

Abstracts of the Immature Beetles Meeting 2021 September 30–October 1, Prague, Czech Republic

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The Immature Beetles Meeting is a traditional two-day get-together of the international coleopterologist community, aimed mainly at beetle larvae. Despite the still ongoing COVID-19 pandemic situation, the meeting took place on 30 September and 1 October in Prague. This year, the special pandemic conditions forced us to bring the meeting partially to the online space to make the attendance possible for all colleagues from all over the world. Therefore, the meeting went on in a hybrid - both personal and online form. The meeting took place, as traditional, at the Faculty of Science, Charles University, and was co-organized by the Department of Entomology, National Museum in Prague, the Crop Research Institute in Prague and the Czech Entomological Society. In total, 22 participants attended the meeting in person (see Fig. 1), while another 11 participants presented their talks in the online meeting form. Many other colleagues also attended the meeting online, but unfortunately, we are not able to provide the full list of these participants. Most of the attendees who arrived personally were from the Czech Republic and other European countries; in the online session, also colleagues from Asia and South America attended the meeting as they presented their talks or posters, but we are also aware of people from North America, or Australia, who appeared in the online space as well.

A total number of 14 contributions were presented, including 4 posters and 10 talks. The topics presented in the meeting covered a large spectrum of beetle families, including Cerambycidae, Coccinellidae, Hydrophilidae, Lucanidae, Lycidae, Phengodidae, Silphidae, Staphylinidae, Tenebrionidae and Trictenotomidae. Topics of general studies of beetle larvae were presented as well, including the modern methods of next-generation sequencing used in barcoding, biological sequence databases, and ecology. Moreover, the meeting continued as usual also in the evening after the session closure, in the informal "Larval Pub", with discussions about beetles and life over a glass of the famous Czech beer. 2021

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Although the meeting was largely affected by the specific conditions caused by the epidemic situation, we are happy that the pandemic couldn't prevent the coleopterists from meeting and sharing interesting research topics again. Hopefully, next years will allow all colleagues to come to Prague in person again, to meet fully with a lot of fascinating presentations and nice (not only) beetle talks during the evening pub gatherings. For more details about the IBM, see the following online address: www.immaturebeetles. eu/ as well as our Facebook page: www.facebook.com/ ImmatureBeetlesMeeting/.

organizers of the Immature Beetles Meeting

Acknowledgements

We are very grateful to all colleagues who supported the organization of the IBM 2021, and who attended the meeting online or in person. We also thank all participants who presented their posters or talks, as well as those who attended the informal beetle discussions in the evening larval pub. Ivan Čepička (Head of the Department of Zoology, Charles University) kindly supported our initiative to organize the meeting and provided the space where the meeting took place. We are also grateful to the Faculty of Science for the possibility to use the Zoom online meeting platform to connect us with the online attendants. The National Museum in Prague, the Crop Research Institute and the Czech Entomological Society supported IBM 2021 as well. The meeting was organized as a part of the research activities of the organizers, partially supported by the grant SVV-260571/2021 (from Charles University) to AFD, OK, and DV, and the grant of the Ministry of Culture of the Czech Republic (DKRVO 2019-2023/5.I.c, National Museum, 00023272) to OK and DV.

List of participants

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The abstracts should be cited as follows:

FERREIRA V. S. & IVIE M. A. 2021: DNA barcoding and collectionsbased life stage associations reveals a hidden phenotypical diversity in the Puerto Rican bank paedomorphic Lycidae (Coleoptera: Elateroidea: Leptolycini). P. 633. In: DAMAŠKAA. F., KOUKLÍK O. & VONDRÁČEK D. (eds): Abstracts of the Immature Beetles Meeting 2021, September 30 – October 1, Prague, Czech Republic. Acta Entomologica Musei Nationalis Pragae 61 (2): 631–638.



Fig. 1. Most participants of the Immature Beetles Meeting 2021 during the lunch (Prague, 30th September).

