How the locust got its stripes: the evolution of density-dependent aposematism

Most of us are familiar with Rudyard Kipling's *Just So Stories*[1], in which he imaginatively explored potential mechanisms to explain bizarre animal traits. Over the years, the yellow and black stripes of juvenile desert locusts (*Schistocerca gregaria*) have proved every bit as intriguing as the camels' hump or the leopards' spots! The yellow and black markings that we all associate with locust nymphs are exhibited only by the high-density *gregaria* morph; the low-density *solitaria* form is cryptic green (Fig. 1). But, why is the *gregaria* morph so conspicuous and why does crowding initiate this colour change? In two new publications, Greg Sword and colleagues[2,3] offer a new explanation for these age-old questions.

The desert locust is a major economic pest throughout much of North Africa, the Middle East and the Indian subcontinent. As such, considerable research effort has been directed at understanding its biology over the past 100 years[4,5]. Much of this continues to explore the evolution of density-dependent phase polyphenism, the process by which one of two extreme phenotypes (or phases) is produced in response to local population density. Accompanying the well documented colour change is a suite of less easily quantified traits, such as swarming behaviour and group cohesion, as well as several physiological and morphological changes. Many of these traits can be explained in terms of adaptations for a migratory lifestyle. However, the functional significance of the yellow and black markings of the high-density morph has thus far remained a mystery. One potential explanation is that they function as visual signals to stimulate nymphal aggregation and to maintain group cohesion during marching. Another explanation is that they have a thermoregulatory role. Until recently, the 'obvious' explanation, that the yellow and black markings act as an aposematic or warning signal to predators of distastefulness, had largely been discounted owing to frequent field observations of desert locusts being consumed by birds and reptiles[6]. However, the work of Sword and colleagues provides a novel slant on this well worn hypothesis, and helps to explain these apparently paradoxical results.

How the hypothesis evolved

Initial insights came while Sword studied a grasshopper in Texas that is closely related to the desert locust *Schistocerca emarginata*. Like its more infamous ancestor, *S. emarginata* exhibits density-dependent phase polyphenism; the *solitaria*-phase nymphs are green, while those in the *gregaria* phase are yellow and black. Sword noticed that in some populations of *S. emarginata*, the nymphs congregate and feed mainly on *Ptelea trifoliata* (Rutaceae), in spite of the presence of numerous other, apparently palatable, host plants. When he offered *gregaria*-phase nymphs that had been reared on *Ptelea* to grasshopper-naive *Anolis* lizards, he found that more than half of them (11 out of 19) either failed to consume the insects or regurgitated them following consumption. This contrasted with no rejections (zero out of 19) for *gregaria* nymphs fed on *Rubus trivialis* (Rosaceae).

Sword was able to show that this unpalatability was mediated by the gut contents of the grasshopper, rather than by any sequestered or manufactured chemicals, by feeding *Ptelea*-reared nymphs on Romaine lettuce for 24 hours. All 14 grasshoppers fed in this way lost their unpalatability. Thus, insects that feed on certain host plants could become unpalatable to potential predators, but do predators learn to associate yellow and black with unpalatability? To answer this question, Sword offered lizards that had previously been fed yellow and black grasshoppers a simultaneous choice between another yellow and black nymph and a green nymph. If the lizards had initially been fed a palatable *Rubus*-fed grasshopper, then they did not discriminate between green and yellow and black nymphs in the choice test (six lizards chose the green nymph and 11 chose the yellow and black). However, if they had previously been given an unpalatable *Ptelea*-fed grasshopper, they overwhelmingly avoided eating another yellow and black nymph (16 out of 17 lizards ate a green nymph in preference). Thus, the yellow and black coloration of *S. emarginata* nymphs produced at high densities can act as a warning signal to potential predators when the insects have been feeding on plants containing compounds toxic to vertebrates. But, can the same explanation be invoked for the colour polymorphism in desert locusts and does yellow and black act as a stronger aposematic signal than green?

