# Blue light-induced immunosuppression in *Bactrocera dorsalis* adults, as a carryover effect of larval exposure

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## Abstract

Detrimental effects of ultraviolet (UV) light on living organisms are well understood, little is known about the effects of blue light irradiation. Although a recent study revealed that blue light caused more harmful effects on insects than UV light and blue light irradiation killed insect pests of various orders including Diptera, the effects of blue light on physiology of insects are still largely unknown. Here we studied the effects of blue light irradiation on cuticular melanin in larval and the immune response in adult stage of *Bactrocera dorsalis*. We also evaluated the effects of blue light exposure in larval stage on various age and mass at metamorphosis and the mediatory role of cuticular melanin in carryover effects of larval stressors across metamorphosis. We found that larvae exposed to blue light decreased melanin contents in their exoskeleton with smaller mass and delayed metamorphosis than insects reared without blue light exposure. Across metamorphosis, lower melanotic encapsulation response and higher susceptibility to *Beauveria bassiana* was detected in adults that had been exposed to blue light at their larval stage, thereby constituting the first evidence that blue light impaired adult immune function in *B. dorsalis* as a carryover effect of larval exposure.

**Keywords:** blue light, immunocompetence, *Bactrocera dorsalis*, melanotic encapsulation, carryover effects

## Introduction

Various methods used for the control of insect pest have a variety of concepts behind their mode of action. Since the negative effects of insecticide application are known and acknowledged far and wide, environment-friendly insect pest control strategies are gaining importance. Insects are known to have a variety of biological responses to various wavelengths of the light spectrum (Tariq *et al.*, 2015). Many insect

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control strategies utilize knowledge of light-dependent behavior of pest insects. Nocturnal insect are attracted towards light (including UV and visible light), which provides the basis for electric insect killers. UV-emitting insect traps effectively attract insects and prevent their entry to greenhouses. This environment-friendly insect pest-control method is being used as a tool in integrated pest management (IPM) programs (Johansen *et al.*, 2011; Ben-Yakir *et al.*, 2013). A plethora of literature reports the negative impact of Ultraviolet (UV) radiation on the biology of pest insects (Tariq *et al.*, 2015). After penetrating cells, UV light induces the production of reactive oxygen species that cause damage to nucleic acids, membrane of lipids, and proteins (Jurkiewicz & Buettner, 1994; Vile & Tyrrell, 1995). A recent study revealed that blue light (400–500 nm) induced more harmful effects than UV light to insect pests of various orders including Diptera (Hori *et al.*, 2014) suggesting that blue light can also be used in IPM programs. However, for insect pest control through blue light, its effects on insect physiology must be understood, though such information is largely lacking.

Animals protect themselves from harmful effect of UV radiation through the deposition of melanin, which is a photo protective compound (Ortonne, 2002). In animals, melanin perform key roles in immune system, camouflage, wound healing and cuticle hardening (Roulin, 2014). Phenoloxidase (PO) enzyme, a part of the pro-PO cascade, is produced by hemocytes, and is responsible for the production and regulation of melanin in insects (Sugumaran, 2002). A recent study revealed that UV light impaired adult immune function of damselfly as a carryover effect of larval exposure, the exposed larvae invested more in cuticular melanin, which caused immunosuppression in adult flies (Debecker *et al.*, 2015). However, it is still unknown whether insects protect themselves from blue light exposure through the deposition of melanin or there is any effect of blue light irradiation on immune function of insects.

Different studies showed that larval stressors can bridge metamorphosis and can severely affect adult fitness-related traits (Pechenik *et al.*, 1998; Pechenik, 2006; Campero *et al.*, 2008*a*). As previous studies documented that blue light is more harmful to insects than UV light (Hori *et al.*, 2014), we propose that blue light exposure may have effect on cuticular melanin synthesis and subsequently cause immunosuppression in *Bactrocera dorsalis*. Larval stressors can also change adult traits with or without affecting metamorphic traits (Marjan De Block, 2005; Allen *et al.*, 2010). However, the effect of blue light exposure at larval stage, on metamorphic traits and adult fitness is poorly understood.

In this study, we evaluated the effect of blue light exposure on cuticular melanin at larval stage and adult immune response of *B. dorsalis*. In addition, we investigated the effects of blue light exposure at larval stage on age and mass at metamorphosis and the mediatory role of cuticular melanin in carryover effects of larval stressors across metamorphosis. The immune response in the adults was evaluated by using melanotic encapsulation technique, which is commonly used to measure immuno-competence in invertebrates (Rantala & Roff, 2005) and by the quantification of hemocytes and PO activity. It is known that natural pathogens trigger immune response more robustly than artificial challenge (Adamo, 2004), and can be used to evaluate the efficiency of insect immune system (Neyen *et al.*, 2014). Thus, adult immune status was directly measured through survival after exposure to a natural pathogen, *Beauvaria bassiana* (Bals.). *Beauveria bassiana*

(Bals.) has been extensively explored against fruit fly (Ekesi *et al.*, 2002; Dimbi *et al.*, 2003; Daniel & Wyss, 2009; Cossentine *et al.*, 2010) as a potent entomopathogen. We assessed the immune performance of adult flies through conidial exposure to *B. bassiana*.