ORAL PRESENTATIONS

DNA barcoding and collections-based life stage associations reveals a hidden phenotypical diversity in the Puerto Rican bank paedomorphic Lycidae (Coleoptera: Elateroidea: Leptolycini)

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The Leptolycini are a group of Lycidae endemic to the West Indies. Leptolycini adult females have been hypothesized to be extreme paedomorphic (i.e., larviform), however, females and larvae of the group are currently unknown. In this study we provide for the first time DNA barcoding life stages associations based on partial cytochrome c oxidase subunit 1 (COI) of winged males and immature stages of specimens from Puerto Rico, altogether with collections-based associations of Leptolycini immatures and one extreme paedomorphic female from the Virgin Islands. To carry out these life-stage associations we prepared an in-depth review of the Leptolycini fauna of the Puerto Rican bank (Puerto Rico and Virgin Islands). We provided morphological diagnosis of the larvae and female found, diagnostic redescriptions of the already described adult males and full descriptions of the new ones. Discussions on the taxonomy, nomenclature and intraspecific variation of many taxa are also provided, and several new taxa and taxonomic arrangements are proposed. The larvae of Leptolycus, Cessator and new Leptolycini genus are described for the first time, and larvae diagnoses of many species are given. The first female of a Leptolycini is diagnosed and illustrated. An updated key to the adults and immature forms of Leptolycini from the Puerto Rican bank and a discussion on the importance of scientific collections in biodiversity studies is also provided.

Using barcoding to explore the diversity of beetles and their larvae in forest leaf litter in Taiwan

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Forest leaf litter habitats are sometimes dubbed 'terrestrial coral reefs', referring to the immense arthropod diversity they host. Soil arthropods, including beetles, are litter transformers and predators, contributing to organic matter decomposition and nutrient cycling, and are often tightly associated with specific forest types or microhabitats. The immense species diversity, which makes them crucial for forest ecosystems, also makes their studies challenging. A limited number of taxonomic experts and a high proportion of unknown species make the identification time-consuming or impossible. Moreover, many specimens sampled by leaf litter sifting are beetle larvae which are even more difficult to identify and hence usually omitted from studies; it remains untested whether this can introduce significant bias to ecological data. Using short DNA cox1 sequences (barcodes) of each specimen or morphospecies (DNA barcoding) or of all specimens in the sample at once (DNA metabarcoding) is often considered a solution for such cases, providing quick data about species diversity. Our goal is to optimize existing DNA barcoding and metabarcoding protocols using the Oxford Nanopore MinION technology for leaf litter beetle samples and use them to intensify the biosystematics and ecological research of species-rich leaf litter beetle communities in Taiwan. Our approach is focused on (1) building a DNA barcoding library of Taiwanese beetles facilitating species identification and discovery, (2) understanding the importance of larval specimens in diversity estimates, and (3) associating larval and adult specimens to understand the biology and larval morphology of leaf litter beetles. We demonstrate our approach on the samples from three localities in the submontane evergreen forests of the Huisun Forest Station in central Taiwan. Preliminary data indicate that each 6-litre sample contains about fifty beetle species, 20% of which are represented by larvae only. As expected, DNA barcodes facilitate species delimitation and discovery, allows for the association of adults with larvae and promote more detailed systematics research. We demonstrate it here by the case of the new species of Oxyomus (Scarabaeidae: Aphodiinae), for which we also got DNA-associated larvae and used them for a detailed description of larval morphology.

Acknowledgements. This project is supported by the Taiwanese Ministry of Science and Technology project MOST 110-2621-B-110-001.

The cryptic, subterranean development of the immature European stag beetle *Lucanus cervus*

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The European stag beetle, *Lucanus cervus* spends 97% of its life cycle in a subterranean habitat while it is developing from an egg into an imago. After emerging from the soil, the imago spends a very limited period above ground before dying. Research during the last twenty years (1990s to the present day), has focused predominantly on the imago after it has emerged above ground. This has

significantly advanced our knowledge of the beetles' behaviour, ecology and distribution. However, little professional attention has been paid to the subterranean period of stag beetle development.

My presentation illustrates the cryptic development period of the immature stag beetle including its obligate dependency on a female-specific yeast species.

