The apparent paradox of colour variation in aposematic poison frogs

by

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SUMMARY

The origin and maintenance of aposmatic polymorphisms is not completely understood. The success of aposmatism as an anti-predator strategy relies on predators’ ability to associate warning signals with unprofitability. Therefore aposmatic species are supposed to have simple colour patterns with minimum variation. However, intraspecific variation in aposmatic colour patterns occurs in many species, bringing the assumptions into question. Recent studies have suggested an interaction between natural and sexual selection as the evolutionary force allowing for the existence of variability in aposmatic signals. Most research on this topic has been done experimentally in the lab on species whose colour patterns vary among populations, but very little is known about the maintenance of variable aposmatic signals within populations in the wild. I studied an aposmatic poison frog with an extensive intrapopulational variation in colour patterns to test in the field some hypotheses about the mechanisms allowing the occurrence of variable aposmatic colour patterns. I investigated the roles of sexual selection, ecological traits and behavioural strategies in the maintenance of the variability on the aposmatic signals of this species. I found that different colour patterns are associated with mating advantages, parental duties, differential use of available microhabitats, different behavioural strategies and the willingness to invado disturbed habitats. My results suggest that the evolutionary forces leading to aposmatic polymorphisms might represent more complex processes than acknowledged to date. Besides predation and sexual selection in the form of mate choice, we must consider aspects of the behaviour and ecology of polymorphic aposmatic species such as escape behaviour, microhabitat selection, activity patterns, alternative colour pattern-behaviour strategies, or the willingness to take advantage of ecological opportunities, as factors enabling the simultaneous existence of such variability in colour patterns while still getting protection from predators.
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Chapter 1

COLOUR PATTERN VARIATION IN APOSEOMATIC SPECIES AND ITS IMPLICATIONS

Abstract. Aposematic species have very different and difficult problems in communicating compared to cryptic species. While natural selection favours the uniformity of aposematic colour patterns, there is increasing evidence that sexual selection could favour their diversity. Up to date, most literature on colour patterns, intraspecific communication and aposematism deal with these topics separately. Here I attempt to bring together the literature on four contexts (predator-prey interactions, mate choice, intra-sexual competition and space use) in which the role of colour patterns has been studied up to date, paying special attention to the situation of aposematic species with variability in their warning signals. I highlight the convenience of studying a system in which the four contexts mentioned above could be addressed simultaneously in order to improve our understanding about the implications of variation in aposematic signals. Finally, I suggest the hypotheses that, in my opinion, would bring a novel approach to research on the origin and maintenance of variation in aposematic colour patterns, using dart-poison frogs as a study system.

Introduction

The role of colour patterns as visual signals in animal behaviour and ecology has been studied extensively in a variety of contexts and taxa, but has been neglected in others. Signals can be addressed to heterospecifics, like predators or prey, or to conspecifics, either of the same or the opposite sex, and vary depending of the context and the audience. Selection is expected to maximize the signal-to-noise ratio (Endler 1992, 1993b; Bradbury & Vehrencamp 1998) while decreasing signal degradation (Endler 1992, 1993b). Likewise, selection tends to favour those signals with a higher efficacy in terms both on how easily transmitted and detected they are, but also on how the receiver’s response to them can increase the sender’s fitness (Gulford & Dawkins 1991; Endler 1993b).
Depending on the receiver, the context in which signal is emitted and the benefits that the sender gets from the receiver’s response, selection might favour either uniformity or diversity in the signals (Bradbury & Vehrencamp 1998). Conspicuous species, therefore, are in an interesting situation as they are subject to almost opposite selective pressures: while both aposematism and sexual selection require bright colours, the former requires constancy and the latter requires variability.

Most of the literature on aposematism, colour patterns and intraspecific communication deals with each topic separately. Moreover, the role of variable colour patterns in intraspecific communication and ecological decision-making has been studied mostly in cryptic species. Here I present a short review of four different contexts in which the role of colour patterns in animal communication has been addressed up to date, and attempt to bring them all together paying special attention to the conflict between constancy and variability in visual signals for conspicuous species and highlighting the convenience of identifying a system that allows for the simultaneous study of the role of colour pattern in different aspects of its behavioural ecology.

Interspecific communication: colours of prey that signal distastefulness to predators

Aposomatic coloration

Aposematism is an anti-predator strategy whereby prey warn predators about their unprofitability (presence of toxins, physical defences such as spines or irritant hairs, or very efficient capture avoidance) by means of specific, simple colour patterns that act as signals (Poulton 1890; Cott 1940; Ruxton et al. 2004). Aposematism may also involve warning odours (Guilford et al. 1987; Siddall & Marples 2011a) or sounds (Owings et al. 2002; Hristov & Conner 2005; Siddall & Marples 2011b). Predators learn the association
between the warning signals and the noxiousness of the prey, and subsequently avoid them (Endler 1991a; Endler & Mappes 2004; Ruxton et al. 2004).

Aposematism can be tested experimentally by using conspicuous vs. cryptic models or live prey, either in controlled environments or in the wild, where the behaviour of potential or known predators is observed and quantified (for example, the ‘Novel world’ (Alatalo & Mappes 1996; Lindstrom et al. 2001; Riihi et al. 2001); see also (Brodie 1993)). Decades of studies on aposematic prey aversion by avian predators have revealed that naïve predators learn to avoid distasteful prey after having being exposed to them based on their colour patterns (Marple et al. 1994; Gamberale-Stille & Guilford 2003; Aronsson & Gamberale-Stille 2008) or the contrast between their colour patterns and their background (Gamberale-Stille 2001; Lindstedt et al. 2011). Nevertheless, some studies have also demonstrated that such aversion could be innate (Schuler & Hesse 1985; Exnerová et al. 2007), most likely as a consequence of neophobia and dietary conservatism (Marple et al. 1998; Marple & Kelly 1999; Lee et al. 2010) rather than only of inherent biases against warning colours or patterns.

The puzzle of aposematic polymorphisms

As a result of stabilising selection, it is expected for aposematic prey to have monomorphic warning signals (Endler 1988; Joron & Mallet 1998; Endler & Mappes 2004; Darst & Cummings 2006). Hence, polymorphisms (the simultaneous occurrence of two or more forms within a population so that the rarest form occurs in frequencies higher than those expected by pure mutation (Ford 1945)), should be selected against in aposematic species (Endler & Mappes 2004). How does this happen?

Several studies with polymorphic, cryptic, palatable prey have shown how visual predators tend to form a ‘search image’ or equivalent behaviour and concentrate on
praying on the most abundant prey available, almost neglecting the rest, simply because it is harder to search at once for different types of prey than to search only for one of them (Bond 2007). As a result, frequency-dependent selection (also called apostatic) is expected to stabilize polymorphisms (Endler 1978, 1991a; Punzalan et al. 2005; Bond & Kamil 2006; Olendorf et al. 2006). The most abundant form is disproportionately preyed upon, making rarer forms more common until they get differentially preyed upon, and so on, making polymorphisms stable (Bond 2007). For aposmatic species, it may not work in this way because signal variability (i.e. aposmatic polymorphisms) may reduce the ability of predators to learn and retain the association between colour patterns and distastefulness (Greenwood et al. 1981; Mallet & Joron 1999; Exnerová et al. 2006). Nevertheless, there are several cases of aposmatic polymorphisms in nature, for example in lady birds (O'Donald & Majerus 1984; Ueno et al. 1998), moths (Nokelainen et al. 2012) and frogs (Myers & Daly 1983). Even though the mechanisms by which these are maintained are not yet fully understood, recent approaches point at an interaction between natural and sexual selection as one possible explanation (Maan & Cummings 2008, 2009; Nokelainen et al. 2012)

Intraspecific communication: colours that attract mates

Colours, patterns, and mate preferences

Brightly coloured, contrasting patches, bands, stripes, spots, and several types of elaborated ornaments either in the body of an individual (males, most of the time), or gathered from its surroundings, are known to have a significant effect on mate choice in a great variety of taxa (Andersson 1994). Females of several species prefer to mate, for example, with more colourful or brighter individuals (Maan et al. 2004; Gomez et al. 2009; Bajer et al. 2010). In some cases, not necessarily the colour but a variety of
different criteria like the number, size or shape of patches or spots can be important factors in mate preferences (Petrie et al. 1991; Pincemyn et al. 2009).

Although in many cases the explanations for mate preferences remain obscure, it has often been found that they are associated with the indirect benefits of ‘good genes’ (Hamilton & Zuk 1982; Milinski & Bakker 1990; Cutrera et al. 2012; Stange & Ronacher 2012). Female preferences could exert directional selection on males’ traits that provide reliable information about their quality because by mating with them females might grant their offspring a higher probability of survival through, for example, parasite resistance (Milinski & Bakker 1990; Barber et al. 2001; Horak et al. 2001; Demuth et al. 2012) or protection from predators (Sheldon et al. 2003; Lancaster et al. 2009).

