   variation in body size, i.e. variation between individuals within a given population. Chown & Gaston

106   (2010) identified that there is a limited information on body size distributions within insect populations

107   and information on geographical clines in these distributions are extremely rare. Some data are also

108   hidden within published studies as Figure 5 in Laiolo et al. (2013). However, recently, a growing interest

109   is observed for this type of studies (e.g. Gouws, Gaston & Chown, 2011; Mega, 2014; Bai *et al.*, 2016).

110   A meta-analysis by Teder, Tammaru & Esperk (2008), based mainly on laboratory case studies, showed

111   that, within species, relative variation in body size is lower in larger insects, in correlation with an

112   increase in environmental quality. Extrapolating these data to predict geographical clines in

113   intrapopulation variation in body size is not straightforward, because in field conditions such patterns

114   are likely to result from a combined action of a diverse set of environmental factors. For example, the

115   study by Rauter *et al.* (2010) showed that a higher intraspecific competition leads to higher relative

116   variations in the larval body mass of the necrophagous beetle *Nicrophorus pustulatus*. Importantly,

117   information about possible differences between sexes in intrapopulation variation in body size along

118   geographical gradients are largely missing.

119   In the present study, we use the carrion beetle *Silpha carinata* to investigate both between-

120   and within-population variation in body size. This species is a highly appropriate model for such study,

121   as it is known for its exceptional variation in body size across its distribution range (Šustek, 1983). First,

122   we examined if adult body size follows an altitudinal cline, and if sexes differ in their responsiveness

123   to elevation. Further, we investigated if intrapopulation variation in body size change with altitude and

124   if there are systematic differences in intrapopulation variation in body size between males and

125   females. Finally, to disentangle local adaptations from phenotypic plasticity, we realized a common

126   garden experiment using parental pairs originating low-altitude and high-altitude sites.

127   Materials and Methods

128   *Study species*

129   *Silpha carinata* Herbst, 1783 is a Palearctic species that is spread in almost whole temperate Eurasia,

130   from lowlands to mountains. *S. carinata* is a habitat generalist that is able to live in various habitats

131   e.g. forest edges, shrub formations and various grasslands, from very dry to very wet habitats. Larvae

132   of this species are predators of smaller arthropods and adults consume various decomposing remains

133   of plants and animals tissues as well as hunt for smaller arthropods (Šustek, 1981; see also evidence

134   for some other *Silpha* species in Ikeda *et al.*, 2007). *S. carinata* is a highly variable species regarding its

135   body size, body shape and colouration. Such high variability led to the description of a huge number

136   of subspecies, which were later merged into three subspecies by Šustek (1983). However, currently,

137   no subspecies in *S. carinata* are distinguished (Růžička, 2002). Some basic patterns of intraspecific

138   variation in *S. carinata*, e.g., decreasing body size with increasing altitude, were described by Šustek

139 (1983). In this study, we go further and investigate intraspecific variation in body size of *S. carinata* in

140 more detail.

141

142 *Body size measurements*

143 Structural body size of all specimens analysed in the present study was measured using digital macro

144 photography followed by an image analysis. Adults, larvae and eggs of *S. carinata* were photographed

145 using Canon 600D camera with Canon EF-S 60mm f/2.8 Macro USM lens. Minimal focusable distance

146 was used in all cases, resulting in 1:1 magnification in all pictures taken. Pictures of all measured

147 specimens were then analysed using ImageJ program (Ferreira & Rasband, 2012). Elytron length and

148 pronotum width were measured for each adult, first tergit width was measured for each analysed 3rd

149 instar larva and two perpendicular diameters were measured for each egg. As elytron length and

150 pronotum width were tightly correlated measures of body size in our dataset, we used only elytron

151 length as a measure of structural body size in following analyses. Two measures of egg diameter for a

152 particular egg were averaged into a single value for the purpose of analyses.

153

154 *Altitudinal patterns in body size of Central European Silpha carinata imagoes*

155 Using a more comprehensive material from Central Europe, we first re-evaluated validity of converse

156 Bergmann's rule, reported for adult *S. carinata* by Šustek (1983) based on animals originating from

157 whole distribution range (comparing animals from geographically very distant sites). Moreover, in

158 parallel, we investigated possible sex-specific responses of body size to altitude. Adult individuals used

159 for the investigation of basic altitudinal patterns in body size of *S. carinata* originated from private

160 collections of two Czech entomologists focused on studying taxonomy and faunistics of carrion beetles

161 (Jan Růžička and Jan Schneider). Just specimens originating from Czech Republic or Slovakia were

162 measured. Specimens without clearly labelled place of collection were omitted to maximize credibility

163 of our dataset. The oldest specimen was collected in 1883, but majority of specimens were collected

164 in late 20th century. In total, 431 individuals (252 females, 179 males) collected from sites spanning

165 altitudinal range from 111 to 1770 m a.s.l. were measured (for details see raw data included in

166 Supporting Information file S2 Dataset). If more than one specimen per sex was present for particular

167 site (a series of animals was collected in the field and preserved), mean elytron length was computed

168 for a given site and a given sex. The final dataset contained data on 93 unique sites for males and 111

169 sites for females.

170 Altitudinal variation in body size was tested by using a generalized linear model (GLM) with a gamma

171 distribution of errors and log link function, because the distribution of errors deviated from a normal

172 distribution. Elytron length was included as a response variable in the model; sex, altitude and

173 interaction between sex and altitude were included as independent variables.

174

175 *Local adaptation vs. phenotypic plasticity*

176 To distinguish between two possible causes of observed altitudinal cline in body size of *S. carinata*

177 (local adaptation or phenotypic plasticity), we performed a laboratory rearing experiment under

178 standardized conditions. Parental individuals of *S. carinata* were collected at two sites in Czech

179 Republic - a lowland site Srbsko (250 m a.s.l., 49°55'53.8"N 14°08'18.9"E) and a mountain site Praděd

180 (1400 m a.s.l., 50°05'00.1"N 17°13'54.4"E) - in late May and early June 2015. Live pitfall traps, emptied

181 each second day, were installed in Srbsko and individual collection using aspirators was employed in

182 Praděd (because of technical complications linked to pitfall trapping on this site). Live adults were

183 transported to the laboratory and parental pairs were established. Each parental pair (one female and

184 one male from the same population) was placed to a plastic transparent container of ca. 13x13x8.5 cm

185 in size. Finally, 12 pairs from Srbsko and 13 pairs from Praděd were selected for the experiment and

186 these beetles were held in a climatic chamber set to a photoperiod of L16/D8, 26 °C during the light

187 phase, 16 °C during the dark phase and relative humidity ca. 60 %). Plastic containers were filled with

188 a ca. 1 cm thin layer of loam soil as substrate. Small pieces of Alaskan cod and sliced meal worm larvae

189 were added as food and water was added in modified Eppendorf tubes stopped with cotton wool, both

190 ad libitum. Eggs laid by parental females were collected every second day when food and water were

191 renewed. Eggs were immediately photographed and were put to a plastic container (max. 8

192 eggs/container) similar to these used for parental pairs. Containers were kept in a climatic chamber

193 under the same conditions as parental pairs. After egg hatching, food and water were added and

194 renewed on each second day. Unfortunately, many of larvae died before pupation so we measured

195 the last (3rd) instar larvae that remained alive (some earlier died unmeasured). Our final dataset

196 included size measures of 5 adult offsprings, 40 (25 Srbsko, 15 Praděd) 3rd instar larvae and 186 eggs

197 (114 Srbsko, 72 Praděd). After finishing the experiment, parental pairs were killed by freezing and

198 photographed for body size measurements. Complete raw data are included in the Supplementary

199 Information (S3 dataset). Differences in body size among parental populations were analyzed using

200 GLM with a gamma distribution of errors and a log link function, with sex as a covariate and population

201 as an independent variable. The effect of parental origin (population) on egg and larval structural size

202 was analysed using generalized linear mixed models (GLMMs) with a gamma distribution of errors and

203 a log link function. Egg diameter or first tergit width of larvae were used as response variables, parental

204 origin (population) as an independent variable and parental pair identity as a random effect. In

205 addition, we were interested in the net effects of mother structural size and origin (population) in

206 determining egg and larval sizes, i.e., whether larger females within particular population produce

207 larger eggs/larvae than smaller females. To investigate this issue, GLM with a gamma distribution of

208 errors and a log link function were applied. In the first model, mother size (elytron length) was used as

209 a covariate and origin (population) as an independent variable. In the second model, origin

210 (population) was used as a covariate and mother size as an independent variable.

211

212 *Intrapopulation variation in body size of S. carinata*

213 To investigate basic patterns of intrapopulation variation in body size of *S. carinata*, large series of

214 animals originating from different populations were measured. Specifically, we were interested

215 whether intrapopulation variation in body size varied systematically with altitude and whether

216 variation in body size differs between sexes within a given population. In total, 13 populations

217   originating from Poland and Czech Republic ranging from 250 to 1400 m a.s.l. were investigated (for

218   details see Supplementary Information S4 dataset). At least 10 specimens from each sex had to be

219   measured for a given population to be included in our intrapopulation analyses. Polish populations

220   were sampled using unbaited pitfall traps filled with formaldehyde as a preservation fluid and Czech

221   populations were sampled using pitfall traps baited with fish and filled with propylene glycol as a

222   preservation fluid (for details see Knapp, Baranovská & Jakubec, 2016). To enable proper comparisons

223   of interpopulation variation in body size among various populations differing in their mean body size,

224   intrapopulation variation was standardized by dividing elytron length of each specimen by population

225   mean. Standardization was made separately for males and females within a given population. Then a

226   variance based on standardized data was computed for each particular population and sex

227   combination. The effect of altitude on intrapopulation variation in body size was analysed using an

228   analysis of covariance (ANCOVA) with sex as a covariate and altitude as an independent variable. To

229   test for systematic sex differences in intrapopulation variation in body size, a paired t-test was applied.

230   Intrapopulation variation of males and females originating from the same population was compared

231   with each other (males and females from the same population forming a pair).

232

233   Results

234   *Altitudinal patterns in body size of Central European* Silpha carinata *adults*

235   Altitude significantly affected structural body size (elytron length) of *S. carinata* (GLM: $F_{1, 203}$ = 206.9, P

236   < 0.001). Adults originating from higher altitudes were smaller than these from lower altitudes (Figure

237   1). There was also a significant difference in structural body size between sexes (GLM: $F_{1, 203}$ = 36.9, P

238   < 0.001), with females being a larger sex (Figure 1). Difference in structural body size between sexes

239   did not change along the altitudinal gradient, i.e., interaction between altitude and sex was not

240   significant (GLM: $F_{1, 203}$ = 0.37, P = 0.54; Figure 1).

241

242   *Local adaptation vs. phenotypic plasticity*

243 Body size of parents, used for breeding of offspring for laboratory experiment, differed significantly

244 between source populations (GLM: $F_{1, 47}$ = 345.2, P < 0.001). Adults collected in high-altitude site

245 (Praděd) were significantly smaller than those collected in low-altitude site (Srbsko; Figure 2a). Eggs

246 produced by females originating from high-altitude site were significantly smaller than eggs produced

247 by females originating from low-altitude site (GLMM: $F_{1, 17}$ = 52.8, P < 0.001; Figure 2b). There was also

248 a significant difference in structural body size (tergit width) of 3rd instar larvae growth from eggs

249 produced by parents from different populations (GLMM: $F_{1, 13}$ = 19.3, P < 0.001). Offspring of low-

250 altitude site individuals were larger than offspring of parents from high-altitude site during 3rd larval

251 instar stage (Figure 2c). Unfortunately, only 2 daughters and 3 sons were reared till adulthood,

252 precluding any statistical analyses. Interestingly, daughters of low-altitude site mothers were about 9

253 % smaller than their mothers whereas daughter of high-altitude site mother was about 14 % larger

254 than her mothers. However, a difference between populations still persisted in adult offspring and

255 daughters of low-altitude site mothers were about 14 % larger than daughter of high-altitude site

256 mother.

