A role for phenotypic plasticity in the evolution of aposematism

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The evolution of warning coloration (aposematism) has been difficult to explain because rare conspicuous mutants should suffer a higher cost of discovery by predators relative to the cryptic majority, while at frequencies too low to facilitate predator aversion learning. Traditional models for the evolution of aposematism have assumed conspicuous prey phenotypes to be genetically determined and constitutive. By contrast, we have recently come to understand that warning coloration can be environmentally determined and mediated by local prey density, thereby reducing the initial costs of conspicuousness. The expression of density-dependent colour polyphenism is widespread among the insects and may provide an alternative pathway for the evolution of constitutive aposematic phenotypes in unpalatable prey by providing a protected intermediate stage. If density-dependent aposematism can function as an adaptive intermediate stage for the evolution of constitutive aposematic phenotypes, differential reaction norm evolution is predicted among related palatable and unpalatable prey populations. Here, I present empirical evidence that indicates that (i) the expression of density-dependent colour polyphenism has differentially evolved between palatable and unpalatable populations of the grasshopper *Schistocerca emarginata* (= *lineata*) (Orthoptera: Acrididae), and (ii) variation in plasticity between these populations is commensurate with the expected costs of conspicuousness.

Keywords: polyphenism; host plant; environmentally determined; density dependent; genetic assimilation

1. INTRODUCTION

Hypotheses for the evolution of aposematism must account for a peculiar evolutionary paradox. When conspicuous deterrent individuals are rare, at frequencies too low to facilitate predator aversion learning, they suffer an increased cost of discovery by predators relative to the cryptic majority (Mallet & Singer 1987; Guilford 1990; Yachi & Higashi 1998; Lindström *et al.* 2001). Traditional allelic substitution models for the evolution of aposematism, in which genes for constitutive conspicuous phenotypes spread through a population of initially cryptic, deterrent individuals, have struggled to account for the frequency- or density-dependent costs of conspicuousness suffered by deterrent prey (Guilford 1990; Mallet & Joron 1999; Rowe & Guilford 2000). We now know, however, that the expression of warning coloration can itself be density dependent and mediated by increases in local prey population density (Sword 1999; Sword *et al.* 2000). These demonstrations of extant instances of density-dependent aposematism raise the possibility that plasticity in prey coloration may have provided an alternative pathway for the evolution of constitutive aposematic phenotypes by providing a protected intermediate stage.

Three distinct lines of evidence in addition to the discovery of density-dependent aposematism lend further support to a role for phenotypic plasticity in the evolution of aposematism in insects. First, density-dependent colour polyphenism has independently evolved a number of times

in unrelated insect lineages (reviewed in Fuzeau-Braesch 1985; Pener 1991; Applebaum & Heifetz 1999) and can affect both larval and adult coloration in hemimetabolous as well as holometabolous insects (Applebaum & Heifetz 1999; Barnes & Siva-Jothy 2000). Importantly, the expression of density-dependent colour polyphenism has recently been linked to density-dependent pathogen resistance, an epidemiological adaptation thought to be widespread in insects (Reeson *et al.* 1998; Wilson & Reeson 1998; Barnes & Siva-Jothy 2000; Wilson *et al.* 2001). These findings indicate that variation for density-dependent colour polyphenism may have been present during the evolutionary past of many insects and that they may have been pre-adapted with a mechanism to generate density-dependent colour change. Second, the expression of density-dependent phenotypes necessarily requires the local aggregation of prey. Aggregation is thought to favour the evolution of aposematism in unpalatable prey in many ways, such as conferring kin or synergistic benefits to group members, dilution of predation risk and enhancement of predator avoidance learning (Guilford 1990; Riipi *et al.* 2001). Finally, the inheritance of density-dependent colour phenotypes can be influenced by an epigenetic component (McCaffery *et al.* 1998; Simpson *et al.* 1999), and epigenetic inheritance is thought to further reduce the barriers of initial rarity to the evolution of aposematism (Brodie & Agrawal 2001). Thus, the possibility that phenotypic plasticity in the form of density-dependent colour change has had an important role in the evolution of aposematic phenotypes in insects clearly warrants further consideration.