## Materials and methods

### Insect cultures

The second-instar larvae of *B. dorsalis* were obtained from the colony reared in our laboratory according to the methods described by Zheng *et al.* (2012). Larvae were transferred to liquid-based artificial diet (Chang *et al.*, 2006) at 25–26°C with 12:12 h light: dark photoperiod and 60–65% RH. The larvae used in this experiment were synchronous and were randomly attributed between treatments (blue light and control), which minimized the effect of size difference and initial instar collection on test results.

### Experimental design

A blue light emitting diode (LED) arrays (light emission surface: 150 × 150 mm; 360 LEDs were equally arranged on a panel; LED type: φ3-mm plastic mould; Langtuo Biological Technology Co. Ltd., Hangzhou, China) that emits radiation in the range of 400–500 nm (center wavelength: 460 nm) was used as the source to irradiate larvae. The distance between the light source and the larvae was 50 cm; the emitted energy of 460 nm was at an intensity of 0.12 W m$^{-2}$ measured by a radiometer (AR823; Digital Lux Meter Co. Ltd., China). Each larvae was confined in a small Petri dish (5 cm diameter) containing 0.5 gram of liquid diet (Hanife, 2008). During measurements, the distance between the light source and the radiometer sensor was approximately the same as it was between the light source and insects in the incubator.

The insects were irradiated inside Petri dishes covered with glass lids. The same glass lid was used to determine the intensity of the light prior to insect radiation. Insects were irradiated inside incubator. The light source was connected with digital timer switches (HTS-AT10, Han-Seung, Daegu, Korea) to allow the treatment of insects for the exact periods of time. The Petri dishes containing larvae were placed directly under the light source during irradiation.

At the start of exposure period, 240 larvae were equally divided into four groups (A, B, C and D). After exposure the group A larvae were used for life history parameters, melanin quantification, encapsulation response, PO activity and hemocyte number counts, and the group B larvae were used at their adult stage for conidial exposure to *B. bassiana*, while the group C and D larvae were kept without exposure, and were used as controls along with the group A and B larvae. The final sample sizes for each response variable are shown in the figures.

The second-instar larvae were randomly assigned to blue light treatments and were individually exposed to radiations for 7 days with the duration of 7 hours per day. The temperature and relative humidity (RH) of the irradiated area was set at 25–26°C and 60–65%, respectively. The control larvae were kept in incubator without exposure. After irradiation, larvae were allowed to continue their life cycles. The late third-instar larvae were allowed to pupate in Petri dishes containing humid sand.

## Life history

Prior to use in experiments and at the end of last exposure period, the larvae were weighted to about 12.5 mg using an electronic balance (Sartorius BSA124S, Germany). The individual larval growth rates were calculated by the following formula (Stoks *et al.*, 2012): (the final wet mass − the initial wet mass)/number of days.

The developmental time of larvae was defined as the number of days to reach the final-instar (Debecker *et al.*, 2015). After eclosion and before immune challenge, the adults were weighted. Four larvae died during the larval stage, two adults did not emerge successfully and two adults showed deformations, and were not further analyzed.

## Melanin quantification

Melanin present in the final-instar's exuviae was measured. The exuviae of successfully emerged flies were collected, the melanin extraction protocol described by Zhou *et al.* (2012) was used. The extraction of melanin was carried out by adding 1 M NaOH/10% DMSO in each individual exuvia, the volume for each exuvia was adjusted (extraction volume in µl = 200 × exuvial mass in mg) as described by Debecker *et al.* (2015). The samples were incubated at 80°C for 2 h so that melanin can dissolve in solvent followed by centrifugation at 12,000 *g* for 15 min. Each sample (20 µl) was loaded onto a 384-well plate with three replications and absorbance was read at 380 nm using microplate spectrophotometer (XMark™, BIO-RAD). Synthetic melanin (Sigma-Aldrich, St. Louis, MO, USA) Cat. No. M8631) was dissolved in 1 M NaOH/10% DMSO to prepare concentration gradients (0–500 µg ml$^{-1}$) for making a standard curve (range of 1–100 µg ml$^{-1}$). Exuvial melanin was measured by extrapolating with standard curve from synthetic melanin. The cuticular melanin content was calculated as the amount of melanin in the exuviae (in µg) per mg larval wet mass.

## Encapsulation assay

Encapsulation rate followed by melanization is an effective innate immune response against parasites. This innate immune response of insects can be triggered by inserting a nylon filament (inert antigen) (Rantala *et al.*, 2000; Therry *et al.*, 2014) in the thorax of the insect. The ability of immune system to encapsulate artificial object (nylon filament) is correlated with the ability to encapsulate parasites (Smilanich *et al.*, 2009). After the adult exoskeleton was hardened a nylon filament (mean length of insert ± SE: 1.00 ± 0.02 mm, diameter: 0.20 mm; rubbed with fine sandpaper) was inserted into the thorax of flies to quantify the encapsulation rate (Nagel *et al.*, 2011). Adults were kept for 24 h in the same conditions, and then encapsulated filaments were removed and stored at −20°C until the quantification of the deposited melanin. The adult flies were also frozen at −80°C for the analysis of PO activity and the hemocyte number. To quantify the melanin deposited on filaments, we used the same protocol used for the extraction of melanin of the exuviae. The melanin deposited on the filaments was desorbed in fixed volume of 50 µl of NaOH/10% DMSO, and the fixed sample was loaded onto a 384-well plate with two replications (2 × 20 µl). Absorbance was read at 380 nm using microplate spectrophotometer (XMark™, BIO-RAD, USA). The degree of melanization could be affected by the length difference of the inserted filaments, so encapsulation rate was expressed as the amount of melanin per unit length (µg mm$^{-1}$) of the inserted filament. The filaments were photographed under microscope (Olympus B × 51, Tokyo, Japan), and the length was measured using Image J. software (US National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/).