Assortative mating with regards to colour

Assortative mating, the non-random pairing of two individuals on the basis of a shared trait, has been broadly documented in a great variety of organisms. It can be associated with morphological, ecological or behavioural traits, and be either positive (matings between the same phenotype) or negative (matings between different phenotypes). Colours seem to play a prominent role in the occurrence of assortative mating. One of the best examples studied to date is that of the cichlid fish of Lake Victoria, where apparently male colouration and the corresponding female preferences appears to have promoted sympatric speciation and enhanced reproductive isolation among co-occurring species (Seehausen & van Alphen 1998; Maan & Seehausen 2011; Terai & Okada 2011). The role of positive assortative mating by colour in speciation has also been established for the aposomatic Heliconius butterflies (Melo et al. 2009) and the Midas cichlid (Elmer et al. 2009). Other examples of positive assortative mating with
respect to colours can be found in crustaceans, insects and birds (Roulin 1999, 2004; Pryke & Griffith 2007; Wang et al. 2009; Calderón et al. 2010).

While positive assortative mating can help in the maintenance of intra-specific geographic variants and lead to speciation in the presence of genetic flow (Kondrashov & Shpak 1998; Maan et al. 2004; Puebla et al. 2007; Terai & Okada 2011; Puebla et al. 2012), negative assortative mating (also called disassortative) is required to maintain different phenotypes within a population (i.e. true polymorphisms sensu Ford 1945; Roulin 2004; Follett et al. 2007; Takahashi & Hori 2008; Hancox et al. 2010)); many studies confuse the mechanisms for inter- and intra-population variation in visual signals.

**Colours can attract both mates and predators**

One of the problems that animals face when they have conspicuous colour patterns is that they may be providing information about their presence and location to both wanted and unwanted receivers. While aposematic species have sorted out this situation by being distasteful, unprotected organisms must find a way to show off, so that they can still attract conspecifics, but not too much, so that they do not attract predators or sneakers (Endler 1978, 1980). This problem might be overcome by using ‘private communication channels’ so that conspicuousness is higher towards conspecifics than it is towards predators (Endler 1978, 1980, 1991b; Schaefer 2010), which would imply that the visual sensitivity of conspecifics is better ‘tuned’ to the characteristics of their visual signals than is the predators’ (Endler 1991b). Alternatively, opposing selective forces can balance out and shape an optimal signal (Maan & Seehausen 2011).
The coloured patches of guppies, for instance, are the product of a balance between natural and sexual selection, whereby males have more conspicuous patches in order to attract females but not too conspicuous in order to enhance predator avoidance (Endler 1978, 1980). Besides guppies, organisms of almost all classes have found to exhibit some degree of interplay between natural and sexual selection in the evolution of their visual signals. For example, the colour patterns of wolf spiders are both cryptic or contrasting with their background depending on where the receiver sees them from: when seen from above by potential predators (birds) their colour patterns blend with those of the leaf litter, whereas when seen from the side by conspecifics their colour patterns are very conspicuous (Clark et al. 2011). Similar trade-offs have been found in other live-bearing fish (Hurtado-Gonzales et al. 2010), lizards (Macedonia et al. 2004), and sparrows (Olsen et al. 2010).

Although in principle aposematic species are well protected due to their defences, those aposematic species with variable colour patterns might face disadvantages in this context given that different morphs can differ in conspicuousness and, in turn, differ in the degree of protection, or because some morphs can attract more mates but attract more predators at the same time. Not surprisingly, for such aposematic species, an interplay between natural and sexual selection might also favour variation in their warning signals (Nokelainen et al. 2012).

Intraspecific communication: colours that signal status

Territoriality, aggression and fighting abilities

Intra-specific aggression has been documented in a wide array of organisms and can occur in different contexts (Lorenz 1966; Riechert 1998). Individuals can behave aggressively against conspecifics when they threaten the survival of their offspring
(Sommer 1987), when they threaten access to potential mates (Andersson 1994), or when they are defending a space that holds resources essential for their survival (i.e. food or shelters) or reproduction. The latter case is normally referred to as territoriality (Kaufmann 1983; Maher & Lott 1995).

Because escalated conflict is costly in terms of energy and implies the risk of being injured (Maynard Smith & Harper 2005), the resolution of conflicts via the exchange of signals is expected to be favoured over physical aggression as an evolutionary stable strategy (Maynard Smith 1982; Maynard Smith & Harper 2005). In fact, the mitigating effect of signalling during agonistic interactions has been tested empirically (Logue et al. 2010). Contestants, therefore, should assess the opponent’s abilities to defeat them, and compare them with their own abilities (Riechert 1998), so that both individuals are at the same time senders and receivers as they exchange information about relative fighting ability and relative resource value. This information can be contained in intrinsic characteristics of individuals such as colours, size, condition or the size or complexity of weapons such as horns or antlers. Alternatively, individuals can give and obtain information on the basis behavioural traits such as the rate at which displays are repeated (Johnstone 1997).

*Colour, patterns and status*

Colouration has been found to be strongly related with status acquisition and/or be an important determinant in conflict resolution for several species of insects, fish, lizards, and birds (Pryke & Andersson 2003a; López et al. 2004; Morimoto et al. 2005; Stuart-Fox & Johnston 2005). There is good evidence that more colourful males, and sometimes females, tend to acquire and retain higher status within a group of individuals, whereas dull-coloured individuals are often subordinate. For example, redder male
firemouth cichlids are more likely to win contests than duller ones (Evans & Norris 1996); sand lizards with more colourful badges are more likely to initiate and win fights (Olsson 1994); territorial Augrabies flat lizard males have a lower UV reflectance than floaters (Whiting et al. 2006) and mountain sheep with darker coats are more dominant (Loehr et al. 2008).

As in mate choice, the colour, size and shape of signals provide information during communication. For example, wasps with more facial spots are more likely to challenge rivals than individuals with no spots (Tibbetts et al. 2010). In contrast, the size of the red spots in the wings of male rubyspot damselflies promotes successful defence of a territory (Grether 1996). Similar trends were found in African red-shouldered widowbirds (Pryke & Andersson 2003b).

*Colour polymorphisms and the formation of hierarchies*

As mentioned in the previous sections, the maintenance of polymorphisms could be explained by negative frequency- or density-dependent selection. In terms of intrasexual competition there are examples of higher aggression of certain individuals towards rivals with their own colour morphs than towards rivals looking different: males of the Lake Victoria cichlid complex *Pundamilia* tend to attack individuals with their own colours more often than other morphs (Dijkstra et al. 2007). Additionally, when confronted with blue males, red males are more likely to win (Dijkstra et al. 2005); a similar pattern was found in the females of another cichlid species, *Neochromis omnicaeruleus*, where each of the three co-occurring morphs exhibits higher aggression towards its own morph (Dijkstra et al. 2008; Dijkstra et al. 2009).

Even though there are few examples of this phenomenon, there are certainly many studies focusing on the relative success of different morphs in polymorphic species in
terms of dominance and the formation of hierarchies (Korzan & Fernald 2007). An interesting example of how hierarchies could originate on the basis of colour polymorphisms can be found in Gouldian finches, which exhibit three different colour morphs (red, yellow and black). Red-headed males are dominant over black-headed males, and both are dominant over yellow-headed ones, as proven during set contests. However, even when the red colouration of dominant males was manipulated by making it darker, the originally red-headed birds kept being dominants, suggesting that a link between head colour and behaviour can explain behavioural differences among the morphs (Pryke & Griffith 2006).

Habitat selection and space use

The distribution of individuals of a species within a particular habitat might be explained by the location of resources or predator refuges, the suitability of different microhabitats or the preferences of conspecific individuals (Alcock 2001). There are at least two ways in which colours or colour patterns can be associated to habitat selection and space use. Firstly, animals might choose a habitat that provides the best blending opportunities in order to minimise detection by predators (Ruxton et al. 2004). That can be achieved either by background matching (Endler 1984; Pellissier et al. 2011), or by mixing with groups of species with similar colouration (Munday et al. 2003). Differential habitat selection has been suggested to be the selective agent facilitating the maintenance of colour polymorphisms in various cryptic species (Merilaita 2001; Perez-Figueroa et al. 2005; Pellissier et al. 2011). Secondly, animals can select microhabitats that increase their own conspicuousness or the conspicuousness of their displays (Endler 1993a; Théry 2001; Thery & Endler 2001; Heindl & Winkler 2003).
Aposematic species are not an example of either of these types of association between colour patterns and habitat selection. Because their strategy to deter predators relies on conspicuousness, aposematic prey do not blend with their background. It is possible that polymorphic aposematic species can benefit from colour pattern-mediated habitat selection as a mechanism to maintain colour pattern variability; each morph could choose a habitat that maximises its conspicuousness. Alternatively, if conspicuousness differed among morphs, less protected individuals could benefit from choosing microhabitats that provide extra protection from predators, for example hiding places. However, to my knowledge, there is no evidence of such type of choice to date.

Aposematic colour patterns may be associated to aspects of space use other than habitat selection. For aposematism to work, prey density must be high (Speed 2000; Ruxton et al. 2004; Mappes et al. 2005), or at least above a certain threshold (Endler & Rojas 2009), so that the frequency of encounters enhances predator learning. Therefore, this strategy is assumed to be more common among species that form spatial aggregations (Gamberale & Tullberg 1996; Tullberg & Hunter 1996; Gamberale & Tullberg 1998; Lindstrom et al. 1999; Gamberale-Stille 2000; Beltrán et al. 2007).

