

Immunocompetence and parasite infestation in a melanistic and normally-coloured population of the lacertid lizard, *Podarcis siculus*

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Abstract. Melanism is the occurrence of individuals that are darker in skin pigmentation than their conspecifics, which is a common colour polymorphism among vertebrates. Due to the pleiotropic effects of the POMC gene that is responsible for melanin-based colouration, dark pigmentation often co-varies with a range of other phenotypic traits. Still, not much is known on the link between melanin-based colouration and immunity in lizards. In this study, we examined and compared the immunocompetence and degree of ectoparasite infestation of *Podarcis siculus* lizards from a fully melanistic population on an islet in the Tyrrhenian Sea, with conspecifics from a ‘normally’-coloured population on the mainland. Our findings show that both males and females from the melanistic population were less parasitized by ectoparasites and had a greater cellular immune response to a phytohemagglutinin injection than normally-coloured conspecifics. This outcome is in line with the “genetic link hypothesis”, which predicts that melanistic individuals will be more resistant to parasites than non-melanistic individuals due to the pleiotropic POMC gene. In addition, we found correlative evidence for a link between ectoparasite load and PHA immune response, but this was only true for males from the normally-coloured population. Immunological data on additional melanistic and non-melanistic populations of *Podarcis siculus* in the Mediterranean basin would provide us better insight into patterns of co-variation between immunity and melanism in lizards.

Keywords: colour polymorphism, immunity, Licosa Islet, melanism, parasites, PHA, *Podarcis siculus*, POMC gene.

Melanism is the occurrence of individuals that are darker in skin pigmentation than their conspecifics (True, 2003; Hoekstra, 2006), which is a common colour polymorphism among ectotherms (Clusella-Trullas et al., 2007), including lizards (e.g. Pérez-Mellado, 1984; Zuffi, 1986; Daniels et al., 2004; Trocsanyi and Korsos, 2004; Korsos and Nagy, 2006; San José et al., 2008; Pereira et al., 2014). Natural selection may promote melanism directly through its effects on thermoregulation (e.g. Clusella-Trullas et al., 2008, 2009), crypsis (e.g. Kettlewell, 1973; Endler, 1984; Rosenblum, 2006), aposematism (e.g. Turner, 1977), sensitivity to UV radiation (e.g. Gunn, 1998), disease resistance (e.g. Wilson et al., 2001), and mate selection (e.g. Wiernasz, 1989). Alternatively,

dark colouration may arise as a by-product of selection on other functions. Due to the pleiotropic effects of the gene responsible for melanin pigmentation in vertebrates (i.e. proopiomelanocortin [POMC] gene), melanin-based colouration often co-varies with a range of physiological and behavioural traits (reviewed in Ducrest et al., 2008). For instance, the POMC gene affects aggressiveness, food intake rate, energy expenditure, and sexual activity, besides pigmentation, so selection for any of these qualities would automatically result in darker colouration (Ducrest et al., 2008). An association between melanin pigmentation and immunity has also been documented, with dark individuals typically having a stronger anti-inflammatory response than lighter individuals. For instance, Roulin et al. (2000, 2001) showed a relationship between the degree of pigmentation and immune response and parasite resistance in barn owls (*Tyto alba*). In tawny owls (*Strix aluco*) and feral pigeons (*Columba livia*), dark individuals can maintain stronger levels of antibody production over longer periods of times compared to pale conspecifics (Gasparini

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et al., 2009; Jacquin et al., 2011). While the link between melanin-based colouration and immunity and parasite load is well documented in birds and mammals (Ducrest et al., 2008), it is less so in lizards (Vroonen et al., 2013; Seddon and Hews, 2016).