How aposematism applies to desert locusts

In a follow-up study in Mauritania, Sword and colleagues[3] used a no-choice test to determine whether host–plant use affects the palatability of desert locusts to its predators. To do this, they first gave fringe-toed lizards (*Acanthodactyus dumerili*) a *gregaria* nymph that had previously been fed on one of four different host plants, all of which were known to be in the diet of *solitaria* desert locusts in recession areas – *Heliotropium bacciferum* (Boraginaceae), *Tribulus terrester* (Zygophyllaceae), *Schouwia purpurea* (Brassicaceae) and *Hyoscyamus muticus* (Solanaceae). They then offered the lizards a second *gregaria* locust, which had been fed on the same food plant, and measured the number of days it took the lizard to attack and consume it. They found that although a high proportion of lizards that initially ate *Heliotropium*-, *Tribulus*- or *Schouwia*-fed locusts consumed another locust (fed the same host plant) the next day (73%, 87% and 94%, respectively), only ten out of 30 lizards (33%) that initially ate a *Hyoscyamus*-fed locust ate another *Hyoscyamus*-fed locust the following day. Indeed, more than a third of these lizards refused to eat another locust even after four days of food deprivation. Thus, even in this simple assay, it is clear that locusts fed *Hyoscyamus* become unpalatable to lizards and that the lizards can learn this fact after a single feeding event. By showing that lizards fed unpalatable locusts would eat a fly the following day, if given the option, Sword and colleagues were able to rule out the possibility that the aversion was a result of a general inappetance because of the toxic chemicals consumed with their prey.

Although these results suggest that the yellow and black coloration of *gregaria* locusts and grasshoppers can act as an effective signal of unpalatability, in order to explain the evolution of density-dependent colour change, it is necessary to show that yellow and black is more effective at deterring predators from subsequent attacks than the plain green exhibited by *solitaria* nymphs. To test this prediction, Sword and colleagues offered green, *solitaria*-phase *Hyoscyamus*-fed locusts as both the initial and subsequent prey in another no-choice experiment. They found that 86% of the lizards (19 out of 22) consumed the green *Hyoscyamus*-fed locusts on both days. This contrasts with just 33% of lizards in the earlier experiment offered yellow and black locusts in both trials. Thus, for desert locusts that have

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become unpalatable to predators by consuming 'toxic' plants, the *gregaria*-phase coloration appears to be a more effective deterrent to predators than *solitaria*-phase coloration.

A further experiment by Sword and colleagues showed that locust colour is not the only cue used by potential predators to assess palatability – olfactory cues are also important. When lizards were offered an unpalatable *Hyoscyamus*-fed nymph followed by a palatable *Heliotropium*-fed one, 73% (21 out of 29) were eaten. This compares with just 33% for lizards offered *Hyoscyamus*-fed locusts on both days (in both experiments *gregaria* phase locusts were used throughout). Thus, *gregaria*-phase coloration is most effective as a deterrent to potential predators when it is combined with olfactory cues associated with nymphal diet.

How density-dependent aposematism evolved

Although the sample sizes in all of these experiments are rather small, taken together Sword's work suggests that in insects that are unpalatable, because they consume plants toxic to vertebrates, density-dependent warning coloration can evolve in response to density-dependent predation pressures. Thus, when unpalatable prey are at low densities, predators might encounter them at such a low rate that they do not learn to avoid them. Under these circumstances, natural selection will favour unpalatable individuals that remain cryptic and minimize their risk of being discovered by predators. As local density increases, and unpalatable prey become more common, natural selection will favour individuals that become conspicuous because this will probably facilitate predator learning. Thus, phenotypic plasticity in nymphal coloration enables unpalatable prey to use a dual antipredator strategy – crypsis at low densities and conspicuousness at high densities.

The observation that birds and other predators have been seen feeding on *gregaria*-phase locusts now becomes easier to understand. One possibility is that the observations were made on locusts in areas where toxic plants did not grow or had previously been defoliated. Alternatively, the observations could have been made on naive predators that had yet to learn to avoid *gregaria* nymphs. More detailed studies are required before these possibilities can be distinguished.