Perhaps the most efficient way to understand the implications of colour pattern variation and its role in the behaviour and ecology of a species would be to identify a study species in which behavioural and ecological aspects such as space use as well as inter- and intraspecific (both within and between the sexes) communication, could be addressed simultaneously in relation to colour patterns. Dart-poison frogs (Dendrobatidae) are an excellent group to work with.
Dart-poison frogs are a good system to study the behavioural, ecological and evolutionary correlates of variability in aposematic signals

*Introducing* dart-poison frogs

Dart-poison frogs (*Dendrobatidae*) are a family of neotropical frogs that occurs from the south of Nicaragua to the north of Brazil, with over 300 species recognised to date (Grant et al. 2006; Santos et al. 2009). Their name comes from the observation that the Emberá indians in the Chocó region (Pacific coast) of Colombia use their toxins to poison the darts they hunt with (Myers et al. 1978), but it is believed that only a few species are (or were) truly used for that purpose. *Dendrobatids* are diurnal (with one exception, *Aromobates nocturnus*; (Myers et al. 1991)) and, although some species can be found in epiphytes at more than 15m high or climbing trees, they are primarily terrestrial (Lötters et al. 2007). Their enormous variation in colours both at the inter- and intraspecific levels along with their fascinating behaviour have made them the target of hobbyists and captive breeders in Europe and North America (Walls 1994; Lötters et al. 2007), and more recently in Asia (Nijman & Shepherd 2010) to the point of being one of the animal groups subject to the highest illegal trade (Gorzula 1996).

Most early studies of poison frogs were focused on the presence of powerful toxins in their skin (Daly & Myers 1967; Tokuyama et al. 1968; Tokuyama et al. 1969; Myers & Daly 1976; Myers et al. 1978), or their unique social and reproductive behaviour (Sexton 1960; Duellman 1966; Crump 1972; Durant & Dole 1975; Wells 1977, 1978b, 1980a, b), which involves a very elaborated parental care

*Figure 1.1* A male of *Ameerega hahneli* carrying tadpoles. In this case, all at once.
(Wells 1981; Weygoldt 1987; Crump 1996). As opposed to most anurans, poison frogs have small clutches of less than 30 eggs (in certain species even less than five) laid in the leaf litter or the axils of bromeliads and (with a few exceptions) periodically looked after by one of the parents (usually the male) in order to avoid desiccation, fungal infections and predation (Wells 1981; Duellman & Trueb 1986; Weygoldt 1987; Crump 1996; Lötters et al. 2007; Wells 2007). Upon hatching, tadpoles are taken by one parent [Fig. 1.1; the male in most of the species (Summers 1989b; Aichinger 1991; Lüddecke 1999; Poelman 2008); for female transport see: (Van Wijngaarden & Bolaños 1992; Pröhl & Hödl 1999; Summers & Earn 1999; Summers et al. 1999b)] either all at once or one by one to small bodies of water (i.e. phytotelmata, like tree holes, axils of bromeliads or palm bracts) where they complete their development (Lötters et al. 2007). Moreover, given the low food availability in these small pools, some species provide their offspring with unfertilised eggs on which tadpoles feed almost exclusively until metamorphosis (Brust 1993; Poelman & Dicke 2007; Brown et al. 2008, 2009, 2010b).

Most poison frogs are relatively easy to find by humans because of their conspicuous acoustic signals which are used both to attract females and as a spacing mechanism among males (Lötters et al. 2007 and references therein). In certain species individual recognition is possible on the basis of their dorsal (Silverstone 1975; Summers 1989a, 1992; Born et al. 2010) or ventral (Ringler et al. 2009) colour patterns, allowing for identification with inexpensive and non-invasive methods. Furthermore, poison frogs have small home ranges compared, for example, to birds or mammals; this means in turn that in comparable areas there is a higher number of individuals than in other vertebrate taxa.
Several species of poison frogs exhibit conspicuous coloration (Santos et al. 2003) and have a wide array of skin toxins (Daly & Myers 1967; Myers & Daly 1976, 1983; Daly et al. 1994; Daly et al. 2002) that have been proven to be at least in part sequestered from their specialised diet (Saporito et al. 2004; Saporito et al. 2007a), which consists mainly of ants, termites, mites and other arthropods found in the leaf litter (Toft 1996; Darst et al. 2005). Toxins, however, vary noticeably within the family in composition, amount and power, but most have in common that they are lipophilic alkaloids. One dendrobatid species, *Phyllobates terribilis* (Fig. 1.2), has the most potent non-proteolytic toxin among vertebrates, batrachotoxin (Myers et al. 1978). Each of these golden yellow or metallic orange frogs can have up to 1.2 mg of the toxin, which could be potentially lethal to humans in a dose as low as 200 μg if in contact with an open wound (Myers et al. 1978).

During the last decade the evolutionary link between colouration and toxicity in dendrobatid frogs has been demonstrated (Summers & Clough 2001; Santos et al. 2003; Summers 2003; Vences et al. 2003; Darst et al. 2005). Some studies have shown that bright colouration has evolved independently at least three times, and diet specialisation, which is in turn linked with higher levels of toxicity (Darst et al. 2005), might have evolved independently at least two and possibly three times within Dendrobatidae (Santos et al. 2003; Vences et al. 2003).

The combination of bright colours and high toxicity in these frogs has been traditionally put forward as a good example of aposematism, even though only until very recently there had not been experimental attempts to prove predator aversion of
colourful dendrobatids. While it has been suggested that some crabs feed on tadpoles (Gray & Christy 2000) and some toxin-resistant snakes might feed on juveniles (Myers et al. 1978), the major predators of adult poison frogs are still unknown, presumably due to their success in deterring predators. As indicated by anecdotal observations in the field both ants, Paraponera clavata (Fritz et al. 1981) and spiders, Cupilliumus coccineus (Szelistowski 1985), reject them as prey.

Experimental evidence obtained in studies with frog models, on the other hand, suggests that poison frogs could be subject of attacks by birds, rodents and crabs, which would concur with at least one observation of a crab feeding on an individual of Oophaga histrionica in the Chocó region of Colombia (A. Vélez, pers. comm.), with observations on some of the traumatic injuries found in Dendrobates auratus in Isla Taboga, Panama (Gray et al. 2002), and with one observation of an adult Rufous Motmot (Baryphthengus martii) consuming one individual of D. auratus with no apparent negative effects (Master 1999). The experiments also showed that colourful models representing local frogs are usually less frequently attacked than dull models or models representing novel morphs, or familiar models placed on novel backgrounds, at least for colours resembling one of the morphs of Oophaga pumilio in Costa Rica (Saporito et al. 2007b; Hegna et al. 2011) and Dendrobates tinctorius in French Guiana (Noonan & Comeau 2009).

Additional support to the role of poison frog colour patterns as an anti-predator strategy has been obtained in recent studies that demonstrate that both Batesian (Darst & Cummings 2006; Darst et al. 2006) and Müllerian (Symula et al. 2001) mimicry do exist among dendrobatids. Given the variability in toxicity and colour patterns within the family, and the co-occurrence of toxic with nontoxic species in wide geographical ranges, it seems likely that more examples of both types of mimicry are still to be uncovered.
Colours are involved in mate choice

Bright colours can be very important signals in mate choice, particularly for diurnal species (like poison frogs) that have a wide range of visual signals used during courtship (Zimmermann & Zimmermann 1988; Hödl & Amézquita 2001). In spite of this, there are hardly any studies on the influence of colouration on mate choice in dendrobatids. The only species in which visual mate choice has been studied extensively to date is Oophaga pumilio (Fig. 1.3), a species with a great inter-populational variation in colour patterns, especially in the Bocas del Toro Archipelago (Panama), where there have been identified at least 15 different forms along different islands (Myers & Daly 1983; Siddiqi et al. 2004). However, with one exception populations of that species are monomorphic and the relative importance of both natural and sexual selection in the origin and maintenance of such diversity is not yet fully understood empirically or theoretically.

In a set of choice experiments with this species in the lab, females presented males of two different populations (i.e. two different colour morphs) were more likely to spend more time with the male of their own population (Summers et al. 1999a; Reynolds & Fitzpatrick 2007). This preference fades away when the choice experiments are done under light filters that hinder differences in colour, suggesting the importance of colour patterns in shaping female preferences. A more recent study, with four populations of the same species, found that female mating preferences are influenced by male colours in such a way that a predilection for native males is favoured, but this preference relies heavily on the phenotype of the second male that is presented in the experiment (Maan & Cummings 2008). Although ventral coloration could be expected to be more important
than dorsal patterns for intraspecific communication (Siddiqi et al. 2004), this study supports the idea that male dorsal coloration is the most relevant trait on which females base their preferences (Maan & Cummings 2008).

*O. pumilio* also shows sexual dimorphism in brightness, a non-chromatic component colour patterns, and females prefer brighter males (Maan & Cummings 2009). This is the first convincing evidence that aposematic signals can also be the subject of directional sexual selection, and not just the stabilising natural selection that predators exert (Maan & Cummings 2009). It would be interesting to determine whether these preferences occur in the field, where choices are actually made, and whether they apply to other species with comparable colour pattern variation (Silverstone 1975; Lötters et al. 2007).

Beyond the actual mating, there is no evidence to date that colours act as honest signals of good genes in poison frogs. It is unknown, for example, whether females obtain indirect benefits when mating with brighter males or males with certain colour patterns and whether that were to enhance the survival of their offspring.

