Condition dependence in animal colouration: tools for a comparative approach

Jessica Ethier Corresp., 1, Emma Despland 1

1 Biology Department, Concordia University, Montreal, Quebec, Canada

Corresponding Author: Jessica Ethier
Email address: jess_ethier@yahoo.ca

The study of condition dependence in animal colour has undergone important changes in recent years. In particular, the focus on carotenoid-based colour traits is a thing of the past and a broader, comparative approach is becoming more common. Here, we refine a previously developed theoretical model describing pigment expression as a saturating function of resource intake and demonstrate its use as a tool to compare patterns of resource assimilation, resource allocation, and condition dependence between groups of organisms. Using a simple equation and non-linear regression analyses, we apply this saturating model to melanin-based colouration in a model insect species. We find that patterns of condition dependence differ between sexes, phenotypes, and populations, suggesting underlying differences in resource allocation priorities. Our results highlight both the usefulness of this method and the value of a comparative approach in identifying proximate mechanisms of condition dependence.
Condition dependence in animal colouration: tools for a comparative approach

Jessica Ethier¹, Emma Despland¹

¹Biology Department, Concordia University, Montreal, Quebec, Canada

Corresponding author:

Jessica Ethier¹

Email address: jess_ethier@yahoo.ca
Abstract

The study of condition dependence in animal colour has undergone important changes in recent years. In particular, the focus on carotenoid-based colour traits is a thing of the past and a broader, comparative approach is becoming more common. Here, we refine a previously developed theoretical model describing pigment expression as a saturating function of resource intake and demonstrate its use as a tool to compare patterns of resource assimilation, resource allocation, and condition dependence between groups of organisms. Using a simple equation and non-linear regression analyses, we apply this saturating model to melanin-based colouration in a model insect species. We find that patterns of condition dependence differ between sexes, phenotypes, and populations, suggesting underlying differences in resource allocation priorities. Our results highlight both the usefulness of this method and the value of a comparative approach in identifying proximate mechanisms of condition dependence.

Introduction

The proximate mechanisms underlying patterns of condition dependence in animal colour traits have been the subject of much research and debate over the last couple decades. Initially, the results of many studies supported the widely accepted theory that pigment type itself is a proximate mechanism behind condition dependence. This ‘pigment-type’ theory held that because carotenoids can only be obtained in the diet, carotenoid ornaments are honest indicators of quality and condition (Houde and Torio 1992; Hill and Montgomerie 1994; Olson and Owens 1998; e.g. Brawner et al. 2000; Hill 2000). In contrast, since melanins are synthesized by the organism from amino acid precursors, it was presumed that melanin ornaments are not costly and
therefore not representative of individual quality and condition (Hill and Brawner 1998; Gonzalez et al. 1999; McGraw and Hill 2000; McGraw et al. 2002; Siefferman and Hill 2005).

By the mid-2000s, however, the validity of the pigment-type theory came under fire from multiple directions (Jawor and Breitwisch 2003; Griffith et al. 2006; Stoehr 2006). Griffith et al.’s (2006) meta-analysis of avian colour traits and condition dependence found no supporting evidence for the pigment-type theory and the authors stressed the importance of a broader, comparative approach in future work on condition dependence of animal colour. Stoehr (2006) challenged the assumed generality of the theory, arguing that while vertebrates may be able to synthesize melanin at low resource cost, there is plenty of evidence that melanism is costly in insects. Stoehr (2006) emphasized the necessity of increased communication between researchers working with different animal taxa, and proposed a basic and broadly applicable theoretical model describing pigment-based ornament expression as a function of resource intake.

These arguments appear to have helped stimulate a departure from the “caroteno-centric” focus in condition dependence research (Kemp et al. 2012), as well as a broadening of scope to include a wider range of experimental systems. Not only is a vast body of evidence accumulating showing that avian melanin ornaments can indeed be condition-dependent (see reviews by McGraw 2008; Guindre-Parker and Love 2014), but studies on avian colour and condition dependence are also investigating pigments other than carotenoids and melanins (e.g. flavonoids; Catoni et al. 2009), structural colour (Griggio et al. 2009; Maia et al. 2012), multicomponent colour traits (Galván 2011), and condition-dependent colour in females (Silva et al. 2008; Murphy et al. 2009; Remeš and Matysioková 2013). Furthermore, a wealth of research has emerged on the condition dependence of different types of colour traits in many other animals,
including insects (e.g. Bezzerides et al. 2007; Tibbetts and Curtis 2007; Lindstedt et al. 2010; Vidal-Cordero et al. 2012; Fabricant et al. 2013; Roff and Fairbairn 2013), fish (Price et al. 2008; Wedekind et al. 2008; Scott 2011; Marie-Orleach et al. 2014; Giery and Layman 2015), reptiles (Molnár et al. 2012; San-Jose and Fitze 2013; Vroonen et al. 2013; Lanuza et al. 2014), and mammals (Bradley and Mundy 2008; Rødven et al. 2009).

