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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

A dark cuticle allows higher investment in immunity, longevity and fecundity in a beetle upon a simulated parasite attack

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Abstract Cuticle melanism in insects is linked to a number of life history traits: a positive relationship is hypothesized between melanism, immune function, fecundity and lifespan. However, it is not clear how activation of the immune system affects trade-offs between life history traits in female mealworm beetles (*Tenebrio molitor*) differing in cuticle melanization. The females with tan, brown and black cuticles examined in the present study did not differ in the intensity of encapsulation response, fecundity and longevity when their immune system was not activated. However, we found that immune activation and cuticle melanization have a significant effect on life history traits. Offspring number and lifespan decreased in females with tan and brown cuticles, while the fecundity and lifespan of black females were not affected. Importantly, we inserted the implants again and found a significant decrease in the strength of encapsulation response in females with tan and brown cuticles. In contrast, black females increased melanotic reactions against the nylon implant, suggesting immunological priming. The results show that cuticle melanization plays an important adaptive role under the risk of being infected, while the lack of these benefits before the insertion of nylon monofilaments suggests that there are costs associated with an activated immunity system.

Keywords Cuticle melanization · Fecundity · Immunological priming · Lifespan · Trade-offs · *Tenebrio molitor*

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Introduction

Melanin is responsible for a major part of the variation in coloration in both vertebrates and invertebrates (Fox 1976; McGraw 2006; Hsiung et al. 2015). This substance has been shown to be involved in a wide range of vital adaptive functions as diverse as camouflage (Kettlewell 1973; Hoekstra et al. 2006), photoprotection (Ortonne 2002), sexual signaling (Jawor and Breitwisch 2003; Svensson and Waller 2013), thermoregulation (Vences et al. 2002), protection against reactive oxygen species (Galván and Solano 2015) and strengthening insect cuticles (Riley 1997). The role of melanin pigmentation has also been demonstrated in immune defense (Wilson et al. 2001; Männiste and Hõrak 2014, but see Contreras-Garduño et al. 2007). For example, in invertebrates, a major aspect of the innate immune

defense system against invading pathogens involves melanin. Within a few hours of infection, the invader is encapsulated in melanin layers (Nappi et al. 1995; Gillespie et al. 1997) and then dies by suffocation, or as a result of toxic compounds released by phenoloxidase (PO) activity (Siva-Jothy et al. 2005; Sugumaran 2002). Melanin itself has antimicrobial activity, which is likely to contribute to its effectiveness in defense (Montefiori and Zhou 1991; Montefiori et al. 1990; Sidibe et al. 1996) and wound healing (Sugumaran 2002).

The heritability of cuticle melanization in arthropods reaches 0.77 (Yurtsever 2000; Lee and Wilson 2006; Cotter et al. 2008; Singh et al. 2009; Roff and Fairbairn 2013). This suggests that the heritability of cuticle melanization is high enough to allow rapid evolutionary change in a phenotype. The relationship between cuticular melanization and several life history traits such as immune function, developmental time and fecundity has been studied extensively in insects. Immune defense is vital for an organism, as it reduces the deleterious impact of other infectious organisms. Many studies report a positive relationship between cuticular darkness and immune function. This includes PO activity (Wilson et al. 2001; Reeson et al. 1998; Cotter et al. 2004; Armitage and Siva-Jothy 2005; Bailey 2011), encapsulation response (Mikkola and Rantala 2010; Bailey 2011; Kivleniece et al. 2010, but see Dubovskiy et al. 2013a, b), hemocyte density (Cotter et al. 2004; Armitage and Siva-Jothy 2005), immune activation with an artificial parasite (Freitak et al. 2005), susceptibility to parasitoids (Wilson et al. 2001), fungal disease (Wilson et al. 2001; Barnes and Siva-Jothy 2000; Krams et al. 2013a) and viral disease (Reeson et al. 1998). However, there is also evidence for a negative relationship between cuticular melanization and immune traits such as antibacterial (lysozyme-like) activity (Cotter et al. 2004), hemocyte density (Rolff et al. 2005), PO activity (Rolff et al. 2005) and susceptibility to a viral disease (Goulson and Cory 1995).