Through the process of genetic assimilation, reaction norm evolution can transform initially plastic phenotypes into genetically determined constitutive phenotypes

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Figure 1. The evolution of aposematism via genetic assimilation. (a) A hypothetical population-level reaction norm for density-dependent colour polyphenism (conspicuousness) representing underlying variable and heritable individual reaction norms. The reaction norm may evolve over time by selection or genetic drift as indicated by the arrows. Although density-dependent colour phenotypes can vary continuously with density (i.e. density-dependent colour polyphenism is not a threshold trait) (Gunn & Hunter-Jones 1952), the shape of the population-level reaction norm can be influenced by spatial distribution of resources, such as host plants, upon which the insects aggregate. Modelling and empirical studies of *Schistocerca gregaria* have shown that, at a given population density, insects have a higher probability of contact and undergoing density-dependent changes when resources are aggregated as opposed to evenly distributed (Collett *et al.* 1998; Despland *et al.* 2000). The population-level reaction norm need not be sigmoidal; the reaction norm depicted here is generalized from Collett *et al.* (1998) and assumes a clumped distribution of host plants. (b) The potential outcomes of selection or drift on the density-dependent colour polyphenism reaction norm.

(Waddington 1942, 1953, 1956, 1961). The recent explosion of phenotypic plasticity investigations has prompted biologists from a number of disciplines to advocate genetic assimilation as a primary mode of phenotypic evolution (Schlichting & Pigliucci 1998). The process of genetic assimilation has been demonstrated in the laboratory, modelled, and invoked to explain many instances of phenotypic evolution (e.g. Waddington 1956; Harvell 1994; Schlichting & Pigliucci 1998; Tardieu 1999). Can genetic assimilation help to explain the evolution of aposematism?

If constitutive aposematic phenotypes can evolve from initially plastic phenotypes via genetic assimilation, reaction norms for density-dependent colour change would be predicted to evolve differentially in closely related palatable and unpalatable prey populations (see figure 1). Reaction norms for constitutive aposematism should be favoured only in unpalatable populations and could result from (i) selection for aposematism independent of prey density, or (ii) consistently high local prey density and loss of plasticity due to drift or selection against plasticity costs (DeWitt *et al.* 1998). Reaction norms for density-dependent (facultative) aposematism should be favoured only in unpalatable populations and could result from selection for both aposematism and crypsis under heterogeneous prey density regimes. Reaction norms for constitutive crypsis could be favoured in both unpalatable and palatable populations. In unpalatable populations, constitutive crypsis could result from drift or selection against costs of plasticity if high-density conditions are rarely encountered. In palatable populations, conspicuousness should always be disadvantageous barring any non-warning function such as Batesian mimicry. Reaction norms for constitutive crypsis in palatable populations could result from (i) selection for crypsis independent of prey density, or

(ii) consistently low population density coupled with the loss of plasticity due to drift or selection against plasticity costs.

In *Schistocerca emarginata* (= *lineata*) grasshoppers as well as the desert locust *S. gregaria*, an increase in local population density mediates a density-dependent shift from cryptic to aposematic juvenile phenotypes (Sword 1999; Sword *et al.* 2000). Deterrence to predators is gut-content-mediated and conferred solely by feeding on toxic host plants (Sword 1999, 2001; Sword *et al.* 2000). Importantly, the environmental cues that mediate colour change, such as direct physical contact among individuals, are unrelated to host-plant chemistry (Pener 1991; Sword 1998; Simpson *et al.* 2001). *Schistocerca emarginata* grasshoppers exhibit developmental variation in host-plant use such that adults tend to be generalist feeders, but the juveniles are host specific (Sword & Dopman 1999). Populations of juvenile *S. emarginata* that express density-dependent aposematism derive protection from vertebrate predators by feeding primarily on *Ptelea trifoliata* (Rutaceae) (Sword 1999, 2001; Sword & Dopman 1999). However, there are other genetically distinct populations of *S. emarginata* juveniles that feed primarily on an unrelated host plant, *Rubus trivialis* (Rosaceae) (Sword & Dopman 1999; Dopman *et al.* 2002). This study characterizes the relationship between host-plant-mediated deterrence to predators and the expression of density-dependent colour polyphenism in *S. emarginata* grasshoppers from *Rubus*- and *Ptelea*-associated populations. Here, I present empirical evidence that indicates (i) the expression of density-dependent colour polyphenism has differentially evolved between palatable and unpalatable populations, and (ii) variation in plasticity between these populations is commensurate with the expected costs of conspicuousness.