In this study, we examined and compared the immunocompetence and degree of ectoparasite infestation of *Podarcis siculus* lizards from a melanistic population with their conspecifics from a 'normally'-coloured population. The melanistic lizards of study originated from a population found on a small island in the Tyrrhenian Sea near the Italian coast, called Licosa Islet. Individuals of both sexes are melanistic and no normally-coloured lizard has ever been observed on the island (Raia et al., 2010; Monti et al., 2013; personal observation). Conspecifics on the mainland have the green-brown dorsal colours typical of the species. Previous research on this particular study system already showed that lizards from the melanistic population of Licosa Islet exhibit increased activity of the POMC gene and its posttranslational products (Raia et al., 2010; Monti et al., 2013; Trapanese et al., 2017), and provided some support for the pleiotropic POMC

effect. Raia et al. (2010) observed more aggressive interactions among melanistic lizards of Licosa Islet than among normally-coloured conspecifics on the mainland, and Monti et al. (2013) established a difference in reproductive output, with melanistic females having a larger clutch mass than normally-coloured females on the mainland. The latter researchers also assessed the lizards' ectoparasite load and found that male lizards from Licosa Islet were more infested than male conspecifics from the mainland. However, these findings are based on a relative low sample size of solely male individuals ($N = 30$). We gathered data on cell-mediated immunity and ectoparasite infestation of a large number of individuals ($N = 106$) from both sexes and from both populations. We hypothesize that due to the pleiotropic effects of the POMC gene, both male and female lizards from the melanistic population of Licosa will be equipped with a greater immune response and will be less parasitized than their normally-coloured conspecifics on the mainland.

In September 2012, we caught 57 adult *Podarcis siculus* lizards (27 males, 30 females) of the melanistic population on the island of Licosa ($40^{\circ}15'05.0''\text{N}$, $14^{\circ}54'00.8''\text{E}$), and 49 normally-coloured lizards on the mainland (23 males, 26 females) near the village Castellabata, in Italy ($40^{\circ}14'59.8''\text{N}$, $14^{\circ}54'37.3''\text{E}$) (fig. 1). Lizards were cap-

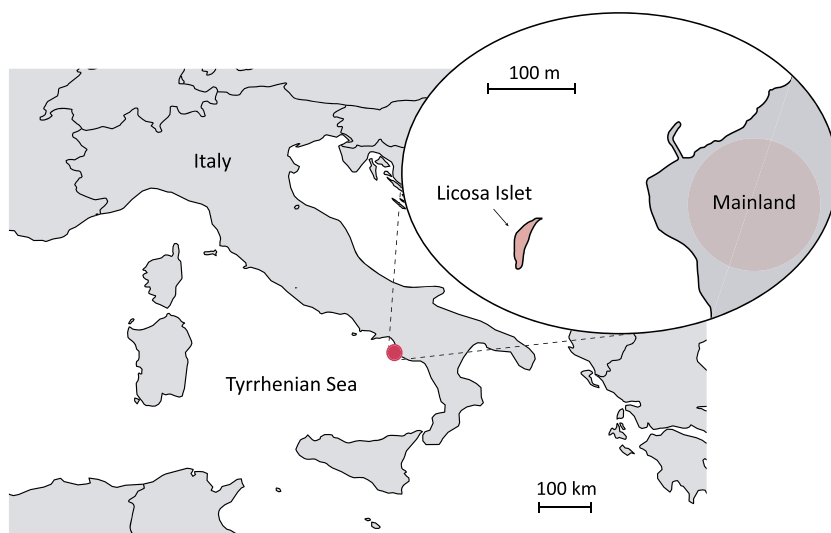


Figure 1. Geographic location of the two populations of study: the melanistic population of *Podarcis siculus* (Licosa Islet), and the 'normally'-coloured population (mainland). Red indicates sampling area.