A positive relationship between cuticular melanization and the strength of immune response suggests the concentration of cuticular melanin as an indicator of immune function, while the negative relationships indicate trade-offs between the efficiency of the immune system and cuticular melanin as predicted by life history theory (Stearns 1989). A number of studies have revealed the costs of immunity (Schmid-Hempel 2003; Sheldon and Verhulst 1996; Roff and Fairbairn 2013; see González-Santoyo and Córdoba-Aguilar 2012 for a review). Evidence shows that there are trade-offs between cuticle melanization and some fitness-related traits (Roff and Fairbairn 2013; Talloen et al. 2004). For example, darker individuals had a slower growth rate and exhibited larger wing asymmetry in a satyrine butterfly *Pararge aegeria* (Talloen et al. 2004). Melanic forms have been found to develop longer and weaker resistance against pathogens (Wilson et al. 2001; True 2003; Cotter et al. 2008; Wittkopp and Beldade 2009; Dubovskiy et al. 2013a, b). A recent report notably suggests that the condition-dependent component of melanin-based coloration is much stronger in invertebrates than vertebrates (Roulin 2015).

On the one hand, trade-offs between cuticle melanization, immune function and other traits may arise because melanin-based cuticular darkening and melanotic encapsulation response share the same melanin production pathway (González-Santoyo and Córdoba-Aguilar 2012). On the other hand, organisms exhibiting different intensities of cuticular melanization may be adapted to different environmental conditions with differing pathogen pressures and thus represent equally fit survival strategies (Galeotti et al. 2003). For example, if there is a linkage disequilibrium between genes coding for dark and pale cuticle coloration, these genes would code not only for a certain concentration of melanin, but also for some physiological features that allow a color morph to outcompete other morphs under specific ecological conditions (Ducrest et al. 2008). However, if the expression of genes coding for the alternate phenotypes is sensitive to environmental factors, individuals expressing different cuticle melanization would achieve a higher fitness only under specific environments. Such local adaptations may show up as a covariation between cuticle melanization and other life history traits only under highly specific conditions (Gonzales et al. 1999). Some studies found that environmental factors affect both the magnitude and the sign of covariations between coloration and life history traits in vertebrates (Gonzales et al. 1999; Fargallo et al. 2007; Piault et al. 2008; Roulin et al. 2008; Moore et al. 2014). Identifying the factors that mediate covariation between cuticle melanization, environmental factors and life history traits is important in order to determine the functional role of cuticle coloration.

Several studies on invertebrates have recently demonstrated the role of ecological conditions on trade-offs between immune function and fecundity (Zerofsky et al. 2005), availability of food (Moret and Schmid-Hempel 2000; Krams et al. 2014a, 2015), especially nitrogen-rich proteins, lifespan (Ye et al. 2009; Krams et al. 2013b) and larval competitive ability (Kraaijeveld and Godfray 1997). Selection for increased cuticular melanization in mealworm beetles *Tenebrio molitor* resulted in an increase in immune response (Armitage and Siva-Jothy 2005). Barnes and Siva-Jothy (2000) found that pathogen resistance is phenotypically plastic in *T. molitor*, where lower mortality, higher degree of cuticular melanization and stronger immune function were found in beetles reared at high larval densities. These findings support a crucial role of environmental conditions in general and pathogens and parasites in particular in life history trade-offs, showing that if there are costs involved with the

maintenance of pathogen resistance, then higher investment in this trait is expected when the risk of pathogenesis is high (Wilson and Reeson 1998). Life history theory predicts different strategies in different sexes, where males are considered to be selected for mating rates, while females are thought to invest more in their immunity or longevity (Stearns 1992). Thus, higher investment in immunity and other fitness-related traits might be expected in females, especially if their coloration is dark, their environment contains pathogens and/or they have been affected by pathogens earlier. However, consistent evidence on positive relationships between cuticular melanization, the strength of immune response and female fitness is still missing.

In the current study, we examined the type of response that *T. molitor* females mount against the insertion of a nylon monofilament in their hemocoel. We also tested whether *T. molitor* females with dark cuticles invest in individual immune priming (Moret 2006; Kivleniece et al. 2010; Krams et al. 2011a; Mikonranta et al. 2014), lifespan and fecundity more than females with brown and tan cuticles. This is important because covariations between cuticle melanization and life history traits can sometimes be detected only under specific conditions, for instance, where one phenotype enjoys the existing ecological benefits while other ones are selected against (Gonzales et al. 1999; Piault et al. 2009; Roulin 2009; Moore et al. 2014).