## Quantification of hemocytes

To quantify the hemocytes, hemolymph was extracted from adult flies by perfusing in 120 µl ice-cold cacodylate buffer (0.01 M Na-Coc and 0.005 M CaCl$_2$) as described by Campero *et al.* (2008*b*). A total of 20 µl of each hemolymph sample was placed into a well of multi-well microscopic slide, stained with 5 µl ethidium bromide (2.5 mM), and incubated at 4°C for 2 h. Afterwards, three images of each well was captured using fluorescence microscope (Olympus B × 51, Tokyo, Japan) at magnification of 400×. Hemocytes were counted by Image Pro-Plus software (Image Pro-Plus 6.0; Media Cybernetics, Silver Spring, MD, USA). The average of the three counts was taken to represent the value of each test sample. The hemocyte number was expressed as the total count per mg of adult wet mass.

## PO assay

The PO activity was measured by spectrophotometric assay (Shi & Sun, 2010). The remaining volume of hemolymph sample (100 µl) was centrifuged at 15,000 *g* for 10 min at 4°C, After centrifugation, 30 µl of the supernatant was mixed with 110 µl milli-Q water, 30 µl phosphate buffered saline (PBS buffer:250 ml distilled water, 2 g NaCl, 50 mg KCl, 360 mg Na$_2$HPO$_4$, 360 mg K$_2$HPO$_4$, and pH 7.2) and 30 µl L-Dopa (Acros Organic, Morris Plains, NJ, USA, 4 mg ml$^{-1}$ ultrapure water) as a substrate, and was kept at 30°C for 90 min, the absorbance was measured at 490 nm every 20 s in duplicate using SpectraMax reader (Molecular Devices Corp., California, USA). The enzyme activity was determined as the slope of the linear part of the reaction curve and expressed in enzyme units (U), one unit represents 1 m*M* dopachrome formed per min per mg adult wet mass.

## Immune efficiency assessment through conidial exposure

The immune competency of adult flies (that had been previously exposed to blue light at their larval stage) was further evaluated through conidial exposure to *B. bassiana*. The adults were treated with LC$_{50}$ [3.12 × 10$^{-6}$ conidia ml$^{-1}$ (De La Rosa *et al.*, 2002)] of *B. bassiana*. One-day old adult flies from group B and D were treated with *B. bassiana*. The entomopathogenic fungus *B. bassiana* strain Bb24 originally isolated from a species of fruit fly was purchased from China General Microbiological Culture Collection Center Beijing, and was used for the experiment. The fungal strains were cultured on Sabouraud dextrose agar medium, in sterilized test tubes and Petri dishes and incubated for 15 days at 27 ± 1°C, 80 ± 5% RH and 14 h photophase. The conidia were harvested after 15 days and stored at 4°C. Before performing the bioassays, the viability of the conidia was confirmed as described by Lane *et al.* (1988). The 55 successfully emerged flies from group B and D were sprayed with 0.5 ml of a suspension of the fungal strain containing 3.12 × 10$^{-6}$ conidia ml$^{-1}$ for 30 s, which is the Mean Lethal Concentration (LC$_{50}$) for fruit flies as reported by De La Rosa *et al.* (2002). The treated flies were placed on absorbent
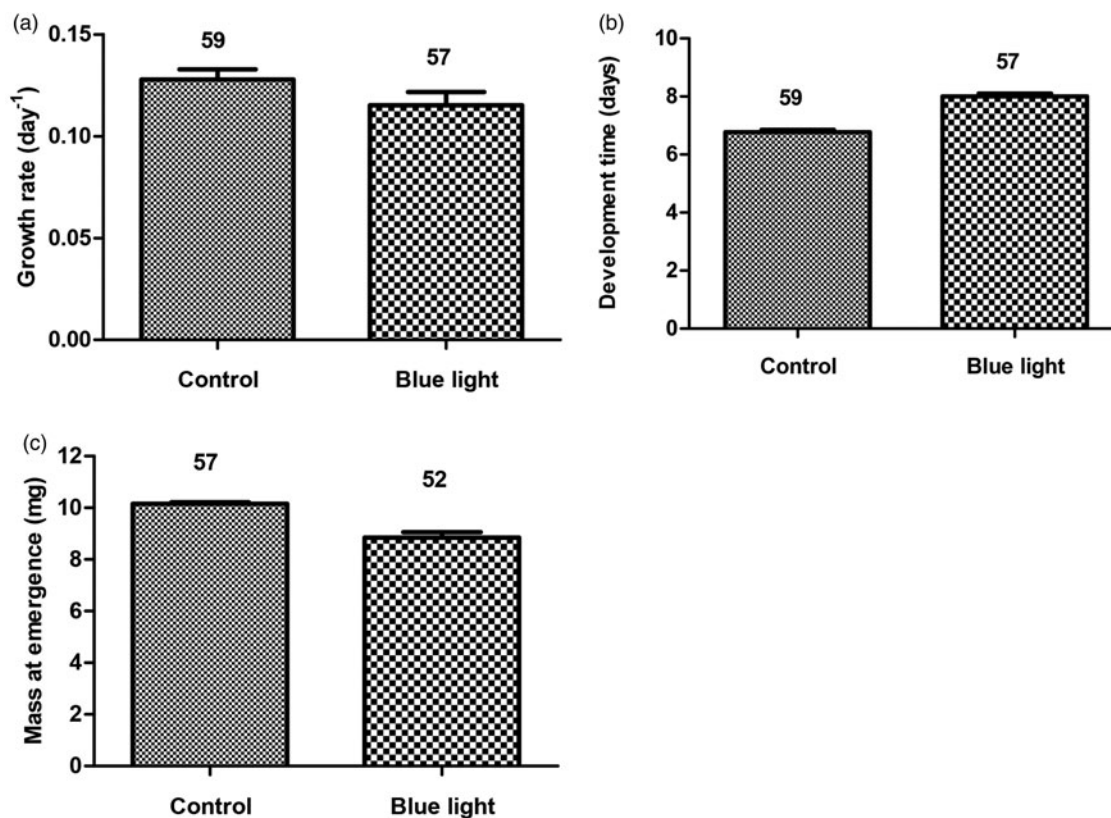
Fig. 1. Mean larval growth rate (A), larval development time (B), and adult mass at emergence (C) as a function of larval blue light exposure in the *B. dorsalis*. Given numbers are observed means ± SE.