A short guidance for Barcode of Life Data System (BOLD) using cerambycid larvae

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The Barcode of Life Data System (BOLD) is an online workbench and database that supports the assembly and use of DNA barcode data. It is a collaborative hub for the scientific community and a public resource for citizens, developed at the Centre for Biodiversity Genomics in Canada. It delivers an online database for the collection and management of specimen, distributional, and molecular data as well as analytical tools to support their validation. In this presentation, by using both larvae and adults of longhorned beetles (Coleoptera: Cerambycidae), I introduce how to submit a microplate with insect tissue to the Centre for Biodiversity Genomics, University of Guelph and further manage and process the data in BOLD. A brief guide on the most useful functions of the platform is also provided.

Insect communities attracted to wild boar carcasses – enumeration of a year-long study

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We performed a year-long study of insect communities attracted to wild boar carcasses. The study took place between August 2020–September 2021 and approximately 70 wild boars of different age, sex, body size were placed in the National Park Bayerischer Wald (Bavaria, Germany). We used different locations for our experiments: pigs were placed into open/wet, open/dry, closed/wet and closed/ dry conditions. The number of insect species and indeed specimens (including larvae) differed greatly based on the pig location and season. Our results indicate that while Dipteran larvae show high abundance from spring (months of April and May), the Coleopteran larvae (represented mainly by the larvae of Silphidae) peak in August. We also report a remarkable discovery of winter-active Diptera larvae that are able to survive under the carcass until the final days of December, indeed under snow

Gondwanan genera of Oxytelinae in the Southern Temperate zone: larval descriptions (Coleoptera: Staphylinidae)

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Seven genera of the staphylinid subfamily Oxytelinae share a supposed Gondwanan origin reflected in the disjunct distributions of these genera today. Larvae are described for *Blediotrogus cordicollis* Broun, 1907, *Teropalpus coloratus* Sharp, 1900, *Sartallus signatus* Sharp, 1871, and the supposed larva of *Homalotrichus newtoni* sp. nov., additional larval characters are given for *Oxypius peckorum* Newton, 1982. The challenges of exotic larval descriptions based on non-reared material are discussed.

Preliminary observations on "trenecitos" (Phengodidae, Coleoptera) from a Mexican temperate forest

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Despite almost 60 phengodid species have been recorded in Mexico (ZARAGOZA-CABALLERO & PÉREZ-HERNÁNDEZ 2014), there is only one published record of phengodid immature stages from the country, which is focused on the feeding behaviour of larvae on millipedes (SÁNCHEZ-ECHE-VERRÍA et al. 2014). We present our preliminary observations on the natural history of two morphospecies of immature Phengodidae, *aka* "trenecitos", from a temperate forest near Morelia, Michoacán, Mexico. Morphospecies of railroad worms showed a high-density population, and we observed them inhabiting different microhabitats within a reforested area. We made field observations on larvae and obtained eggs, first instars of larvae, pupae, male and female adults from laboratory rearing. For the first time, we report phengodid larvae preying upon Coleoptera and Lepidoptera larvae.

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Larval morphology of *Oiceoptoma thoracicum* (Coleoptera: Silphidae) and its comparison with immature stages of other carrion beetles

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Carrion beetles of the genus *Oiceoptoma* Leach, 1815 are an important group of necrophagous insects, with great potential for forensic entomology. The genus is Holarctic in distribution, thus offering forensic applications throughout the temperate zone. However, proper identification of its larvae and determination of particular instars has not yet been described. Therefore, *Oiceoptoma* cannot be fully utilized in forensic investigations. The aim of our project is to describe all larval instars of *Oiceoptoma thoracicum* (Linnaeus, 1758), a common and widespread Palaearctic species, in detail, and create an identification key to its larval instars. We used the methods of conventional morphology (employing light microscopy and scanning electron microscopy) and morphometrics. All three instars differ consistently in a colour pattern on a lateral part of protergum. Proportions of body length, head width, protergum length, metanotum width, length of basal segment of urogomphi, and length ratio of urogomphi segments are also significantly different between instars. Furthermore, we compare our results with immature stages of three other European carrion beetle species, *Heterotemna tenuicornis* (Brullé, 1836), *Thanatophilus rugosus* (Linnaeus, 1758), and *T. sinuatus* (Fabricius, 1775), which were recently redescribed in detail, using similar methods. This work was funded by the Internal Grant Agency of the Faculty of Environmental Sciences, University of Life Sciences Prague (no. 2020B0046).