257   No significant effect of mother size on egg size was observed within populations (GLM: $F_{1, 190}$ =

258 0.01, P = 0.93). However, there was a highly significant difference in egg size between populations

259 (mother's size used as covariate; GLM: $F_{1, 190}$ = 12.4, P < 0.001; Supplementary Information S1 Figure).

260

261 *Intrapopulation variation in body size of S. carinata*

262 Altitude did not significantly affect intrapopulation variability in body size in *S. carinata* (ANCOVA: $F_{1,}$

263 $_{23}$ = 0.41, P = 0.53; Figure 3). There was a trend towards higher intrapopulation variation in males

264 compared to females, but the effect was marginally nonsignificant (paired t-test: t = -1.74, df = 12, P =

265 0.11; Figure 3).

266

267 Discussion

268   We found that *S. carinata* follows the converse Bergmann's cline along altitudinal gradient within

269   geographically limited area, which is in agreement with the previous findings of Šustek (1983) made

270   on a dataset based on specimens from geographically distant areas. Newly, we analysed also a change

271   in sexual size dimorphism along an altitudinal gradient. The response of males and females to altitude

272   seems to be similar and thus no significant changes in sexual size dimorphism were observed. As shown

273   by our common garden experiment, altitudinal clines in *S. carinata* are at least partially caused by a

274   local adaptation. Interestingly, no significant change in intrapopulation variation in *S. carinata* body

275   size was observed along altitudinal gradient and there is no significant difference between body size

276   variation between males and females from the same population, but male's body size tend to vary a

277   little bit more.

278   Many insect species show some variation in body size along geographic gradients, whereas

279   both increases and decreases in body size with increasing altitude/latitude are known (Blanckenhorn

280   & Demont, 2004). Increasing altitude or latitude could represent not only a gradient of temperature

281   experienced by given populations, but also a gradient of shortening season length. Large terrestrial

282   univoltine species are supposed to suffer from limited time for their development more than smaller

283   multivoltine species which can compensate for reduced season length by lowered number of

284   generations (Blanckenhorn & Demont, 2004; Horne *et al.*, 2015; but see Kivelä *et al.* 2011). A similar

285   decrease of body size with increasing altitude as in *S. carinata* has been observed in another large and

286   univoltine carrion beetle, *Silpha longicornis* (Nishikawa, 2010). An alternative explanation to shortened

287   season could be a varying food availability or quality along an altitudinal gradient (Stillwell *et al.*, 2007).

288   Ikeda *et al.* (2012) found that body size of a large ground beetle, *Carabus tosanus*, decreased with

289   increasing altitude and they attributed the decline in body size to lowered abundance of earthworms

290   at higher altitudes. Sexual size dimorphism has been proposed to vary between populations in several

291   insect species (e.g. Blanckenhorn *et al.*, 2006; Stillwell & Fox, 2009; Laiolo *et al.*, 2013; Levy & Nufio,

292   2015), however the absence of geographical variation in sexual size dimorphism has also been

293   repeatedly reported (Blanckenhorn *et al.*, 2006; Eweleit & Reinhold, 2014; Levy & Nufio, 2015). Both

294 local adaptations as well as sex specific phenotypic plasticity could be responsible for variation in

295 sexual size dimorphism between populations (Stillwell *et al.*, 2010), however at least sex specific

296 phenotypic plasticity seems to be less frequent in beetles than in other insect orders (Teder &

297 Tammaru, 2005). This kind of phylogenetic constrain could be particularly responsible for absence of

298 altitudinal cline in sexual size dimorhism in *S. carinata* observed in this study.

299   Two distinct mechanisms could generate geographical clines in body size (Stillwell, 2010). As

300 environmental conditions (e.g. temperature, growth season length or humidity) frequently show

301 systematic changes along altitudinal or latitudinal gradients, body size change could be a plastic

302 response to experienced conditions, i.e. phenotypic plasticity. Such plastic responses could be even

303 non-adaptive (Ghalambor *et al.*, 2007). Alternatively, variation in body size could be a result of local

304 adaptations, when body size optimizing fitness differs between sites (Kawecki & Ebert, 2004).

305 Mechanisms generating altitudinal clines in insects have been investigated for several species using

306 common garden experiments (e.g. Karl, Janowitz & Fischer, 2008; Stillwell & Fox, 2009; Tsuchiya *et al.*,

307 2012; Keller *et al.*, 2013; Okuzaki, Sugawara & Sota, 2015; Sniegula, Golab & Johansson, 2016). In

308 general, published studies have confirmed the existence of local adaptation in body size, whereas only

309 a part of them have tried to investigate possible effects of phenotypic plasticity (i.e., common garden

310 experiment with several treatments; Karl *et al.*, 2008; Stillwell & Fox, 2009; Tsuchiya *et al.*, 2012;

311 Sniegula *et al.*, 2016). In this study, we provide evidence that altitudinal variation in carrion beetle

312 *Silpha carinata* is also, at least partly, caused by local adaptation: size of eggs and larvae reared under

313 standardized laboratory conditions differed between populations originating from high and low

314 altitudes. Smaller egg sizes at higher altitudes have been observed also in non-insect ectotherms in

315 nature, e.g. *Rana sylvatica (Davenport & Hossack, 2016)*. Smaller egg size is probably a way how to

316 produce a similar number of offspring with a limited energy budget. Interestingly, Sniegula *et al.* (2016)

317 found that smaller dragonflies from higher latitudes produced larger eggs than larger dragonflies from

318 lower latitudes, while the pattern of larval size was already consistent with this observed for adults,

319 i.e., smaller larvae were found at higher latitudes. Egg size does not seem to be limited by female size

320 per se, which is also confirmed by our analysis, showing that larger females within a particular

321 population do not produce larger eggs than smaller females. Unfortunately, high mortality during late

322 larval and pupal stages in our experiment precludes a rigorous comparison of adult body sizes reached

323 in nature with those reached under standardized laboratory conditions. Based on a few successfully

324 reared adults, differences between populations persisted after rearing in the laboratory, but seem to

325 be smaller than in the nature, i.e., phenotypic plasticity could be also partly responsible for the

326 altitudinal clines observed in nature.

327   In the last part of our study, we investigated within population variation in body size of *Silpha*

328 *carinata*. This issue seems to be surprisingly unexplored in insects (Gouws *et al.*, 2011). Studies directly

329 focused on geographical clines in intrapopulation variation in body size are almost missing and some

330 data are published as by-product of investigation of geographical variation in mean body size (e.g.

331 variances shown in Figure 7 in Chown & Gaston, 2010; Figure 5 in Laiolo *et al.*, 2013). We found no

332 significant relationship between altitude and relative intrapopulation variation in body size. This

333 observation is partly surprising, as one can expect that more stressful environmental conditions, e.g.

334 limited resources at higher altitudes, could cause an altitudinal cline in intrapopulation variation. When

335 resources are limited, less successful individuals could be penalized relatively more than under

336 conditions of resource surplus, as shown by Rauter *et al.* (2010) for *Nicrophorus pustulatus* larvae. Such

337 expectation is also supported by the meta-analysis performed by Teder *et al.* (2008) showing that

338 relative variation in body size in insects tends to increase with decreasing environmental quality, being

339 highest in most stressful conditions. In addition, we found a marginally nonsignificant trend towards

340 higher relative intrapopulation variation in body size of males than of females from the same

341 population. A possible mechanism responsible for this pattern is a higher canalization of body size in

342 females, as females suffer from a higher fitness disadvantage from extremely lowered body size than

343 males (Honěk, 1993; Stearns & Kawecki, 1994). However, this explanation would assume increasing

344 sexual size dimorphism with increasing altitude, which was not observed.

345　　　　In conclusion, converse Bergmann's cline observed in *Silpha carinata* corresponds to what can

346　　be predicted for a large, univoltine species. A sharp decline in body size with increasing altitude (ca.

347　　15% of elytron length lost per 1 km of elevation) could be given by poor dispersal abilities of this

348　　species, which could facilitate process of local adaptation (such pattern has also been reported by Levy

349　　& Nufio, 2015 in orthopterans). Results of our common garden experiment suggest that geographical

350　　variation in body size of *S. carinata* is partly due to local adaptation, however a high mortality during

351　　preimaginal development precluded precise quantification of the effects of local adaptation and

352　　phenotypic plasticity and future investigation of this issue may be worth of effort. Comparisons of body

353　　size variation at the intrapopulation level revealed a tendency to higher variation in males compared

354　　to females. Investigation of intrapopulation variation in body size in insects is an unexplored area and

355　　this issue deserves attention in future ecological and evolutionary research.

356

364

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501   <u>Figures</u>

502   **Figure 1**

503   **Body size variation of Central European *Silpha carinata* adults along an altitudinal gradient.** Black

504   triangles represent males and grey circles represent females. When more individuals of the same sex

505   were available from the same site, their mean body size is shown in the graph. Body size was measured

506   as elytron length.

507

508
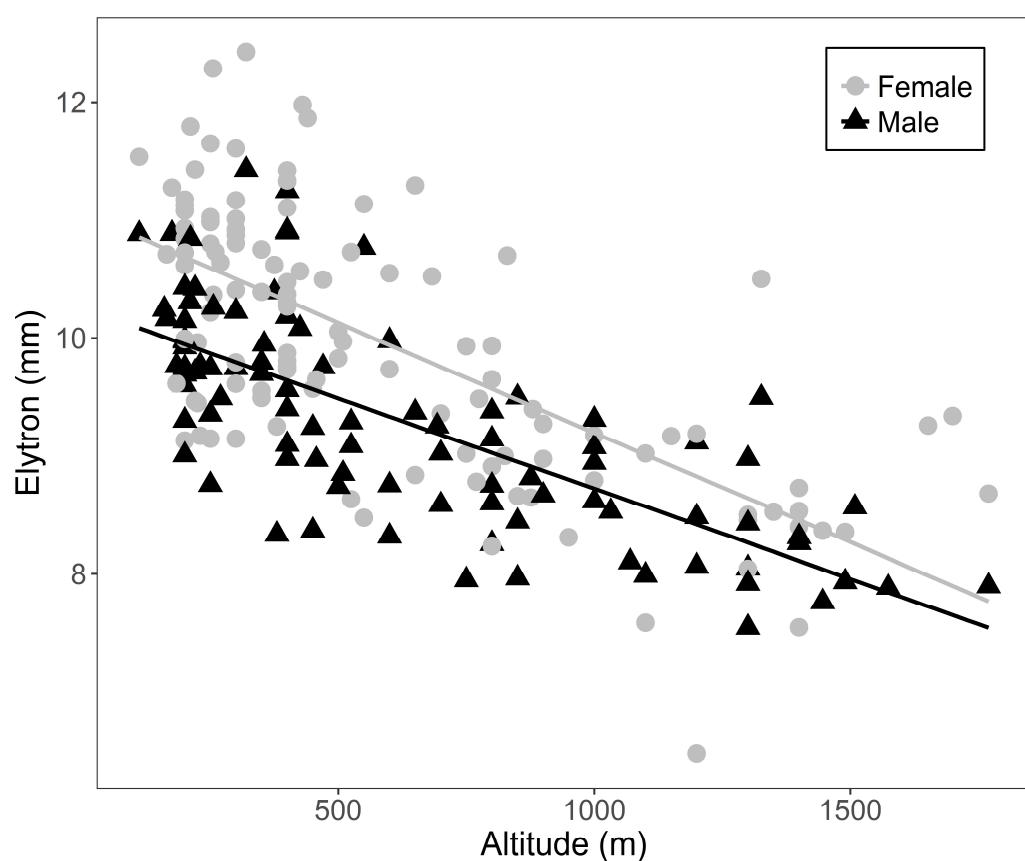
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511   **Figure 2**

512   **Body size of *Silpha carinata* parental individuals, originating from low- (grey bar) and high- (white**

513   **bar) altitude populations, and their offspring reared under standardized laboratory conditions.** A)

514   body size (elytron length in mm) of parental individuals collected in the nature; B) size of eggs

515   (diameter in mm) produced by parental females in laboratory; C) body size of last larval instar (tergit

516   width in mm) reared under standardized laboratory conditions. Mean values ± SEM are shown.