Materials and methods

Insects

To avoid inbreeding effects (Polkki et al. 2012), we mixed beetles taken from a long-term, over 10-year laboratory population maintained at the University of Tennessee, Knoxville (60 %), with beetles obtained from Big Apple Pet Supply (30 %) (Boca Raton, FL, USA) and those obtained from a natural population (10 %). We used the next generation of beetles for this study, which was maintained on a diet of chick starter mash supplemented with occasional vegetables and fruit, such as carrots, apples and potatoes. The beetles were kept at 24 ± 2 °C. We removed pupae from the culture on the day of pupation. They were weighed and their sex was determined by examining genitalia on the eighth abdominal segment (Bhattacharya et al. 1970). The pupae and newly emerged adult females were kept individually in 200 ml plastic containers filled with food ad libitum. Only individuals with no visible abnormalities were used in the experiments. All of the experimental trials were performed in winter 2014/2015.

Study design

We weighed 12 days old virgin females to the nearest 0.1 mg (mean body weight ± SD = 109.46 ± 7.02 mg). The beetles were randomly assigned to experimental and control groups (Fig. 1). In the treatment group, each female was placed on ice and received one sterile nylon monofilament implant (2 mm length, 0.18 mm diameter, knotted at one end) through their pleural membrane between the third and fourth abdominal sternite (Rantala et al. 2002; Krams et al. 2011a, b; Daukšte et al. 2012) for 6 h at 24 ± 0.5 °C. Females of the control group were handled similarly, but their cuticle was not punctured and these animals were not implanted.

The treatment females were further divided into three subgroups: breeding, repeated implantation and survival subgroups (Fig. 1). The control group was divided into two subgroups: breeding and survival subgroups. Each of these subgroups (3 subgroups in the treatment group and 2 subgroups in the control group) consisted of females of three different types of cuticle (elytra) coloration: 'black', 'brown' or 'tan' (Figs. 1, 2).

Treatment group, breeding subgroup: on day 17 after imaginal eclosion (5 days after the insertion and removal of the implants), we placed two males to each of 30 females of each elytra color class (90 individuals in total) for 24 h. These females were left alone in their boxes to lay eggs for 7 days. We counted the number of larvae on day 55 after imaginal eclosion (Fig. 1).

Treatment group, survival subgroup: after the removal of the implants, 30 females of each color class (90 individuals in total) were provided with bran and fresh apple. Their survival was verified on a daily basis. These females were not allowed to reproduce (Fig. 1).

Treatment group, repeated implantation subgroup: 30 females of each of elytra color class (90 individuals in total) received the second nylon implant for 6 h 5 days after the removal of the first implant (day 17 after imaginal eclosion). In this way, we checked whether the first activation of the immune system resulted in an increased immune response 5 days later and whether this possible individual immune priming was related to cuticle melanization (Fig. 1).

Control group, breeding subgroup: 30 females of each elytra color group (90 individuals in total) were paired with two males on day 17 after imaginal eclosion for 24 h, and we counted offspring number on day 55 after imaginal eclosion (Fig. 1).

Control group, survival subgroup: 30 females of each elytra color group (90 individuals in total) were kept separately with food ad libitum and we checked for their survival until the last individual died (Fig. 1).