Case report: Tenebrionidae immatures and adults feeding on corn and wheat cereal at Beirut port after August 4 blast

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Forensic stored product entomology is the field of forensic entomology that deals with insect pests of raw and processed cereals, seeds, dried fruits, nuts, and other types of dry food commodities (RIVERS & DAHLEM 2014). It addresses issues of both civil and criminal nature (RIVERS & DAHLEM 2014). On August 4, 2020, approximately 3,000 tons of ammonium nitrate which were kept at the Port of Beirut detonated forming one of the worst nonnuclear manmade explosions and the 3rd largest explosion in modern time (CHEAITO & AL-HAJJ 2020). This blast totally harmed the port main grain silos which contained more than 80% of Lebanon's imported grains (CHEAITO & AL-HAJJ 2020). An entomological analysis of the grain still present one year after the blast should determine its possible pest infestation.

After the permission given by the general security forces of Lebanon, two people inspected the place of destroyed silos on June 7, 2021 and collected adults and immature insects, and took photos of the place and of the disintegrating cereals that are still found at the port. Adult beetles were reared on wheat flour and burnt corn during the summer of 2021 and they were kept to breed in order to study the immatures and the life cycle. Three Tenebrionidae genera were identified, viz. *Opatrum*, *Opatroides* and *Tribolium*. Species determination will be confirmed later. In the reared samples, the latter taxon was the most abundant, represented by larvae and pupae. They did infest the wheat flour after an approximate of one month. Also, a second generation of adults was also collected in August 2021.

When the population density of a given insect exceeds some unacceptable subjective threshold level beyond which economic damage occurs, a pest status is achieved (CHEAITO & AL-HAJJ 2020). *Tribolium castaneum* is considered a major pest of both wheat and rice flour and was reported on corn (GERKEN & CAMPBELL 2020). It has high fecundity on wheat flour (GERKEN & CAMPBELL 2020). When it infests the host diets, it decrease their biomass and food quality and they will be contaminated (NASERI et al. 2017). Understanding the pattern and rate of development of insects on the diets they infest is critical to developing management tools (GERKEN & CAMPBELL 2020). Stored product insects are one of the major concerns of food production and storage across the globe and the defect action levels are defined by FDA and should be monitored for the safety of consumption (RIVERS & DAHLEM 2014). The grains that are still found in Beirut port could be used for feeding insects in certain growth chambers and then harvesting them for producing food; however, there are strict regulations existing which includes not feeding spoiled grains to insects for producing humans' food. Eventually, food safety measures should be taken.

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Larval niche partitioning of two longhorn beetles, *Batocera rufomaculata* De Geer and *Glenea multiguttata* Guérin-Méneville (Coleoptera: Cerambycidae) in mango

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Mango, Mangifera indica L., considered as the king of fruits, is the leading fruit crop of India cultivated in an area of 2.3 million hectares with an annual production of 21.28 million metric tonnes during 2019-2020. Mango trees are infested by several insect pests among which mango hoppers, stem borers, fruit flies and stone weevil are of major economic importance. The stem borers, of late, have been emerging as a serious threat to mango orchards. They attack the trunk and branches disrupting the xylem and phloem conduction of the plant thus leading to the death of productive trees. There are several stem borer species belonging to Cerambycidae and Buprestidae, of which longhorn beetles are serious economic pests. The species commonly associated with mango are Batocera rufomaculata De Geer, Batocera rubus (L.), Glenea multiguttata Guérin Méneville, and Coptops sp. Of these, B. rufomaculata, commonly known as mango stem borer, is the most destructive one. It is prevalent across the country while B. rubus is found mostly in North India. In South India, another species, G. multiguttata is also found affecting mango trees. The larval stages are the most damaging ones that bore into the stem and tunnel into the pith of the trunk or branch of the tree. Though they have a similar pattern of feeding inside the stem, rarely different species are found together at the same part of the tree thus giving an indication of niche partitioning. Hence a study has been undertaken in selected mango orchards at Bengaluru, Karnataka, India to investigate the feeding and damaging pattern of larvae of B. rufomaculata and G. multiguttata on mango. The trunks and branches of the trees showing stem