517

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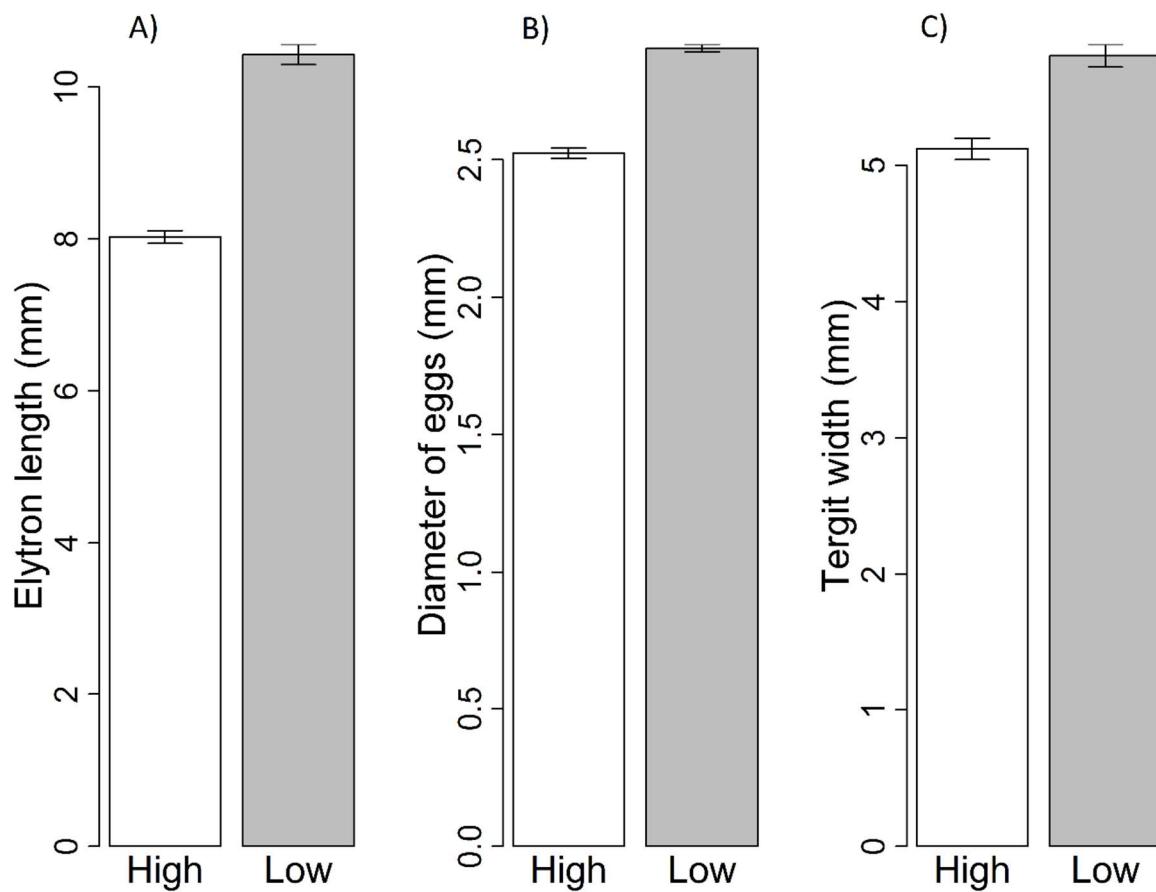
Figure 3

Effect of altitude on standardized intrapopulation variation in body size of *Silpha carinata* adults.

Males are represented by black triangles and females are represented by grey circles. The difference

between intrapopulation variation in males and females within a particular population was

marginally non-significant (P = 0.11). Intrapopulation variation was expressed as variance computed

for standardized data (elytron length of each specimen was divided by respective population mean;

standardization was made separately for males and females within a given population).
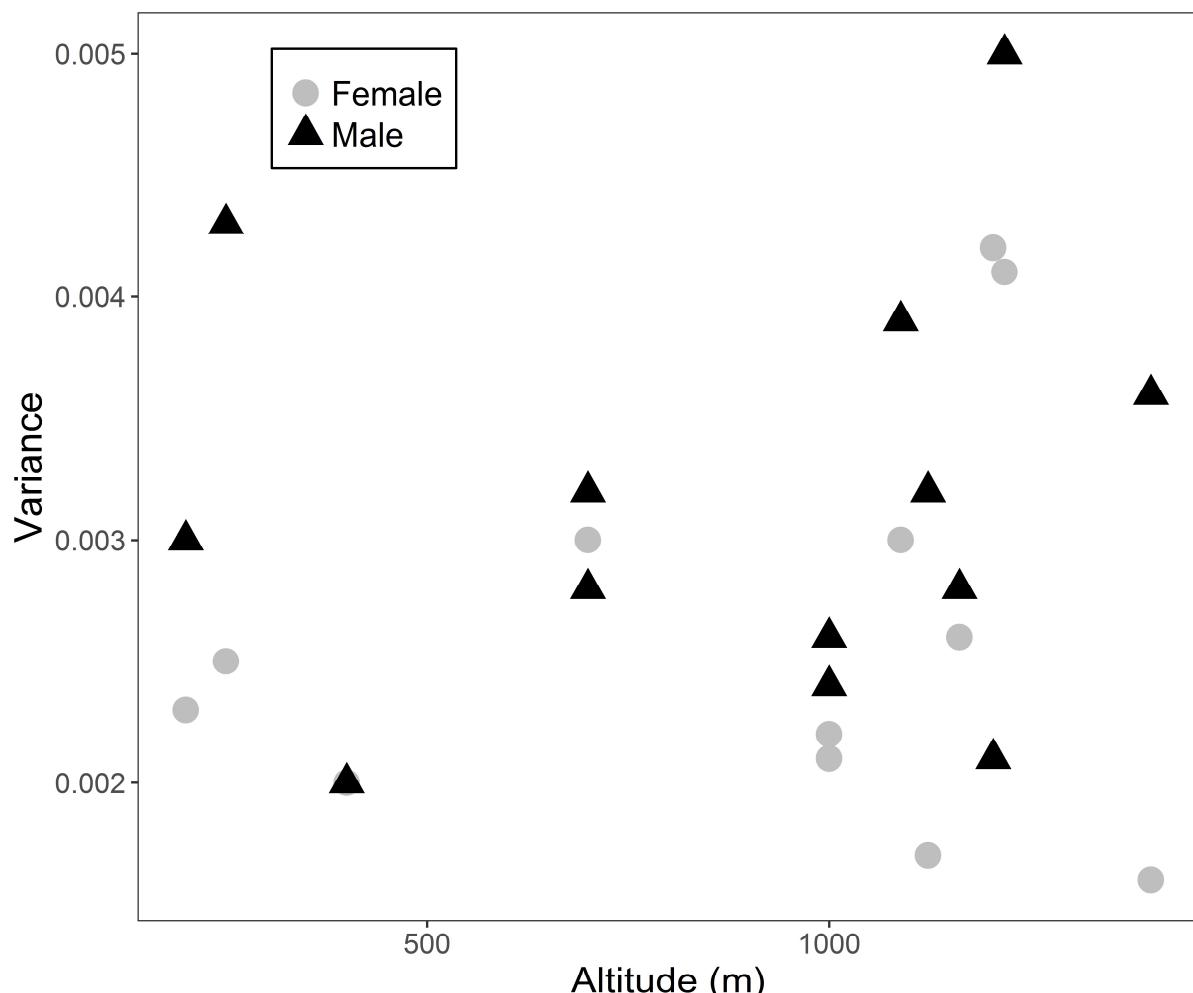
528   **Figure S1**

529   **Effect of mother size on egg diameter in *Silpha carinata*.** Two perpendicular diameters were

530   measured for each egg produced by parental females in laboratory and averaged for a single value.

531   Measured eggs were 24 to 48 hours old.

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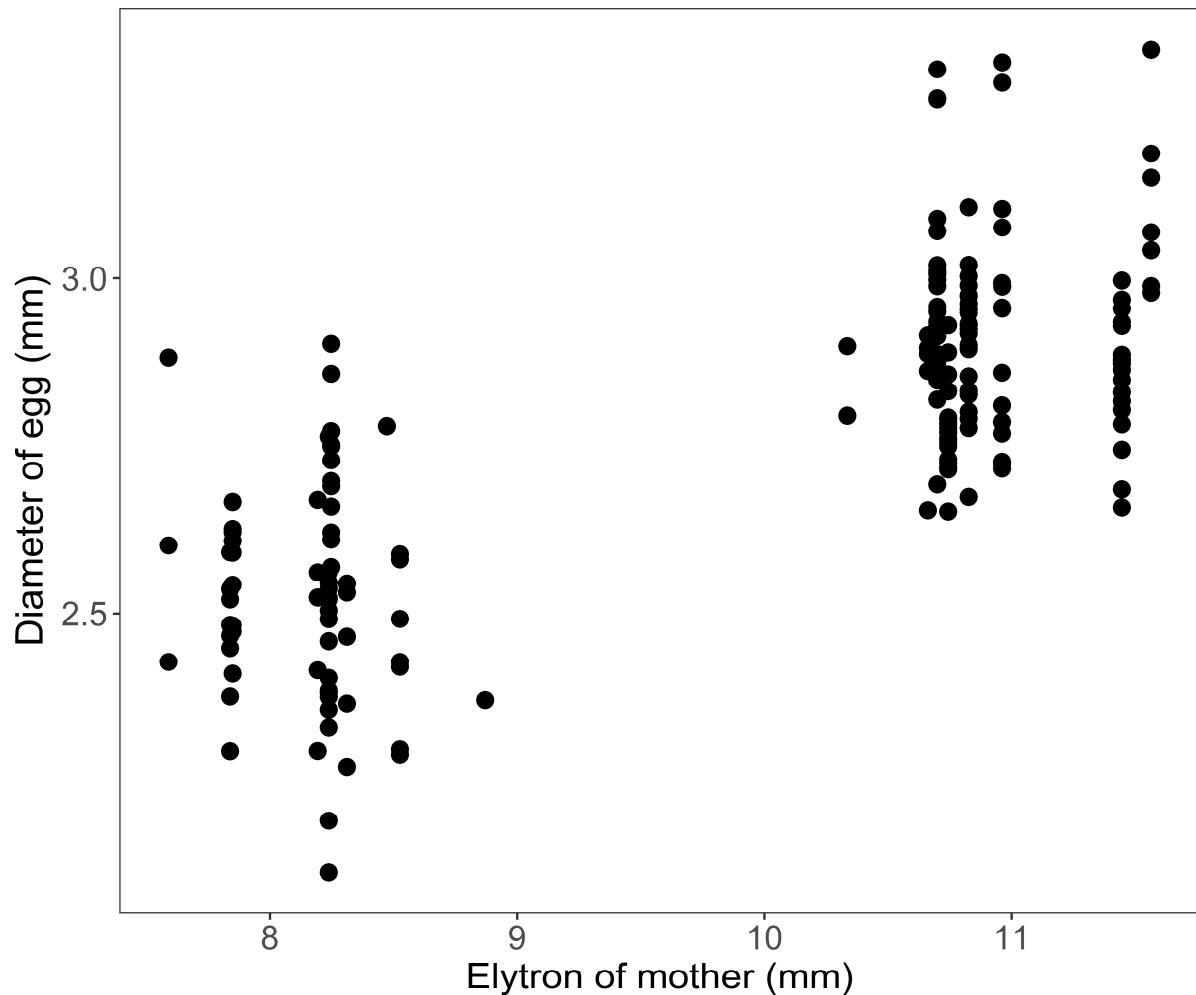
S2 Dataset

List of measured (elytron length in mm) individuals of *Silpha carinata* from collection.

