*New Idea***Biased sex ratios and aposematic polymorphism in African butterflies: A hypothesis****Eihab Idris¹ and Sami Saeed M. Hassan**

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Abstract

In East and Central Africa, the nymphalid butterflies *Danaus chrysippus*, *Acraea encedon* and *A. encedana* are involved in a Müllerian mimicry complex. Unusually for aposematic mimetic organisms, the three species show extensive colour pattern polymorphism. Within the same geographic zone, the three species show female-biased sex ratios as a consequence of infection by maternally-inherited, male-killing bacteria. The co-occurrence of biased sex ratios and aposematic polymorphism within these three sympatric, mimetic butterfly species has led to the speculation that invasion by the male-killing bacteria and the subsequent female-biased sex ratios are the underlying reason for the maintenance of colour polymorphism in these species, following its establishment by periods of allopatry and monomorphism and hybridisation. In this paper, we present a novel hypothesis that describes a mechanism by which such causal link might have taken place; in our view, positive frequency-dependent selection favouring the most common colour form in the species is disrupted as a consequence of the recurrent extinction-recolonisation cycles undergone at the level of the species populations following the spread of the male-killer. Likewise, extinctions and recolonisations taking place in the other mimics randomly change the direction of selection on each species, potentially leading to multiple selective pressures favouring different colour patterns in the mimicry complex. Thus, selection for

monomorphism within each species as well as between the mimetic species will be continuously randomised by the enhanced metapopulation dynamics and the state of polymorphism will be maintained consequently.

Keywords: Müllerian mimicry, male-killing, metapopulation dynamics, *Acraea encedon*, *Acraea encedana*, *Danaus chrysippus*

Background

Aposematism is a prey defence mechanism that relies on the inedibility of the prey tissues and is characterised by the possession of bright and contrasting colour patterns (Barnard 2004). Aposematism works when a naïve predator encounters the prey and learns to associate the inedibility of this prey type with its characteristic colouration (Poulton 1890). Learning is facilitated when this colouration is conspicuous, but this leads to a cost, that is, the predation attempts of naïve predators, whether successful or not. The magnitude of this cost depends on several factors, such as the proportion of naïve predators in the total predator population, the relative abundance of the prey species compared with the predator species, the number of trials required to develop the avoidance response (which is a function of both the predator's learning capacity and the prey's tissue noxious qualities), and, finally, the number of different colour patterns that predators should learn to

avoid. If the prey species is monomorphic, predators will need to experience only one colour form to avoid all the members of the species. On the contrary, naïve predators of polymorphic species need to experience each colour form independently to develop the avoidance response towards that particular form, implying that the cost of predation is proportional to the extent of polymorphism. This is why natural selection is expected to favour colour monomorphism in aposematic prey species (Fisher 1930, Ford 1964, Greenwood et al. 1981). Likewise, aposematic species involved in a Müllerian mimicry complex are driven towards morphological convergence by two selective forces, both induced by predation: first, selection for colour monomorphism within each species, and second, selection for colour pattern convergence between the species of the mimicry complex (Owen 1970, Matthews 1977, Joron 2005). It is to be noted, however, that some predators may be able to generalize over several similar prey phenotypes (Duncan and Sheppard 1965); these predators would not select for mimetic monomorphism.

A wide variety of cytoplasmic endosymbionts of arthropods are known to manipulate host reproduction to improve their transmission rate down the female line (O'Neill et al. 1997). One strategy of reproductive manipulation is early-acting male killing in which the endosymbiont kills the infected males during their early development but remains harmless to infected females (Majerus 2003). From an evolutionary perspective, such a male killer would spread only if infected females gain a fitness advantage over uninfected females. Species that experience high sibling competition and/or sibling cannibalism, and in which eggs are laid in large groups represent ideal targets for male-killing endosymbionts, because these life history traits maximize the fitness advantage gained by infected females through resource reallocation from their dead male siblings. The equilibrium prevalence of male killers in their host species are determined by the magnitude of the fitness advantage and the efficiency of male killer's vertical transmission (Hurst 1991, Hurst and Majerus 1993).

For most sexually-reproducing organisms, the production of 1:1 sex ratio by parents is regarded as an evolutionary stable strategy (ESS), due to the negative frequency-dependent selection favouring the rare sex (Fisher 1930). Invasion by a male killer is likely to bias the equal sex ratio towards females as a result of the production of all-female progeny by infected females. Theoretical models predict that perfectly-transmitted sex ratio distorters with high fitness advantage and no fitness cost to their favoured sex and with full expression in their unfavoured sex, would spread to fixation, driving their host species ultimately towards extinction as the sex ratio approaches 0:1 (e.g. Hamilton 1967). Models also predict that male killers with sufficient fitness advantage but with less than perfect vertical

transmission can cause frequent extinction events at the population level, thus enhancing the metapopulation dynamics of the host species (Heuch 1978).

Danaus chrysippus is a nymphalid butterfly that belongs to the subfamily Danainae and is distributed throughout the old world tropics and subtropics. *Acraea encedon* and its sibling species, *A. encedana*, are Afro-tropical butterflies of the sub-family Heliconiinae. In Africa, *A. encedon* and *A. encedana* occupy the same geographic range as *D. chrysippus* except that they are absent north of the Sahara (Owen 1971). The three species are open-country butterflies that are rapidly becoming adapted to human disturbance, and are common in grasslands, agricultural areas and gardens (Owen 1970). They are aposematic butterflies with characteristic warning colouration (Reichstein et al. 1968, Owen 1970, Rothschild et al. 1975).

