F-1.2.2 Individual variation in parasite resistance

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Abstract Males of the damselfly *Mnais costalis* (Odonata: Calopterygidae) are morphologically and behaviourally polymorphic, typically existing as clear-winged non-territorial 'sneaks' and orange-winged territorial 'fighters'. The amount of orange pigment in the wing, as measured with a chromameter, varied between individuals, and decreased as the reproductive season progressed. Individuals maintained in the laboratory on high and low nutrient diets differed in the amount of pigment in the wing. Males in the high nutrient group developed darker wings faster than those in the low nutrient group. Young adults of both sexes and morphs were fed ^{14}C radiolabelled tryptophan or tyrosine (precursors of the pigments ommochrome and melanin respectively). Ommochrome was restricted to the pseudopterostigma of the males of both morphs and was not present in females. The presence of tyrosine in the wing cells of orange males but not of clear males indicated that the orange pigment is at least partly constituted from melanin. These data show that at least some pigment levels must be continuously maintained in the wings of orange males, and that maintainance is costly since it is compromised at low nutrient levels. The results suggest that any trade-off, which may operate between the strategy of each male morph, may do so via the immune system and the expression of secondary sexual characters. This is important in the context of conservation biology because it shows that the insect immune system is plastic and can be adjusted according to environmental conditions. It means that insects in stressed environments may divert resources from their immune-systems, so that the presence of insects in a given environment may not mean that the population is healthy.

Key Words Dimorphism; immune system; Odonata; reproductive success; *Mnais costalis*

1. Introduction

Colour change in insects may be short term and physiological or longer term and morphological. Physiological changes involve the formation of new pigments as, for example, occurs when insects darken at night[1]. Morphological changes involve pigment metabolism and may be under hormonal control[2] or, presumably, influenced by the nutritional status of the insect.

Damselflies of the *Mnais* species group (Odonata: Calopterygidae) are polymorphic, males existing in orange- and clear-winged forms[3),4)]. The orange wing is probably a sexually selected signal, since females are clear-winged and of the males, the orange-winged morph is territorial, whilst clear-winged males are usually non-territorial [3),4)]. Orange males have been behaviourally classified as 'fighters' and clear males as 'sneaks'[4)]. The chemical nature of the orange wing pigment is unknown. Wing size varies both within and between morphs and clear-winged males live for longer than the orange wings[4)]; moreover, individuals vary in the 'darkness' of their orange wings (unpublished data). In

this report we ask (1) does the orange colour in 'fighter' males vary temporally in the same individual; (2) does nutritional status affect wing colour; and (3) what is the pigment base to the orange colour?

2. Material and Methods

The study site was a 50m stretch of a mountain stream in and 7 July 1997 all adults of this population were captured and individually marked on the hind wings with enamel pens. Teneral (newly emerged) individuals could be accurately aged since they have soft shiny cuticle for two days after eclosion; colour develops in the wings of orange males in the first few days after eclosion. In both male morphs a discrete area of five or six cells, the pseudopterostigma, is coloured with a dark red pigment. In females the pseudopterostigma is coloured white whilst the rest of the wing is clear. We collected tenerals from the field and housed them separately in the laboratory in plastic pots (20 cm diameter, 10 cm deep) with a water source and perch, under a 16L : 8D photoperiod, at 20℃.

We measured colour in the field every two to three days with a portable chromameter (Minolta CR 321). For measurement the insect was held against a white card and the wings held under transparent plastic. The chromameter measured (in a 3mm diametre circle) three parameters defined by the International Commission on Illumination –[L*, a* and b*] hereafter referred to for simplicity as values representing 'transmission, 'red' and 'yellow' respectively.