ID	Elytron	Pronotum	Sex	Altitude	Locality
1	8.523	6.762	F	1350	Labská louka (Krkonoše)
2	7.851	6.107	M	1770	Dolské mlýnice (Vysoké Tatry - mlýnická dolina)
3	9.746	7.548	F	800	Paseky nad Jizerou
4	9.140	7.677	M	800	Paseky nad Jizerou
5	9.673	7.502	F	800	Paseky nad Jizerou
6	8.909	7.046	F	800	Dobrá na Šumavě
7	9.947	7.791	F	800	Paseky nad Jizerou
8	10.066	8.120	F	800	Paseky nad Jizerou
9	9.157	7.460	M	800	Paseky nad Jizerou
10	9.521	7.888	M	800	Paseky nad Jizerou
11	9.601	7.654	F	800	Paseky nad Jizerou
12	8.862	7.011	F	800	Paseky nad Jizerou
13	10.259	8.131	F	800	Paseky nad Jizerou
14	9.071	7.434	M	800	Paseky nad Jizerou
15	8.908	7.617	M	800	Paseky nad Jizerou
16	10.385	7.971	F	800	Paseky nad Jizerou
17	9.988	7.795	F	800	Paseky nad Jizerou
18	9.266	7.714	M	800	Paseky nad Jizerou
19	8.937	7.397	M	800	Paseky nad Jizerou
20	9.526	8.079	F	800	Paseky nad Jizerou
21	8.768	7.434	F	800	Paseky nad Jizerou
22	8.602	7.216	F	800	Paseky nad Jizerou
23	9.868	7.641	F	800	Paseky nad Jizerou
24	8.118	6.273	F	1770	Dolské mlýnice (Vysoké Tatry - mlýnická dolina)
25	7.937	6.375	M	1770	Dolské mlýnice (Vysoké Tatry - mlýnická dolina)
26	9.235	6.914	F	1770	Dolské mlýnice (Vysoké Tatry - mlýnická dolina)
27	10.899	8.609	F	200	Dolní Věstonice
28	11.155	9.064	F	400	Řevnice
29	11.486	9.050	F	400	Řevnice
30	11.118	9.025	F	200	Kamenný most n. Hr. (Sk)
31	9.187	7.478	M	693	Žlutice (Vladař)
32	9.250	7.027	F	900	Malá Fatra (SK)
33	8.996	7.241	F	1100	H. Kvilda
34	8.769	6.943	F	230	Hodonín
35	9.417	7.988	F	230	Hodonín
36	11.383	9.047	F	150	Lednice
37	9.422	7.976	M	230	Hodonín
38	8.843	6.775	F	230	Hodonín
39	9.044	7.220	F	1100	H. Kvilda
40	8.828	7.117	F	900	Malá Fatra (SK)

41	9.318	7.538	M	693	Žlutice (Vladař)
42	8.750	7.441	M	600	Křížlice (9km N of jilemnice) Krkonoše
43	8.664	6.995	M	900	Malá Fatra (SK)
44	8.844	6.871	F	900	Malá Fatra (SK)
45	9.708	8.109	M	220	Louny
46	9.041	7.898	F	220	Louny
47	10.937	8.344	F	220	Louny
48	9.493	7.697	M	1326	Malý Sněžník
49	11.647	9.289	F	250	Seleška (SK)
50	11.098	8.562	F	400	Litovel
51	9.076	7.680	M	1000	Stachy
52	8.659	6.677	F	1300	Krkonoše (Dvorská bouda)
53	8.617	6.934	F	1300	Krkonoše (Dvorská bouda)
54	8.213	6.720	F	1300	Krkonoše (Dvorská bouda)
55	8.203	6.544	F	1300	V. Tatry (Štrbské pleso)
56	11.331	8.505	F	400	Zadielská dolina
57	11.977	9.861	F	430	Bratislava
58	9.923	7.710	M	200	Káraný
59	12.161	9.184	F	300	Pálava
60	8.313	6.869	M	600	Trutnov
61	10.498	7.949	F	1326	Malý Sněžník
62	8.062	6.626	M	1200	Jeseník
63	7.916	6.711	M	1300	V. Tatry (Štrbské pleso)
64	11.289	8.591	F	650	Dobšiná
65	8.299	6.455	F	1150	V. Tatry (Koprova dolina)
66	9.511	7.170	F	1150	V. Tatry (Koprova dolina)
67	9.690	7.345	F	1150	V. Tatry (Koprova dolina)
68	12.125	9.103	F	300	Koda u Berouna
69	10.868	9.145	F	300	Loštice
70	7.873	6.917	F	1300	V. Tatry (Štrbské pleso)
71	11.161	8.831	F	300	Žloukovice
72	10.222	8.592	M	300	Žloukovice
73	9.471	6.967	F	300	Koda u Berouna
74	8.363	6.599	M	1400	Praděd
75	12.324	9.648	F	220	Trnová
76	10.417	8.672	M	220	Trnová
77	8.613	6.853	M	1000	Jeseník Vernířovice (Wermsdorf)
78	11.146	8.771	M	550	Čebovce (Šošar)
79	11.129	8.757	F	550	Čebovce (Šošar)
80	10.181	8.481	M	550	Čebovce (Šošar)
81	10.523	8.618	F	220	Trnová
82	7.542	6.281	M	1300	Špindlerův mlýn
83	10.892	8.724	F	200	Čelákovice
84	9.507	8.079	M	200	Čelákovice
85	10.738	8.828	M	400	Řevnice

86	11.243	8.647	M	400	Všenory
87	9.289	7.632	M	200	Čelákovice
88	10.688	8.432	F	320	Pavlovské Vrchy
89	10.963	8.808	M	550	Čebovce (Šošar)
90	10.426	8.494	M	200	Rohatec (Morava)
91	11.168	8.747	F	200	Rohatec (Morava)
92	11.422	9.155	M	320	Kobylí
93	8.942	7.827	M	1000	Kralický Sněžník
94	11.040	8.591	F	250	Šardice (Morava)
95	8.490	6.777	F	1400	Praděd
96	7.977	6.466	M	1400	Praděd
97	8.054	6.855	M	1400	Praděd
98	10.002	8.251	M	200	Čelákovice
99	10.892	8.547	M	400	NP Podyjí (Havraníky)
100	10.698	8.535	F	250	Šardice (Morava)
101	11.684	9.580	F	220	Čejč
102	8.392	7.258	F	1400	Kotelné jámy
103	9.338	6.750	F	1700	Bukovská dolina (N. Tatry)
104	11.194	8.277	F	250	Šardice (Morava)
105	8.423	7.336	M	1300	Muráň (Sk)
106	8.259	6.701	M	1400	Vysoký Jeseník (Ovčárna Praděd)
107	8.479	7.570	M	1200	Růžomberok
108	7.792	6.754	F	1400	Praděd
109	9.180	7.274	F	1400	Praděd
110	8.656	6.909	F	1400	Praděd
111	8.335	6.407	M	1400	Praděd
112	10.131	8.634	F	500	Dobříš
113	11.289	8.338	F	200	Lednice prostřední rybník
114	9.487	8.152	M	200	Lednice prostřední rybník
115	7.759	6.169	M	1446	V. Jeseník (Petrův kámen)
116	7.933	6.359	F	1446	V. Jeseník (Petrův kámen)
117	8.331	6.689	F	1446	V. Jeseník (Petrův kámen)
118	8.812	6.791	F	1446	V. Jeseník (Petrův kámen)
119	8.758	7.200	M	1400	Praděd
120	9.258	7.168	F	1652	N. Tatry Prašivá
121	8.104	6.415	M	1400	Praděd
122	8.508	6.448	M	1400	Praděd
123	10.725	8.462	F	260	Lištice (Beroun)
124	9.731	8.098	F	400	Račice - Předonín (Roudnice n.L.)
125	10.945	9.137	F	500	Dobříš
126	10.911	8.558	F	500	Dobříš
127	12.420	9.333	F	320	Kobylí
128	9.133	7.377	F	220	Čejč
129	9.188	7.395	F	1200	Růžomberok
130	9.653	7.765	F	230	Hodonín

131	10.740	8.930	F	250	Hovorany (Morava)
132	10.802	8.854	F	250	Hovorany (Morava)
133	10.840	8.754	F	250	Hovorany (Morava)
134	11.073	8.840	M	400	Řevnice
135	11.865	9.319	F	440	Tábor
136	11.255	8.683	F	400	Bílichov
137	10.108	7.885	M	230	Hodonín
138	10.532	8.651	F	200	Lednice prostřední rybník
139	10.528	8.428	M	200	Lednice prostřední rybník
140	10.975	8.260	F	200	Lednice prostřední rybník
141	10.507	8.558	F	200	Lednice prostřední rybník
142	11.576	8.711	F	400	Bílichov
143	9.629	7.890	M	200	Lednice prostřední rybník
144	9.629	8.675	F	200	Lednice prostřední rybník
145	10.300	8.560	M	200	Lednice prostřední rybník
146	10.239	8.817	M	160	0.8 km NW of Ladná
147	9.447	7.537	F	225	1 km E of Mendlov
148	10.918	8.724	M	175	1 km SW of Uherčice
149	11.270	8.756	F	175	1 km SW of Uherčice
150	10.840	8.767	M	175	1 km SW of Uherčice
151	9.731	7.336	F	750	1 km E of Prachatice
152	8.655	6.858	F	850	1.5 km S of Moldava (K.H.)
153	11.065	7.929	F	165	1.9 km W of Velké Němčice
154	10.535	8.320	M	165	1.9 km W of Velké Němčice
155	10.347	8.597	F	165	1.9 km W of Velké Němčice
156	9.782	7.537	M	165	1.9 km W of Velké Němčice
157	10.301	8.492	M	210	2.7 km N of Přibice
158	10.126	7.753	F	750	2 km E of Prachatice
159	9.881	7.814	F	865	2 km W of Loučná - Jiřík (K.H.)
160	9.549	7.256	F	350	2 km N of Ralsko (VVP Ralsko)
161	10.470	8.312	F	400	6 km NW Zbiroh - Třebnuška
162	10.179	8.141	M	400	6 km NW Zbiroh - Třebnuška
163	8.844	7.237	F	750	Adolfov (Kruš.H.)
164	9.193	7.279	F	750	Adolfov (Kruš.H.)
165	7.947	7.253	M	750	Adolfov (Kruš.H.)
166	9.087	7.589	M	700	Albrechtice v Jizer. Horách - Křížek
167	9.016	7.253	F	700	Albrechtice v Jizer. Horách - Křížek
168	9.701	7.200	F	700	Albrechtice v Jizer. Horách - Křížek
169	8.881	7.247	M	700	Albrechtice v Jizer. Horách - Křížek
170	9.098	7.227	M	700	Albrechtice v Jizer. Horách - Křížek
171	10.616	8.233	F	375	Beroun - Kosov
172	10.379	8.735	M	375	Beroun - Kosov
173	10.653	8.492	M	375	Beroun - Kosov
174	10.124	8.027	M	375	Beroun - Kosov
175	8.597	7.021	M	800	Boleboř - Svahová (K.H.)