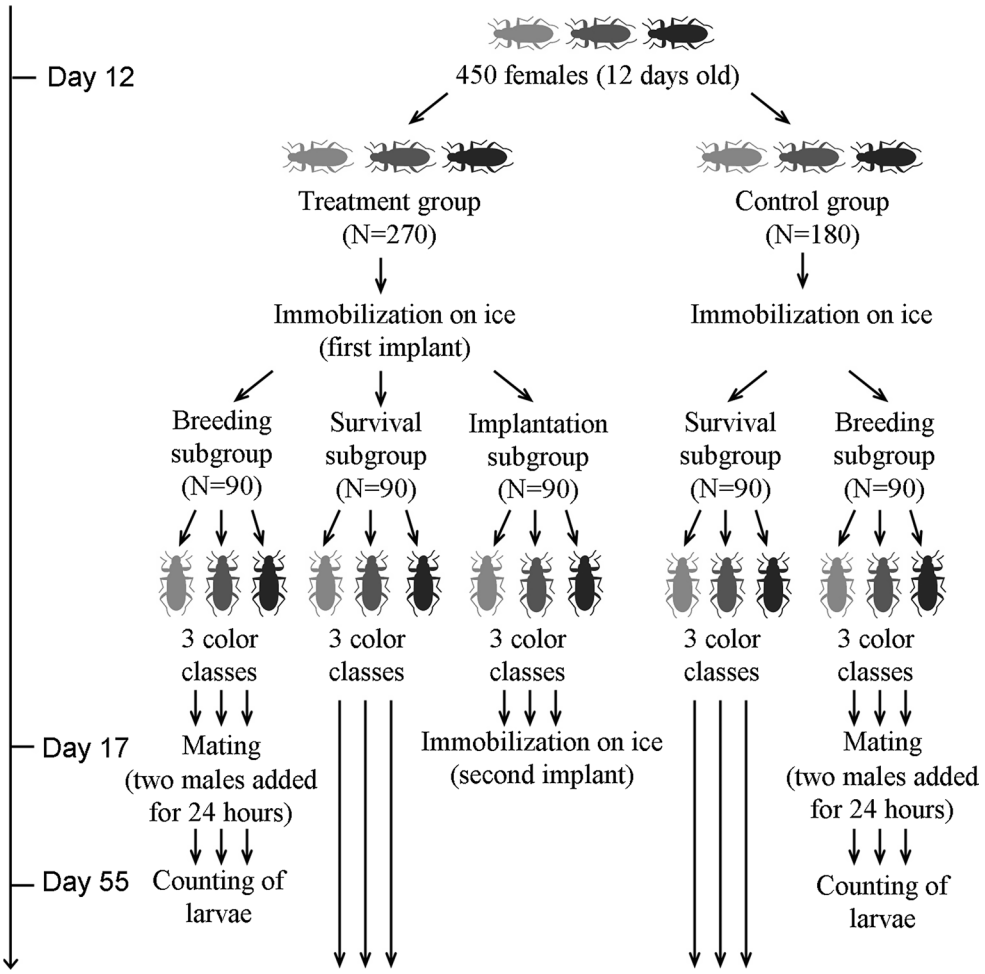

Fig. 1 The experimental protocol used to study immune response, fecundity and lifespan of female mealworm beetles

Fig. 2 The elytra melanization classes (*tan*, *brown*, *black*) of female mealworm beetles (color figure online)

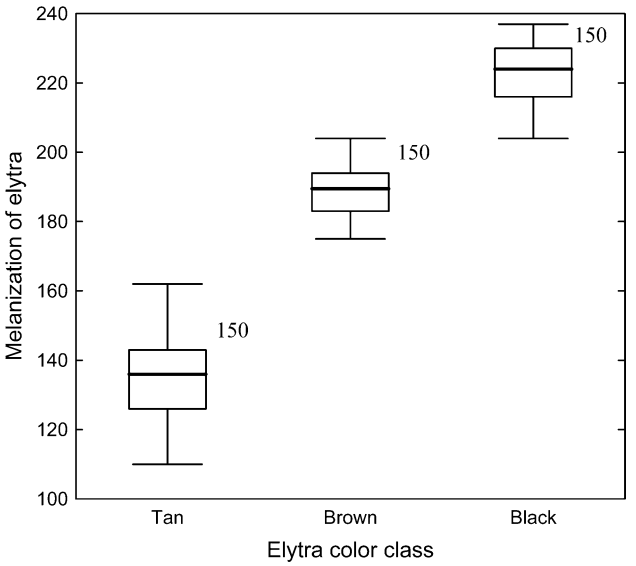

Fig. 3 Median cuticle melanization in three elytra color classes of mealworm beetles; the *box* indicates the 25–75 % percentiles, the whiskers indicate the min–max range, the *numbers* indicate the sample size

Cuticle coloration

To assess the cuticle melanization, we followed the recommendations by Barnes and Siva-Jothy (2000). In brief, we assessed the beetle coloration under a Nikon stereo

microscope with LED illumination. 'Tan' females were easy to tell from the rest of the groups, because their elytra was light brown (Fig. 2). The elytra of 'brown' beetles was dark brown and easily distinguishable from 'tan' beetles (Fig. 2). Discrimination of 'brown' and 'black' beetles was done by including into the 'black' group only females with no traces of brown coloration to their elytra even under higher zoom positions (Fig. 2). We also took digital images of the beetles and analyzed them using image analysis software (Image J, Abramoff et al. 2004). The elytra melanization was expressed as a grayscale value between 0 (white) and 240 (black). We found a significant effect of coloration on the darkness grayscale values obtained (ANOVA: $df = 2$, $F = 3628.10$, $P < 0.0001$, Fig. 2). The grayscale values were significantly different between each elytra color category ('tan' = 135.40 ± 11.45, 'brown' = 188.52 ± 6.98, 'black' = 223.25 ± 7.93) (Tukey tests: all $P < 0.001$, Fig. 3).