tured by noose and transported individually in cotton bags to the field lab on the mainland. In the lab, the degree of ectoparasite infestation of each lizard was assessed by counting the number of ticks (Ixodidae) and mites (Trombiculidae) on the lizard's skin surface. The cloth bags were also checked for possible ectoparasites, which the lizard may have lost during transport. Mite count and tick count were later pooled into one measure ('ectoparasite count') and used in further statistical analyses. Lizards were, thereafter, kept per population (sexes separately) in large meshed cages (1.2 × 0.5 × 0.7 m). Water and food (wax moth larvae, *Achroia grisella*) were provided *ad libitum*. To estimate the immunocompetence of each lizard, we quantified its delayed cutaneous hypersensitivity response (Belliere et al., 2004; Oppliger et al., 2004) by injecting one foot of every individual with a 20 μ L solution containing 30 mg of phytohaemagglutinin (PHA; Sigma-Aldrich, L-8754) in 5 mL phosphate buffered saline (PBS). PHA influences a variety of cell types and, therefore the response to PHA injection is complex, but can serve as an index for heightened immune cell activity (Kennedy and Nager, 2006; Martin et al., 2006). Thickness of the foot was measured before injection and 24 hours later using a digital calliper (Mitutoyo; accuracy: 0.01 mm). The other foot was treated in the same way, but injected with 20 μ L of PBS serving as a control. The immune response was calculated as the change in thickness of the PHA injected foot minus the change in the control foot. Larger localized swelling indicated an increased immune activity at the cellular level. The snout-vent length (SVL) and body mass (Balance EC 100; accuracy: 0.01 g) of each lizard was also measured. Body condition was estimated as the residuals of a linear regression of body mass on SVL. Prior to any statistical analyses in R, variables were \log_{10}

(SVL, mass) or square root (ectoparasite count) transformed to meet assumptions of normality (Shapiro-Wilks test: $W \geq 0.95$). Linear regressions were used to explore patterns of variation in ectoparasite load, immune response, SVL, and body condition between sexes and populations.

The proportion of lizards infested with ectoparasites was greater in the normally-coloured mainland population (100%) than the melanistic island population (81%) (Z -score = 3.25, $P = 0.001$). The degree of infestation was also significantly higher in normally-coloured lizards than in melanistic lizards ($F_{1,103} = 115.82$, $P < 0.001$; fig. 2a). In the normally-coloured population, but not in the melanistic population, males were more severely infested than females (population × sex interaction; $F_{1,102} = 9.66$, $P = 0.002$; fig. 2a). Further, melanistic lizards exhibited a significantly greater PHA immune response than normally-coloured lizards ($F_{1,103} = 19.93$, $P < 0.001$, fig. 2b). There were no sex differences in immune response ($F_{1,103} = 0.74$, $P = 0.392$, fig. 2b). In normally-coloured males, ectoparasite load was positively correlated with PHA immune response ($F_{1,17} = 4.93$, $P = 0.040$). In the other groups, the relationship was not