Transmission represents the 'darkness' of the wing pigment, i.e. a higher transmission value would indicate a lighter wing. The red value represents reflectance in the green-red part of the spectrum, i.e. 520 to 780nm. The yellow value measures reflectance from blue to yellow, i.e. 410 to 560nm. Using these measurements we were able to track wing colour changes in the field. Field measurements are the average colour measurement of the left and right forewings of each animal. The chromameter was positioned between the nodus and the pseudopterostigma of the wing, such that the wing vein radius 4 passed through the centre of the circle of measurement. We tested the repeatability of our measurement technique by removing and replacing the insect from under the plastic and remeasuring. The measurements for each parameter were highly repeatable (ANOVA, transmittance, R=0.96, F=779.8, d.f.=74, p<0.0001; red, R=0.97, F=1048.7, d.f.=74, p<0.0001; yellow, R=0.98, F=1992.3, d.f.=74, p<0.0001).

To test whether nutrient condition affects wing colour, we took twenty teneral orange males into the laboratory and randomly split them into two groups. The high nutrient group (n=10) was hand fed six live adult chironomid flies from a monospecies culture twice a day; the low nutrient group (n=10) received two flies twice a day. We measured the three colour parameters (transmission and red and yellow reflectance) at 17.00h every day with the chromameter described above. Right and left fore and hindwings were colour measured and an average taken. The left hindwing length (mm) of each individual was also measured to assess the relationship between wing size and colour.

Pigments in insect wings belong to four major classes: melanins, ommochromes, pterins and flavonoids[5]. Since flavonoids must be derived from plants[6], we can eliminate this class of pigments as a candidate for the wing colour of *M. costalis*. Although pterins occur throughout the insecta, as wing pigments they have only been found in a few species of Lepidoptera[5], and we do not consider them further here. This leaves melanins and ommochromes. To distinguish between these two pigment classes we fed tenerals with ^{14}C radiolabelled isotopes of specific precursors of melanin and ommochrome. The amino acid tyrosine is a precursor of melanin, and tryptophan is a precursor of ommochrome [5), 7)]. We

fed five females and five individuals of each morph, 5 ml of ^{14}C-tyrosine or ^{14}C-tryptophan containing approximately 1 mSv radioactivity and reared them in the laboratory, hand feeding them twice a day with three live flies (Chironomidae). After ten days all animals were killed and their wings stored at room temperature on a sealed autoradiograph plate. The autoradiograph was developed one month later. The presence of either of the ^{14}C radiolabelled isotopes of these amino acids in the wings of our experimental animals would indicate the nature of the pigment.

3. Results

Variation in wing colour throughout the season in the wild. Transmittance values increase with time, that is, wings fade. At the beginning of the experiment there is no significant difference in transmittance or red or yellow reflectance (transmittance, *t*- test, t=1.24, d.f.=18, p=0.229; red, t=0.074, d.f.=18, p=0.942; yellow, t=0.069, d.f.=18, p=0.945) but all three parameters are significantly different after six days on high or low nutrients (transmittance, *t* -test, t=2.75; d.f.=18, p=0.013; red, t=2.13, d.f.=18, p=0.047; yellow, t=2.11, d.f.=18, p=0.049). Wings got darker and accumulated pigment faster at high compared to low nutrient conditions.

The change in transmittance is significant (Sign test, p=0.041; Sign tests compared the difference between the first and last measurement compared to a random change). The decline in red and yellow reflectance measurements is also significant (Sign test, red, p=0.019; (c) yellow, p=0.003), but note that some individuals were able to recover. There is variation in wing colour between and within individuals throughout the season.

Pigment accumulation at high and low nutrient levels. The wings of orange-winged males became darker and did so faster at high compared to low nutrient conditions. There were significant relationships between wing size and all three colour parameters both at day zero (the beginning of the experiment) and at day six (the end): longer wings were darker (day 0, r^2=0.381, n=20, p=0.004; day 6, r^2=0.346, n=20, p=0.006) and gave higher red (day 0, r^2=0.408, n=20, p=0.008; day 6, r^2=0.347, n=20, p=0.006) and yellow measurements (day 0, r^2=0.346, n=20, p=0.006; day 6, r^2=0.227, n=20, p=0.034). In other words, bigger individuals had darker wings with more reflectance in the red and yellow parts of the spectrum and the difference held throughout the experiment.