176	9.932	7.347	F	800	Boleboř - Svahová (K.H.)
177	9.761	7.718	F	400	Bílina - Kaňkov
178	9.096	7.366	M	400	Bílina - Kaňkov
179	8.569	7.726	M	380	Bořeň kopec (České S.)
180	8.907	7.462	M	380	Bořeň kopec (České S.)
181	8.844	7.262	M	380	Bořeň kopec (České S.)
182	8.328	6.628	F	380	Bořeň kopec (České S.)
183	8.925	6.883	F	380	Bořeň kopec (České S.)
184	9.410	7.066	F	380	Bořeň kopec (České S.)
185	8.978	6.633	F	380	Bořeň kopec (České S.)
186	9.567	7.559	F	380	Bořeň kopec (České S.)
187	10.341	7.664	F	380	Bořeň kopec (České S.)
188	8.225	6.592	M	380	Bořeň kopec (České S.)
189	8.873	7.342	M	380	Bořeň kopec (České S.)
190	8.768	7.006	M	380	Bořeň kopec (České S.)
191	9.198	6.831	F	380	Bořeň kopec (České S.)
192	9.079	7.418	M	400	Boreč (České s.)
193	10.084	8.122	F	400	Boreč (České s.)
194	10.336	7.976	F	400	Boreč (České s.)
195	10.463	7.941	F	400	Boreč (České s.)
196	8.919	7.316	M	400	Boreč (České s.)
197	9.668	7.438	F	400	Boreč (České s.)
198	9.340	7.227	F	400	Boreč (České s.)
199	9.529	7.296	F	400	Boreč (České s.)
200	9.691	7.368	F	400	Boreč (České s.)
201	8.939	7.762	M	400	Boreč (České s.)
202	10.138	7.482	F	400	Boreč (České s.)
203	8.811	7.342	M	400	Boreč (České s.)
204	9.138	6.891	F	400	Boreč (České s.)
205	9.130	7.519	M	400	Boreč (České s.)
206	10.334	8.087	F	400	Boreč (České s.)
207	9.744	7.727	M	250	Brekov
208	10.520	8.204	F	683	Buková hory (České středohoří)
209	8.363	7.489	M	450	Brník pr. Mnichov (České s.)
210	9.117	7.088	M	1200	Bukovské Vrchy - Nová Sedlica
211	9.851	7.471	F	880	Cínovecký hřeben
212	8.940	6.777	F	880	Cínovecký hřeben
213	10.299	8.513	M	200	Bulhary
214	10.602	8.323	F	200	Bulhary
215	11.073	8.286	F	200	Bulhary
216	9.647	8.027	M	200	Bulhary
217	10.700	8.450	F	200	Čejč
218	9.689	8.120	M	200	Čejč
219	11.238	8.527	F	200	Čejč
220	10.574	8.756	F	200	Čejč

221	11.188	8.493	F	200	Čejč
222	12.197	9.476	F	200	Čejč
223	11.907	9.146	F	200	Čejč
224	9.729	8.181	F	200	Čejč
225	10.901	8.727	F	200	Čejč
226	9.146	7.886	F	250	Čejkovice - PR Špidláky II
227	11.255	8.578	F	200	Čelákovice
228	9.516	7.537	M	650	Dešenice (Šumava), Městiště
229	9.224	7.494	M	650	Dešenice (Šumava), Městiště
230	10.079	8.733	M	425	Dobříš
231	10.266	8.354	F	425	Dobříš
232	6.469	5.808	F	1200	Čihadlo mt. (Krkonoše)
233	9.146	7.184	F	300	Divoká Šárka
234	9.389	7.425	M	876	Fláje (K.H.)
235	8.268	6.950	M	876	Fláje (K.H.)
236	9.007	7.218	M	876	Fláje (K.H.)
237	8.621	7.124	M	876	Fláje (K.H.)
238	8.552	7.347	M	876	Fláje (K.H.)
239	8.823	6.874	M	876	Fláje (K.H.)
240	8.989	7.099	F	876	Fláje (K.H.)
241	8.606	7.150	M	876	Fláje (K.H.)
242	8.296	6.617	F	876	Fláje (K.H.)
243	9.183	7.700	M	876	Fláje (K.H.)
244	8.838	7.875	M	876	Fláje (K.H.)
245	10.236	7.978	F	200	Dolní Věstonice
246	11.380	8.512	F	200	Dolní Věstonice
247	8.789	6.717	F	1000	Horní Mísečky
248	9.271	7.244	F	900	H. Vltavice - Šumava
249	9.696	7.933	M	350	Frýdek
250	11.004	8.625	F	300	Hnanice - Znojmo
251	7.962	6.771	M	850	Hora sv. Šebestiána
252	8.415	6.501	F	1490	Hrubý Jeseník
253	8.146	6.851	M	1490	Hrubý Jeseník
254	8.580	6.442	F	1490	Hrubý Jeseník
255	8.040	6.218	M	1490	Hrubý Jeseník
256	7.602	6.681	M	1490	Hrubý Jeseník
257	8.053	6.246	F	1490	Hrubý Jeseník
258	9.482	7.416	F	775	Jelení vrch - Kadaň
259	8.471	7.328	F	550	Horní stropnice - Hojná voda
260	8.098	6.891	M	1070	Churáňov
261	9.825	7.554	F	500	Chlum - Šumava
262	9.378	7.597	M	800	Hrhov (SK)
263	7.492	6.215	M	1300	Jeseníky
264	7.492	6.237	F	1300	Jeseníky
265	8.840	6.643	F	1300	Jeseníky

266	8.967	6.319	F	1300	Jeseníky
267	8.511	6.661	F	1300	Jeseníky
268	8.734	6.573	M	1300	Jeseníky
269	7.912	6.367	M	1300	Jeseníky
270	7.582	6.231	F	1100	Jeseníky (Červenohorské sedlo)
271	7.985	6.875	M	1100	Jeseníky (Červenohorské sedlo)
272	10.213	8.140	F	250	Kamenica - n. Hron
273	8.754	7.220	M	250	Kamenica - n. Hron
274	9.086	7.563	M	525	Kadaň - NPR Úhošť
275	8.629	6.902	F	525	Kadaň - NPR Úhošť
276	9.568	8.210	F	450	Koněpruské jeskyně
277	10.272	8.409	M	200	Kochánky
278	10.522	8.356	F	200	Kochánky
279	10.695	7.425	F	830	Kopec Podmileská výšina (Kruš.H. - 0,5 km S Rusová)
280	9.130	8.127	F	200	Káraný
281	9.946	8.358	M	355	Kamýk - pod Plešivcem
282	10.838	8.477	M	211	Kyjov
283	11.794	9.053	F	211	Kyjov
284	7.543	6.264	F	1400	Krk - Dívčí kameny (Krkonoše)
285	9.733	7.258	F	600	Krušné hory - Louchov
286	9.241	7.709	M	450	Košice
287	9.960	8.198	F	225	Košátky (stř. Čechy)
288	10.144	8.481	M	200	Mikulov
289	9.332	6.814	F	650	Liptovský Ján
290	8.337	6.429	F	650	Liptovský Jan
291	11.157	8.406	F	400	Libochovany - Církvice
292	9.370	7.271	F	400	Libochovany - Církvice
293	9.550	8.025	M	400	Libochovany - Církvice
294	9.348	7.830	M	400	Libochovany - Církvice
295	9.292	7.627	M	400	Libochovany - Církvice
296	8.414	8.010	F	300	Louny
297	8.251	6.752	M	800	M. Fatra - Vrátna dolina
298	10.401	7.823	M	200	Milovice (VVP Mladá)
299	9.349	7.486	M	200	Milovice (VVP Mladá)
300	9.277	7.434	M	200	Milovice (VVP Mladá)
301	9.275	7.913	M	200	Milovice (VVP Mladá)
302	10.122	8.124	F	200	Milovice (VVP Mladá)
303	8.905	6.943	F	200	Milovice (VVP Mladá)
304	10.491	8.055	F	200	Milovice (VVP Mladá)
305	10.101	7.710	F	200	Milovice (VVP Mladá)
306	9.310	7.593	F	200	Milovice (VVP Mladá)
307	10.389	7.790	F	200	Milovice (VVP Mladá)
308	9.696	8.333	F	200	Milovice (VVP Mladá)
309	10.317	8.014	F	200	Milovice (VVP Mladá)
310	10.803	8.452	F	200	Milovice (VVP Mladá)

311	10.033	7.754	M	200	Milovice (VVP Mladá)
312	9.734	7.732	F	200	Milovice (VVP Mladá)
313	10.091	8.057	F	200	Milovice (VVP Mladá)
314	10.173	8.388	F	200	Milovice (VVP Mladá)
315	9.216	7.236	M	200	Milovice (VVP Mladá)
316	10.618	8.216	F	200	Milovice (VVP Mladá)
317	8.550	7.440	M	200	Milovice (VVP Mladá)
318	9.052	7.776	M	200	Milovice (VVP Mladá)
319	10.294	8.094	F	200	Milovice (VVP Mladá)
320	9.716	7.959	M	200	Milovice (VVP Mladá)
321	10.190	7.737	F	200	Milovice (VVP Mladá)
322	8.965	7.846	M	200	Milovice (VVP Mladá)
323	9.204	7.984	M	200	Milovice (VVP Mladá)
324	9.863	7.692	F	200	Milovice (VVP Mladá)
325	8.872	7.464	F	200	Milovice (VVP Mladá)
326	9.145	7.442	M	200	Milovice (VVP Mladá)
327	9.854	7.871	F	200	Milovice (VVP Mladá)
328	8.730	7.457	M	200	Milovice (VVP Mladá)
329	10.635	8.466	F	270	Milovický les - PR Milovická stráň
330	9.490	7.712	M	270	Milovický les - PR Milovická stráň
331	12.280	9.489	F	255	Mutěnice
332	9.614	8.090	M	470	Mlynářův luh, Křivoklátsko
333	9.740	8.312	M	470	Mlynářův luh, Křivoklátsko
334	10.590	8.037	F	470	Mlynářův luh, Křivoklátsko
335	10.393	8.473	F	470	Mlynářův luh, Křivoklátsko
336	9.905	8.193	M	470	Mlynářův luh, Křivoklátsko
337	10.050	8.647	F	500	Mníšek pod Brdy
338	10.361	9.960	F	256	Mladá Boleslav - Radouč
339	10.255	8.062	M	256	Mladá Boleslav - Radouč
340	8.578	6.874	F	950	Nízké Tatra - Telgart
341	8.028	6.780	F	950	Nízké Tatra - Telgart
342	8.440	6.521	M	850	Nízké Tatry - Donovaly
343	8.278	6.605	F	1400	Nízké Tatry - Čertovica
344	8.779	6.989	F	1400	Nízké Tatry - Čertovica
345	9.558	7.813	F	1000	Nízké Tatry - Janecká dolina
346	8.913	7.001	F	1000	Nízké Tatry - Janecká dolina
347	9.051	7.186	F	1000	Nízké Tatry - Janecká dolina
348	9.309	7.905	M	1000	Nízké Tatry - Janecká dolina
349	8.999	7.150	F	825	Novodomské rašeliniště (Krušné hory)
350	9.784	7.950	F	184	Nymburk- Zvěřínek
351	9.762	7.697	M	184	Nymburk- Zvěřínek
352	9.439	7.417	F	184	Nymburk- Zvěřínek
353	9.027	7.545	M	509	Oblík
354	9.533	7.959	F	509	Oblík
355	8.669	7.005	M	509	Oblík