borer damage were wrapped with a nylon mesh to observe the emergence of the adult beetles. The covered trees were monitored at fortnightly intervals to record the adult emergence. It was observed that B. rufomaculata emerged from the main trunk while G. multiguttata emerged from lateral branches. Thus it can be inferred that B. rufomaculata mainly infests the main trunk while G. multiguttata preferred the lateral branches thus avoiding interspecific competition and the niche partitioning was evident. The number of B. rufomaculata was low (1-2 per tree) compared to that of G. multiguttata (4–5 per tree). The larval damage of G. multiguttata was more prominent with extensive tunnelling in the branches, which lead to quick drying and wilting. It was further observed that the partially dried branches were more prone to G. multiguttata attack, thus the other stem or trunk borer infestation might be acting as a predisposal factor for G. multiguttata infestation. The exit holes of adult beetles of G. multiguttata in the lateral branches of the mango tree was observed to be of 3-4 mm diameter while that of *B. rufomaculata* were in the range of 5–7 mm. From the studies, it is evident that the selection of infestation sites by the two cerambycids within a tree is correlated with the body size of larvae as they grow. Batocera rufomaculata larva being relatively larger in size preferred main trunk and G. multiguttata being relatively smaller confined to lateral branches. Besides the body size, the duration of the larval stage of these cerambycid borers might also influence the niche partitioning in relation to resource availability, which may be further explored.

POSTERS

Unlocking the biological secrets of the genus *Autocrates* Thomson, 1860 (Coleoptera: Trictenotomidae)

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The family Trictenotomidae Blanchard, 1845 is one of the smallest families of the tenebrionoid beetle superfamily Tenebrionoidea, with 17 valid species in two genera – *Trictenotoma* Gray, 1832 and *Autocrates* Thomson, 1860 (TELNOV & DRUMONT 2021). They are widely distributed in the Oriental Region, and also in the eastern Palaearctic

Region restricted to China and South Korea. Adult Trictenotomidae, often mistaken for longhorn beetles or stag beetles, has received much attention due to their large size and unusual appearance. The larval form of *Trictenotoma childreni* Gray, 1832 and *T. formosana* Kriesche, 1919 described by GAHAN (1908) and HU et al. (2020) clearly indicated that this group belongs to Tenebrionoidea. The natural history of *T. formosana* was published by LIN & HU (2018, 2019). However, nothing is known about the immature stages and biology of the genus *Autocrates* for 160 years.

The first three authors collected two females of Autocrates maqueti Drumont, 2006 using light traps from its easternmost occurrence, South Korea in early June 2021. Following successful oviposition, bionomic characteristics such as oviposition behavior, larval behavior and food under artificial conditions are observed for the first time. As of August 29, the larvae have grown to the 8th instar. DNA barcoding of the species is also obtained. The morphological, molecular and ecological analysis beyond the scope of this preliminary study will improve our understanding of the phylogeny and biology of the genus *Autocrates* and related groups.

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Notes of the ecology and immature forms of *Odontolabis* sp. (Coleoptera: Lucanidae) beetle in Mt. Pantaron, Cabanglasan, Bukidnon, The Philippines

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Notes on the ecology and immature stages of the *Odontolabis* sp. stag beetle are provided. The habitat and the immature forms – larvae and pupae are described and documented from specimens collected in Mt. Pantaron, Cabanglasan, Bukidnon, Philippines from last February 4, 2020, to May 20, 2021 during fieldwork and reared in the

rearing laboratory of the University Museum, CMU, Musuan, Bukidnon, Philippines. The habitat of *Odontolabis* sp. is represented by fallen decaying logs in the montane forest and larvae and pupae were successfully reared to adults in the laboratory.