Despite this impressive amount of progress in the field of condition dependence research, the theoretical model of pigment-based colour ornament expression proposed by Stoehr (2006) has, to our knowledge, yet to be used empirically. Stoehr’s (2006) model predicts a saturating relationship between trait expression (size or intensity of pigmented area) and resource intake, with the slope of the relationship depending on processing efficiency. We first propose a refining of the model, as Stoehr’s (2006) definition of processing as “everything that happens between resource acquisition and ornament expression” actually encompasses two stages of resource processing: assimilation and allocation. By opening the ‘black box’ of resource processing, the comparative approach can be further elaborated and the mechanisms behind condition dependence better understood. Resource availability, assimilation efficiency, and patterns of allocation can all differ not only between pigment types, species, and taxa, but also within species. For instance, populations experience different environments (resource types and availability, temperature, etc.) and selective balances (parasitism rates, predation pressure, sexual selection, etc.); colour phenotypes within populations may have different resource-based trade-offs or life history strategies; males and females experience different suites of selection pressures, and may or may not have similar colour traits; and multiple colour traits can be present in individuals.
Methods

Fitting the model

We developed the following equation to fit the relationship between trait expression and resource intake to the predicted saturating model:

\[ y = a \left( 1 - e^{-\frac{x}{b}} \right), \quad (1) \]

where \( y \) is trait expression, \( x \) is resource intake, \( a \) is the asymptote (maximum), and \( b \) is the slope. Non-linear regression analyses using this equation will quantify patterns of condition dependence and provide both the parameter values and 95% confidence intervals for the slope, asymptote, and fit of the model, thus allowing for comparison between groups of organisms.

The slope value \( (b) \) is most important in describing trait expression when resources are limiting (i.e., at low levels of resource intake, before the asymptote is reached). Differences in slope values will reflect differences in resource assimilation efficiency, patterns of resource allocation, or some combination of both. Thus, a higher slope value could be due to more efficient resource assimilation, increased resource allocation to colour, or both.

Asymptote values \( (a) \) represent the approximate maximal level of trait expression (i.e., intensity of pigmentation or size of pigmented area), and may be of particular interest in systems with colour polymorphism or sexual colour dimorphism. The asymptote also has contextual value in determining what levels of resource intake should be considered as ‘high’ (not limiting) and ‘low’ (limiting); that is, any level of resource intake that results in submaximal trait expression can be considered limiting with respect to colour. Asymptotes may not always be reached in natural systems, but can nevertheless provide useful information. For example, a sexually-
selected conspicuous colour trait may have a higher asymptote in a population with reduced predation pressure even if individuals from both populations mainly occur in their respective slope (pre-asymptote) regions.

The model fit value \( r^2 \) indicates how well this non-linear saturating model fits observed data. A low model fit could be due to a number of reasons. If the positive relationship between resource intake and colour trait expression is linear, the model cannot predict where an asymptote may occur. Conversely, linear data with a slope of zero suggests that colour is uniform and not dependent on the measured resource over the range of resource values tested. Finally, no apparent relationship between resource intake and colour trait expression (highly scattered data) suggests colour expression is either not condition dependent or depends on a resource other than that studied.

**Study organism**

The saturating condition dependence model was tested on *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), the forest tent caterpillar. Two distinct melanin-based colour phenotypes occur in the adult moth: the melanic phenotype is dark brown and only expressed in males, while the less heavily pigmented typical phenotype is light brown or tan and occurs in both sexes (Lorimer 1979; Ethier and Despland 2012). As is typical for folivorous insects, nitrogen is a highly limiting nutrient for *M. disstria* (Hemming and Lindroth 1995; Colasurdo et al. 2009). This provides a unique opportunity to compare patterns of condition dependence within a population in two ways: a) between sexes that differ in the suite of selection pressures acting upon them, but share a colour phenotype (i.e. typical males and females); and b) between
same-sex individuals that may differ in resource-based trade-offs due to colour phenotype (i.e. melanic males and typical males).