*Territoriality and aposematic signals as indicators of fighting abilities*

Even though most frogs rely on acoustic signals for communication (Fig. 1.4), visual signals have proven to play an important role in both inter and intra-sexual communication in several species (Hödl & Amézquita 2001). For example, in poison frogs intra-sexual visual communication occurs in the context of agonistic behaviour. Nearly all anurans defend small, short-term individual spaces during the breeding season, for
example because they confer a particular advantage for the propagation of mating signals or a better access to mates (Wells 2007 and references therein). Dendrobatid frogs seem to be a remarkable exception to this generalisation, as all of the species studied to date exhibit some degree of long-term territoriality (Pröhl 2005). They defend multi-purpose territories that aid in mate attraction (Wells 2007) and subsequent breeding, but what resource(s) individuals (males, females or both) actually defend in their territory varies from species to species. Given their complex parental care, territory defence is most frequently related to the access to reproductive resources such as egg laying or tadpole deposition sites (Pröhl 2002; Poelman & Dicke 2008) or access to mating opportunities (Summers 1989a, 1992).

Because territory size/quality is one of the criteria females use in order to choose a mate, the successful acquisition and subsequent maintenance of a territory can be directly related to male mating success (Roithmair 1992, 1994a, b; Pröhl & Hödl 1999; Ursprung et al. 2011). Resident dendrobatids advertise territory ownership to intruders with a combination of acoustic and visual signals (Zimmermann & Zimmermann 1988; Hödl & Amézquita 2001), and in some cases physical combats can occur until the intruder is chased away, or gains the territory over the resident. Some species have been proven to need both acoustic and visual signals (like the inflation-deflation of the vocal sac) in order to trigger agonistic behaviour (Narins et al. 2003; de Luna et al. 2010). Thus, the importance of visual signals and the potential role of colouration and colour patterns becomes particularly interesting in species that emit very soft sounds (Dendrobates leucomelas, D. truncatus and D. auratus; (Erdtmann & Amézquita 2009)) or lack an advertisement call (Dendrobates tinctorius; Born et al. 2010, pers. observ.).

If colour pattern is an honest signal of the fighting abilities of an individual, as shown in other taxa, one would expect brighter or more colourful individuals to win contests more often than the dull ones, and hence be more successful in acquiring and
maintaining high-quality territories. To date there is only one study: *Oophaga pumilio* males call towards and approach brighter males more frequently in a lab experiment. Interestingly, a male’s own brightness also predicts his own behaviour: brighter males approach stimulus frogs faster and call more towards bright individuals (Crothers et al. 2011). So brightness might just be correlated with overall male condition or quality.

Both male territorial behaviour and male-male aggression have been well documented in *Dendrobatids* (Pröhl 2005). Aggression between females, on the other hand, has been poorly studied and, with one exception (*Oophaga pumilio*, Meuche et al. 2011), reports on its occurrence remain a matter of side observations in the course of studies of male mating behaviour (Wells 1978a; Summers 1989a, 1992). Female aggression has been reported for at least three colourful species, namely *O. pumilio*, *D. auratus* (Fig. 1.5) and *D. leucomelas* (Wells 1978a; Summers 1989a, 1992; Meuche et al. 2011), and a cryptic species in which females have a coloured throat, *Mannophryne trinitatis* (Sexton 1960; Wells 1960b). Given that females do not vocalise at all, their means of acquiring, defending and maintaining a territory are still obscure. Hence, species with territorial females constitute an excellent choice for examining the importance of colour patterns and other visual signals during intra-sexual interactions.

*Colours, habitat preferences and space use*

According to predictions from studies in other aposematic taxa, spatial aggregations facilitate predators’ association between colour and distastefulness (Tullberg & Hunter 1996; Gamberale & Tullberg 1998; Lindstrom et al. 1999; Gamberale-
Stille 2000; Beltrán et al. 2007). While colourful dendrobatids are also highly toxic, their strong territorial behaviour makes them unlikely to be aggregated. They are generally spaced bringing into question the aggregation assumption of aposematism. It is presently unknown whether or not the territories of conspicuous species are more aggregated than those of dull-coloured species. This prediction arises because predators are expected to make decisions based on the encounter rate of prey (Endler & Rojas 2009).

Another way to approach the same conceptual problem would be to conduct studies comparing populations of a colourful species that differ in abundance. There is good theoretical support in favour of the idea that spatial variation in selective pressures might be a relevant factor shaping the evolution of aposematic signals, and that the emergence of novel signals can be a frequency-dependent process (Endler & Mappes 2004; Endler & Rojas 2009). To date, however, there is only one empirical study examining this in poison frogs (Comeault & Noonan 2011). Clay models were used to compare the attack rate of different morphs of Dendrobates tinctorius in two populations differing in the abundance of the local morph. This study provides evidence that selective pressures affecting the survival rate of a ‘protected’ morph vary in accordance with its relative abundance in the population. In other words, an aposematic signal that is successful at high densities might not be so when the densities are low.

An increase in aposematic brightness enhances predator learning (Prudic et al. 2008), and changes in brightness can be associated to differences in colour (Maan & Cummings 2009). Therefore, at a smaller scale, one could expect a pattern of microhabitat choice such that species with duller coloration, or duller individuals within the same species, were more frequently found in places where they are less conspicuous or more sheltered, whereas bright, colourful species would either choose microhabitats that enhance their conspicuousness or show no preference at all. The
same principle could apply to aposmatic species with variable colour patterns that differ in conspicuousness, specially if differences in conspicuousness imply differences in chemical protection (i.e. more conspicuous individuals are more toxic and vice versa; (Maan & Cummings 2012))

Aposmatic species with high variation in their colour patterns are excellent models to understand the ways in which natural and selection interact in nature. For the reasons presented above, dart-poison frogs are excellent for testing hypotheses about the origin, evolution and maintenance of such variation. In this thesis I take advantage of the extensive variation in the aposmatic colour patterns of the dart-poison frog *Dendrobates tinctorius* (Fig. 1.6) in order to test in the wild different hypotheses about its maintenance. More specifically, my aims are: (1) Determining whether there is sexual dimorphism in colour patterns; (2) Testing for differential microhabitat selection in relation to colour patterns; (3) Evaluating the role of sexual selection via mate choice and assortative mating on the maintenance of colour pattern variation; (4) Establishing whether colour patterns are associated with movement and activity; and (5) Examining the role of colour patterns and resource availability in the invasion of unoccupied habitats.

Figure 1.6 *Dendrobates tinctorius*. Les Nouragues Reserve, French Guiana
Chapter 2
SEXUAL DIMORPHISM AND DIFFERENTIAL MICROHABITAT USE REFLECT COLOUR PATTERN VARIATION IN THE APOSEMATIC FROG DENDROBATES TINCTORIUS

Abstract. Aposematism, the link between conspicuous signals and chemical or physical defences that make prey unprofitable, is a well-known anti-predator strategy that relies on predators learning that association. Predator learning is facilitated by simple patterns with low variability, and yet many aposmatic species exhibit high within-population variation in their colour patterns. The maintenance of such variation has been suggested to be the result of the interaction between sexual and natural selection, but other selective forces that might be operating are largely unexplored. Here I evaluate the consequences of within-population variability in aposmatic signals in a natural population of the aposmatic frog Dendrobates tinctorius. I found that colour patterns differ between the sexes, and that there is differential microhabitat use associated with colour patterns. I argue that parental care can work synergistically with aposmatism to select for yellower males and that differential microhabitat use could reflect differences in conspicuousness among the different colour patterns.

Introduction
Aposematism is an anti-predator strategy by which some animals warn their predators about their unprofitability with conspicuous colours or patterns (Poulton 1890; Ruxton et al. 2004). Because variation in aposmatic signals makes it difficult for predators to learn and retain the association between colour patterns and distastefulness, warning signals are expected to be simple and uniform (Endler 1988; Joron & Mallet 1998; Endler & Mappes 2004; Darst et al. 2006). However, variation in aposmatic species occurs in many different taxa such as moths (Nokelainen et al. 2012), ladybirds (O’Donald & Majerus 1984; Ueno et al. 1998), butterflies (Mallet & Joron 1999) and frogs (Myers & Daly 1983; Wollenberg et al. 2008), suggesting that signal
variation may serve other purposes or respond to different selective pressures. Here I explore the function of variation in aposematic species.

Recent studies have suggested that an interaction between natural and sexual selection might be responsible for colour pattern variation in some aposematic species. Different components of sexual selection (intra-sexual competition, female choice, etc.) might select for individuals with certain patterns to become more attractive to conspecifics (Ueno et al. 1996; Maan & Cummings 2009; Nokelainen et al. 2012), possibly leading to sexual dimorphism in the aposematic signals (Maan & Cummings 2009). This trend can be balanced out by natural selection by allowing for individuals with certain colour patterns to avoid predation more successfully than others (Nokelainen et al. 2012) thus aiding in the maintenance of aposematic polymorphisms.

Because variation in aposematic signals might also imply differences in conspicuousness among the morphs, it would be expected that individuals suffered morph-specific attack rates (Endler 1988, 1991a); therefore, aposematism as an antipredator strategy might be less effective for individuals with some colour patterns than for others. In order to offset this disadvantage, the ‘least protected’ individuals might benefit from compensatory behaviours that provide them with extra protection. These differences in colour patterns could lead, for example, to differential microhabitat use. In such case, regardless of the availability of diverse niches for all types of colour patterns, only individuals with certain colour patterns would be favoured in one specific microhabitat (Gray & McKinnon 2007) either by increased conspicuousness or because it offers the best hiding or escaping opportunities (i.e. less exposed).