Immune assays

To quantify the strength of encapsulation response to a foreign body, we analyzed the lightness of each nylon insert. It is widely acknowledged that insect immune systems respond to the insert by attempting to encapsulate the foreign body as though it were a parasitoid or fungal invasion (e.g., Rantala et al. 2000; Dubovskiy et al. 2013a, b). The resulting melanization correlates with the level of immune system response (e.g., Sadd et al. 2006; Krams et al. 2011a, 2013a, b; Rantala et al. 2000, 2002). The ability to encapsulate a synthetic substrate is also positively related to the encapsulation of parasites (Paskewitz and Riehle 1994; Gorman et al. 1998) and to the ability to resist an entomopathogenic fungal disease (Rantala and Roff 2007; Dubovskiy et al. 2013a, b; Krams et al. 2013a). Overall, high levels of melanization or darkening of the inserts indicates increased levels of immune system activity and response (Yourth et al. 2001, 2002; Krams et al. 2013a, b). However, it is important to note that a number of studies failed to find a positive correlation between melanization of artificial inserts and the ability to encapsulate real parasites (e.g., Schwartz and Koella 2002; Mallon et al. 2003; Honkavaara et al. 2009; Nagel et al. 2014).

To quantify lightness, we photographed each of the removed inserts from two directions under constant light conditions using a Nikon stereo microscope. We then analyzed the digital images using image analysis software (Image J, Abramoff et al. 2004). We marked the area of that portion of the insert that had been within the beetle's body and the program calculated the lightness value. Since increasing melanization indicated a stronger immune response in this study, we calibrated reflectance of an implant before the insertion to zero level.

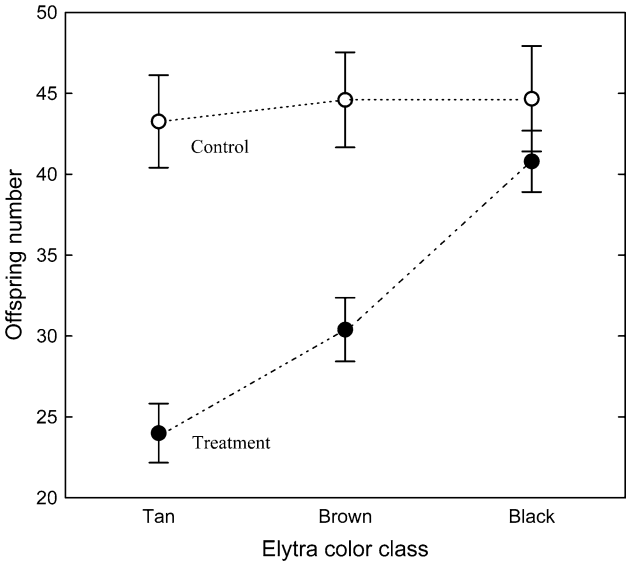

Fig. 4 Average offspring number (±95 % CIs) of three elytra color classes in the control (*open circles*) and treatment (*black circles*) groups of mealworm beetles

Statistics

The strength of the encapsulation response was distributed normally in all groups (Kolmogorov–Smirnov tests: all $P > 0.20$). We used ANOVA to test differences in offspring numbers across control and treatment groups and elytra melanization classes. Lifespan was analyzed using a Cox proportional hazards model, while we used a linear mixed model with female identity as a random factor to find possible treatment and elytra color effects on the activation of the immune system. All statistical tests used in this study were two-tailed.