Within certain regions in East and Central Africa, female-biased sex ratios have been observed in the wild populations of the above three species (Poulton 1914, Owen and Chanter 1968, Gordon 1984a). The distortion in the population sex ratio was attributed to the occurrence of all-female broods. Investigation of the causative factor using molecular screening and breeding experiments has led to the identification of male-killing bacterial endosymbionts in the three species: *Spiroplasma* in *D. chrysippus* and *Wolbachia* in both *A. encedon* and *A. encedana* (Jiggins et al. 1998, Hurst et al. 1999, Jiggins et al. 2000b, 2000c). The bacterial prevalences in the wild populations of these butterflies were found to be unusually high, particularly for the two *Acraea* species; in some Ugandan populations of *A. encedon*, more than 95% of females were infected with *Wolbachia* (Jiggins et al. 2002). The extreme sex ratio distortion in *Acraea* butterflies has led to a reversal in their ancestral mating system; in the two sibling species, females were found to form leks and compete for access to males as they have become the limiting factor of reproduction due to their rarity (Jiggins et al. 2000a). In *D. chrysippus*, considerable prevalences have been reported in the wild, although the life history characteristics of this species have not been thought to allow resource reallocation (Jiggins et al. 2000c). Recently, Ireri et al. (unpublished obs.) found evidence that females, in a population infected 74% by *Spiroplasma*, lay eggs disproportionately on isolated host plants compared with plants growing in clumps. Thus, resource reallocation may occur in *D. chrysippus*.

The problem

In East and Central Africa, the three aposematic butterfly species *D. chrysippus*, *A. encedon* and *A. encedana* comprise a Müllerian mimicry complex with unusual ecology. The surprising aspect of their ecology is that they are all extensively polymorphic; each

species has multiple colour forms that resemble parallel forms in the mimetic species (Owen and Smith 1993, Owen et al. 1994). The maintenance of aposematic and mimetic polymorphism in the three species represent an evolutionary paradox that requires satisfying explanation.

To understand the difficulties that face the evolution of polymorphism in a mimetic complex, let us imagine a new colour pattern allele entering the prey population through mutation or migration; initially, individuals with the novel colour pattern will represent only a tiny minority in the population, while individuals with the original colour pattern will represent the absolute majority. Moreover, the sympatric populations of the mimetic species will contain abundant Müllerian mimics of the original, but not of the new colour form. As a consequence, the probability that any individual predator has experienced the original colour form will be much greater than the probability that it has experienced the new colour form, implying a higher level of protection for common forms. Natural selection would act to eliminate rare forms from the population as they suffer higher predation rates than common forms, thus leading to a state of colour monomorphism. Then, the question would be: why does this scenario not apply to the case of *D. chrysippus*, *A. encedon* and *A. encedana*?

Two categories of hypotheses are usually provided for this evolutionary puzzle. In the first category, predation is considered as the only selective force affecting colour pattern, and thus polymorphism is regarded as the output of predation pressure under special conditions. In the second category, it is assumed that factors other than predation are involved in the maintenance of polymorphism. We will discuss one hypothesis from each category.

The 'mimetic load' hypothesis

This hypothesis states that the load of Batesian mimics is the subtle factor that maintains colour polymorphism among the Müllerian mimics. This argument is as follows: because Batesian mimics are edible, they tend to dilute the aposematic signal of their model; if a naïve predator encounters a model for the first time, it will subsequently avoid potential prey with the aposematic signal, thus protecting the mimic. On the other side, if the first encounter was with the mimic, the predator will subsequently target potential prey with the aposematic colour pattern, thus threatening the model. As a result, mimics should be much less frequent than their models for mimetic resemblance to be protective; otherwise predators will actively search for the aposematic signal rather than avoiding it. If an aposematic colour form is heavily loaded by edible Batesian mimics to the extent that it is targeted rather than avoided, it would be beneficial to diverge from this form and gain protection

from predators' unfamiliarity. In this case, natural selection would favour the development of colour polymorphism among the model species (Owen 1971, Allen 1988, Smith et al. 1993).

The mimetic complex of *D. chrysippus*, *A. encedon* and *A. encedana* is loaded by several Batesian mimics including some of the female-limited forms of *Papilio dardanus* (Papilionidae), two forms of *Pseudacraea poggei* (Nymphalidae: Nymphalinae), *Mimacraea marshalli* (Lycaenidae), *Euryphene iris* (Nymphalidae: Satyrinae) and, most notably, the female diadem butterfly *Hypolimnas misippus* (Nymphalidae), whose female has four different colour forms that show impressive resemblance to the four colour forms of *D. chrysippus* (Smith 1976, Gordon 1987). Thus, it has been suggested that polymorphism in the three Müllerian mimics might have developed as an adaptive response to extensive Batesian mimicry. The general geographic distribution of the colour forms of *D. chrysippus* appears to support this view, because the butterfly is polymorphic in East and Central Africa, where it is heavily loaded by Batesian mimics, while it is monomorphic in West Africa, where *D. chrysippus* is mimicked only by the form *alcippoides* of female *H. misippus* (Edmunds 1969, Gordon 1987).