Field studies offer an unusual opportunity to investigate the link between the ecology of a species and the evolutionary processes it has undergone. Here I evaluate the direct consequences in the wild of intra-populational variability in aposematic signals. I studied a natural population of the aposematic, highly polymorphic poison frog
*Dendrobates tinctorius* to test the hypotheses that (1) males would benefit from more conspicuous aposmatic signals (sexual dimorphism) given their prolonged exposure to predators due to parental duties and that (2) there is colour-pattern mediated use of microhabitats as a way to compensate for variation in aposmatic signals and sexual dimorphism in predator exposure.

**Methods**

*Study species and study site*

*Dendrobates tinctorius* is one of the largest species of the Neotropical family Dendrobatidae, with a body length (from snout to end of the urostyle; hence referred to as SUL) that ranges between 37 and 53 mm in adult individuals (this study) at the study site.  

![Colour pattern variation in Dendrobates tinctorius at the study site.](image)

It is distributed along the Eastern Guiana Shield and is associated to canopy gaps in primary forests between 0 and 600 m.a.s.l. (Noonan & Gaucher 2005; Wollenberg et al. 2006; Löfters et al. 2007; Born et al. 2010). The population density at the study site is about 4.3 individuals/100m² (Devillechabrolle 2011). Like most dendrobatid species, *D. tinctorius* is diurnal and exhibits an elaborated parental care that consists of clutch attendance and tadpole transport, both performed by males (Löfters et al. 2007; pers. observ.). In contrast to all other dendrobatids, males of this species seldom vocalise during courtship, and when they do they produce very soft calls, usually when the
courting female is out of their sight (Rojas, unpubl. data). \textit{D. tinctorius} has skin toxins (Summers & Clough 2001) and bright colour patterns that seem to signal unprofitability to potential aerial predators according to field experiments with plasticine models (Noonan & Comeault 2009; Comeault & Noonan 2011). These colour patterns vary significantly within (pers. observ.; Fig. 2.1) and among populations (Wollenberg et al. 2008).

This study was done at a lowland forest next to Camp Pararé, Les Nouragues Reserve, French Guiana (3°59’N, 52°35’W, approximately 120 m.a.s.l.), over three field seasons between January and February 2009, January and March 2010, and January and June 2010. These months of the year correspond to part of the breeding season of the species. Data on microhabitat use were collected only during 2010.

\textit{Sexual dimorphism in colour patterns}

At the beginning of the study gender was identified on the basis of behaviour (i.e. individuals vocalising were recognised as males) during courtship. Both courting individuals were caught when possible and their snout-urostyle length and disc width (third finger of the left hand) were measured. These data (from 36 males and 36 females) were used to construct a gender index from a discriminant function analysis so that all subsequent individuals could be assigned to male or female on the basis of their measurements (100% of individuals in the training set were classified correctly).

Frogs were found during daily surveys of a 1.5 Km transect. Upon capture, every frog (N=321) was photographed against graph paper for size calibration. Snout-urostyle length and disc size were measured on the photos using the software ImageJ. The back of each frog was cropped out from the photographs for subsequent analyses of colour patterns with a new method (Endler in press) that uses transects across colour patterns,
and allows the estimation of parameters like pattern complexity, pattern elongation, and proportion of a particular colour based on the number of transitions between adjacent colours (in this case yellow and black). These analyses were done with the software MATLAB. Besides the parameters mentioned above, I recorded the number of yellow patches and the number of interruptions of yellow in the back (modified from Wollenberg et al. 2008), and calculated a value of dorsal contrast by multiplying the proportion of yellow x the proportion of black; a larger product means more contrast since if either colour is rarer the pattern has less contrast than if both are equally frequent. I estimated the rank proportion of blue and yellow covering both the arms and the hind limbs by choosing by eye one of five values between 0 (no blue/yellow at all) to 1 (completely blue/yellow) representing the approximate proportion covered with each colour (0, 0.25, 0.5, 0.75, and 1). Ranks were used instead of direct measurements because arms and legs are difficult to photograph in a standard way. Because ventral patterns were not as variable as dorsal ones, I took ventral photographs of approximately of 1/3 (N=115) of the individuals used for dorsal pattern analysis in order to estimate the coloured proportion of the ventral side (blue in most cases) and, more specifically, of the throat region. The presence of shadows and the variable position of the frogs in the ventral photos made it impossible to use the automated method for measuring colour proportions. Therefore the rank proportion of ventral coloration was estimated in the same way as that of the limbs. The proportion of colour for the throat was estimated with the software ImageJ, and the throat contrast was calculated in the same way as dorsal contrast.

With the exception of pattern elongation none of the parameters of colour patterns were normally distributed even after transformations, so I used non-parametric analyses (Mann-Whitney) in order to test for differences in colour patterns characteristics between
<table>
<thead>
<tr>
<th>Variable</th>
<th>Population</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snout-urostyle length (mm)</td>
<td>43.32 ± 1.97 (35.48 -)</td>
<td>40.89 ± 1.97 (35.48 -)</td>
<td>46.19 ± 2.55 (36.96 -)</td>
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<tr>
<td>N=311</td>
<td>44.66</td>
<td>44.66</td>
<td>52.54</td>
</tr>
<tr>
<td>Disc size (mm)</td>
<td>2.49 ± 0.41 (1.52 -)</td>
<td>2.81 ± 0.24 (2.02 -)</td>
<td>2.11 ± 0.20 (1.52 -)</td>
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<tr>
<td>N=311</td>
<td>3.35</td>
<td>3.35</td>
<td>2.61</td>
</tr>
<tr>
<td>Proportion of arms covered with yellow</td>
<td>0.28 ± 0.33 (0 - 1)</td>
<td>0.24 ± 0.34 (0 - 1)</td>
<td>0.33 ± 0.33 (0 - 1)</td>
</tr>
<tr>
<td>N=320</td>
<td>N=173</td>
<td></td>
<td>N=147</td>
</tr>
<tr>
<td>Proportion of arms covered with blue</td>
<td>0.37 ± 0.38 (0 - 1)</td>
<td>0.37 ± 0.38 (0 - 1)</td>
<td>0.30 ± 0.36 (0 - 1)</td>
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<tr>
<td>N=320</td>
<td>N=173</td>
<td></td>
<td>N=147</td>
</tr>
<tr>
<td>Proportion of legs covered with yellow</td>
<td>0.02 ± 0.08 (0 - 1)</td>
<td>0.03 ± 0.09 (0 - 1)</td>
<td>0.02 ± 0.07 (0 - 1)</td>
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<td>N=173</td>
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<tr>
<td>Proportion of legs covered with blue</td>
<td>0.49 ± 0.25 (0 - 1)</td>
<td>0.50 ± 0.24 (0 - 1)</td>
<td>0.47 ± 0.25 (0 - 1)</td>
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<tr>
<td>Number of yellow patches in the back</td>
<td>1.65 ± 1.10 (1 - 7)</td>
<td>1.62 ± 0.98 (1 - 6)</td>
<td>1.69 ± 1.23 (1 - 7)</td>
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<td>Number of dark patches within yellow</td>
<td>0.97 ± 0.64 (0 - 3)</td>
<td>0.95 ± 0.63 (0 - 2)</td>
<td>0.99 ± 0.66 (0 - 3)</td>
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<tr>
<td>Number of interruptions of dorsal yellow</td>
<td>1.08 - 1.44 (0 - 7)</td>
<td>1.12 ± 1.31 (0 - 6)</td>
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<td>N=174</td>
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<tr>
<td>Proportion of dorsal yellow</td>
<td>0.30 ± 0.09 (0.07 -)</td>
<td>0.32 ± 0.08 (0.09 -)</td>
<td>0.29 ± 0.09 (0.07 -)</td>
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<tr>
<td>N=321</td>
<td>0.60</td>
<td>0.60</td>
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<tr>
<td>Pattern elongation</td>
<td>2.46 ± 0.37 (1.51 -)</td>
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<td>2.45 ± 0.36 (1.60 -)</td>
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<td>N=321</td>
<td>3.62</td>
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<td>Pattern complexity</td>
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<td>N=321</td>
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<td>Dorsal contrast</td>
<td>0.20 ± 0.03 (0.06 -)</td>
<td>0.21 ± 0.03 (0.08 -)</td>
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<td>N=321</td>
<td>0.25</td>
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<tr>
<td>Proportion of ventral</td>
<td>0.46 ± 0.22 (0 - 1)</td>
<td>0.49 ± 0.20 (0.25 -)</td>
<td>0.42 ± 0.24 (0 - 1)</td>
</tr>
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</table>
the sexes. Pattern elongation was compared between males and females by means of a one-way Anova.

**Microhabitat use**

During the field season of 2010 each of 103 captured frogs was assigned to one of four microhabitats according to where they were first seen: leaf litter (when frogs were on a relatively open patch of leaf litter without any obvious structure in a 1m radius), fallen logs (when frogs were visibly exposed on top of the log), fallen branches (when individuals were in fallen tree crowns) and tree/palm roots (when the frogs were within the exposed roots or next to them, or inside hollow trunks).

**Figure 2.2** Scatterplots showing the relationships among colour pattern characteristics. See Table 2.2 for details on statistics.
Table 2.2. Non-parametric correlation matrix for body size and colour pattern characteristics. [SUL (snout-urostyle length), pYA (proportion of arms covered with yellow), pYB (proportion of arms covered with blue), nY (number of yellow patches in the back), nDP (number of dark patches within yellow), nLY (number of interruptions of yellow), pVC (coloured proportion of the ventral side), pDY (proportion of dorsal yellow), PC (pattern complexity), PE (pattern elongation), DC (dorsal contrast), pYL (proportion of legs covered with yellow) and pBL (proportion of legs covered with blue)]. Values given are Spearman rho and sample size (in brackets). Values in bold letters denote significant relationships at the 0.05 (*) or 0.01(~~) significance level.