Results

Offspring number

Cuticle darkness had no effect on offspring number in the control group ($F_{2,87} = 0.29$, $P > 0.05$, Fig. 4). In contrast, we found a significant effect of cuticle melanization on offspring number after the activation of the immune system in beetles of the treatment group, revealed as cuticle melanization group and treatment interaction ($F_{2,174} = 20.22$, $P < 0.001$, Fig. 4). All three cuticle melanization groups differed significantly from each other in the number of offspring produced after the insertion of the nylon monofilament. The offspring number of tan females was smaller than that of brown females (Tukey HSD: $P = 0.004$), and brown females produced less offspring than black females ($P < 0.001$, Fig. 4). The decrease in the number of offspring

was found to be greatest among tan females, while the fecundity of black females was not impaired by the activation of their immunity system: the offspring number of the black females of the treatment group did not differ from that of the black females in the control group (Tukey HSD: $P > 0.05$, Fig. 4).

Lifespan

Cuticle color was not related to the longevity of females in the control group ($\chi^2 = 0.90$, $P > 0.05$, Fig. 5). In the treatment group, the insertion of implants significantly decreased lifespan, as revealed by a significant interaction between elytra color and treatment ($\chi^2 = 48.92$, $P < 0.001$, Fig. 5). While the activation of the immunity system via the insertion of the nylon monofilament did not affect the lifespan of the females with black elytra ($z = -0.03$, $P > 0.05$), the implantation significantly decreased the lifespan of the females with tan ($z = 5.29$, $P < 0.001$) and brown cuticles ($z = 6.85$, $P < 0.001$, Fig. 5).

Encapsulation response

The first activation of the immune system via the insertion of the nylon monofilament did not show any difference in the intensity of implant melanization among the female groups (ANOVA: $F_{2,87} = 1.19$, $P > 0.05$, Fig. 6). The second implantation reflects the investment into encapsulation ability done by females between the first and second implantations. We found the encapsulation response to be significantly different among cuticle color groups ($F_{2,122} = 63$, $P < 0.001$, Fig. 6). Females with black elytra increased the strength of their encapsulation response ($t_{122} = 2.34$, $P = 0.021$), suggesting immune priming of melanotic reactions. In contrast, the strength of the encapsulation response decreased in females with brown ($t_{122} = -9.92$, $P < 0.001$) and tan cuticles ($t_{122} = -9.53$, $P < 0.001$).

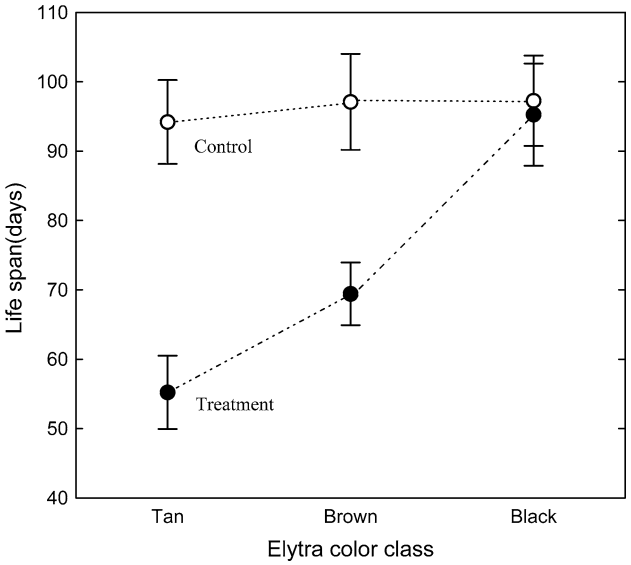

Fig. 5 Average lifespan (±95 % CIs) of three elytra color classes in the control (*open circles*) and treatment (*black circles*) groups of mealworm beetles

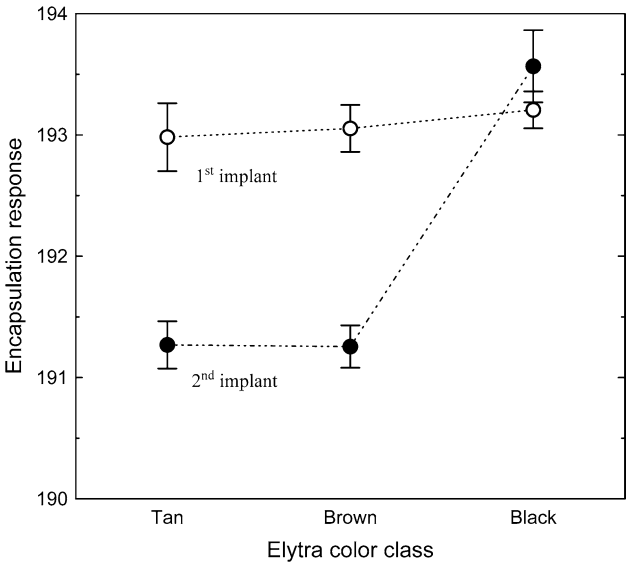

Fig. 6 Strength of encapsulation response against the first and second implantation in tan, brown and black elytra color classes of female mealworm beetles