The major difficulty with this hypothesis is that the geographic distribution of mimics and colour forms does not consistently follow the expected patterns. For example, in Khartoum (Sudan), *D. chrysippus* was found to show considerable polymorphism despite the occurrence of only one Batesian mimetic form (form *misippus* of female *H. misippus*); this observation contradicts the expectations of the mimetic load hypothesis (Idris and Hassan 2012). Furthermore, the distribution of the mimetic forms does not always follow the distribution of their respective model forms, contrary to the predictions of the Batesian mimicry theory. For example in West Africa only one form of the model species occurs (*alcippus* form of *D. chrysippus*), but all the four forms of female *H. misippus* are found, thus three of them are effectively non-mimetic in this area. Even more paradoxical is that the one mimetic form (*alcippoides*) is relatively rare (Clarke et al. 1995). Overall, these observations raise considerable doubt on the view that Batesian mimicry is the major force in the selective environment affecting colour pattern in the three species.

The 'hybrid zone' hypothesis

There are two classes of observations suggesting that the co-occurrence of biased sex ratios and aposematic polymorphism might not be coincidental: first, the two phenomena show tight phylogenetic association across unrelated insect taxa such as Coleoptera and Lepidoptera, as many species that show aposematic

polymorphism were found to yield positive results when screened for male-killers' infection (e.g. the ladybird beetles *Adalia decempunctata* and *Harmonia axyridis* and the nymphalid butterflies *A. encedon*, *A. encedana* and *D. chrysippus*) (Majerus 2003). This presumed association is based on anecdotal observations and requires formal phylogenetic meta-analysis; however, attributing it to pure chance seems highly unlikely. Far more convincing is the geographic association between the two phenomena in the case of *D. chrysippus*, because there appears to be a close correlation between polymorphism and biased sex ratios in East and Central Africa (Owen and Chanter 1968, Smith et al. 1997, 1998); populations occurring outside this particular geographic zone are monomorphic and show normal sex ratios (Lushai et al. 2003).

These observations have initiated an alternative theory to account for the origin of aposematic polymorphism in *D. chrysippus*. In this theory, colour forms are viewed as allopatric sub-species that arose through past geographic isolation (or invasions from outside Africa at different times) rather than sympatric morphs of a polymorphic species. The region of East and Central Africa represents a giant 'hybrid zone' in which sub-species of *D. chrysippus* co-occur sympatrically. Hybridization takes place despite partial pre-zygotic reproductive isolation as a result of the differential infection of sub-species with the male-killing bacteria *Spiroplasma*; females from the highly infected sub-species are forced to accept hybrid mating with males from the less infected sub-species due to the lack of males with their own colour pattern. As a consequence, gene flow was restored between the sub-species of *D. chrysippus* and thus the speciation process was interrupted (e.g. Lushai et al. 2003, 2005, Smith et al. 2010).

The 'hybrid zone' hypothesis is supported by three lines of evidence: first, both breeding experiments and field data suggest that assortative mating takes place between the different colour forms of *D. chrysippus* (e.g. Smith 1984, Gordon 1984b). In addition, field observations have shown that intermediate colour forms are frequently produced in nature (e.g. Smith et al. 1975, 1980, 1998). The occurrence of assortative mating and hybrid colouration among the colour forms of *D. chrysippus* agrees with the 'allopatric sub-species' idea but not with the 'sympatric morphs' view. Second, the geographic distribution of the colour forms is consistent with the hypothesis as it shows the predicted morph ratio clines throughout Africa (Smith et al. 1997). Third, there is some field and experimental data suggesting that susceptibility to *Spiroplasma* infection varies with colour pattern (Smith 1975, Herren et al. 2007).

On the other side, the distribution of the colour forms of *A. encedon* and *A. encedana* does not show apparent geographic vicariance and therefore the

hypothesis does not apply to them. In our personal view, the simultaneous occurrence of a rare phenomenon within three sympatric mimetic butterfly species intuitively suggests that whatever the cause in one species, it is also the cause in the other species. Unfortunately, the hybrid zone idea is limited to the case of *D. chrysippus*.

Moreover, a considerable amount of data appears to be inconsistent with this hypothesis. For example, both the field investigation of population sex ratio and the molecular analysis of bacterial infection indicate that Khartoum is a *Spiroplasma*-free zone (Hassan 2008, Idris and Hassan 2012); however, *D. chrysippus* shows significant polymorphism there (Idris and Hassan 2012). In Uganda, a thorough three-year investigation has been conducted for male-killing and aposematic polymorphism in *D. chrysippus*; no association between the two phenomena was detected, because colour forms did not show a significant variation with the sex ratio or the *Spiroplasma* prevalence (Hassan et al. in prep.). These findings challenge earlier reports on the variable susceptibility of *D. chrysippus* colour forms to *Spiroplasma* infection (Smith et al. 1993, 1997).

In this paper, we provide a new hypothesis on the maintenance of aposematic polymorphism in *D. chrysippus*, *A. encedon* and *A. encedana*. This hypothesis assumes the existence of indirect causal link between sex ratio distortion and colour pattern polymorphism, mediated by the host metapopulation dynamics.