356	10.407	7.516	F	509	Oblík
357	9.057	7.014	M	509	Oblík
358	8.566	7.135	M	509	Oblík
359	8.922	7.549	M	509	Oblík
360	9.014	7.821	M	400	Obory - Na Závisti (okr. Příbram)
361	10.102	8.097	M	400	Obory - Na Závisti (okr. Příbram)
362	10.365	8.358	F	400	Obory - Na Závisti (okr. Příbram)
363	9.785	7.419	F	650	Paseky nad Jizerou (chata Bosna)
364	10.103	7.804	F	525	Pavlovské vrchy - Pálava
365	9.287	8.225	M	525	Pavlovské vrchy - Pálava
366	10.678	8.398	F	525	Pavlovské vrchy - Pálava
367	11.099	8.715	F	525	Pavlovské vrchy - Pálava
368	11.050	8.551	F	525	Pavlovské vrchy - Pálava
369	11.047	8.481	F	300	Pálava
370	10.915	8.389	F	300	PP Pramen Pšovky (Kokořínsko)
371	9.274	7.666	M	200	Pouzdřany
372	10.302	8.716	M	200	Pouzdřany
373	9.900	8.861	M	200	Pouzdřany
374	9.508	7.892	M	200	Pouzdřany
375	9.352	7.619	M	250	Poříčany (stř. Čechy)
376	10.613	8.233	F	200	Písty u Nymburka
377	10.611	8.073	F	200	Písty u Nymburka
378	8.930	6.712	F	1491	Praděd
379	8.363	6.599	M	1465	Praděd - Vysoká hole
380	9.300	6.772	F	1465	Praděd - Vysoká hole
381	9.748	7.764	F	350	Praha - Motol
382	9.139	7.287	M	350	Praha - Motol
383	8.142	6.356	M	350	Praha - Motol
384	8.867	7.115	F	350	Praha - Motol
385	8.684	7.194	F	350	Praha - Motol
386	10.240	7.657	F	350	Praha - Motol
387	9.909	7.804	F	350	Praha - Motol
388	8.544	7.439	M	350	Praha - Motol
389	9.791	8.291	F	300	Radovesice - Bílé stráně
390	9.745	7.804	M	300	Praha 6
391	9.836	7.700	F	457	Raná
392	9.253	7.351	M	457	Raná
393	9.192	7.214	M	457	Raná
394	8.457	7.762	M	457	Raná
395	9.455	7.520	F	457	Raná
396	8.747	7.153	M	800	Rejvíz
397	10.095	8.191	M	600	Silická planina
398	9.857	8.161	M	600	Silická planina
399	10.546	7.743	F	600	Rudohor - Švedlar
400	10.401	7.890	F	300	Slapská přehrada

401	8.526	6.926	M	1032	Slovenský ráj-Geravy
402	11.595	8.684	F	250	Srbsko
403	11.337	8.994	F	250	Srbsko
404	11.202	8.569	F	250	Srbsko
405	9.937	7.771	F	250	Srbsko
406	10.746	9.128	F	350	Srbsko - NPR Koda
407	9.791	8.500	M	350	Srbsko - NPR Koda
408	9.006	7.138	M	200	Stará Boleslav
409	8.406	6.617	F	800	Starý Rejvíz -Jeseníky
410	8.054	6.805	F	800	Starý Rejvíz -Jeseníky
411	10.959	8.621	F	111	Štúrovo
412	11.377	9.351	M	111	Štúrovo
413	11.254	9.427	M	111	Štúrovo
414	11.437	9.114	F	111	Štúrovo
415	11.571	8.667	F	111	Štúrovo
416	12.541	9.725	F	111	Štúrovo
417	10.000	8.630	M	111	Štúrovo
418	11.090	8.989	F	111	Štúrovo
419	11.597	9.071	F	111	Štúrovo
420	10.386	8.690	F	350	Trenčianska Jastrabie
421	9.946	8.504	M	850	Šumava - Špičák
422	9.045	7.851	M	850	Šumava - Špičák
423	9.804	8.412	F	400	Ústí n. L.
424	7.881	6.747	M	1574	Velká Fatra - Křížná
425	8.736	7.602	M	500	Vižňov pr. Meziměstí
426	8.582	7.545	M	700	Vítkovice v Podkrkonoší
427	8.975	7.296	M	1300	Vysoké Tatry - Bielá voda
428	8.778	7.193	F	770	Žebrácký roh (K.H.)
429	8.562	7.331	M	1509	Vysoké kolo - Krkonoše
430	10.298	7.876	F	400	Voznice - Dobříš
431	9.610	8.001	F	300	Zádiel (SK)

536

537

S3 Dataset

List of body size (elytron length in mm) of parental individuals collected in the nature.

ID	Label	Elytron	Pronotum	Sex	Population	Altitude
1	J1	7.982	6.384	Male	Praděd	High
2	J1	7.617	6.325	Female	Praděd	High
3	J2	7.563	6.196	Male	Praděd	High
4	J2	8.870	6.402	Female	Praděd	High
5	J3	7.605	5.962	Male	Praděd	High
6	J3	8.339	6.671	Female	Praděd	High
7	J4	8.154	6.822	Male	Praděd	High
8	J4	7.838	6.298	Female	Praděd	High
9	J5	8.854	6.795	Male	Praděd	High
10	J5	7.848	6.169	Female	Praděd	High
11	J6	7.905	6.410	Male	Praděd	High
12	J6	8.192	6.341	Female	Praděd	High
13	J7	7.859	6.315	Male	Praděd	High
14	J7	8.247	6.696	Female	Praděd	High
15	J8	8.520	6.870	Male	Praděd	High
16	J8	8.136	6.521	Female	Praděd	High
17	J9	7.667	6.543	Male	Praděd	High
18	J9	8.237	6.817	Female	Praděd	High
19	J10	7.150	6.023	Male	Praděd	High
20	J10	7.589	6.194	Female	Praděd	High
21	J11	7.815	6.598	Male	Praděd	High
22	J11	8.311	6.635	Female	Praděd	High
23	J12	7.378	6.130	Male	Praděd	High
24	J12	8.472	6.289	Female	Praděd	High
25	J13	8.017	6.651	Male	Praděd	Low
26	J13	8.525	6.646	Female	Praděd	Low
27	S1	9.356	7.629	Male	Srbsko	Low
28	S1	10.961	8.247	Female	Srbsko	Low
29	S2	10.168	8.886	Male	Srbsko	Low
30	S2	10.661	8.441	Female	Srbsko	Low
31	S3	9.044	7.557	Male	Srbsko	Low
32	S3	10.962	8.965	Female	Srbsko	Low
33	S4	9.980	8.621	Male	Srbsko	Low
34	S4	11.341	8.828	Female	Srbsko	Low
35	S5	10.548	8.620	Male	Srbsko	Low
36	S5	11.446	8.760	Female	Srbsko	Low
37	S6	9.681	8.090	Male	Srbsko	Low
38	S6	9.920	8.049	Female	Srbsko	Low
39	S7	9.983	8.157	Male	Srbsko	Low
40	S7	10.826	8.347	Female	Srbsko	Low

41	S8	10.531	8.673	Male	Srbsko	Low
42	S8	10.335	8.294	Female	Srbsko	Low
43	S9	10.049	8.242	Male	Srbsko	Low
44	S9	11.564	8.726	Female	Srbsko	Low
45	S10	10.739	8.696	Male	Srbsko	Low
46	S10	10.715	8.138	Female	Srbsko	Low
47	S11	10.397	8.470	Male	Srbsko	Low
48	S11	10.743	8.500	Female	Srbsko	Low
49	S12	9.585	7.663	Male	Srbsko	Low
0	S12	10.699	7.663	Female	Srbsko	Low

540

541 **List of egg sizes (diameter and elytron length of their mother, both in mm) produced by parental**

542 **females in laboratory**.

ID	Mother	A	B	Diameter	Population	Size of mother	Altitude
1	S1	3.170	2.815	2.993	Srbsko	10.961	Low
2	S1	2.885	2.569	2.727	Srbsko	10.961	Low
3	S1	2.864	2.707	2.786	Srbsko	10.961	Low
4	S1	2.855	2.683	2.769	Srbsko	10.961	Low
5	S1	3.157	2.755	2.956	Srbsko	10.961	Low
6	S1	2.915	2.705	2.810	Srbsko	10.961	Low
7	S1	2.755	2.678	2.717	Srbsko	10.961	Low
8	S1	2.915	2.803	2.859	Srbsko	10.961	Low
9	S1	2.776	2.670	2.723	Srbsko	10.961	Low
10	S1	2.821	2.631	2.726	Srbsko	10.961	Low
11	S3	3.284	2.924	3.104	Srbsko	10.962	Low
12	S3	3.516	3.130	3.323	Srbsko	10.962	Low
13	S3	3.439	3.146	3.293	Srbsko	10.962	Low
14	S3	3.287	2.865	3.076	Srbsko	10.962	Low
15	S3	3.129	2.780	2.955	Srbsko	10.962	Low
16	S3	2.894	3.081	2.988	Srbsko	10.962	Low
17	S5	2.952	3.041	2.997	Srbsko	11.446	Low
18	S5	2.939	2.819	2.879	Srbsko	11.446	Low
19	S5	3.057	2.800	2.929	Srbsko	11.446	Low
20	S5	3.023	2.845	2.934	Srbsko	11.446	Low
21	S5	2.961	2.948	2.955	Srbsko	11.446	Low
22	S5	2.724	2.909	2.817	Srbsko	11.446	Low
23	S5	2.978	2.681	2.830	Srbsko	11.446	Low
24	S5	2.976	2.750	2.863	Srbsko	11.446	Low
25	S5	2.933	2.763	2.848	Srbsko	11.446	Low
26	S5	2.964	2.807	2.886	Srbsko	11.446	Low
27	S5	2.940	2.667	2.804	Srbsko	11.446	Low
28	S5	2.843	2.902	2.873	Srbsko	11.446	Low