paper towels to absorb excess fungal suspension. Adult flies of each group were kept in plastic flasks and maintained under 25–26°C with 12:12 h light: dark photoperiod and 60–65% RH. Sterile water and food were provided to flies daily as described by Zheng *et al.* (2012). Flies survival was scored on daily basis for 10 days post-inoculation. The Kaplan–Meier survival test was used to calculate the survival rates.

### Statistical analysis

The effects of blue light exposure on different response variables were tested by running ANOVA using R software (R CoreTeam, 2013). Data for development time and encapsulation rate were log-transformed and square root-transformed, respectively, and the growth rate and hemocyte number were $\log(x + 1)$-transformed. The effects of blue light on mass at adult emergence could be well explained by the difference in cuticular mass between treated and un-treated flies. ANOVA was run on the sum of the masses (mass at emergence and cuticular mass). Survival statistics were performed using log rank analysis and the Gehan–Breslow–Wilcoxon test using GraphPad Prism v. 5.0 (GraphPad Software, USA).

### Results

Blue light exposure did not affect significantly larval growth rate (ANOVA, $F_{1,114} = 1.56$, $P = 0.12$, fig. 1A). However, blue light-irradiated animals showed longer

development time ($F_{1,114} = 9.4$, $P = 0.0001$, fig. 1B) and lower mass at emergence ($F_{1,107} = 6.18$, $P = 0.0001$, fig. 1C) as compared with animals reared without blue light exposure. The results revealed that blue light significantly inhibited larval cuticular melanin. The blue light-exposed larvae incorporated lower melanin in their cuticle ($F_{1,110} = 15.4$, $P = 0.0001$, fig. 2A), and at adult stage they showed reduced encapsulation of nylon filaments ($F_{1,107} = 30.8$, $P = 0.0001$, fig. 2B). The adults that emerged from blue light-exposed larvae showed lower PO activity ($F_{1,110} = 21.03$, $P = 0.0001$, fig. 2C) however, no significant change in hemocyte number was detected ($F_{1,110} = 1.23$, $P = 0.21$, fig. 2D) as compared with the control animals.

The immune competency of blue light-treated and non-treated flies was further measured at larval stage through conidial exposure of *B. bassiana*. Survival assays showed a significant ($P = 0.0001$) increase in susceptibility of blue light-exposed insects to *B. bassiana* over control individuals (fig. 3).