The hypothesis

Assumptions

Invasion by an efficient male killer is expected to bias the host population sex ratio towards females because infected females produce only daughters. Initially, the sex ratio change would have a positive or neutral impact on host reproductive rate. The reason is that males have higher reproductive potential than females (Trivers 1985), and thus a minority of males can easily fertilize all the females in the population. However, as the sex ratio distortion develops to extreme levels, population reproduction becomes sperm limited as many females may die without leaving offspring because they did not encounter a male during their life time. Under such conditions, local populations would frequently undergo extinction due to the lack of males. Habitat batches that are emptied following population extinctions are then recolonised by new migrants from surrounding populations. Thus, the metapopulation dynamics of the host species (i.e. the rate of extinction and recolonisation of sub-populations) can undergo substantial acceleration following invasion by the male-killing endosymbiont. The occurrence of recurrent population extinctions and recolonisations in *A. encedon* has been previously

hypothesized to explain the maintenance of polymorphism with respect to the male-killing trait during evolutionary time (i.e. the maintenance of the category of uninfected females) (Heuch 1978). Here, we assume that population extinctions and recolonisations driven by the spread of male killers occur frequently in the wild populations of *D. chrysippus*, *A. encedon* and *A. encedana*.

As discussed previously, natural selection acting on the colour forms of an aposematic polymorphic species is expected to show a positive frequency-dependent nature, favouring the most abundant colour form in the species. Likewise, within a Müllerian mimicry complex composed of multiple species, selection is expected to favour the colour form that happens to be more abundant in the species group. The important thing about frequency-dependent selection is that it is not based on any inherent property of the favoured trait; rather, it favours on the base of mere abundance. Due to this feature, the direction of frequency-dependent selection is determined by the initial allelic frequency in the population founders. Whichever allele happens to be initially more abundant will come to dominate the population, as the initial difference in frequency (slight as it may be) is magnified through generations of the action of natural selection. As a consequence, selection on colour pattern is expected to show extreme spatial heterogeneity; although monomorphism will be selected for everywhere, the particular colour form that is favoured by selection will vary between populations depending on the initial allelic frequency in each population. Here we assume that colour pattern frequencies are spatially heterogeneous in *D. chrysippus*, *A. encedon* and *A. encedana* due to the combined effects of population histories and natural selection described above.

If positive frequency-dependent selection acting on heterogeneous populations is the only force affecting the colour pattern, the expected outcome from this selective system would be monomorphism on the local scale (i.e. the population level), with every individual population showing a single, dominant colour pattern, but polymorphism on the regional scale (i.e. the metapopulation level), with different populations dominated by different colour patterns. Indeed, this is not the case in any of the three species, given that multiple colour forms occur sympatrically in natural populations. It appears as if there is a subtle factor that interferes with the act of natural selection in the populations of *D. chrysippus*, *A. encedon* and *A. encedana*, thus maintaining the non-adaptive state of within-population polymorphism. If this is so, what is the nature of this factor? And how does it affect selection on colour pattern?

Mechanism

Selection within the aposematic species

Consider a polymorphic population of one species, for example, *A. encedon*. At any point in time, frequency-dependent selection will be driving the most frequent colour pattern allele towards fixation. Indeed, the frequency of the favoured colour pattern at that point in time would be higher than its initial frequency in the founding population, representing the effect of past selection. There would be several other populations of *A. encedon* in the surrounding vicinity; each of them moving towards monomorphism by positive frequency-dependent selection. However, both the direction of current selection (i.e. the identity of the favoured colour pattern) and the output of past selection (i.e. the increase in the frequency of the favoured colour pattern relative to its initial frequency) are likely to vary between populations, reflecting different population histories. The populations of *A. encedon* at a particular region are connected through occasional dispersal of individuals between habitat batches, thus representing a metapopulation.

Now consider the invasion of the *A. encedon* population by a highly efficient male-killing endosymbiont. In the beginning, the male-killer would have low frequency in the population; however, due to the fitness advantage of infected females over uninfected ones, the male-killer would spread gradually in the population, thus leading to a growing state of sex ratio distortion and, consequently, to lower reproductive rate. As the male killer achieves extremely high prevalence, the percentage of males will decline to critical levels that threaten the population with extinction. If the male killer reaches fixation, the ratio of males to females in the population will drop to '0:1' and extinction will follow thereafter. Importantly, the male-killer prevalences at any point in time are expected to show spatial heterogeneity, with different populations suffering variable levels of infection. The factors underlying this population variability are the differences in the history of infection, differences in the initial male killer's frequency and, most importantly, differences in the rate of male killer's spread, because the vertical transmission of bacterial male killers in natural populations might be affected by local environmental factors.

When an event of extinction strikes an *A. encedon* population at a given habitat patch as a result of male-killer-induced sex ratio distortion, migrational movements from other populations will lead to the recolonisation of that empty habitat batch. The few

migrant butterflies that have recolonised the site would undergo population expansion through successive generations, ultimately leading to a new population replacing the extinct one. During recolonisation, uninfected females have a reproductive advantage over infected ones because their progeny can mate with siblings; Heavily-infected migrants are likely to undergo extinction within few generations due to the lack of males, leaving the site open for a new wave of migrants. Thus, it is predicted that the new population would have considerably lower male-killer prevalences than the old, extinct population.