With respect to genetic melanic polymorphisms, the asymptotic maximum of pigmentation (trait expression) should be genetically-based and higher for melanic phenotypes. As discussed above, variation in the slope of the relationship between pigmentation and resource intake (i.e. nitrogen) will result from differences in either resource assimilation, allocation, or both. Some Lepidoptera larvae, including *M. disstria*, regulate post-ingestive protein assimilation when fed moderately unbalanced (i.e. nitrogen-rich or nitrogen-poor) diets; as a result, nitrogen concentration in the adult body remains constant despite differences in dietary protein-carbohydrate ratios (Lee et al. 2002; Colasurdo et al. 2009). Since *M. disstria* moths are completely non-feeding (Fitzgerald 1995), this applies to the pupal stage as well. This allows pupal mass to be used as a proxy measure of nitrogen intake, as it proportionally represents the entire nitrogenous resource pool an individual has to allocate towards its adult structures. Finally, since the pupal stage effectively separates the steps of assimilation and allocation in time, any variation in the slope of the relationship between pigmentation and pupal mass (nitrogen intake) will be due to differences in resource allocation.

**Part 1: Testing the model**

To directly test the applicability of the saturating condition dependence model, larval nitrogen intake was manipulated by modifying the protein content of artificial agar-based diet and feeding larvae either high- or low-protein diet. Data from both diet treatments were pooled to have individuals with a wide range of pupal masses, and hence, a gradient of nitrogen intake from low to high (for further details on insect rearing, refer to the methods reported by Ethier et al. (2015);
the pigmentation and pupal mass data used here were collected from all 12 families of insects in
the laboratory experiment).

Part 2: Further applications

To further test the model as a comparative tool, it was also applied to females obtained from two
different source populations, reared under identical conditions. Egg masses were collected in
spring 2011 from a population near St. Esprit in Quebec, Canada (45°55'59.2"N 73°41'57.7"W)
and a population near Prince George in British Columbia, Canada (53°49'48.0"N
123°08'24.0"W). These insects were hatched and reared in the laboratory on a balanced artificial
agar-based diet; all other rearing conditions were identical to those used in the protein-
manipulation experiment for Part 1. All experiments conformed to the relevant regulatory
standards concerning animal welfare.

Independent samples two-tailed \( t \)-tests were used to compare mean pupal masses (mg) between
groups of interest. All \( t \)-tests and nonlinear regressions were performed in SPSS v.20.0.0 (IBM
2011).

Results and Discussion

Part 1: Testing the model

The expected saturating relationships between pigmentation and pupal mass (nitrogen intake) are
clearly seen in Fig. 1; results of the non-linear regressions and mean pupal masses (mg) are
reported in Table 1. Using the 95% confidence intervals to interpret significance (an overlap of
approximately 1/4 of the confidence interval or less; see Cumming and Finch 2005 for details),
these results show both a sex-based difference and a phenotypic difference in patterns of resource allocation and condition dependence in this species.

Typical males and (typical) females have similar asymptote values (i.e. similar maximal pigmentation), but the significantly lower slope of females suggests that females allocate proportionally fewer resources to melanin synthesis under nitrogen limitation. Melanic males have significantly higher asymptote and slope values than typical males, but the two types of males do not differ significantly in mean pupal mass (nitrogen intake) \((t_{230}=-0.871, P=0.385)\).

This indicates that melanic and typical males have similar resource intakes but different resource allocation patterns, such that melanics allocate proportionally more to pigment synthesis regardless of nitrogen intake. The low model fit \((r^2)\) for melanic males is due to the scarcity of individuals in the slope region combined with the highly scattered data (Fig. 1), which suggests that colour is only weakly condition dependent in this group of organisms.

In this study system, it is possible that severely limiting levels of nitrogen intake (pupal mass < 200 mg) do not allow such small melanic males to survive, but that all surviving melanic males reach maximal or near-maximal pigmentation due to their high allocation to pigmentation. In typical males, colour was moderately condition-dependent: only the smallest, lowest-quality individuals were incapable of reaching maximal pigmentation. Conversely, colour was strongly condition-dependent in females. Female fecundity, and therefore quality, is strongly predicted by pupal mass (Fitzgerald 1995; Parry et al. 2001). Here, it can be seen that only the largest, highest quality females were capable of maximal pigmentation.

**Part 2: Further applications**
Both the asymptote and slope values were significantly higher for females from the Prince George population compared to females from the St. Esprit population (Table 1). Thus, the Prince George females allocate proportionally more resources to pigmentation at all levels of nitrogen intake. Females from Prince George also had a slightly, but significantly, higher mean pupal mass than those from St. Esprit (equal variances not assumed; $t_{865.4}=-2.11, P=0.035$), despite being reared under identical conditions in the laboratory. These results could be indicative of important genetic differences between the two populations. As the Prince George population is much farther north than the St. Esprit population, differences in female pigmentation and size could be related to thermal melanism (Ethier 2013).