### Discussion

Hori *et al.* (2014) showed for the first time that blue light induced more harmful effects than UV light to insects, and blue light irradiation killed insect pests of various orders including Diptera. However, the effects of blue light on the physiology of insects are still largely unknown. In the present study we found that blue light irradiation inhibited cuticular melanin in the larvae of *B. dorsalis*, and exposed-larvae showed longer
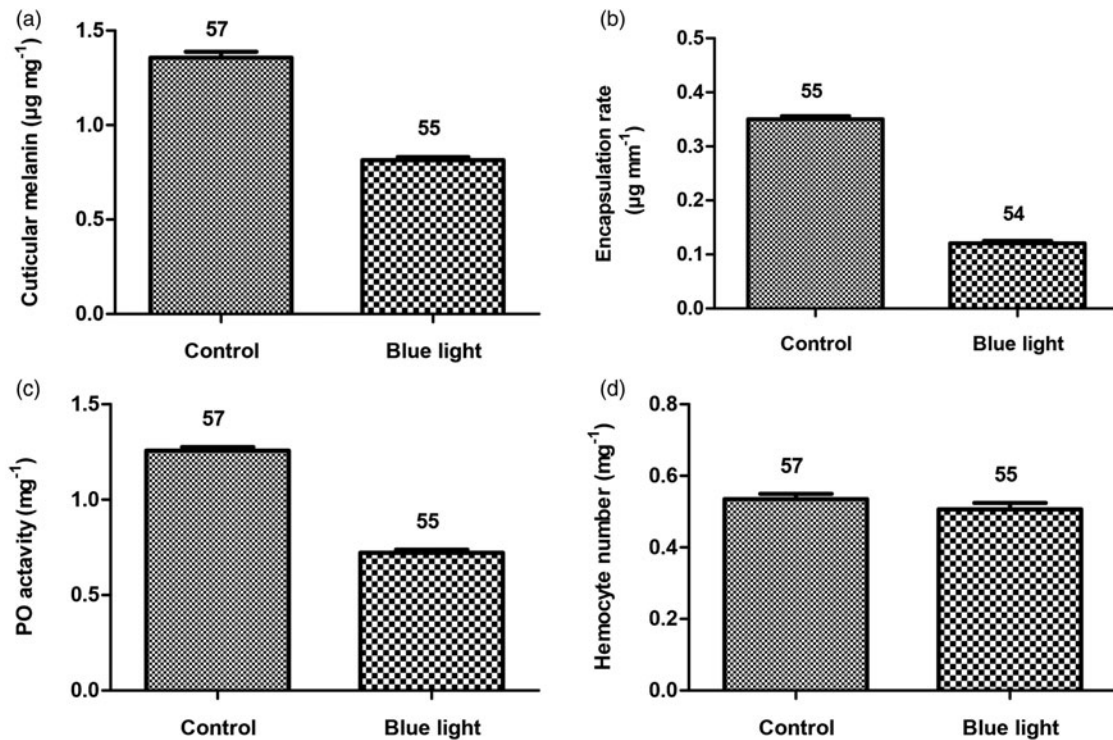
Fig. 2. Larval cuticular melanin content (A), adult encapsulation rate (B), adult PO activity (C), and adult hemocyte number (D) as a function of larval blue light exposure in the *B. dorsalis*. Given numbers are observed means ± SE.
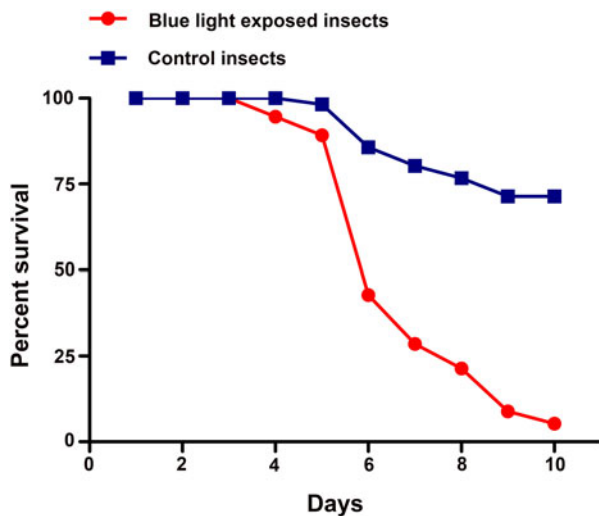


Fig. 3. Insects exposed to blue light at their larval stage are more susceptible to *B. bassiana* infections. Adults of *B. dorsalis* were challenged with *B. bassiana* (strain Bb24). Graph represents percent survival as calculated by the Kaplan–Meier method. The log rank analysis and Gehan–Breslow–Wilcoxon Test indicated that the survival of blue light exposed insects was significantly different than control flies ($P < 0.0001$).

development time, with a lower mass at emergence. The carryover effects of larval blue light exposure were observed in the adult stage in terms of reduced melanotic encapsulation response, and lower PO activity, while no significant change was detected in hemocytes numbers. The blue light exposure resulted in lower encapsulation response and higher susceptibility of adult flies to *B. bassiana* clearly suggesting that blue light impaired the adult immune function of *B. dorsalis*.

Melanin performs several key functions in insects including immune responses (Ortonne, 2002; Roulin, 2014; Debecker *et al.*, 2015). The biosynthesis of melanin was inhibited in melanoma cells that were irradiated with blue light (Ohara *et al.*, 2004). Moreover, a recent study revealed that blue light exposure decreased the viability of epithelial cells and ultimately lower melanin content (Chiarelli-Neto *et al.*, 2014). Our results showed that blue light-exposed larvae incorporated lower melanin in their exoskeletons suggesting the effect of blue light on cell vitality (Chiarelli-Neto *et al.*, 2014), inhibition of DNA synthesis, and cell division (Ohara *et al.*, 2004). Debecker *et al.* (2015) reported that UV exposed-larvae of damsel fly invested more in melanin synthesis which caused impair immune function of adult flies. We found that exposure of *B. dorsalis* larvae to blue light also impaired adult immune function but the mode of impairment was different from that with UV. Unlike UV, blue light inhibited larval cuticular melanin and impaired immune function at adult stage. Several studies reported the associations between cuticular melanization and immune function (Robb *et al.*, 2003; Hagen *et al.*, 2006; Prokkola *et al.*, 2013) but they are largely unknown in the context of blue light exposure.

PO enzyme regulates the formation of melanin in insects (Sugumaran, 2002). Reduced PO activity was detected in adult flies that had been exposed to blue light at their larval stage; the low PO activity may be cause of reduced cuticular melanin. In insects, melanization is an innate

immune response against pathogens in terms of encapsulation (Balasubramanian *et al.*, 2009), and PO activity is a key immune function parameter (Siva-Jothy *et al.*, 2005; González-Santoyo & Córdoba-Aguilar, 2012). Lower cuticular melanin and PO activity recorded in this study might explain the reduced encapsulation response and higher mortality against *B. bassiana* in blue light-exposed insects. Furthermore, blue light exposure strongly inhibited melatonin biosynthesis (Bonmati-Carrion *et al.*, 2014), which regulates a wide range of physiological functions including immune system (Carrillo-Vico *et al.*, 2013) this inhibitory effect could well explain the impaired immune function in blue light-exposed animals.