Now, let us consider the impact of extinction and subsequent recolonisation on the morph ratio of a polymorphic population of *A. encedon*. Based on our previous assumption that morph ratios show considerable spatial heterogeneity, it is expected that the extinction of a population and its replacement by a new one originated through migration from another population will result in both random and substantial change in the morph ratio at the habitat patch. The morph ratio change is described as substantial because it involves the total substitution of the original morph ratio by another one, and is described as random because the percentages of colour patterns that happen to occur in the migrants are unrelated to these in the extinct population. This will affect the positive frequency-dependent selection acting on the colour pattern in two ways: first, it will eliminate the output of past selection as is represented by the adaptive increase in the frequency of the favoured colour pattern, and second, it will shift the direction of future selection from the most abundant colour pattern in the original population to the most abundant one among the migrants.

It is easy to see that extinction-recolonisation cycles have immense disrupting influence on natural selection for colour monomorphism. Every time a male killer induces an extinction-recolonisation event, it also eliminates the effect of generations of frequency-dependent selection that were taking place at the habitat batch. Following recolonisation, selection for monomorphism would have to restart from the scratch, with a new combination of colour form frequencies and a new favoured colour form. If cycles of extinction and recolonisation occur recurrently at the level of the metapopulation, then polymorphism will be restored regularly through the flow of migrants between subpopulations. At any given time, there will be populations acting as 'colour sources', exporting mainly migrants of a particular colour form to empty habitat patches, as they happen to have a high frequency of that colour form. At a later point in time, extinctions and recolonisations would shuffle the exportation roles between populations, with populations at other habitat batches playing the 'colour source' role instead of the old, extinct populations, which, in turn, might be

replaced by new populations exporting a different colour form. Natural selection will continue to favour monomorphism at any single population, but the direction of selection will fluctuate extensively over space and time responding to extinction-recolonisation cycles so that the non-adaptive state of colour polymorphism is maintained within populations.

To conclude, we are proposing the occurrence of a recurring series of bottleneck/founder effects in the populations of the three species; stochastic drift in colour pattern genes is expected to occur whenever a new colony is founded by a small number of immigrants and the resulting change in gene frequencies would be entirely random.

Selection between the mimetic species

So far, we have considered the effect of extinction-recolonisation cycles taking place within a single species. Now let us consider the effect of these cycles at the level of the mimicry complex. Consider the case of a population of *D. chrysippus* in a habitat patch which also hosts sympatric populations of *A. encedon* and *A. encedana*. It is easy to see that the morph ratios of the two *Acraea* species would have a selective influence on the morph ratio of *D. chrysippus*, with the most abundant mimetic forms in *Acraea* selecting for their parallel *Danaus* forms as their numerical superiority implies that more predators will be avoiding any prey with these characteristic colour forms. As a consequence, the frequency-dependent selection on the mimetic colour forms will act at the level of the whole mimicry complex rather than the level of individual species (e.g. form *daira* of *A. encedon* and form *dorippus* of *D. chrysippus* will be selected as a single colour form as they mimic each other). Through generations of natural selection, it is predicted that the colour form with higher initial frequency in the mimicry complex will come to dominate the sympatric populations of the three mimetic species, ultimately leading to a state of colour monomorphism; this hasn't happened.

The species *D. chrysippus*, *A. encedon* and *A. encedana* are all infected with male-killing bacterial endosymbionts. Thus, in a habitat patch where the three species co-exist, a similar pattern of extinction-recolonisation cycles will occur in the three sympatric populations. However, it should be noted that the population dynamics of the male killer and, consequently, the rate of extinctions and recolonisations is independent in each mimetic species; unlike the case of morph ratio changes, the bacterial prevalence in one population does not affect the prevalence in the sympatric population of the other species. The reason is that the male-killing strains are transmitted vertically; horizontal transmission between species occurs rarely and thus does not contribute to infection levels in the

ecological time scale (Majerus 2003). Thus, it is likely that a complex of the three mimetic species at a given habitat patch will experience unsynchronized population extinctions that take place at variable rates, depending on the efficiency of the male killer in each species.

If a population of one species, for example *D. chrysippus*, has survived in the habitat patch for a considerable span of time, selection induced by the sympatric populations of *A. encedon* and *A. encedana* would shift the original morph ratio in the population towards homogeneity with the morph ratios of the mimetic populations (the same homogenizing effect results from selection induced by *D. chrysippus* on *Acraea* species). Selection could be described as pushing the 'species morph ratio' to match the 'whole complex morph ratio'. When extinction strikes the population, the morph ratio may be replaced by a new one that has never been selected to match other mimics in the site. Thus, it is likely that the most frequent colour form in the new population, and thus the favoured form, will be different from the most frequent, thus favoured, colour form in the mimetic species. As a result, rare forms within one mimetic species might be protected from predation because of the abundance of their parallel forms in the newly-recolonised population and thus will maintain its frequency, against the direction of selection within their own species.