2. METHODS

(a) *Palatability assay*

The palatability of *Rubus*-reared *S. emarginata* juveniles collected from *Rubus*-associated populations was determined using insectivorous *Anolis carolinensis* lizards in a bioassay identical to that employed in Sword (1999, 2001). Field-collected first instar grasshoppers from *Rubus*-associated populations at Altair and Lake Whitney State Park (LWSP), Texas (Sword & Dopman 1999), were reared exclusively on *Rubus* in groups at 30 °C under constant light. Ten insects from Altair and eight from LWSP were used in the palatability assay as mid-fourth and fifth instars.

Lizards were maintained in the laboratory in 1 m³ screen sleeve cages with 150 W incandescent lamps provided as heat sources on a 14 L : 10 D photoperiod. Lizards were daily offered an *ad libitum* diet of water and crickets, *Acheta domestica* (Orthoptera: Gryllidae), occasionally supplemented with non-aposematic grasshoppers (no *Schistocerca*). Prior to testing, lizards were isolated in 3.5 l clear plastic tubs with plastic mesh-covered lids, a stick for roosting and a water dish. Cages were maintained in a walk-in environmental chamber at 30 °C, 14 L : 10 D. Lizards were then offered a standardization meal of one adult cricket to control for motivational state. Feeding trials were conducted one day following consumption of the standardization meal by placing the lizards in a 1 m³ screen cage housed in the same environment chamber with a 150 W incandescent lamp provided overhead for basking. Lizards were allowed to settle and then offered a live grasshopper inserted through a hole at the base of the cage. Lizard feeding trials were conducted for a maximum of 3 h and continuously observed. Uneaten insects were removed from the cage at the end of the testing period. Lizards that did not attack were tested in subsequent trials on successive days until an attack occurred. Each lizard was used only once. Acceptance was scored as consumption without regurgitation within 24 h. Rejection was scored as attacking and releasing or regurgitation within 24 h.

(b) *Coloration assay*

Field-collected, newly hatched first-instar *S. emarginata* from a *Ptelea*-associated population at the University of Texas Brackenridge Field Laboratory (BFL) and a *Rubus*-associated population at Altair, TX (Sword & Dopman 1999) were reared in the laboratory under isolated and crowded rearing conditions. Isolated insects were individually reared in cylindrical 946 cm³ clear plastic cages with white plastic lids. Cages had a wire mesh floor 2 cm above the base with a 1 cm × 8 cm rectangular hole below the floor for air flow and faeces removal. Insects were visually isolated from each other with white paper dividers and olfactorily isolated by individually ventilating the cages with ca. 164 cm³ min⁻¹ fresh compressed air through a 5 mm diameter hole in the lid. Crowded cages for each population consisted of 40 individuals reared in 8 litre clear plastic tubs with wire mesh lids. Crowded and isolated cages were maintained at BFL in separate buildings at 30 °C, 14 L : 10 D. Insects were fed Romaine lettuce and wheat bran.

Colour patterns were analysed in the final nymphal instar. Cuticular melanization was quantified by digital image analysis performed on a Macintosh computer using public domain NIH Image software (developed at the US National Institutes of Health and available at <http://rsb.info.nih.gov/niH-image/>). Grasshoppers were anaesthetized under CO₂ and digitally photographed in lateral view. The overall black proportion of the pronotum, wing pad, femur and abdomen of each insect was

determined. Background colour was qualitatively assessed as yellow, green or other. Three insects intermediate between yellow and green were randomly classified as green or yellow to avoid bias.

3. RESULTS

Rubus and *Ptelea*-associated populations of *S. emarginata* differ substantially in the protection from predators that they derive from feeding on their respective host plants. *Rubus* consumption failed to confer unpalatability to *S. emarginata* nymphs from *Rubus*-associated populations. Insectivorous lizards accepted all 18 *Rubus*-fed grasshoppers. These results contrast with earlier findings using the same bioassay in Sword (1999) in which 11 of 19 *Ptelea*-fed *S. emarginata* from a *Ptelea*-associated population were rejected (Fisher's exact test, $p < 0.0001$), whereas 19 *Rubus*-fed grasshoppers were accepted by lizards.