Autoradiographs. We made positive autoradiographs of wings from the orange and clear morphs and females of individuals fed with (a) ^{14}C-labelled tryptophan and (b) ^{14}C-labelled tyrosine. In the autoradiographs, the black areas indicate the presence of the isotope. In females the pseudopterostigma is clear in both cases, indicating the absence of the isotope. The pseudopterostigma of both male morphs, however, contains tryptophan; the pigment in this discrete set of 4 or 5 cells of the wing therefore contains ommochrome. ^{14}C-labelled tyrosine is present in the blood of both morphs (insect blood circulates through the veins of the wings), but tyrosine is particularly evident in the cells of the orange wing, suggesting that a major component of orange colour is melanin. Note the 'patchiness' in the autoradiograph of the orange wing of a male fed with ^{14}C tyrosine.

4. Discussion

Orange wing colour 'fades' with time in the field as well as in the laboratory. Young animals maintained on a low nutrient diet showed a slower increase in wing colour

compared to those raised on a high nutrient diet. The radio-labelling experiment revealed the presence of tyrosine in the wing cells of orange males, indicating that melanin is an important constituent of the absorbance and reflectance spectra of the wings.

There is some debate concerning the function of sexually selected signals, and particularly about the importance of condition-dependent expression of such signals. Whilst variation in secondary sexual signals has been reported in cricket song [8], this is the first description of a secondary sexual morphological signal in an insect which (a) is not fixed at maturity and (b) varies with nutrient status and age. Our results indicate that when orange-winged *M. costalis* are nutrient-deprived, the colouration in the wings is reduced. Visual inspection of the wings sometimes revealed areas where pigment distribution becomes clumped and patches appear in the wings, which may be related to nutrient deprivation. Such patches can be seen in the autoradiograph of the orange wing of a male fed with ^{14}C tyrosine. We fed the animals six flies a day, an amount probably less than their natural intake, which may account for the irregular deposition of colour in the wings.

Several factors indicate that orange male *M. costalis* have a shorter lifespan than clear males, a difference which may arise as a consequence of different energy budgets. Orange males must fight to gain and defend a territory, an energetically extremely costly activity[9], whereas clear males do not fight for territories and so do not, in general, have to pay this cost. Moreover, orange males have a higher daily copulation rate than clear males[4]. These factors probably contribute to the shorter lifespan of orange males. But since after capture clear males live for longer than orange males even in the laboratory[4], where activity levels are the same, there is probably another factor influencing differences in lifespan.

In the current study, orange colour decreases at low nutrient levels, suggesting that there may be a physiological cost of being orange in addition to any behaviourally mediated life history costs. In the flour moth *Ephestia kuehniella*, nonmelanic females live significantly longer than females of the melanic genotype, a difference which may be explained by the higher activity rates of melanic compared to nonmelanic genotypes[10]. Such lifespan differences may arise, however, because melanic and nonmelanic genotypes will differ in the precise way the pathway of melanization is regulated[10]. In other words, it is likely that there are physiological differences underlying 'phenotype-level' differences.

The physiological differences, if any, between orange- and clear-winged males are unknown. However, there may be a cost of maintaining the orange pigment paid by the immune system. Melanin is a key component of the insect humoral immune system[11),12)], and since tyrosine is a precursor of melanin, it is not surprising to find it in the blood of females and both male morphs. In the bee Bombus terrestris, increased foraging effort decreased the strength of the encapsulation response[13]. In another Japanese calopterygid damselfly, *Matrona basilaris japonica*, the encapsulation response was reduced after reproductive activity[14]. One as yet untested explanation for such decreases is that the increased energetic expenditure of the insect decreased the amount of melanin available for immune defence. In *Drosophila melanogaster* the strength of the encapsulation response is related to nutritional status[15]. If a similar relationship holds for *M. costalis*, the orange wings of 'fighter' males would qualify as a 'handicap'[16] since they use resources which could otherwise be used for immune defence. Current work is addressing this question, and investigating the genetic basis of the polymorphism.

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