If the extinction-recolonisation event is followed by long-term demographic stability, natural selection would act slowly and gradually to restore the homogeneity between different species' morph ratios in the mimicry complex. However, because the episodes of extinctions and recolonisations take place recurrently within the sympatric mimetic populations, the dominant colour forms and, consequently, the favored colour forms, will show extensive temporal variations in all the three species. As a consequence, there will be no time available for selection to restore homogeneity with other species because those other species themselves are undergoing random changes in the morph ratio. The expected outcome would be a permanent state of multiple selective pressures favouring multiple colour forms, thus maintaining colour polymorphism in the mimicry complex.

To conclude, the enhanced host metapopulation dynamics contribute to the maintenance of colour polymorphism at two distinct levels: first, at the level of individual species, because extinctions and recolonisations eliminate the outcome of past selection on colour pattern and second, at the level of the multi-species mimicry complex, because extinctions and recolonisations shift the direction of selection within one species away from the direction of selection within the rest of the mimetic species, thus maintaining heterogeneous selective pressures on colour pattern. The recurrent extinctions and recolonisations at the first level counter-

act selection for monomorphism, while at the second level they induce positive selection towards polymorphism.

Discussion

In this paper, we suggest a causal relationship between male killing and colour pattern polymorphism in the aposematic mimetic butterflies *D. chrysippus*, *A. encedon* and *A. encedana*. The relationship is mediated by the enhancement in the natural metapopulation dynamics of host butterflies in response to the female-biased population sex ratios caused by male-killers. The enhanced rate of population extinctions and recolonisations within each species both destroys the products of past selection and randomly changes the direction of current selection, thus leading to substantial reduction in the efficiency of natural selection for colour monomorphism. Moreover, recurrent extinctions and recolonisations taking place in the three sympatric populations of these mimetic species maintain a continuous state of multiple selective pressures on colour pattern at the level of the mimicry complex, such that selection positively favours colour polymorphism.

A third critical assumption of the hypothesis presented here (the first two assumptions are mentioned earlier) is that colour pattern does not affect the butterfly susceptibility to male-killer infection or its dispersal potential. If colour forms vary in the degree to which they are 'susceptible' and 'resistant', then populations with a high percentage of 'susceptible' forms will undergo extinction more frequently than populations composed mainly of 'resistant' forms. There is some evidence that this is the case in *D. chrysippus* because morph *dorippus* was found to be more resistant to *Spiroplasma* infection than morph *chrysippus* (Smith 1975b). Under this condition, a sort of group selection will act against the susceptible forms, thus reducing the extent of colour polymorphism in the species. Likewise, if colour pattern affects the dispersal behaviour, with certain colour forms being more active in dispersal than others, then the average representation of 'active' forms among successful colonisers will be higher than their representation in the species as a whole. As a consequence, recurrent extinctions and recolonisations will increase the frequency of active forms rather than maintaining colour forms in a stable polymorphism. Again this has been suggested in the case of *D. chrysippus* (Smith and Owen 1997).

Importantly, this hypothesis explains the maintenance but not the origin of colour pattern polymorphism. As previously noted, the enhanced metapopulation dynamics can affect selection on the colour pattern only if colour form frequencies show extensive spatial heterogeneity; if morph ratios are largely homogeneous within the metapopulation, then the recolonisation of

one sub-population at a particular site by migrants originated from other sub-populations would have only a minor influence on the morph ratio at that site. We believe that the origin of colour polymorphism in the three species as well as the initial spatial heterogeneity in the morph ratios was driven by the load of Batesian mimics and/or the 'hybrid zone' effect. Later, recurrent extinctions and recolonisations have become a major force in the maintenance of colour pattern polymorphism. To conclude, the three hypotheses on the subject of aposematic polymorphism may reinforce each other and thus are not mutually exclusive.

Testing the Hypothesis

The hypothesis presented here states that extinctions and recolonisations occur frequently in the three species, causing extensive and random morph ratio changes. These expectations can serve as a base for testing this hypothesis against field data. In a thorough investigation for population sex ratios, male-killer prevalences and morph ratios in *D. chrysippus*, *A. encedon* and *A. encedana* conducted in Uganda (Hassan et al. 2012a, 2012b, in prep.), two temporal patterns were found, which are highly consistent with theoretical predictions. Firstly, comparing the bacterial prevalences recorded during (2005–2007) with those recorded a few years later (1998–1999) (Jiggins et al. 2000a, 2000b, 2000c) for the three species has shown that the male killer undergoes extensive population dynamics. Importantly, in both *Acraea* species, populations that have shown high prevalences in the earlier collection were found to experience marked decline in prevalence during the later collection. This pattern is exactly what would be expected if populations with high prevalence went extinct and were recolonised by migrants with initially low prevalence. Secondly, population morph ratios were found to show substantial temporal changes during the three-year period of the study (2005–2007) that did not coincide with seasonal changes. Moreover, comparing morph ratio estimations in previous collections (e.g. 1964–1966, 1991, 1998–1999) (Smith et al. 1993, Owen et al. 1994) with the recent estimation (2005–2007) has revealed that colour form frequencies experience considerable annual fluctuations that do not display any detectable trend. The observed lack of pattern in morph ratio fluctuations agrees with our hypothesis, because the presumed changes induced by extinction-recolonisation cycles are essentially random in nature.