Colour patterns of grasshoppers from unpalatable (*Ptelea*-associated) and palatable (*Rubus*-associated) populations responded differently to rearing density, as indicated by the significant interaction effect between rearing density and population of origin on the degree of cuticular melanization (2×2 factorial ANOVA: population × density; $F_{1,116} = 11.15$, $p = 0.0011$; figure 2a). This population-level genotype × environment interaction arose because unpalatable *S. emarginata* were more extensively melanized independent of rearing density and exhibited a greater mean increase in melanization in response to increased rearing density (30–52%) than did the palatable *S. emarginata* (2–16%).

The background colour of grasshoppers from both populations was also affected by rearing density (Monte Carlo $R \times C$ contingency table analysis (available at <http://www.wisc.edu/genetest/CATG/pstat/index.html>), unpalatable isolated versus crowded, $p < 0.0001$; palatable isolated versus crowded, $p < 0.0001$; figure 2b). However, under isolated conditions, the unpalatable *S. emarginata* were much more likely to be yellow than their commonly green palatable counterparts (Monte Carlo $R \times C$ contingency table analysis, isolated unpalatable versus palatable, $p < 0.0001$; figure 2b). Furthermore, under crowded conditions the unpalatable individuals were invariably yellow, while the palatable insects exhibited significantly more variation in colour (Monte Carlo $R \times C$ contingency table analysis, crowded unpalatable versus palatable, $p = 0.003$; figure 2b). See figure 3 for examples of the extreme phenotypes that can be generated by isolated and crowded rearing conditions.

4. DISCUSSION AND CONCLUSIONS

Both the unpalatable (*Ptelea*-associated) and palatable (*Rubus*-associated) *S. emarginata* populations exhibited density-dependent colour polyphenism, but differed substantially in the final phenotypes they produced. On the one hand, unpalatable grasshoppers can benefit from the expression of warning coloration and accordingly exhibited an extreme density-dependent colour change, producing phenotypes that ranged from cryptic green to conspicuous yellow and black. On the other hand, palatable grasshoppers should not benefit from warning color-

Figure 2. Density-dependent colour polyphenism in unpalatable and palatable *Schistocerca emarginata* populations. (a) Effect of rearing density on cuticular melanization. Box plots depict the 10th, 25th, 50th, 75th and 90th percentiles of the blackened proportion of individual grasshoppers reared under isolated and crowded conditions. (b) Effect of isolated and crowded rearing conditions on background coloration. Pie charts depict the number of individuals in each colour category (black segments, green; white segments, yellow; grey segments, other). Phenotypes classified as ‘other’ included individuals that were tan or brown, as well as heterogeneously coloured individuals with orange heads and tan, brown or yellow bodies.

Figure 3. Examples of the extreme density-dependent phenotypes produced by *Schistocerca emarginata* from unpalatable *Ptelea*-associated and palatable *Rubus*-associated populations.

ation. As expected, palatable individuals exhibited a reduced phenotypic response to rearing density and their crowd-reared phenotypes were less conspicuous than those of their unpalatable counterparts (figures 2 and 3). To the degree that these plastic responses are fitness related, their genetic variability should be reduced in stable environments due to continuous selection (Fisher

1930). Phenotypic variability can reflect underlying genetic variability and may provide insight into the history of selection acting in these populations (Pigliucci *et al.* 1995; Hoffman & Merila 1999). The patterns of phenotypic variability within the isolated and crowded rearing treatments were opposite between the unpalatable and palatable populations. Among the unpalatable grass-

hoppers, phenotypic variability was reduced for crowded as opposed to isolated rearing density phenotypes (figure 2a,b). This indicates that natural selection in the field may have occurred more often for conspicuous yellow and black warning coloration at high local population density. Among the isolation-reared unpalatable grasshoppers, 9 of the 14 least-melanized individuals were also green. This resulted in a mixture of relatively cryptic (green) and conspicuous (yellow and black) individuals. The high frequency of yellow and black phenotypes produced under isolated conditions among unpalatable *S. emarginata* may reflect either (i) selection for conspicuousness regardless of prey density, (ii) consistently high densities in the field and a loss of plasticity due to drift or costs (DeWitt *et al.* 1998), or possibly (iii) the presence of parental effects (Islam *et al.* 1994; McCaffery *et al.* 1998).