Generally, negative correlation between cuticular coloration and immuno-competence has been observed (Robb *et al.*, 2003; Hagen *et al.*, 2006). Our mechanistic observations regarding the carryover effects of blue light-exposed larvae on adult immune function are therefore considered to be general, widespread and may express important cost of blue light exposure in an insect. While *in vitro* experiments showed that blue light inhibited melanin contents (Ohara *et al.*, 2002; Ohara *et al.*, 2004; Chiarelli-Neto *et al.*, 2014), our study reveals for the first time, this effect *in vivo* in an insect.

Blue light exposure affected age and mass at metamorphosis, the delayed metamorphosis with smaller mass negatively affected the fitness of adult damselflies (Thompson *et al.*, 2011; Stoks & Cordoba-Aguilar, 2012). Blue light irradiation cause stress and can be lethal for insect pests of various orders including Diptera (Hori *et al.*, 2014). To reduce the detrimental effects, the organism may prolong its developmental time (Zhang *et al.*, 2011). We found that blue light-exposed larvae had longer development time and a lower mass at metamorphosis. The prolonged developmental time could be a strategy to reduce the harmful effects of radiations. The prolonged development time was observed in *B. dorsalis* after the inhibition of PO activity occurred (Bai *et al.*, 2014) indicating the lowered PO activity may prolong development time in blue light-exposed larvae. Blue light had no significant effect on larval growth rate which seems to be in conflict with delayed metamorphosis at a smaller mass. Based on the growth rates of the larvae measured during the 7-day of blue light exposure, the growth rate does not seem to be affected during the exposure period but some days later after the exposure.

In life history study, the theory was that larval stressors could be carried over to the adult stage through age and mass at metamorphosis (Rowe & Ludwig, 1991; Abrams *et al.*, 1996; Relyea, 2007), although it was not upheld in current study, but there was little evidence in our study that described the mediatory role of the developmental time and mass at metamorphosis to the carryover effects of irradiated-larvae for the adult stage.

In conclusion, our study provides the first evidence that blue light exposure inhibits cuticular melanin, PO activity and impaired immune function in an insect. Furthermore, research revealed that blue light-exposed larvae had the carryover effects in adult stage, in terms of developmental time and mass at metamorphosis. It was documented that stressors such as temperature (Prokkola *et al.*, 2013), food shortage (Lee *et al.*, 2008), crowding (Hagen *et al.*, 2006) and drought (Talloen *et al.*, 2004) can affect cuticular melanin. Our results highlight that, for future consideration, melanin serving as a mediator of larval carryover effects for adult stage may provide better insight that how larval stressors shape adult fitness (Pechenik, 2006; Stoks & Cordoba-Aguilar, 2012; O'Connor *et al.*, 2014), which is still largely enigmatic to date.

## References

**Abrams, P.A., Leimar, O., Nylin, S. & Wiklund, C.** (1996) The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. *American Naturalist* **147**, 381–395.

**Adamo, S.A.** (2004) How should behavioural ecologists interpret measurements of immunity? *Animal Behaviour* **68**, 1443–1449.

**Allen, B.G.V., Briggs, V.S., McCoy, M.W. & Vonesh, J.R.** (2010) Carry-over effects of the larval environment on postmetamorphic performance in two hylid frogs. *Oecologia* **164**, 891–898.

**Bai, P.-P., Chen, E.-H., Shen, G.-M., Wei, D., Wei, D.-D. & Wang, J.-J.** (2014) Inhibition of phenoloxidase activity delays development in *Bactrocera dorsalis* (Diptera: Tephritidae). *Florida Entomologist* **97**, 477–485.

**Balasubramanian, N., Hao, Y.-J., Toubarro, D., Nascimento, G., Simões, N.** (2009) Purification, biochemical and molecular analysis of a chymotrypsin protease with prophenoloxidase suppression activity from the entomopathogenic nematode *Steinernema carpocapsae*. *International Journal of Parasitology* **39**, 975–984.

**Ben-Yakir, D., Antignus, Y., Offir, Y. & Shahak, Y.** (2013) Optical manipulations: an advance approach for reducing sucking insect pests. pp. 249–267 in *Advanced Technologies for Managing Insect Pests*. Netherlands, Springer.

**Bonmati-Carrion, M.A., Arguelles-Prieto, R., Martinez-Madrid, M.J., Reiter, R., Hardeland, R., Rol, M.A. & Madrid, J.A.** (2014) Protecting the melatonin rhythm through circadian healthy light exposure. *International Journal of Molecular Sciences* **15**, 23448–23500.

**Campero, M., De Block, M., Ollevier, F. & Stoks, R.** (2008a) Metamorphosis offsets the link between larval stress, adult asymmetry and individual quality. *Functional Ecology* **22**, 271–277.

**Campero, M., Block, M.D., Ollevier, F. & Stoks, R.** (2008b) Correcting the short-term effect of food deprivation in a damselfly: mechanisms and costs. *Journal of Animal Ecology* **77**, 66–73.

**Carrillo-Vico, A., Lardone, P.J., Álvarez-Sánchez, N., Rodríguez-Rodríguez, A. & Guerrero, J.M.** (2013) Melatonin: buffering the immune system. *International Journal of Molecular Sciences* **14**, 8638–8683.