The field observations listed above are consistent with our hypothesis, but they do not clearly distinguish it from alternative hypotheses. This is attributed to the fact that these same observations can be interpreted in alternative ways; the male-killer dynamics might have resulted from natural fluctuations in the vertical transmission of male-killing endosymbionts induced by

unknown environmental factors. In addition, the morph ratio dynamics could reasonably be attributed to the occurrence of regular migrational movements in the host species not related to population extinctions. Further data are needed before the validity of this hypothesis can be formally demonstrated.

We suggest that the hypothesis presented here could be critically evaluated in three ways: First, according to this hypothesis, the metapopulation dynamics of the sympatric populations of the other Müllerian mimics is a major factor in the maintenance of aposematic polymorphism. Thus, it is to be expected that in regions where only one mimetic species occur, polymorphism is less developed than regions inhabited by a complex of two or three of the Müllerian mimics. Second, a critical assumption of our hypothesis is that colour forms do not affect susceptibility to male killing, while a critical assumption of the 'hybrid zone' hypothesis is that colour forms do indeed vary in susceptibility. Thus, a clear test for both hypotheses would be to investigate the relationship between colour pattern and *Spiroplasma* infection in *D. chrysippus* through a large study conducted within substantial spatial and temporal scales. Third, the most direct approach to test the idea that male-killer-induced extinctions and recolonisations occur in the wild and that they alter population morph ratios is a long-term monitoring study of individual populations at particular sites to assess the potential association between the population dynamics of the host and that of the male killer, as well as the impact of this presumed association on host morph ratio. Finally, developing a formal mathematical model that describes the effect of recurrent extinctions and recolonisations on selection for colour pattern is a necessary step toward assessing the power of this hypothesis and obtaining clear predictions from it.

Criticism

One argument against the present hypothesis is that the male-killer-driven population extinctions are merely a speculation that has never been directly confirmed from the field. It is thus possible that male killers do not spread to fixation in wild populations but reach equilibrium prevalences, and if this is so, then the category of uninfected females would be maintained indefinitely in infected populations. Another possibility is that extinction-recolonisation cycles really take place in the field but within a scale of hundreds or thousands of years. If this is the case, selection for colour homogeneity can easily overwhelm the disrupting influence of the enhanced metapopulation dynamics.

The major difficulty, however, lies in the dispersal behaviour of the three butterfly species. In *Acraeas*, most dispersal is performed by females. As a consequence, heavily-infected populations (i.e. highly female-

biased populations) export more migrants than less-infected populations, thus leading to higher infection prevalences among colonisers. An even more serious issue is the differences in dispersal zones between *D. chrysippus* on one side and the two *Acraeas* on the other side. While *Acraeas* have highly aggregative/colonial distribution, *D. chrysippus* is widely dispersive and migratory species such that spatial structuring occurs only over vast distances (e.g. Owen et al. 1994). It is hard to see, then, how the morph ratio heterogeneity can be maintained in the face of the homogenizing influence of the extensive dispersal of *D. chrysippus*.

Since *D. chrysippus* is more abundant and more widely dispersed than the *Acraeas*, it follows that the *Acraea* colonies are nested within much broader zones of *D. chrysippus* abundance. Thus, the migrating butterflies of any species will pass through habitats where *Danaus* is abundant but *Acraeas* are rare and thus will be selected to match the prevailing *Danaus* colour pattern. In the long term, this may lead to the dominance of the prevailing *Danaus* colour form among all the mimetic species in the region. In other words, the higher abundance and wider dispersal of *D. chrysippus* compared with that of *A. encedon* and *A. encedana* implies that the Müllerian selection will be driving the *Acraeas* to match *Danaus* colouration rather than driving *Danaus* to match *Acraeas* colouration. If this is the case, the enhanced metapopulation dynamics induced by male killers would not be able to maintain polymorphism at the regional scale.

The details of the mimetic association between the three species further complicate the overall picture; the two *Acraeas* differ fundamentally in their mimicry of *D. chrysippus* (D.A.S. Smith, personal communication): *Acraea encedana* is a convincing mimic throughout most of its geographic range. On the other side, *A. encedon* more often represents an unconvincing mimic, and where it is a good mimic (Tanzania, Owen and Smith 1991), *A. encedana* is absent. Moreover, three of the *Acraea* forms are universally non-mimetic (*lycia*, *suffused lycia*, and *sganzini*) but are nevertheless extremely abundant and widely dispersed (Owen and Smith 1993, Owen et al. 1994). It follows, then, that the simple idea of multiple mimetic colour patterns occurring within three sympatric populations ignores the imperfections in the mimetic association that prevail within most of the mimicry zone.

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Response to referees

In this paper, we have conducted a theoretical investigation of the intriguing problem of mimetic polymorphism in three African butterflies: *Danaus chrysippus*, *Acraea*

encedon and *Acraea encedana*. We started from the assumption that the observed correlation between male killing and colour polymorphism in the three species is not coincidental, which is the same view held by Smith DAS, Gordon IJ and their colleagues. However, we proposed that their specific interpretation (i.e. the hybrid zone hypothesis) is not the only mechanism by which such association between polymorphism and male-killing could have evolved and we proceeded to develop an alternative scenario that explains such association without reference to the hybrid zone idea. It is important to stress that we did not present our hypothesis as the only or even the true explanation of polymorphism in these species; rather, our aim was to improve the understanding of field and experimental data regarding this phenomenon through exploring further theoretical possibilities not previously appreciated.