<table>
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<td>pBA</td>
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<tr>
<td>nY</td>
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<td>-0.462**</td>
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<td>nDP</td>
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<td>-0.567**</td>
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<td>0.032</td>
<td>0.879**</td>
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<td>pVC</td>
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<td>-0.273**</td>
<td>0.238**</td>
<td>-0.233**</td>
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<td>-0.476**</td>
<td>0.476**</td>
<td>-0.515**</td>
<td>0.280**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>-0.115*</td>
<td>0.031</td>
<td>0.007</td>
<td>-0.052</td>
<td>0.216**</td>
<td>-0.019</td>
<td>0.127</td>
<td>-0.080</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE</td>
<td>-0.035</td>
<td>-0.109</td>
<td>0.139**</td>
<td>-0.344**</td>
<td>-0.018</td>
<td>-0.361**</td>
<td>0.040</td>
<td>0.022</td>
<td>0.204**</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DC</td>
<td>-0.080</td>
<td>0.322**</td>
<td>-0.125*</td>
<td>-0.477**</td>
<td>0.477**</td>
<td>-0.515**</td>
<td>0.276**</td>
<td>1.000**</td>
<td>-0.081</td>
<td>0.024</td>
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</tr>
<tr>
<td>pYL</td>
<td>-0.044</td>
<td>0.457**</td>
<td>-0.286**</td>
<td>-0.175**</td>
<td>0.162**</td>
<td>-0.172**</td>
<td>-0.041</td>
<td>0.239**</td>
<td>-0.021</td>
<td>0.095</td>
<td>0.388**</td>
<td>1.000</td>
</tr>
<tr>
<td>pBL</td>
<td>-0.079</td>
<td>-0.414**</td>
<td>0.575**</td>
<td>-0.164**</td>
<td>0.081</td>
<td>-0.125**</td>
<td>0.458**</td>
<td>0.018</td>
<td>0.038</td>
<td>0.131*</td>
<td>0.017</td>
<td>-0.389**</td>
</tr>
</tbody>
</table>

Frogs were only included in the analyses once (recaptures of the same individual were excluded in order to avoid pseudoreplication).

I tested for differences in microhabitat use between the sexes by means of a Chi-Square test, and for the relationship between colour patterns and microhabitat use by means of Manova. All statistical analyses were done with the software SPSS 19.0 for Mac.
Results

*Intra-populational variation in colour patterns*

There is much variation in the characteristics of colour patterns studied in this population (Fig. 2.1; see Table 2.1 for descriptive statistics). In general, the background dorsal colour is black, with between one and seven yellow patches that might be uninterrupted and take between 7% and 60% of the dorsal area. As opposed to some other populations where individuals have a completely yellow back (Lötters et al. 2007; Noonan & Comeault 2009), in the study population even the yellower individuals have a black patch within the yellow area, and some individuals have two or three. The limbs have also a black reticulated pattern with blue or a few scattered yellow patches. The ventral side has most often a reticulated pattern of black and blue, but can be almost entirely black. Aside from pattern complexity, none of the characteristics of the colour patterns measured was correlated with body size (Table 2.2). Table 2.2 provides a summary of the significant correlations among the colour pattern characteristics measured. Individuals with yellower arms have a larger proportion of dorsal yellow (Fig. 2.2a) distributed in fewer patches. Individuals with bluer arms have less yellow in their back (Fig. 2.2b), more elongated patterns and a larger proportion of their

<p>| Table 2.3. Differences between the sexes in colour pattern characteristics. Values in bold letters denote significant relationships at the 0.05 (*) or 0.01(**) significance level after the corresponding Sequential Bonferroni correction (Rice 1989) |</p>
<table>
<thead>
<tr>
<th>Variable</th>
<th>U or F</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of arms covered with yellow</td>
<td>10623.0</td>
<td>320</td>
</tr>
<tr>
<td>Proportion of arms covered with blue</td>
<td>15009.0**</td>
<td>320</td>
</tr>
<tr>
<td>Proportion of legs covered with yellow</td>
<td>13097.0</td>
<td>320</td>
</tr>
<tr>
<td>Proportion of legs covered with blue</td>
<td>13640.5</td>
<td>320</td>
</tr>
<tr>
<td>Number of yellow patches in the back</td>
<td>13094.5</td>
<td>320</td>
</tr>
<tr>
<td>Number of dark patches within yellow</td>
<td>12468.0</td>
<td>320</td>
</tr>
<tr>
<td>Number of interruptions of dorsal yellow</td>
<td>14202.0</td>
<td>320</td>
</tr>
<tr>
<td>Proportion of dorsal yellow</td>
<td>15723.5**</td>
<td>321</td>
</tr>
<tr>
<td>Pattern elongation</td>
<td>F(3,320)=0.025</td>
<td>321</td>
</tr>
<tr>
<td>Pattern complexity</td>
<td>13134.0</td>
<td>321</td>
</tr>
<tr>
<td>Dorsal contrast</td>
<td>15717.5**</td>
<td>321</td>
</tr>
<tr>
<td>Proportion of ventral side coloured</td>
<td>10623.0</td>
<td>115</td>
</tr>
<tr>
<td>Proportion of throat coloured</td>
<td>F(1,115)=1.865</td>
<td>115</td>
</tr>
<tr>
<td>Throat contrast</td>
<td>2166.0</td>
<td>115</td>
</tr>
</tbody>
</table>
ventral side coloured (Fig. 2.2c).

Pattern elongation is positively correlated with pattern complexity (Fig 2.2d), and negatively correlated with the number of interruptions of yellow (Fig. 2.2e). Individuals with dark patches within the yellow area have a higher contrast (Fig. 2.2f) and a higher pattern complexity (Fig. 2.2g), whereas those individuals with the largest number of interruptions of yellow had the lowest dorsal contrast (Fig. 2.2h).

Sexual dimorphism

Males are smaller than females (Table 2.1) and can be distinguished from the latter on the basis of a combination of body size and the size of their finger discs. Males have wider discs in proportion to their body size than females (Ancova: SVL: $F_{(1,310)}=75.57, p<0.001$; SVL*Sex: $F_{(1,310)}=4.84, p=0.029$, Sex: $F_{(1,310)}=0.014$, NS; Fig. 2.3a). According to the discriminant analyses, based on SUL (Discriminant function coefficient = -0.884) and disc size (Discriminant function coefficient = 0.985), 100% of the individuals were classified correctly for sex (Canonical correlation=0.932, Wilks’ Lambda=0.132, $\chi^2=621.28$, df=2, $p<0.001$; Fig. 2.3b), indicating that gender can be identified reliably from morphometrics.
Figure 2.4 Differences in colour pattern characteristics between males and females

Table 2.3), but both dorsal (Fig. 2.4b) and throat contrast (Fig. 2.4c) were more variable in females than in males (Dorsal: Levene statistic $= 10.071, p=0.002$; Throat: Levene statistic $= 5.916, p=0.016$). The coloration of the arms also differs significantly between the sexes; females have yellower arms whereas males tend to have bluer arms (Table 2.1; Table 2.3; Fig. 2.4d). The differences in throat contrast and proportion of arms covered with yellow do not hold after a sequential Bonferroni correction.

There are no differences between the sexes in pattern complexity, pattern elongation, number of interruptions of yellow patches, coloration of the hind limbs and the coloured (blue or yellow) proportion of the ventral side (Table 2.3). Males have a significantly larger proportion of dorsal yellow (Table 2.1; Table 2.3; Fig. 2.4a) and a higher dorsal and throat contrast (Table 2.1; Table 2.3).
Colour patterns and microhabitat use

More individuals, regardless of the sex, were found in the leaf litter ($\chi^2=31.796$, $df=3$, $p<0.001$, $N=103$; Fig. 2.5). There are no differences between the sexes in microhabitat use ($\chi^2=6.496$, $df=3$, NS, $N=103$). Individuals found in roots had significantly simpler patterns than individuals found in fallen branches, but not in other types of microhabitat (MANOVA on microhabitat: Pillai’s trace $F_{5,103}=1.419$; Wilk’s Lambda $F_{5,103}=1.423$, $p=0.18$ in both cases; Proportion of yellow $F_{5,103}=1.51$, NS; Pattern elongation $F_{5,103}=0.56$, NS; Pattern complexity $F_{5,103}=2.776$, $p=0.045$; Tukey HSD Mean difference = 0.098, $p=0.038$). There were no differences among microhabitats in any other aspect of colour patterns.

Discussion

The selective pressures that lead to the origin and maintenance of variation in aposematic signals are still poorly understood. Dendrobates tinctorius shows sexual dimorphism in colour patterns and the colour pattern variation is associated with differential microhabitat use. This suggests that selection pressures for aposematism differ among microhabitats and also between the sexes. Aposematism does not necessarily work the same way in all habitats and both sexes.