29	S5	2.822	2.549	2.686	Srbsko	11.446	Low
30	S5	2.878	2.612	2.745	Srbsko	11.446	Low
31	S5	3.090	2.664	2.877	Srbsko	11.446	Low
32	S5	2.924	2.393	2.659	Srbsko	11.446	Low
33	S5	3.162	2.774	2.968	Srbsko	11.446	Low
34	S5	2.910	2.655	2.783	Srbsko	11.446	Low
35	S2	2.981	2.793	2.887	Srbsko	10.661	Low
36	S2	2.993	2.730	2.862	Srbsko	10.661	Low
37	S2	3.123	2.706	2.915	Srbsko	10.661	Low
38	S2	3.039	2.752	2.896	Srbsko	10.661	Low
39	S2	2.848	2.461	2.655	Srbsko	10.661	Low
40	S7	3.014	2.845	2.930	Srbsko	10.826	Low
41	S7	2.867	2.714	2.791	Srbsko	10.826	Low
42	S7	3.020	2.829	2.925	Srbsko	10.826	Low
43	S7	2.864	2.690	2.777	Srbsko	10.826	Low
44	S7	3.023	2.840	2.932	Srbsko	10.826	Low
45	S7	3.184	2.856	3.020	Srbsko	10.826	Low
46	S7	3.082	2.867	2.975	Srbsko	10.826	Low
47	S7	3.145	2.861	3.003	Srbsko	10.826	Low
48	S7	3.033	2.945	2.989	Srbsko	10.826	Low
49	S7	3.040	2.866	2.953	Srbsko	10.826	Low
50	S7	3.065	2.770	2.918	Srbsko	10.826	Low
51	S7	3.053	2.925	2.989	Srbsko	10.826	Low
52	S7	2.819	2.844	2.832	Srbsko	10.826	Low
53	S7	3.078	2.722	2.900	Srbsko	10.826	Low
54	S7	3.280	2.933	3.107	Srbsko	10.826	Low
55	S7	3.056	2.736	2.896	Srbsko	10.826	Low
56	S7	2.941	2.711	2.826	Srbsko	10.826	Low
57	S7	2.912	2.751	2.832	Srbsko	10.826	Low
58	S7	3.188	2.709	2.949	Srbsko	10.826	Low
59	S7	3.069	2.778	2.924	Srbsko	10.826	Low
60	S7	3.013	2.775	2.894	Srbsko	10.826	Low
61	S7	3.077	2.847	2.962	Srbsko	10.826	Low
62	S7	3.055	2.891	2.973	Srbsko	10.826	Low
63	S7	2.977	2.624	2.801	Srbsko	10.826	Low
64	S7	2.504	2.845	2.675	Srbsko	10.826	Low
65	S7	2.940	2.767	2.854	Srbsko	10.826	Low
66	S9	2.963	2.994	2.979	Srbsko	11.564	Low
67	S9	3.104	3.197	3.151	Srbsko	11.564	Low
68	S9	3.096	3.042	3.069	Srbsko	11.564	Low
69	S9	3.245	3.128	3.187	Srbsko	11.564	Low
70	S9	3.060	3.024	3.042	Srbsko	11.564	Low
71	S9	3.378	3.306	3.342	Srbsko	11.564	Low
72	S9	2.971	3.005	2.988	Srbsko	11.564	Low
73	S11	3.003	2.775	2.889	Srbsko	10.743	Low

74	S11	2.857	2.683	2.770	Srbsko	10.743	Low
75	S11	2.988	2.674	2.831	Srbsko	10.743	Low
76	S11	2.823	2.756	2.790	Srbsko	10.743	Low
77	S11	2.860	2.640	2.750	Srbsko	10.743	Low
78	S11	2.872	2.987	2.930	Srbsko	10.743	Low
79	S11	2.623	2.901	2.762	Srbsko	10.743	Low
80	S11	2.888	2.666	2.777	Srbsko	10.743	Low
81	S11	2.689	2.616	2.653	Srbsko	10.743	Low
82	S11	2.862	2.599	2.731	Srbsko	10.743	Low
83	S11	2.861	2.852	2.857	Srbsko	10.743	Low
84	S11	2.852	2.669	2.761	Srbsko	10.743	Low
85	S11	2.902	2.682	2.792	Srbsko	10.743	Low
86	S11	2.880	2.686	2.783	Srbsko	10.743	Low
87	S11	2.803	2.707	2.755	Srbsko	10.743	Low
88	S11	2.854	2.583	2.719	Srbsko	10.743	Low
89	S11	2.739	2.692	2.716	Srbsko	10.743	Low
90	S11	2.732	2.718	2.725	Srbsko	10.743	Low
91	S11	2.729	2.815	2.772	Srbsko	10.743	Low
92	S12	3.170	3.008	3.089	Srbsko	10.699	Low
93	S12	2.979	2.878	2.929	Srbsko	10.699	Low
94	S12	2.915	3.104	3.010	Srbsko	10.699	Low
95	S12	3.109	2.903	3.006	Srbsko	10.699	Low
96	S12	2.901	3.014	2.958	Srbsko	10.699	Low
97	S12	3.094	2.882	2.988	Srbsko	10.699	Low
98	S12	2.993	2.777	2.885	Srbsko	10.699	Low
99	S12	3.007	2.766	2.887	Srbsko	10.699	Low
100	S12	3.005	2.895	2.950	Srbsko	10.699	Low
101	S12	2.913	2.988	2.951	Srbsko	10.699	Low
102	S12	3.144	2.832	2.988	Srbsko	10.699	Low
103	S12	3.132	2.695	2.914	Srbsko	10.699	Low
104	S12	3.338	3.287	3.313	Srbsko	10.699	Low
105	S12	3.150	2.866	3.008	Srbsko	10.699	Low
106	S12	2.854	2.881	2.868	Srbsko	10.699	Low
107	S12	3.139	2.879	3.009	Srbsko	10.699	Low
108	S12	3.147	2.892	3.020	Srbsko	10.699	Low
109	S12	2.966	2.728	2.847	Srbsko	10.699	Low
110	S12	3.211	3.327	3.269	Srbsko	10.699	Low
111	S12	3.109	2.760	2.935	Srbsko	10.699	Low
112	S12	3.355	3.180	3.268	Srbsko	10.699	Low
113	S12	3.020	2.729	2.875	Srbsko	10.699	Low
114	S12	2.714	2.672	2.693	Srbsko	10.699	Low
115	S12	2.972	2.665	2.819	Srbsko	10.699	Low
116	S12	3.103	2.919	3.011	Srbsko	10.699	Low
117	S12	3.171	2.970	3.071	Srbsko	10.699	Low
118	S12	2.813	2.900	2.857	Srbsko	10.699	Low

119	S12	3.115	2.880	2.998	Srbsko	10.699	Low
120	S8	2.914	2.676	2.795	Srbsko	10.335	Low
121	S8	2.964	2.833	2.899	Srbsko	10.335	Low
122	J2	2.388	2.353	2.371	Praděd	8.870	High
123	J4	2.614	2.428	2.521	Praděd	7.838	High
124	J4	2.420	2.332	2.376	Praděd	7.838	High
125	J4	2.442	2.631	2.537	Praděd	7.838	High
126	J4	2.705	2.478	2.592	Praděd	7.838	High
127	J4	2.624	2.273	2.449	Praděd	7.838	High
128	J4	2.576	2.358	2.467	Praděd	7.838	High
129	J4	2.439	2.148	2.294	Praděd	7.838	High
130	J4	2.553	2.413	2.483	Praděd	7.838	High
131	J5	2.654	2.310	2.482	Praděd	7.848	High
132	J5	2.601	2.346	2.474	Praděd	7.848	High
133	J5	2.831	2.351	2.591	Praděd	7.848	High
134	J5	2.726	2.491	2.609	Praděd	7.848	High
135	J5	2.632	2.454	2.543	Praděd	7.848	High
136	J5	2.704	2.540	2.622	Praděd	7.848	High
137	J5	2.811	2.442	2.627	Praděd	7.848	High
138	J5	2.875	2.459	2.667	Praděd	7.848	High
139	J5	2.915	2.418	2.667	Praděd	7.848	High
140	J5	2.611	2.209	2.410	Praděd	7.848	High
141	J6	2.626	2.422	2.524	Praděd	8.192	High
142	J6	2.655	2.468	2.562	Praděd	8.192	High
143	J6	2.502	2.328	2.415	Praděd	8.192	High
144	J6	2.572	2.016	2.294	Praděd	8.192	High
145	J6	2.831	2.509	2.670	Praděd	8.192	High
146	J7	3.009	2.795	2.902	Praděd	8.247	High
147	J7	2.857	2.643	2.750	Praděd	8.247	High
148	J7	2.914	2.801	2.858	Praděd	8.247	High
149	J7	2.825	2.719	2.772	Praděd	8.247	High
150	J7	2.685	2.635	2.660	Praděd	8.247	High
151	J7	2.586	2.553	2.570	Praděd	8.247	High
152	J7	2.798	2.706	2.752	Praděd	8.247	High
153	J7	2.709	2.534	2.622	Praděd	8.247	High
154	J7	2.780	2.601	2.691	Praděd	8.247	High
155	J7	2.820	2.638	2.729	Praděd	8.247	High
156	J7	2.787	2.609	2.698	Praděd	8.247	High
157	J7	2.725	2.496	2.611	Praděd	8.247	High
158	J9	2.574	2.343	2.459	Praděd	8.237	High
159	J9	2.479	2.564	2.522	Praděd	8.237	High
160	J9	2.566	2.526	2.546	Praděd	8.237	High
161	J9	2.507	2.300	2.404	Praděd	8.237	High
162	J9	2.625	2.449	2.537	Praděd	8.237	High
163	J9	2.623	2.423	2.523	Praděd	8.237	High

164	J9	2.468	2.515	2.492	Praděd	8.237	High
165	J9	2.276	2.104	2.190	Praděd	8.237	High
166	J9	2.449	2.358	2.404	Praděd	8.237	High
167	J9	2.599	2.929	2.764	Praděd	8.237	High
168	J9	2.590	2.161	2.376	Praděd	8.237	High
169	J9	2.628	2.141	2.385	Praděd	8.237	High
170	J9	2.560	2.515	2.538	Praděd	8.237	High
171	J9	2.573	2.087	2.330	Praděd	8.237	High
172	J9	2.828	2.296	2.562	Praděd	8.237	High
173	J9	2.543	2.374	2.459	Praděd	8.237	High
174	J9	1.893	2.333	2.113	Praděd	8.237	High
175	J9	2.477	2.286	2.382	Praděd	8.237	High
176	J9	2.525	2.484	2.505	Praděd	8.237	High
177	J9	2.428	2.285	2.357	Praděd	8.237	High
178	J10	2.627	2.228	2.428	Praděd	7.589	High
179	J10	2.977	2.786	2.882	Praděd	7.589	High
180	J10	2.766	2.438	2.602	Praděd	7.589	High
181	J11	2.514	2.217	2.366	Praděd	8.311	High
182	J11	2.863	2.200	2.532	Praděd	8.311	High
183	J11	2.329	2.211	2.270	Praděd	8.311	High
184	J11	2.796	2.294	2.545	Praděd	8.311	High
185	J11	2.788	2.143	2.466	Praděd	8.311	High
186	J12	2.886	2.673	2.780	Praděd	8.472	High
187	J13	2.424	2.169	2.297	Praděd	8.525	High
188	J13	2.396	2.181	2.289	Praděd	8.525	High
189	J13	2.728	2.449	2.589	Praděd	8.525	High
190	J13	2.521	2.334	2.428	Praděd	8.525	High
191	J13	2.590	2.394	2.492	Praděd	8.525	High
192	J13	2.732	2.430	2.581	Praděd	8.525	High
193	J13	2.539	2.301	2.420	Praděd	8.525	High

543

List of larval body sizes (tergit width and elytron length of their mother, both in mm). Larvae were

reared under standardized laboratory conditions and measured in last (3rd) larval instar.