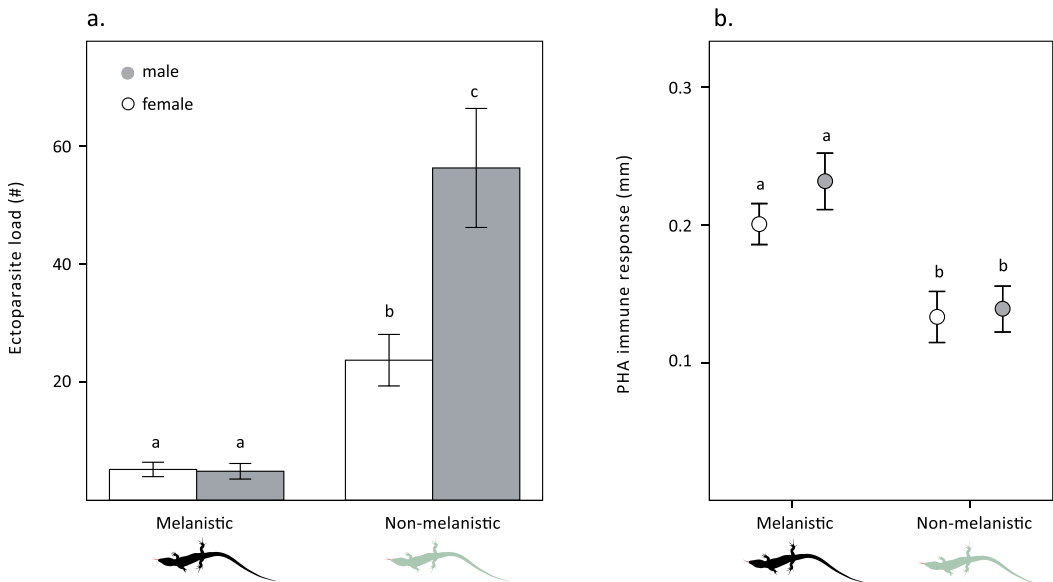


Figure 2. Mean (a) ectoparasite infestation and (b) cellular immune response to PHA injection in males (grey) and females (white) lizards from a melanistic population and 'normally'-coloured (i.e. non-melanistic) population of *Podarcis siculus*. Error bars represent SE. The same letters above the bars denote that means are not significantly different from each other.

significant (normally-coloured females: $P = 0.221$; melanistic males: $P = 0.927$; melanistic females: $P = 0.416$). Lizards on the island of Licosa did not differ in SVL from their conspecifics on the mainland ($F_{1,103} = 0.48$, $P = 0.482$). While males were larger than females in both populations ($F_{1,103} = 31.82$, $P < 0.001$), the degree of sexual size dimorphism was higher in the normally-coloured population (population \times sex interaction; $F_{1,102} = 4.78$, $P = 0.031$). On average, normally-coloured lizards had a higher body condition than melanistic lizards ($F_{1,103} = 34.79$, $P < 0.001$). Body condition was higher in males than females, and this sex difference was most pronounced in the normally-coloured population (population \times sex interaction; $F_{1,102} = 5.75$, $P = 0.018$). For neither sex nor population, we found a significant relationship between body condition and ectoparasite load (all $F < 1.4$, $P > 0.24$), and immune response (all $F < 2.4$, $P > 0.13$).

Our findings show that *Podarcis siculus* lizards from the melanistic population of Licosa Islet were less parasitized by ectoparasites and had a greater cellular immune response to PHA injection than normally-coloured conspecifics on the nearby mainland, and this was true for both males and females. This outcome is in line with the “genetic link hypothesis” (Jacquin et al., 2011), which predicts that melanistic individuals will be more resistant to parasites than non-melanistic individuals due to the pleiotropic effects of the POMC gene (Ducrest et al., 2008). Similar findings have been documented in birds and mammals (Roulin and Ducrest, 2011), although the few studies that have focused on lizards show equivocal results on the link between melanism and immunity. In *Zootoca vivipara*, males (but not females) with a higher degree of ventral melanin pigmentation mounted a stronger PHA-induced immune response than males of the same population that have less pigmentation (Vroonen et al., 2013). No significant relationship was found between degree of pigmentation and tick in-

festation (Vroonen et al., 2013). Seddon and Hews (2016) studied two populations of *Sceloporus occidentalis* lizards, which differed substantially in their degree of melanisation. Male lizards originating from the darkly-coloured population were found to be more infested with mites than males from the pale-coloured population. However, within population variation in the degree of melanin pigmentation was not significantly related with mite load (Seddon and Hews, 2016). Part of these discordant findings among studies might be attributed to the large variation in melanisation among the lizards of study and/or the timing of sampling. Firstly, our study compares two populations that drastically differ in melanisation (complete melanistic vs. complete non-melanistic), while previous studies compared lizards that only vary slightly in their degree of pigmentation (such as Vroonen et al., 2013, and Seddon and Hews, 2016). It would be interestingly to gather immunological data on several full melanistic and non-melanistic populations of *Podarcis siculus* in the Mediterranean basin in order to gain a better insight into patterns of co-variation between immunocompetence and melanism in lizards. Secondly, while the aforementioned studies by Vroonen et al. (2013) and Seddon and Hews (2016) were conducted early in reproductive season (May-June), we surveyed our study site near the end of the season (September). Temporal variation in parasite abundance (which can moreover differ among localities; Brito et al., 2014), but also lizard immune function (Huyghe et al., 2009), might obscure clear patterns of covariance between melanisation and immunity. Clearly, future studies should invest in collecting information at multiple times throughout the reproductive season.