**Chang, C.L., Vargas, R.I., Caceres, C., Jang, E. & Cho, I.K.** (2006) Development and assessment of a liquid larval diet for *Bactrocera dorsalis* (Diptera: Tephritidae). *Annals of the Entomological Society of America* **99**, 1191–1198.

**Chiarelli-Neto, O., Ferreira, A.S., Martins, W.K., Pavani, C., Severino, D., Faião-Flores, F., Maria-Engler, S.S., Aliprandini, E., Martinez, G.R., Di Mascio, P., Medeiros, M.H.G., Baptista, M.S.** (2014) Melanin photosensitization and the effect of visible light on epithelial cells. *PLoS ONE* **9**, e113266.

**Cossentine, J., Thistlewood, H., Goettel, M., Jaronski, S.** (2010) Susceptibility of preimaginal western cherry fruit fly, *Rhagoletis indifferens* (Diptera: Tephritidae) to *Beauveria*

*bassiana* (Balsamo) Vuillemin Clavicipitaceae (Hypocreales). *Journal of Invertebrate Pathology* **104**, 105–109.

Daniel, C. & Wyss, E. (2009) Susceptibility of different life stages of the European cherry fruit fly, *Rhagoletis cerasi*, to entomopathogenic fungi. *Journal of applied entomology* **133**, 473–483.

Debecker, S., Sommaruga, R., Maes, T. & Stoks, R. (2015) Larval UV exposure impairs adult immune function through a trade-off with larval investment in cuticular melanin. *Functional Ecology* **29**, 1292–1299.

De La Rosa, W., Lopez, F.L. & Liedo, P. (2002) *Beauveria bassiana* as a pathogen of the Mexican fruit fly (Diptera: Tephritidae) under laboratory conditions. *Journal of Economic Entomology* **95**, 36–43.

Dimbi, S., Maniania, N.K., Lux, S.A., Ekesi, S. & Mueke, J.K. (2003) Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, to three adult fruit fly species: *Ceratitis capitata* (Weidemann), *C. rosa* var. *fasciventris* Karsch and *C. cosyra* (Walker) (Diptera : Tephritidae). *Mycopathologia* **156**, 375–382.

Ekesi, S., Maniania, N., Lux, S. (2002) Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. *Biocontrol Science and Technology* **12**, 7–17.

González-Santoyo, I. & Córdoba-Aguilar, A. (2012) Phenoloxidase: a key component of the insect immune system. *Entomologia Experimentalis et Applicata* **142**, 1–16.

Hagen, S.B., Sørlibråten, O., Ims, R.A. & Yoccoz, N.G. (2006) Density-dependent melanism in winter moth larvae (Lepidoptera: Geometridae): a countermeasure against parasitoids? *Environmental Entomology* **35**, 1249–1253.

Hanife, G. (2008) Modified agar-based diet for small scale laboratory rearing of olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae). *Florida Entomologist* **91**, 651–656.

Hori, M., Shibuya, K., Sato, M. & Saito, Y. (2014) Lethal effects of short-wavelength visible light on insects. *Scientific Reports* **4**, 7383.

Johansen, N., Vänninen, I., Pinto, D.M., Nissinen, A. & Shipp, L. (2011) In the light of new greenhouse technologies: 2. Direct effects of artificial lighting on arthropods and integrated pest management in greenhouse crops. *Annals of Applied Biology* **159**, 1–27.

Jurkiewicz, B.A. & Buettner, G.R. (1994) Ultraviolet light-induced free radical formation in skin: an electron paramagnetic resonance study. *Photochemistry and Photobiology* **59**, 1–4.

Lane, B.S., Humphreys, A.M., Thompson, K. & Trinci, A.P.J. (1988) ATP content of stored spores of *Paecilomyces farinosus* and the use of ATP as a criterion of spore viability. *Transactions of the British Mycological Society* **90**, 109–111.

Lee, K.P., Simpson, S.J. & Wilson, K. (2008) Dietary protein-quality influences melanization and immune function in an insect. *Functional Ecology* **22**, 1052–1061.

Marjan De Block, R.S. (2005) Fitness effects from egg to reproduction: bridging the life history transition. *Ecology* **86**, 185–197.

Nagel, L., Mlynarek, J.J. & Forbes, M.R. (2011) Immune response to nylon filaments in two damselfly species that differ in their resistance to ectoparasitic mites. *Ecological Entomology* **36**, 736–743.

Neyen, C., Bretscher, A.J., Binggeli, O. & Lemaitre, B. (2014) Methods to study Drosophila immunity. *Methods* **68**, 116–128.

O'Connor, C.M., Norris, D.R., Crossin, G.T. & Cooke, S.J. (2014) Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. *Ecosphere* **5**, art28.

Ohara, M., Kawashima, Y., Watanabe, H. & Kitajima, S. (2002) Effects of blue-light-exposure on growth of extracorporeally circulated leukemic cells in rats with leukemia induced by 1-ethyl-1-nitrosourea. *International Journal of Molecular Medicine* **10**, 407–411.

Ohara, M., Kobayashi, M., Fujiwara, H., Kitajima, S., Mitsuoka, C. & Watanabe, H. (2004) Blue light inhibits melanin synthesis in B16 melanoma 4A5 cells and skin pigmentation induced by ultraviolet B in guinea-pigs. *Photodermatology, Photoimmunology & Photomedicine* **20**, 86–92.