ID	Mother	Tergit	Population	Size of mother	Altitude
1	J10	4.911	Praděd	7.589	High
2	J4	4.850	Praděd	7.838	High
3	J4	4.795	Praděd	7.838	High
4	J4	4.715	Praděd	7.838	High
5	J5	5.078	Praděd	7.848	High
6	J5	4.963	Praděd	7.848	High
7	J6	5.267	Praděd	8.192	High
8	J7	5.498	Praděd	8.247	High

9	J7	5.265	Praděd	8.247	High
10	J7	5.191	Praděd	8.247	High
11	J7	5.015	Praděd	8.247	High
12	J7	5.734	Praděd	8.247	High
13	J7	5.530	Praděd	8.247	High
14	J7	5.255	Praděd	8.247	High
15	J9	4.726	Praděd	8.237	High
16	S1	5.757	Srbsko	10.961	Low
17	S11	6.007	Srbsko	10.743	Low
18	S11	5.386	Srbsko	10.743	Low
19	S12	6.126	Srbsko	10.699	Low
20	S12	5.533	Srbsko	10.699	Low
21	S12	5.906	Srbsko	10.699	Low
22	S2	6.067	Srbsko	10.661	Low
23	S2	5.696	Srbsko	10.661	Low
24	S3	5.704	Srbsko	10.962	Low
25	S3	6.745	Srbsko	10.962	Low
26	S5	5.480	Srbsko	11.446	Low
27	S7	6.099	Srbsko	10.826	Low
28	S7	5.958	Srbsko	10.826	Low
29	S7	6.015	Srbsko	10.826	Low
30	S7	5.119	Srbsko	10.826	Low
31	S7	5.716	Srbsko	10.826	Low
32	S7	6.444	Srbsko	10.826	Low
33	S7	5.260	Srbsko	10.826	Low
34	S7	5.656	Srbsko	10.826	Low
35	S8	4.951	Srbsko	10.335	Low
36	S8	5.726	Srbsko	10.335	Low
37	S8	5.825	Srbsko	10.335	Low
38	S9	5.421	Srbsko	11.564	Low
39	S9	6.308	Srbsko	11.564	Low
40	S9	6.136	Srbsko	11.564	Low

546

547 **S4 dataset**

548 **List of intrapopulation variance for various *Silpha carinata* populations.**

ID	Variance	Sex	Population	Number	Altitude	Locality	Country
1	0.0016	Female	1400P	31	1400	Praděd	Czech Republic
2	0.0036	Male	1400P	20	1400	Praděd	Czech Republic
3	0.0041	Female	1218TV	50	1218	Terebowiec Valley – Szeroki Wierch	Poland
4	0.0050	Male	1218TV	50	1218	Terebowiec Valley – Szeroki Wierch	Poland
5	0.0042	Female	1204SR	39	1204	Suche Rzeki Valley – Szeroki Wierch	Poland
6	0.0021	Male	1204SR	22	1204	Suche Rzeki Valley – Szeroki Wierch	Poland
7	0.0026	Female	1162TV	11	1162	Terebowiec Valley – Szeroki Wierch	Poland
8	0.0028	Male	1162TV	18	1162	Terebowiec Valley – Szeroki Wierch	Poland
9	0.0017	Female	1123TV	30	1123	Terebowiec Valley – Szeroki Wierch	Poland
10	0.0032	Male	1123TV	22	1123	Terebowiec Valley – Szeroki Wierch	Poland
11	0.0030	Female	1089SR	50	1089	Suche Rzeki Valley – Szeroki Wierch	Poland
12	0.0039	Male	1089SR	50	1089	Suche Rzeki Valley – Szeroki Wierch	Poland
13	0.0022	Female	1000K	50	1000	Krušné hory	Czech Republic
14	0.0026	Male	1000K	52	1000	Krušné hory	Czech Republic
15	0.0021	Female	1000G	26	1000	Krkonoše	Czech Republic
16	0.0024	Male	1000G	13	1000	Krkonoše	Czech Republic
17	0.0030	Female	700K	16	700	Krušné hory	Czech Republic
18	0.0028	Male	700K	12	700	Krušné hory	Czech Republic
19	0.0030	Female	700J	50	700	Jeseníky	Czech Republic
20	0.0032	Male	700J	27	700	Jeseníky	Czech Republic
21	0.0020	Female	400G	50	400	Krkonoše	Czech Republic
22	0.0020	Male	400G	50	400	Krkonoše	Czech Republic
23	0.0025	Female	250S	50	250	Srbsko	Czech Republic
24	0.0043	Male	250S	25	250	Srbsko	Czech Republic
25	0.0023	Female	200M	16	200	Milovice	Czech Republic
26	0.0030	Male	200M	11	200	Milovice	Czech Republic

549

Závěry a další směřování výzkumu

I když je proměnlivost ve velikosti těla hmyzu poměrně často studována, výzkumy jsou zaměřeny téměř výlučně na velké prostorové měřítko. Výzkumy změn ve velikosti těla v kratším časovém horizontu nebo na malé prostorové škále jsou poměrně zanedbávány a článků zabývající se proměnlivostí velikosti těla u střevlíků a především mrchožroutů je nedostatek. Přitom střevlíci jsou vhodnými modelovými organismy ke studiu proměnlivosti ve velikosti těla na malé i velké prostorové škále i v průběhu roku, jelikož odpovídají na změny prostředí. Obzvláště v agroekosystémech mohou sloužit jako bioindikátory (Kromp, 1999). Na druhou stranu, velikost těla některých druhů mrchožroutů je často vázána na jiné faktory prostředí. Například velikost mršiny, na které se juvenilní jedinci vyvíjí, může odrážet velikost těla dospělých brouků (Smith *et al.*, 2000). Avšak *Silpha carinata* (Herbst, 1783) je poměrně velký druh, který má pouze jednu generaci do roka a v dospělosti se živí zbytky hmyzu (larva je predátor) a proto je vhodným organismem pro studium změn ve velikosti těla.

Z mého výzkumu vyplývá, že velikost těla *S. carinata* se s rostoucí nadmořskou výškou zmenšuje. Tedy brouk následuje konverzní Bergmannovo pravidlo. Výsledek je i v souladu s výsledky meta-analýzy, kterou prováděli Horne *et al.*, (2015) a zjistili, že zpravidla větší druhy suchozemského hmyzu, které mají pouze jednu generaci potomků za rok, následují toto pravidlo. Ultimátní mechanismy generující geografickou variabilitu ve velikosti těla u *S. carinata* jsou pravděpodobně lokální adaptace (Stillwell, 2010). Ačkoliv se mi provedení zahradního experimentu nevydařilo zcela dle představ, jelikož jsem nedochovala dostatek dospělých jedinců druhé generace, tak přesto lze s určitou mírou opatrnosti konstatovat, že rozdíly mezi populacemi z vyšší a nižší nadmořské výšky byly zachovány a lze je tedy alespoň částečně připsat adaptaci dané populace k lokálním podmínkám prostředí. Samice z nižší nadmořské výšky kladly větší vajíčka a i z nich vylíhlé larvy si zachovaly větší velikost těla než larvy pocházející od matek z vyšší nadmořské výšky. Je zajímavé, že vztah mezi nadmořskou výškou a variabilitou ve velikosti těla uvnitř populace nevyšel průkazně (viz 4. manuskript článku). Obecně samci bývají více variabilní ve velikosti těla než samice (Stillwell *et al.*, 2010). I když v mé studii rozdíly ve variabilitě mezi pohlavími nebyly signifikantní, tak z grafu (viz obr. č. 3, 4. Manuskript článku) je patrný alespoň trend, že i u *S. carinata* je vnitropopulační variabilita nepatrně vyšší u samců. Je možné, že samičí tělo je vystaveno tzv. kanalizaci znaků, protože samice více trpí nevýhodou menší velikosti těla, jelikož menší samice mají významně sníženou plodnost (Honěk, 1993; Stearns & Kawecki, 1994). Je ale nutné podotknout, že míra pohlavního dimorfismu ve velikosti těla *S. carinata* je podél gradientu nadmořské výšky neměnná. Pokud by velikost těla

samců, měla více reagovat na měnící se přírodní podmínky, tak by měli mít samci strmější sklon podél gradientu nadmořské výšky ve srovnání se samicemi (Blanckenhorn *et al.*, 2006). Jelikož s rostoucí nadmořskou výškou klesá teplota a zkracuje se délka sezóny, lze předpokládat snižující se kvalitu prostředí. Ve výsledku by se měl s rostoucí nadmořskou výškou zvětšovat rozdíl ve velikosti těla mezi samci a samicemi. Na druhou stranu Tsuchiya *et al.* (2012) zjistil interakci mezi nadmořskou výškou a pohlavím u střevlíka *C. tosanus*, kde samice jsou větším pohlavím. V tomto případě se naopak pohlavní dimorfismus s rostoucí nadmořskou výškou snižoval. Proto jsem se rozhodla zaměřit své další zkoumání na zjištění vnitropopulačních a mezipopulačních rozdílů ve variabilitě velikosti těla u několika druhů střevlíků a podívat se, jestli tyto rozdíly jsou konzistentní podél gradientu nadmořské výšky a mezi pohlavími.

Velikost těla i tělesná kondice se nemění pouze v prostoru, ale i v čase. Existuje málo studií, které by vyšetřovaly vztah mezi časovou i prostorovou variabilitou v tělesné velikosti i v tělesné kondici (Östman, 2005; Baranovská & Knapp, 2014). Östman (2005) zjistil, že jedinci nemusí být v trvale lepší tělesné kondici nebo tělesné velikosti na jednom poli než na jiném. Proto je důležité provádět studie tělesné kondice a tělesné velikosti déle než jeden rok. Tělesná kondice a tělesná velikost se může měnit v průběhu let vlivem abiotických i biotických faktorů a může se tedy významně lišit mezi jednotlivými roky (Östman, 2005).

Pravděpodobně největší vliv na změny tělesné kondice střevlíků v průběhu sezóny mají strategické faktory, které jsou začleněny do životního cyklu jedinců. Jedinci *Anchomenus dorsalis* (Pontoppidan, 1763) byli v lepší tělesné kondici před přezimováním, jelikož před hibernací zvyšují příjem potravy, aby měli větší šanci přežít zimu. Také pohlavně specifické rozdíly v tělesné kondici byly zaznamenány. Samice byly v lepší tělesné kondici ve srovnání se samci. Zjištění není zcela překvapující, jelikož samice vydávají mnohem více energie do rozmnožování a tedy na samice působí větší selekční tlak. Na druhou stranu vliv faktorů prostředí zaznamenala malou část celkové variability v tělesné kondici *A. dorsalis*. Faktory prostředí v tomto případě mají pouze omezený význam na tvarování variability v tělesné kondici u *A. dorsalis*.

Existuje několik faktorů (např. potravní nabídka, teplota, disturbance prostředí), které mohou mít významný vliv na variabilitu ve strukturální velikosti těla. Tyto faktory jsou poměrně málo prozkoumané, ale již existuje několik studií, které se tímto problémem zabývají (Smith *et al.*, 2000; Laparie *et al.*, 2010; Giglio *et al.*, 2011). Z klimatických faktorů nejen teplota ale i vlhkost (Stillwell *et al.*, 2007) je důležitým činitelem, který může generovat proměnlivost ve velikosti těla. V současné době, kdy můžeme na

vlastní kůži pocítit působení klimatických změn a zvyšující se období such, je důležité zjišťovat, jak je hmyz připraven na tyto výzvy. Zejména zemědělská krajina a její organismy mohou být citlivé na sucho. Například populace střevlíků, kteří jsou významnou součástí agroekosystémů jako predátoři škůdců, mohou být významně narušeny působením různého abiotického stresu. Proto jsem svůj výzkum v posledním roce zaměřila na působení nedostatku vody na vybrané druhy střevlíků pocházejících z různých biotopů.

Závěrem bych chtěla říci, že i když je variabilita ve velikosti těla značně zkoumána, vystává zde mnoho dalších otázek, na které by se měly dále hledat odpovědi. Například: Jaké proximátní mechanismy generují geografické gradienty ve velikosti těla hmyzu? Jaké faktory ovlivňují pohlavní dimorfismus ve velikosti těla u hmyzu? Jsou teplota a potravní nabídka jedinými činiteli, kteří způsobují variabilitu ve velikosti těla na malém prostorovém měřítku a proměnlivost ve velikosti těla v krátkém časovém horizontu u hmyzu? Liší se vnitropopulační proměnlivost ve velikosti těla u hmyzu mezi populacemi stejného druhu podél geografických gradientů?

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