Conclusions

Using our equation for Stoehr’s (2006) model (Eqn 1), we found that patterns of condition dependence differed between colour phenotypes within a sex, between sexes sharing a colour phenotype, and between populations. We suggest that the phenotype-based differences in resource allocation to pigmentation reflect different life history costs for melanic and typical males; this may have phenotype-specific effects on male fitness and reproductive success, which might be density-dependent in this outbreaking species (see Ethier et al. 2015). The sex-based differences in the condition dependence of melanin-based colour indicate different resource allocation priorities in *M. disstria*: whereas colour appears to be a high priority for males of both phenotypes, females preferentially allocate limited resources elsewhere. However, the differences in resource allocation to pigmentation between females from different populations suggest that pigmentation may be a higher priority for females from more northern populations.
These results confirm that patterns of condition dependence should not be over-generalized; ecological and life history differences can lead to different patterns of condition dependence at all levels of study. Our equation for Stoehr’s (2006) saturating model is an extremely useful tool for comparing patterns of condition dependence in many other situations as well as those investigated here, such as between species using the same type of pigment for their colour ornaments, between pigment types within individual organisms, and so on.

In general, simple non-linear regression analyses based on our equation will quantify the relationship between colour trait expression and resource intake, thus allowing for comparison of slope, asymptote, and model fit values between groups of organisms. Different asymptotes may indicate different genetic limits to colour expression or different selective balances on colour traits, while comparison of slope values can provide valuable information on patterns of resource allocation and/or resource assimilation when resources are limiting. The overall fit of the model will indicate the relative strength of condition dependence in groups of interest. This method will facilitate the comparative approach by identifying relevant factors in diverse study systems, thus clarifying the proximate mechanisms of condition dependence in animal colouration.

Acknowledgements

The authors would like to thank Michael Gasse for insect rearing.
Literature Cited


Ethier J. 2013. Costs and Benefits of Melanism in the *Malacosoma disstria* Moth: Investigating the Maintenance of a Stable Polymorphism. Department of Biology. Concordia University, Montreal, Quebec, Canada.


Scott R.J. 2011. Reflectance characteristics are correlated with male condition in a population of threespine stickleback (*Gasterosteus aculeatus*) that has lost the species typical nuptial signal. Environmental biology of fishes 91: 287–294.


Table 1. Summary of nonlinear regression results for pigmentation vs. pupal mass in melanic male, typical male, and female *Malacosoma disstria* moths.

<table>
<thead>
<tr>
<th>Adult Type</th>
<th>Sample size (n)</th>
<th>Pupal mass (mg) mean (95% CI)</th>
<th>Asymptote (maximum) a (95% CI)</th>
<th>Slope b (95% CI)</th>
<th>Model fit r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanic males</td>
<td>130</td>
<td>267.6 (260.8-274.4)</td>
<td>158.6 (151.1-166.1)</td>
<td>1.96 (1.38-2.54)</td>
<td>0.050</td>
</tr>
<tr>
<td>Typical males</td>
<td>102</td>
<td>262.8 (254.2-271.5)</td>
<td>141.7 (129.8-153.5)</td>
<td>1.20 (0.94-1.47)</td>
<td>0.236</td>
</tr>
<tr>
<td>Females</td>
<td>194</td>
<td>414.8 (401.0-428.7)</td>
<td>130.3 (123.0-137.6)</td>
<td>0.64 (0.56-0.72)</td>
<td>0.343</td>
</tr>
</tbody>
</table>

Part 1: Testing the model¹

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SE females³</td>
<td>503</td>
<td>356.9 (350.8-363.0)</td>
<td>123.8 (119.9-127.8)</td>
<td>0.80 (0.73-0.87)</td>
<td>0.218</td>
</tr>
<tr>
<td>PG females⁴</td>
<td>377</td>
<td>366.1 (360.1-372.1)</td>
<td>137.3 (133.6-141.1)</td>
<td>0.97 (0.88-1.06)</td>
<td>0.206</td>
</tr>
</tbody>
</table>

¹ Part 1 shows the results from the protein-manipulation experiment, used to test the model.

² Part 2 applies the model to females obtained from two distinct populations and reared in the laboratory under identical conditions.

³ SE: St. Esprit, Quebec, Canada.

⁴ PG: Prince George, British Columbia, Canada.
Figure 1. Degree of pigmentation (255 – greyscale score) vs. pupal mass (mg) for melanic male (n=130), typical male (n=102), and female (n=194) *Malacosoma disstria* moths from Part 1 (Testing the model). Increased pigmentation scores indicate darker colouration and increased melanin production. Fit lines are the predicted y-values from the nonlinear regression analyses (solid line spans actual data; dashed line shows full theoretical curve).