We agree completely with the conclusion of Gordon (2013) that the mimetic polymorphism in the three species represents a complex phenomenon that varies extensively over space and time and is affected by many factors other than the metapopulation dynamics. However, it is this same conviction that motivates us to disagree with his remark that only *A. encedon* could be a potential target of the mechanism described in this paper. Due to the large size and the habitat heterogeneity of East and Central Africa, the absence of the assumed metapopulation structure at a particular area or within the populations of a particular species does not imply that such structure does not occur in other regions or within other populations of the same species.

In *D. chrysippus*, it is true that the species has higher abundance and dispersal than the two *Acraea* species; however, this is not necessarily the case everywhere. For example, in arid regions such as Sudan (as well as much of East and Central Africa), the vegetated habitats are separated by extended desert or semi desert belts, which makes dispersal between habitat patches difficult for any butterfly species regardless to its dispersal ability. Moreover, in arid regions, the overall density of butterflies is much lower than that at savannah regions. Due to the lower butterfly abundance and the naturally-fragmented habitats in Sudan, *D. chrysippus* can show a metapopulation structure over much lower distances than that observed in Uganda, for example. It is also necessary to point out that migration does not occur everywhere in East and Central Africa; for example, migration has never been recorded for *D. chrysippus* populations in Sudan and the colour form frequencies in this country show considerable seasonal stability (pers. observ.). It is easy to see that when the population density and the dispersal pattern are inherently limited by the habitat structure and where seasonal migration does not occur, the metapopulation scale of *D. chrysippus* will shrink to match those of *A. encedon* and *A. encedana*.

As we noted earlier in this article, the extinction-recolonisation cycles that act to maintain polymorphism are of two *distinct* types; those within the species and those within its Müllerian mimics. Importantly, our metapopulation mechanism can still work if only one of these two cycles takes place but it would be more powerful with the two cycles reinforcing each other. We suggest that the case of *D. chrysippus* could be explained by extinctions-recolonisations occurring only *outside* the species while the case of *A. encedon* is an example of extinctions-recolonisations occurring only *inside* the species. Finally, the case of *A. encedana* represents an example of extinctions-recolonisations taking place both *inside* and *outside* the species. This could be justified as follows:

- 1) In *D. chrysippus*, there is no evidence for extreme *Spiroplasma* prevalence or severe sex ratio distortion from wild populations, thus making the extinction-recolonisation scenario somehow unlikely. However, recurrent random fluctuations in the morph ratio of the mimetic *Acraea* populations (driven by the male-killer-induced population extinctions) will result in recurrent and random shifts in the direction of the Müllerian selection imposed on *D. chrysippus* populations thus maintaining polymorphism. In other words, the morph ratio of *D. chrysippus* will be “dragged” by the extinction-recolonisation cycles in the *Acraea* populations even if the species itself does not experience any of these cycles.
- 2) In *A. encedon*, Müllerian mimicry is not an important force in the ecology of this species since the mimetic forms are mostly poor mimics and many wild populations are dominated by totally non-mimetic forms, thus, the metapopulation dynamics of the mimetic species may have a little influence on the morph ratio of *A. encedon*. However, even if Müllerian mimicry does not occur at all, extinction-recolonisation cycles within *A. encedon* can still resist selection for monomorphism through inducing recurrent random fluctuations in the population morph ratio. Mimicry is not a necessary condition for our model to work; as long as there is an aposematic polymorphic species with spatially heterogeneous morph ratio, any enhancement of the metapopulation dynamics will prevent random forms from undergoing extinction, thus maintaining the non-adaptive state of polymorphism.
- 3) In *A. encedana* the male killers have very high prevalences and the host shows extremely female-biased sex ratios, implying that the species is susceptible to the population extinctions induced by the spread of the male-killing *Wolbachia*. Moreover, Müllerian mimicry is highly developed between *A. encedana* and *D. chrysippus*. Thus, polymorphism in *A. encedana* seems to be maintained by extinction-

recolonisation cycles taking place both *inside* and *outside* the species populations.

In our view, the enhanced metapopulation dynamics represent only one factor in the complex ecological background that maintains colour polymorphism in the three butterfly species; the other factors include the load of Batesian mimics and the evolutionary history of *D. chrysippus*. We criticized the two theories earlier in this paper not to refute them but to demonstrate that they are not fully sufficient to explain the aposematic polymorphism in these species. As Gordon (2013) and Smith (2013) rightly emphasize, the assumptions of our model (such as the similar dispersal patterns for the three species and that the colour form has no influence on male-killing susceptibility) are not met in all regions and all populations. Indeed, the differences in dispersal patterns between *D. chrysippus* and the two *Acraea* species represent a serious difficulty that we could only explain in an unsatisfying way. Moreover, the detection of lower susceptibility of f. *dorippus* to *Spiroplasma* infection that was reported at Dar es Salaam, Tanzania (Smith 1975b, Smith, pers. comm.) further complicates the application of our hypothesis to the case of *D. chrysippus* (but see Hassan et al. 2013). More research, including theoretical, field, and experimental investigations are strongly recommended in order to resolve this complex subject.

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