Phylogeny of water scavenger beetles (Coleoptera: Hydrophilidae) based on the study of traditional morphology and the sensory system of larvae

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Larvae of water scavenger beetles (Coleoptera: Hydrophilidae) are adapted to a wide variety of aquatic habitats, and this diversity is reflected by both morphology and the sensory system. The study of Hydrophilidae larvae has proven to be a source of reliable characters at different taxonomic levels. However, most of these analyses focus on small groups such as subfamilies or tribes (e.g. ARCHANGELSKY 2004, 2008; ARCHANGELSKY et al. 2020; MINOSHIMA et al. 2013). Here we assessed the higher-level relationships of water scavenger beetles based on larval characters. With this purpose, a phylogenetic analysis of 329 characters (179 derived from sensory system, 143 morphological, 7 ecological) scored for 103 taxa was performed. Resulting data matrix was analyzed using parsimony under implied weights (K=20). Hydrophilidae was recovered as monophyletic with high statistical support, which is consistent with previous molecular and morphological studies. Subfamilies and tribal relationships usually showed weak support, except for a few well-supported clades such as Acidocerinae and Enochrinae, Hydrophilini, Hydrobiusini, Megasternini, and some groups of genera. The tribe Laccobini was non-monophiletic. The *Paracymus* and *Tormus* genera of the *Paracymus*-group were found nested within Chaetarthriini with low support, and *Tritonus* was recovered as a sister group to Hydrobiusini with relatively high support. The phylogeny of the family was difficult to reconstruct due to the combined effect of convergent evolution and the number of derived character states given the selective pressure of the environment. These two conditions affect clade relationships, especially at subfamily and tribe level. However, many characters derived from the external sensory system or from traditional morphology have proven useful in defining the relationships between certain groups.

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Larval nutrition-induced plasticity affects reproduction in ladybird beetles

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In the environment resources often become limiting and the environment is frequently heterogeneous in terms of space and time and life history theory explains the behaviours and tactics used by different organisms to enhance their reproductive fitness in different environmental conditions. Consequently, animals should evolve in their morphology, function or behaviour so as to maximize their fitness under differential resources. The adults emerging from juvenile/immature stages facing favourable nutritional conditions have higher fitness. However, the environment-matching hypotheses suggest that fitness is not only limited to juvenile nutrition but to the continuation of a favourable environment in the adult stages as well. For this experiment, newly hatched larvae were subjected to one of the two food regimes, i.e. abundant and scarce till pupation in Propylea dissecta (Mulsant). The newly emerged adults from each food regime were further split into two postemergence food regimes, viz. abundant and scarce, and continued to be reared on the same food regime until they become 10 days old. On the 10th day of postemergence, individuals were paired together in the following combinations: (i) Abundant (A \bigcirc) × Abundant $(A \stackrel{\bigcirc}{\downarrow}), (ii)$ Abundant $(A \stackrel{\bigcirc}{\multimap}) \times$ Scarce $(S \stackrel{\bigcirc}{\downarrow}), (iii)$ Scarce $(S \stackrel{\bigcirc}{\multimap})$ × Abundant (A $\stackrel{\bigcirc}{+}$) and (iv) Scarce (S $\stackrel{\bigcirc}{-}$) × Scarce (S $\stackrel{\bigcirc}{+}$). The results revealed that the food availability at different life stages, viz. larval and adult diet plays an important and differential role in shaping behavioural and reproductive traits in adults. Food conditions experienced during early life stages may result in developing adaptations for future environments. However, this condition dependent response

is not limited to the larval stage only as the adults are also under constant selection pressure. Thus, the present study reveals that: (i) food is considered of paramount importance and any change in food supply will affect an individual's reproductive performances and success, (ii) food stress in early life stages exert a strong effect on reproductive fitness, which might result in small body size and reduced fecundity, (iii) fecundity was affected by the abundant diets, not the sequence. As long as the ladybird beetles had abundant food regime at any two stages in either one of the three feeding stages, the stress of scarce food regime could be effectively dealt with by the females.

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