![Figure 2.6 Box plots showing the distribution of values of pattern complexity among four different microhabitats. Letters above the boxes represent significant differences among groups.](image-url)
Sexual selection may affect the sexes of aposmotic species differently. For example, in the poison frog *Oophaga pumilio* sexual dimorphism in brightness could be a consequence of sexual selection via female choice, given that females prefer brighter males (Maan & Cummings 2009), suggesting that the correlated response to selection across the sexes is small. Sexual dimorphism in the bright coloration of *Papilio* butterflies, on the other hand, seems most likely to be the result of natural selection for the warning coloration of females (Kunte 2008) which, according to Wallace (1889; cited in Kunte 2008), are more vulnerable to predation because of the weight of their eggs and their less effective escape flight. In my study species (*D. tinctorius*) there is an advantage for females in being yellower; yellower females, but not males, are more frequently engaged in courtship (Chapter 3). Thus, it seems unlikely that the male-biased sexual dimorphism in colouration in this species is the result of directional sexual selection via female choice. It is more likely due to differential natural selection for protective colouration of males. Males are likely to experience more predation because they provide parental care, and while they transport the tadpoles they are exposed more frequently and for a longer time than females. There is evidence in other species that an increase in aposmotic brightness enhances predator learning (Prudic et al. 2007), and that changes in colour (hue) may cause concomitant changes in brightness (Maan & Cummings 2009), so males could benefit from being yellower in order to quickly educate their predators and protect not only themselves, but also their offspring. Males may have yellower backs than females as a result of a synergy between sexual selection in the form of parental care and natural selection in the form of enhanced aposematism. To my knowledge, this is the first study to consider the role of parental care as a selective force affecting the way in which aposematism works in a polymorphic species.

Individuals of *D. tinctorius* make differential use of four available microhabitats depending on their colour patterns. Habitat segregation according to colour patterns is
known to occur in non-aposematic species of different taxa (Endler 1984; Morey 1990; Sandoval 1994; Merilaita & Jormalainen 1997; Ahnesjo & Forsman 2006; Capula et al. 2009) where individuals tend to select the microhabitat in which they are least visible in order to avoid differential predation risk. If an aposematic species is variable, then each colour form should select the microhabitat or visual background that maximizes its conspicuousness. Alternatively, individuals with patterns that are predicted to be, in general, duller or more difficult for predators to learn or remember could be associated to microhabitats that convey a higher protection in terms of hiding or escaping possibilities (i.e. lower exposure). A trend for higher exposure linked to higher conspicuousness has been reported for Oophaga pumilio, a species that exhibits great geographic variation in colour patterns. Males of the most conspicuous populations tend to choose more exposed perches for vocalisation than their less conspicuous counterparts (Pröhl & Ostrowski 2011; Rudh et al. 2011).

There are intrinsic differences in the protection that individual D. tinctorius can get from each of the four microhabitats considered in this study. Individuals in open patches of leaf litter are more exposed than individuals moving around fallen branches or tree roots, therefore I would have expected duller and more complex-pattemed individuals to be associated more often with roots and branches, where they would be less exposed. These results do not support my predictions completely. Although individuals with more complex patterns are more often found in fallen branches as predicted, individuals with simpler colour patterns (which are supposed to be learnt and remembered more easily by visual predators) are found most frequently associated with roots. Such association could mean that individuals with simpler patterns achieve higher levels of conspicuousness in that particular habitat, but that hypothesis requires further testing.

We must not assume that aposematism functions the same way for individuals with different colour patterns in all habitats and both sexes. This study suggests that the
attempts to understand the maintenance of aposematic signal variability must consider selective forces other than predation and mate choice as the only active component of sexual selection. Parental care, as a component of sexual selection, could work in synergy with aposematism to select for differences in colour patterns between the sexes. Additionally, colour-pattern mediated differences in aspects of the behaviour and ecology of polymorphic aposematic species such as escape behaviour, microhabitat selection, activity and the willingness to take advantage of ecological opportunities might work jointly to allow for the existence and maintenance of intra-populational variation in aposematic colour patterns.
Chapter 3

BLONDES HAVE MORE FUN: MATING PATTERNS IN A NATURAL POPULATION OF THE APOSEMATIC, COLOUR PATTERN POLYMORPHIC FROG DENDROBATES TINCTORIUS

Abstract. Aposematism is an anti-predator strategy that combines conspicuous colouration with unpalatability. This strategy favours signal uniformity in order to enhance predator learning, but signal variability could promote the attractiveness of some forms over others, suggesting an interaction of natural and sexual selection in the evolution of aposmatic signals. Positive assortative mating might aid in the maintenance of geographic variants within a species, whereas negative assortative mating can maintain intraspecific variation within populations. I studied a population of an aposmatic frog with highly variable aposmatic signals to test the potential role of sexual selection in the maintenance of such variation by investigating whether individuals mated disassortatively and whether individuals of certain colour patterns had any advantages over others. There is no evidence of negative (or positive) assortative mating, but yellower females are more often engaged in courtship. I suggest that other components of sexual selection besides mating preferences, as well as ecological and behavioural characteristics of the species need to be considered in order to understand how variability in aposmatic signals is maintained.

Introduction

Aposematism is a strategy to deter predators by combining warning signals with unpalatability (reviewed in Ruxton et al. 2004). Because predators learn the association between the signals and the noxiousness of the prey, and subsequently avoid them, aposmatic signals are likely to evolve under natural selection (Endler 1991a; Endler & Mappes 2004). Although there is robust evidence supporting this, recent approaches have demonstrated that not only natural, but also sexual selection play a relevant role in the evolution of aposmatic signals (Maan & Cummings 2008, 2009; Melo et al. 2009; Nokelainen et al. 2012).
The interplay between natural and sexual selection in the evolution of aposomatic signals is particularly interesting in species in which the variability of the signal challenges the uniformity assumption of aposematism. Whilst a non-variable signal within a population is expected in order to favour predator avoidance learning (Endler 1988; Joron & Mallet 1998; Mallet & Joron 1999; Endler & Mappes 2004; Darst et al. 2006; Exnerová et al. 2006; Mallet 2010), a variable signal among individuals—without losing its conspicuous nature—could be associated with variable attractiveness among individuals (Ueno et al. 1998; Maan & Cummings 2009; Nokelainen et al. 2012). For this reason, aposmatic species with a high within-population phenotypic variability (i.e., polymorphic) are excellent models to test how both natural and sexual selection affect the evolution and design of warning signals, specifically as a result of frequency-dependent selection or assortative mating.

Recent studies have shown that geographic variability in the aposmatic signals of the strawberry poison frog, Oophaga pumilio, is the result of the combined action of natural and sexual selection. Predators avoid warningly coloured plasticine models in the field (Saporito et al. 2007b; Hegna et al. 2011) and experiments in controlled setups have shown that not only females prefer to mate assortatively with males of their own morph (Summers et al. 1999a; Reynolds & Fitzpatrick 2007; Maan & Cummings 2008) but also prefer overall males with brighter colouration (Maan & Cummings 2009). These studies have contributed largely to our understanding of the existence and maintenance of the intraspecific variability in aposmatic signals of parapatric or allopatric populations, but to date no studies have focused on elucidating whether this occurs where significant variability in aposmatic colour patterns occurs within populations rather than in narrow zones between parapatric geographic variants. Here I consider a natural population of the aposmatic, polymorphic dart poison frog Dendrobates tinctorius to test in the field whether the extensive within population variation in its colour patterns can be explained
by the occurrence of negative assortative mating, and whether there is any indication that individuals with certain colour patterns have a mating advantage over others. Unlike *O. pumilio*, which is only marginally polymorphic in a narrow zone of overlap between parapatric forms, *D. tinctorius* shows extensive within-population colour pattern variation over a fraction of its geographic range. Given that aposmatic theory predicts that aposmatic species should be monomorphic, this system requires understanding of the factors favouring both aposmatism and polymorphism.

**Methods**

**Study species and study site**

*D. tinctorius* is a diurnal, large (37-53mm; Chapter 2) frog of the neotropical family Dendrobatidae which exhibits striking within- and among-population variation in colour patterns (Löfters et al. 2007; Wollenberg et al. 2008). Although extremely variable in colour patterns within the population studied, *D. tinctorius* does not exhibit a classical polymorphism (*sensu* Ford 1945) because there are no large gaps among the colour variants (Fig. 3.1). It inhabits canopy gaps in primary forests in the Eastern Guiana Shield from sea level up to 600 m (Noonan & Gaucher 2006; Wollenberg et al. 2006; Born et al. 2010). Like all other
Dendrobates, it has toxic alkaloids in its skin (Summers & Clough 2001), and its bright colours appear to signal unpalatability to potential aerial predators, which avoid attacking clay models with their colours (Noonan & Comeault 2009; Comeault & Noonan 2011). In the field pairs lay clutches of 4-5 eggs that hatch after approximately two weeks (pers. observ.). Tadpoles are then carried by the male, one or two at a time, to tree holes or palm bracts at variable heights (P. Gaucher, pers. comm.; Rojas, unpubl. data). Courtship consists of lengthy paired movements by male and female throughout the forest floor alternated with an exchange of visual signals and tactile interactions (Fig. 3.2).

Courtship can be initiated by females, which play a very active role in the whole process by frequently stroking the male’s back, head and limbs until eggs are laid. As opposed to most Dendrobatids, males of D. tinctorius seldom vocalise during courtship. When they do they produce very soft calls, most of the times when the courting female is out of their sight (pers. observ).