Discussion

Life history theory asserts that the schedule and duration of key events in an organism's lifetime are shaped by natural selection to produce the largest possible number of surviving offspring (Stearns 1992). Males of many species tend to increase the number of copulations (Bateman 1948), and in case of terminal investment in reproduction male individuals may increase their sexual attractiveness at the expense of their longevity (Krams et al. 2014b, 2015). In females, fitness is often positively linked to lifespan (Trivers 1972), while longevity largely depends on investment into immune function (Lin et al. 1998; Krams et al. 2014a).

It has been recently shown that melanin pigmentation is linked with the ability to cope with infections: darker melanic individuals usually have a lower infection intensity and a greater immune response than paler individuals (Jacquin et al. 2011; Prokkola et al. 2013). Darker melanic individuals can even reduce the fecundity of parasites (Roulin et al. 2001). Two hypotheses have been suggested to explain the covariation between the intensity of melanin pigmentation and the expression of life history traits. The

genetic link hypothesis states that positive links between melanin-based coloration and the strength of immune function could be explained by the pleiotropic effects of genes coding for melanin pigmentation on the immune system (Ducrest et al. 2008; Gasparini et al. 2009). The exposure hypothesis posits that melanin-based pigmentation and immunity are linked, because melanin pigmentation develops and may be exploited in habitats that differ in parasite exposure (Galeotti and Rubolini 2004; Roulin 2004). In *T. molitor*, beetles reared at high larval densities showed a higher degree of cuticular melanization and lower mortality against an entomopathogenic fungus than those reared solitarily (Barnes and Siva-Jothy 2000). Larvae and pupae of *T. molitor* can assess conspecific densities using both mechanical and chemical cues (Tschinkel and Willson 1971; Kotaki and Fujii 1995). This indicates that perceived pathogen exposure did not differ among *T. molitor* individuals used in this study, because all the beetles grew under similar larval densities. Thus, our results are likely to support the genetic link hypothesis, because females with black elytra showed signs of immune priming, while tan and brown females mounted a weaker encapsulation upon second challenge. Furthermore, these effects cannot be attributed to a density-dependent prophylactic response to rearing density.

The genetic link and the exposure hypotheses are considered to be competing explanations of melanism. Disentangling their mechanisms is important for understanding the adaptive function of melanin-based pigmentation. However, our results cannot provide any decisive evidence on the distinction of the two hypotheses. First of all, the strength of the immune response, the number of offspring and lifespan did not differ among females with different cuticle melanization before the activation of the immune system, suggesting costs of cuticle coloration. The benefits of the dark cuticle appeared only when the immune responses against artificial infection were induced. This demonstrates that the innate immunity of adult insects may be adjusted to changes in the risk of infection by individual priming, which increased the lifespan of the females with black cuticles. This provides support for the parasite exposure hypothesis, since *T. molitor* females needed an environmental cue to prime their innate immunity. Thus, the exposure and the genetic link hypotheses might not be mutually exclusive explanations of the adaptive role of cuticle melanization in *T. molitor*. To provide further tests for these hypotheses, different levels of larval densities and parasite exposures should be manipulated in future work. This is important in order to test whether darker or paler cuticles are associated with adaptations to one highly specific environment and represent the lack of adaptations in other circumstances (Kawecki and Ebert 2004). This is a key aspect when considering the role of environmental heterogeneity in the maintenance of polymorphism (Piault et al. 2009; Roulin 2009).