Although the hypothesis presented by Sword and colleagues is appealing, one or two mysteries remain. For example, why is diet not used as a cue for colour change? It seems probable that the full benefits of aposematism will be realized only by
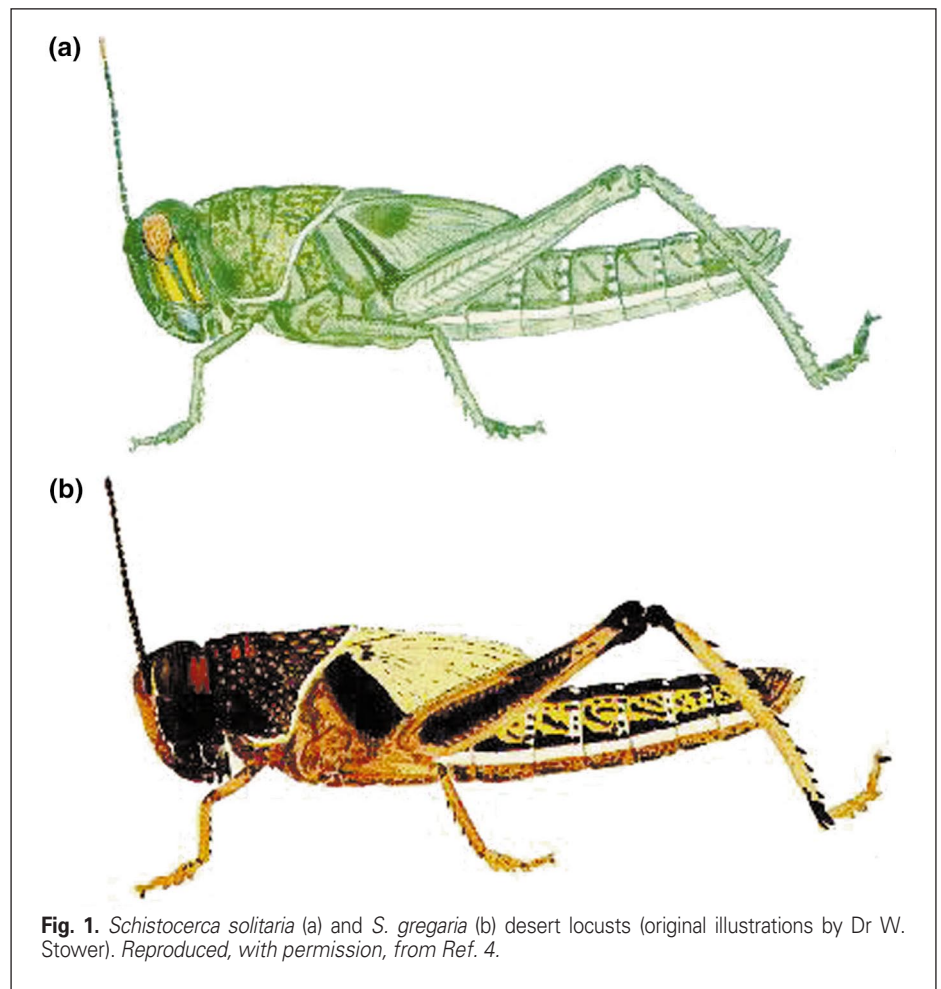

Fig. 1. *Schistocerca solitaria* (a) and *S. gregaria* (b) desert locusts (original illustrations by Dr W. Stower). *Reproduced, with permission, from Ref. 4.*

responding to the combined cues of density and diet. Changing colour in response to diet alone would probably be punished at low densities because individuals feeding on toxic plants would become more conspicuous and thus suffer an increased risk of discovery by predators relative to conspecifics feeding on nontoxic plants that stayed green. Similarly, using local density alone as a cue for colour change would probably be punished when toxic host plants are absent from the environment. In these circumstances, crowded individuals feeding on nontoxic plants that falsely displayed the 'unpalatability' signal would soon be discovered as cheats by predators and thus be at greater risk than conspecifics that remained cryptic.

If cheats are found out, then natural selection should result in individuals having a lower propensity for becoming conspicuous at high densities when they live in habitats where toxic plants are absent. At sites in Texas where *Ptelea* was lacking, *S. emarginata* grasshoppers fed on *Rubus* and were palatable to predators (G.A. Sword, PhD thesis, University of Texas, 1998). Grasshoppers from these populations responded to crowding in the laboratory by remaining green and cryptic more often than did those from Sword's main study site where the grasshoppers primarily fed

on *Ptelea*. Thus, via natural selection, the propensity to exhibit density-dependent colour change in the two populations has altered to match the average rewards and costs of being conspicuous at high densities. Similar geographic variation in the propensity to change colour has been recorded in *S. gregaria*[5]. However, as yet there is no evidence to suggest that this is linked with variation in the distribution of toxic plants.