Ortonne, J.P. (2002) Photoprotective properties of skin melanin. *British Journal of Dermatology* **146**, 7–10.

Pechenik, J.A. (2006) Larval experience and latent effects – metamorphosis is not a new beginning. *Integrative and Comparative Biology* **46**, 323–333.

Pechenik, J.A., Wendt, D.E. & Jarrett, J.N. (1998) Metamorphosis is not a new beginning. *Bioscience* **48**, 901–910.

Prokkola, J., Roff, D., Kärkkäinen, T., Krams, I. & Rantala, M.J. (2013) Genetic and phenotypic relationships between immune defense, melanism and life-history traits at different temperatures and sexes in *Tenebrio molitor*. *Heredity* **111**, 89–96.

Rantala, M.J., & Roff, D.A. (2005) An analysis of trade-offs in immune function, body size and development time in the Mediterranean Field Cricket, *Gryllus bimaculatus*. *Functional Ecology* **19**, 323–330.

Rantala, M.J., Koskimìki, J., Taskinen, J., Tynkkynen, K. & Suhonen, J. (2000) Immunocompetence, developmental stability and wingspot size in the damselfly *Calopteryx splendens* L. *Proceedings of the Royal Society of London B: Biological Sciences* **267**, 2453–2457.

Relyea, R.A. (2007) Getting out alive: how predators affect the decision to metamorphose. *Oecologia* **152**, 389–400.

Robb, T., Forbes, M.R. & Jamieson, I.G. (2003) Greater cuticular melanism is not associated with greater immunogenic response in adults of the polymorphic mountain stone weta, *Hemideina maori*. *Ecological Entomology* **28**, 738–746.

Roulin, A. (2014) Melanin-based colour polymorphism responding to climate change. *Global Change Biology* **20**, 3344–3350.

Rowe, L. & Ludwig, D. (1991) Size and timing of metamorphosis in complex life cycles: time constraints and variation. *Ecology* **72**, 413–427.

Shi, Z.-H. & Sun, J.-H. (2010) Immunocompetence of the red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Curculionidae, Scolytinae): variation between developmental stages and sexes in populations in China. *Journal of Insect Physiology* **56**, 1696–1701.

Siva-Jothy, M.T., Moret, Y. & Rolff, J. (2005) Insect immunity: an evolutionary ecology perspective. *Advances in Insect Physiology* **32**, 1–48.

Smilanich, A.M., Dyer, L.A. & Gentry, G.L. (2009) The insect immune response and other putative defenses as effective predictors of parasitism. *Ecology* **90**, 1434–1440.

Stoks, R. & Cordoba-Aguilar, A. (2012) Evolutionary ecology of Odonata: a complex life cycle perspective. *Annual Review of Entomology* **57**, 249–265.

Stoks, R., Swillen, I. & De Block, M. (2012) Behaviour and physiology shape the growth accelerations associated with predation risk, high temperatures and southern latitudes in Ischnura damselfly larvae. *Journal of Animal Ecology* **81**, 1034–1040.

Sugumaran, M. (2002) Comparative biochemistry of eumelanogenesis and the protective roles of phenoloxidase and melanin in insects. *Pigment Cell Research* **15**, 2–9.

**Talloen, W., Van Dyck, H. & Lens, L.** (2004) The cost of melanization: butterfly wing coloration under environmental stress. *Evolution; International Journal of Organic Evolution* **58**, 360–366.

**Tariq, K., Noor, M., Saeed, S. & Zhang, H.** (2015) The effect of ultraviolet-A radiation exposure on the reproductive ability, longevity, and development of the *Dialeurodes citri* (Homoptera: Aleyrodidae) F1 generation. *Environmental entomology* **44**, 1614–1618.

**Therry, L., Nilsson-Örtman, V., Bonte, D. & Stoks, R.** (2014) Rapid evolution of larval life history, adult immune function and flight muscles in a poleward-moving damselfly. *Journal of Evolutionary Biology* **27**, 141–152.

**Thompson, D.J., Hassall, C., Lowe, C.D. & Watts, P.C.** (2011) Field estimates of reproductive success in a model insect: behavioural surrogates are poor predictors of fitness. *Ecology Letters* **14**, 905–913.

**Vile, G.F. & Tyrrell, R.M.** (1995) UVA radiation-induced oxidative damage to lipids and proteins in vitro and in human skin fibroblasts is dependent on iron and singlet oxygen. *Free Radical Biology and Medicine* **18**, 721–730.

**Zhang, C.-Y., Meng, J.-Y., Wang, X.-P., Zhu, F. & Lei, C.-L.** (2011) Effects of UV-A exposures on longevity and reproduction in *Helicoverpa armigera*, and on the development of its F1 generation. *Insect Science* **18**, 697–702.

**Zheng, W., Peng, T., He, W. & Zhang, H.** (2012) High-Throughput sequencing to reveal genes involved in reproduction and development in *Bactrocera dorsalis* (Diptera: Tephritidae). *PLoS ONE* **7**, e36463.

**Zhou, J., Shang, J., Ping, F. & Zhao, G.** (2012) Alcohol extract from *Vernonia anthelmintica* (L.) willd seed enhances melanin synthesis through activation of the p38 MAPK signaling pathway in B16F10 cells and primary melanocytes. *Journal of Ethnopharmacology* **143**, 639–647.