It is known that melanogenesis involves the formation of melanin pigments and toxic by-products from the action of PO on quinone precursors, and occurs primarily in the cuticular structures, midgut epithelium and hemolymph (Cerenius and Söderhäll 2004). However, the strength of the encapsulation response did not differ between black and paler females before their immune system was activated via the insertion of the nylon monofilament. The absence of excess activation in females with black elytra can be explained by energetic costs and oxidative stress of permanently activated immune system (Freitak et al. 2003; Krams et al. 2014b). The damaging action of chemical radicals produced during the activation of the immune response against intruders can also harm the host cells and tissues (von Schantz et al. 1999; Finkel and Holbrook 2000; Metcalfe and Alonso-Alvarez 2010). Melanic insect morphs exhibit an unusually high concentration of cuticular melanin (Barnes and Siva-Jothy 2000; Wilson et al. 2001, 2002), and the immune system of black females potentially generates too harmful a response. It is probably more adaptive for the immune system to avoid the permanent harm to self and respond just when the host is under attack by infection, even if the response is delayed.

In insects, melanin pigments and their precursors are important as structural and protective components of the cuticle. In the greater wax moth (*Galleria mellonella*), the cuticle of melanic larvae is shown to be substantially thicker than in a non-melanic morph. The cuticle of melanic larvae of the greater wax moth can generate a short burst of enhanced cuticular PO activity during the early stages of fungal penetration (Dubovskiy et al. 2013a, b). A thicker cuticle and slower penetration of the intruder allows sufficient time for the insect to activate its defenses, such as encapsulation of the intruder (Sweeney et al. 1983; Butt et al. 1988). It is important to note that a high concentration of cuticle melanin plays a significant role in wound healing after damage by intruders, as seen in mosquito midguts following penetration by the malaria parasite (Shiao et al. 2006). This might be especially important in explaining the greater offspring number and the longer lifespan of black *T. molitor* females after the activation of the immune system. However, future research is needed to test whether dark and pale *T. molitor* differs with respect to wound healing ability, the energetic costs of wound healing and whether any possible differences in wound healing are responsible for the higher fitness of the darker female *T. molitor* beetles.

In this study, we did not find any costs related to melanin pigmentation of the females with black elytra. However, a number of studies revealed many trade-offs between immunity and other life history traits in *T. molitor*. Also in the greater wax moth, the heavy defense investments

made by melanic insects result in a lower body mass, decreased longevity and lower fecundity in comparison with the non-melanic morph. One possible explanation for the similar fecundity of darker and paler females before the activation of the immune system is that the egg-laying period of brown and tan females was longer than that of black females, and exceeded the period of 7 days that was allowed to oviposit.

To conclude, the present study shows some benefits that the melanized cuticle brings to *T. molitor* females. While fecundity, the strength of encapsulation response and longevity do not differ between darker and paler individuals, highly melanized females can prime their immune response, increase their lifespan and not decrease their fecundity upon parasitoid-like attacks (here, the insertion of the nylon implant). Although cuticular darkening and encapsulation response may compete for the same limiting resources necessary for melanin synthesis, such as tyrosine, we did not find any costs associated with immune response, fecundity and longevity in females with black elytra. The availability of food is an important predictor of survival and reproductive strategies in *T. molitor* (Krams et al. 2015). However, the access to tyrosine, a food-derived melanin precursor, was likely the same for dark and pale females in this study. It is possible that female beetles differed in their ability and efficiency to transform tyrosine and properly invest it into cuticular and immunity-related melanin. We also do not know whether melanin deposited in cuticle could be re-invested into immediate immune response. Importantly, relationships between cuticle darkening, fecundity, immune response and longevity of *T. molitor* females cannot be estimated without finding possible trade-offs between these life history traits. Cuticle melanization provides a widespread source of pigmentation in insects, yet the relationship between cuticle melanin and its use in immune response appears to be more complex than previously thought.

Our results emphasize the fact that the benefit of being melanic is accrued only under specific environmental conditions, a phenomenon observed in several vertebrates (e.g., Gonzales et al. 1999; Piault et al. 2009). This raises the possibility that the covariation between the degree of melanism and other phenotypes can be detected only under specific conditions (Roulin 2009). Finally, our study shows that males and females may differ in trade-offs between parameters involved in immune response and reproductive strategies, which suggests a more important role of sex- and hormone-related regulation of immune function and senescence in *T. molitor*.

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Author contribution statement IK, T.K. and M.J.R. conceived and designed the study and participated in the drafting of the manuscript. I.K., T.K. R.K., S.L. and G.T. performed the study, and collected and extracted the data. A.K., G.T., G.M.B. and I.K. analyzed the data. M.J.R., T.K., S.L. and R.K. participated in data analysis, results interpretation and drafting the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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