Changing colour in response to population density is a good idea for individuals that live in habitats where there is a reliable supply of toxic host plants. Similarly, when these plants are absent, it is a good idea not to change colour in response to crowding. However, it is difficult to envisage the benefits of using nymphal density alone as a cue for colour change for highly mobile species, such as the desert locust, which range over a patchwork of habitats where the growth of toxic plants is unpredictable. One would imagine that a better strategy would be either to use chemical cues from the host plant or to forgo any colour change in favour of a cryptic defence strategy. For highly mobile insects such as locusts, perhaps individuals feeding on plants that fail to confer unpalatability are effectively acting as Batesian mimics of distasteful conspecifics

feeding on neighbouring plants that do confer unpalatability (i.e. perhaps they are automimics[7]). Alternatively, density-dependent aposematism might have evolved in stable, non-swarming locust populations in recession areas, where the supply of toxic host plants is predictable. Under this scenario, responding to local density alone would generate cheats only within mobile hopper bands.

How the hypothesis applies to other species

Although Sword and colleagues[2,3] restrict their hypothesis to *Schistocerca*, it is tempting to extrapolate their ideas to other insects. Density-dependent colour changes are observed in many other insect taxa, including phasmids[8] and Lepidoptera[9]. Most of these species do not exhibit the classic aposematic colour patterns seen in *gregaria*-phase locusts (i.e. the juxtaposition of strongly contrasting colours), although they are perhaps equally conspicuous (and memorable) to predators. Typically, colour changes in these taxa tend to be restricted to the uniform deposition of melanin in the cuticle, giving the high-density morph a darker appearance. For example, the high-density form of the African armyworm *Spodoptera exempta* is more or less uniformly black at high densities, but green or pale brown at low densities. As with locusts, birds have frequently been observed feeding on *Sp. exempta* and this has led armyworm biologists (like locust biologists before them) to discount aposematism as a potential mechanism favouring colour change, and alternative explanations for it have been explored[10]. However, *Sp. exempta* larvae also feed on toxic plants that could confer unpalatability, such as the widespread cyanogenic grass *Cynodon dactylon*. Therefore, it is possible that the melanism expressed at high density acts as a warning signal to potential predators in much the same way as the yellow and black stripes of locusts appear to. However, until the appropriate experiments are conducted it remains to be seen whether density-dependent aposematism represents a universal explanation for crowding-induced colour changes or simply becomes another *Just So* story.

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Evolution in subdivided populations

Population geneticists often wryly lament that there has been nothing fundamentally new in their field since the groundbreaking work of Fisher, Wright and Haldane. There might be an element of truth in this, but, recently, perspectives have certainly been changing in both theoretical and empirical domains of population genetics. Advances in molecular biology are allowing us to view, in detail, the material upon which evolutionary and demographic processes have left their mark, and the conceptual innovations of coalescent theory are giving us a handle on the statistical properties of genetic samples from wild populations[1,2]. Moreover, elements of ecological realism are increasingly finding their way into population genetics. Together, these advances form threads that ran through a recent workshop on spatially structured populations*. The workshop brought together both theoreticians and empiricists who reported recent research on subjects as varied as the spatial distribution of human skin pigmentation alleles (Rosalind Harding, University of Oxford, UK), clinal variation in toad hybrid zones (Beate Nürnberger, University of Munich, Germany; Tim Vines, University of Edinburgh, UK), the evolution of virulence in host–parasite interactions (Margaret Mackinnon, University of Edinburgh), and spatial models of plant competition and colonization (Ben Bolker, University of Florida, USA).

The central idea behind the meeting was that genes, or genetic markers, occur within a context that is probably structured (such as populations that are spatially subdivided into discrete subpopulations or demes) and that such structure can have important population genetic consequences. This was a theme that coloured much of Sewall Wright's work. However, the fate of mutations might depend as much on their position in the genome as on where they happen to occur geographically. Indeed, as Darlington (in Ref. 3) noted, '[t]he really important small populations are the little bits of chromosomes […] within which recombination cannot occur'. Hidden in Darlington's statement is the analogy between recombination and migration that was drawn repeatedly in the workshop. Just as migration can move genes from one geographic background to another, so recombination can move genes between genetic backgrounds. Both events can alter a gene's evolutionary fate.

Constant size of demes or genetic backgrounds

The effect of population subdivision depends on the way in which spatial or genetic backgrounds change in size over time. If demes remain constant in size, or if the frequency of genotypes maintained by selection is constant, population subdivision caused by low migration or low recombination rates is expected to increase diversity at neutral or weakly selected loci[2,4]. This is because the coalescence of a pair of alleles can only occur within the same (spatial or genetic) background or the same class of a subdivided population; low migration or low recombination implies that alleles sampled from different classes have to wait a considerable time before