The converse pattern of phenotypic variability occurred among palatable *S. emarginata* (figure 2a,b). Variability was much lower among isolation-reared individuals and indicates that selection may have commonly been for cryptic green phenotypes at low local population densities. The production of alternative crowd-reared phenotypes among palatable *S. emarginata* could be consistent with selection for crypsis independent of density, if the crowd-reared phenotypes are producing weak signals and not distinguished by predators from the cryptic phenotypes produced under isolated rearing conditions (Lindström *et al.* 1999). Plasticity in this population may be accounted for by either (i) the expression of a hidden reaction norm maintained in the absence of costs, (ii) selection for alternative high-density phenotypes, the functions of which remain unidentified, or (iii) as a by-product of some other adaptive density-dependent response such as density-dependent pathogen resistance (Reeson *et al.* 1998; Barnes & Siva-Jothy 2000; Wilson *et al.* 2001).

The differential expression of density-dependent colour polyphenism between palatable and unpalatable *S. emarginata* populations is in accordance with the expected costs and benefits of conspicuousness. This demonstration of different population-level reaction norms in naturally occurring populations is consistent with the predictions of the evolution of aposematism via genetic assimilation (see figure 1). This study is not, however, a direct demonstration of genetic assimilation in action, and alternative explanations for the observed differences between populations should be considered. Inherited parental effects are known to influence the colour of hatchlings in the closely related *S. gregaria* (Islam *et al.* 1994). Adult crowding during mating and oviposition can result in hatchlings that are primarily black as opposed to green. This does not appear to be a likely source of variation in this study, however, because *S. emarginata* hatchlings from both populations are always green, and adult mating and oviposition in the field appear to occur in isolation. Differences in the expression of colour change between populations could also potentially arise by environmentally induced shifts in the threshold of susceptibility to change colour (see Emlen 1997), but because insects from both populations were reared under the same environmental conditions, one population should not have been differentially exposed to potentially confounding environmental conditions. Therefore, it is most likely that the difference between populations in their propensity to change colour with changes

in population density constitutes an additional genetic difference between these populations (Dopman *et al.* 2002).

In the unpalatable *S. emarginata* grasshoppers considered here, the high frequency of yellow and black individuals produced under isolated rearing conditions indicates that the reaction norm for colour plasticity may be tending towards constitutive expression of aposematism independent of local population density (figure 2a,b). As evidenced by the spectacular crossing of the Atlantic Ocean by *S. gregaria* swarms from Africa in 1988, the ongoing adaptive radiation of *Schistocerca* in the New World could be the result of one or more transatlantic colonization events by a *S. gregaria*-like ancestor with the ability to express density-dependent colour change (Ritchie & Pedgley 1989; Kevan 1989). If so, variation for the expression of density-dependent colour polyphenism may be the ancestral state in this lineage, and indeed, many extant *Schistocerca* species do retain the ability to express some degree of density-dependent colour change (Duck 1944; Harvey 1981; R. F. Chapman and G. A. Sword, unpublished data). This study indicates that ecological conditions, such as host-plant availability and distribution, prey population dynamics and predator behaviour, can interactively affect the evolution of density-dependent colour polyphenism on a local scale. Constitutive aposematism, as well as crypsis, could feasibly evolve in this lineage via genetic assimilation, simply through the local fine-tuning of existing reaction norms for density-dependent colour polyphenism.

Among insects in general, the evolution of aposematism has been notoriously difficult to explain. Given that density-dependent colour polyphenism is widespread, this study indicates that plasticity in prey phenotypes could have had an important role in the evolution of aposematism by providing a protected intermediate stage. The prevailing allelic substitution models for the evolution of aposematism must all contend with the density-dependent barriers to the spread of aposematic phenotypes. Density-dependent plasticity in coloration should substantially reduce these barriers and relax the conditions under which aposematism can evolve. Considering prey plasticity and genetic assimilation in conjunction with existing hypotheses, such as predator psychology models (Lindström 1999; Rowe & Guilford 2000; Servedio 2000; Speed 2001), may reveal that aposematism can evolve as a matter of convention rather than a paradox.

I thank L. Gilbert and M. Singer for their support at the University of Texas (UT) and the Brackenridge Field Laboratory. Discussions with C. Schlichting and J. Mallet helped to refine this study. C. Schlichting and D. Branson provided useful comments on the manuscript. Permission to work in Texas State Parks was granted by Texas Parks and Wildlife Department Permit no. 13-96. This project was supported by grants from the Orthopterists' Society Research Fund, Sigma Xi, Lorraine I. Stengl Endowment to the UT Department of Zoology, and USDA/NRICGP award no. 98-35302-6823.

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