Why would melanism have evolved in lizards on Licosa Islet? Atypical pigmentation on islands has been explained as an adaptive response to thermal conditions (Ortega and Pérez-Mellado, 2016), to differential predation pressures (Cooper and Pérez-Mellado, 2012), or a combination of both (Bittner and King, 2003;

Tanaka, 2007). In this scenario, the thermal benefits of a dark pigmentation (Clusella-Trullas et al., 2007, 2008, 2009) combined with the loss of the need for camouflage (Gibson, 1978; Vervust et al., 2007) may have driven the evolution of melanism on Licosa Islet, and the accompanied high immunocompetence would have then arose as a by-product ('spandrel') due to the pleiotropic POMC gene. While the "genetic link hypothesis" is one explanation on why we found differences in ectoparasite load and immunocompetence between lizards from Licosa Islet and lizards from the mainland, there are, at least, four alternative explanations. Firstly, while our findings indicate that melanistic lizards carry a lower number of parasites because they have a better immunocompetence than normally-coloured lizards, we can not rule out that the observed differences in parasite infestation between populations might be the result of differences in parasite abundance. There are cases in birds and mammals, where inter-population variation in parasite abundance has been reported as an important driver of variation in parasite infestation (Poulin, 2006; Santiago-Alarcon et al., 2008). Since parasite communities on islands often occur in low densities (Nieberding et al., 2006), the low degree of parasite infestation in Licosa lizards might, therefore, be the result of overall low parasite abundance on Licosa Islet. Moreover, we were unable to find correlative evidence for a direct link between parasite load and immunocompetence in our lizards of study, with the exception of mainland males where we established a significant positive correlation. The direction of correlation was, however, not as expected, as we predicted that the immune response to PHA-injection would be negatively (not positively) correlated with the intensity of parasite infection, e.g. as recorded in *Podarcis muralis* (Amo et al., 2005). However, a positive correlation has also been observed, i.e. in males of the lizard *Podarcis melisellensis* (Huyghe et al., 2009). The latter researchers argue that this result could be a priming effect, in which lizards with high

loads of ectoparasites may already be primed for immunological responses (PHA swelling) because their immune system is not constantly challenged. Those with decreased ectoparasite loads may then exhibit a lowered swelling response, as their immune systems have not been primed in the same way. It would be interesting to evaluate the link between endoparasite load (e.g. haemogregarines, coccidian, nematodes) and PHA swelling response, and whether it varies (or not) with ectoparasite load. Secondly, differences in immunocompetence between populations could also be ascribed to divergent levels of corticosterone due to differences in stress. Elevated levels of corticosterone as a result of stress can suppress immune activity (Berger et al., 2005) and increase parasite infection (Oppliger et al., 1998). One can imagine that lizards on the mainland are exposed to higher levels of stress than island lizards, due to high predation pressure (from both natural and domestic predators) on the mainland in comparison to the island (e.g. Pérez-Mellado et al., 1997; Vervust et al., 2007), or due to the high level of interspecific competition for resources on the mainland relative to the island (Case and Bolger, 1991). This hypothesis could be tested by comparing corticosterone levels between lizards of both populations (Huyghe et al., 2009). Thirdly, the evolution of melanism on Licosa Islet might be a by-product on selection for immunocompetence. If parasite resistance is more important (relative to other functions) on Licosa Islet than on the mainland, insular melanism may arise as a secondary result. However, this scenario is highly unlikely, as parasite abundance is generally lower on islands than on the mainland (Nieberding et al., 2006). Fourthly, diversity in pigmentation and/or immunity can also occur through factors such as genetic drift, founder effects, and inbreeding (e.g. Runemark et al., 2010), factors that are typically more common in island than mainland populations (Nevo, 1978).

In conclusion, the findings of this study show differences in immunity and parasite infestation

between lizards from a melanistic and non-melanistic population. While the “genetic link hypothesis” is one potential explanation for this outcome, additional research is necessary to determine the true underlying mechanism. We hope that this short note encourages further research on the link between melanin-based colouration and immunity in lizards.

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