This study was conducted in a lowland forest in the vicinity of Camp Pararé, Les Nouragues Reserve, French Guiana (3°50’N, 52°35’W, approximately 120 m.a.s.l.), where there is a fairly large population of Dendrobates tinctorius (approx. 4.3 individuals/100m², Devillechaabrolle 2011). Data collection took place over three field seasons (9 January to 20 February 2009, 16 January to 20 March 2010, and 17 January to 8 June 2011), during the breeding season of this species.

Data collection

Courting pairs were found during daily walks over a 1.5 Km transect that was surveyed twice a day (approximately 2.5 hours in each direction). Two individuals were
considered to be in courtship when they were less than 1m apart (as in Pröhl 2002) and one was clearly following and stroking the other (pers. observ.) for at least 15 min. A 15 min waiting time was chosen on the basis of the time threshold for choice in previous studies of mate choice and assortative mating in captive Dendrobatids (Maan & Cummings 2008, 2009). Pairs were followed for as long as they were visible, or until oviposition took place, and both males and females were captured (when possible) when they were about to disperse. Upon capture, each individual was photographed with a digital camera both dorsally and ventrally against graph paper for scale calibration, and subsequently released exactly where captured.

**Colour pattern analyses**

Using each dorsal photograph I measured the snout-urostyle length (SUL) to the nearest mm, and the total dorsal area, yellow dorsal area, head area, and yellow area in the head to the nearest mm². 

![Figure 3.3 Variables measured in the image analyses of the colour patterns of Dendrobates tinctorius (see text for details)](image)

From those measurements, a proportion of dorsal, rear and head yellow was calculated. I calculated the dorsal and head contrast by multiplying the proportion of yellow times the proportion of black in each area, and used the perimeter/area ratio of the yellow dorsal region in order to calculate a value of ‘pattern complexity’. I also recorded the number of uninterrupted yellow patches in the back, the presence/absence and number
of dark patches within the dorsal yellow region, the presence/absence and number of interruptions in the yellow region in both back and head, and the presence/absence of a ‘tail’ in the pattern (Fig. 3.3). Some of these variables were modified from those used in a previous study on the geographic variation in colour patterns of this species (Wollenberg et al. 2008).

Because the value of proportion of yellow gives information only about the total amount of yellow in the back of an individual, but not about how the yellow is distributed, I measured the length of the yellow patches in three different longitudinal transects of the frog’s body (left, centre and right). For each transect I also measured its width and then calculated a variable called ‘elongation’ by dividing length by width (Fig. 3.3). If the yellow on the back is homogenously distributed, there should be no differences between the elongation values for these three transects.

All these variables give information about the frog colouration from a dorsal perspective. However, for the purposes of intraspecific communication the individual’s frontal appearance can be as important (or more) as the dorsal one. Therefore, I also calculated a value of ‘frontal yellowness’ by combining in one variable the proportion of yellow in the head with the amount of yellow in the forelimbs, which are clearly visible when the frogs are facing each other. The chest and throat region can be visible too in the latter context, but they were not considered in the calculations of ‘frontal yellowness’ as they very rarely have yellow. Because of the difficulty of standard photography of the limbs, their amount of yellow and blue was ranked by eye with five discrete values from 0 (no blue/yellow at all) to 1 (completely blue/yellow) representing the approximate proportion of the limbs covered with each colour (0, 0.25, 0.5, 0.75, and 1). Frontal yellowness was thus calculated as $X=\frac{p_{\text{Yellow Forelimbs}}+p_{\text{Yellow Head}}}{2}$, and provided values between 0 (no yellow at all) and 1 (totally yellow).
In the ventral photographs, I estimated the total proportion covered with colour (either blue, or yellow or both, no blue dorsally) using the same rank proportional values used for the colour coverage in the limbs. I measured the throat area and amount of blue (and yellow, when that was the case) to the nearest mm² in order to calculate the coloured proportion of the throat. All the pattern analyses were done with the software ImageJ v. 1.44.

Statistical analyses

In order to test for assortative mating with respect to colour patterns, I ran analyses where the pair (and not the individual) was the statistical unit. I used parametric correlations to compare continuous variables for members of courtship pairs and of random pairs separately; I used chi-square tests for comparing categorical variables between the same two groups. I ran a second analysis with all individuals as statistical units in order to test whether all the males and females found in courtship differed from those taken from a random sample in their colour patterns; this included some individuals not included in the pairwise analysis because the other member of the pair was not caught or measured. By means of a Manova I tested for the effect of sex, courtship status (either courting pairs or random pairs), and the interaction between sex and courtship status on the continuous variables. I used non-parametric (Mann-Whitney) tests when

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<tr>
<td>Rear yellow</td>
<td>F(1,119)</td>
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<td>5.550*</td>
</tr>
<tr>
<td>Pattern complexity</td>
<td>F(1,119)</td>
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</tr>
<tr>
<td>Pattern elongation</td>
<td>F(1,119)</td>
<td>2.036</td>
<td>0.001</td>
</tr>
</tbody>
</table>
variables did not meet the assumptions of normality and homoscedasticity even after being transformed. All statistical analyses were done with SPSS 19.0 for Mac.

Results

Fourty-seven pairs were found in courtship during the study (10 in 2009, 14 in 2010 and 23 in 2011), consisting of 40 different males and 39 different females courting different individuals between 1 and 3 times (Females: MEAN±SD=1.20±0.47 courtships; Males: MEAN±SD=1.17±0.45 courtships). Nine pairs were not included in the assortative mating analysis because at least one of the individuals in the pair was not captured and eight additional pairs were excluded because at least one of the individuals had been seen in courtship with a different individual before (we scored only the first observed mating), leaving 30 pairs suitable for the analyses. Those pairs were compared to 23
pairs taken randomly from all frogs recorded but not seen to mate. Thirty-five males and 35 females found in courtship, and 27 males and 22 females chosen randomly were included in the analysis of individuals.

Pairs found in courtship were not different from pairs taken randomly with respect to the colour patterns. There is no evidence of assortative mating based on yellowness (Dorsal: Courting pairs Pearson r = 0.230, NS, N=30; Random pairs Pearson r = -0.119, NS, N=23; the correlation coefficients are not significantly different: z=1.20, p>0.05; Fig.3.4a; Frontal: Courting pairs Pearson r = 0.233, NS, N=30; Random pairs Pearson r = -0.050, NS, N=23; the correlation coefficients are not significantly different: z=0.97, p>0.05 Fig. 3.4b) or on any other characteristic of the patterns.

![Box plot showing variation in frontal yellowness between males and females](image)

**Figure 3.6** Variation in frontal yellowness (transformed as Log(x) (frontal yellowness)) between males and females of *D. tinctorius* found in courtship, and those in pairs formed randomly.

When considered individually and not in pairs, neither males nor females found in courtship differ from individuals drawn from a random sample in terms of pattern complexity, average elongation of the yellow patches, proportion of dorsal yellow or proportion of yellow in the head region (Table 1). There were no differences in visual contrast (Head+Throat contrast: Males: Mann-Whitney U=360.0, NS, N=60; Females: Mann-Whitney U=367.0, NS, N=53; Dorsal contrast: Males: Mann-Whitney U=437.0, NS, N=62; Females: Mann-Whitney U=426.0, NS, N=57) or proportion of throat coloured (Males: Mann-Whitney U=360.0, NS, N=60; Females: Mann-Whitney U=296.0, NS, N=53).
Females found in courtship have dorsal dark patches surrounded by yellow significantly more often than females taken from a random sample ($\chi^2=6.700$, df=1, p=0.010, N=57; Fig.3.5 Left). Males found in courtship, on the other hand, have dark patches surrounded by yellow as often as males taken from a random sample ($\chi^2=1.796$, df=1, NS, N = 62; Fig.3.5 Right). Similarly, females in courtship seem to have primarily a single, uninterrupted yellow patch ($\chi^2=6.263$, df=2, p=0.044, N=57), whereas males in both categories seem to have the same number of yellow patches in their dorsal patterns ($\chi^2=0.840$, df=2, NS, N=62).

Courting females have significantly higher values of frontal yellowness (MEAN ± SD=0.52±0.21) than both random females (MEAN ± SD=0.40±0.24) and males from both categories (Random: MEAN ± SD=0.42±0.23; In courtship: MEAN ± SD=0.36±0.21; Table 1; Fig. 3.6). Frontal yellowness also seems to be a good predictor of courtship success (number of courtships) for females (Linear regression: F=10.466, p=0.002, N=49), but not for males (Linear regression: F=0.111, NS, N=47; Fig. 3.7). No other variable was related with courtship success for either sex. Males in general have significantly greater values of rear yellow (MEAN ± SD=0.24±0.07) than females in both categories (MEAN ± SD=0.21±0.06; ANOVA Sex: $F_{(1,119)}=5.550$, p=0.020; Courtship Status: $F_{(1,119)}=0.241$, NS; Sex*Courtship Status: $F_{(1,119)}=0.114$, NS). Size was not related to the proportion of yellow calculated for any region, in any sex (Table 2).
Discussion

This study shows that being yellower represents an advantage for female *D. tinctorius*. Females with a yellower front (not back; Fig 3.8) not only are found in courtship more often than duller females, but also seem to have a higher courtship success. However, I found no evidence of either positive or negative assortative mating for colour patterns, suggesting that the mating system is not the selective factor maintaining the colour pattern polymorphism, unless the advantage of yellow in females is exactly balanced by other forms of selection against yellow in females.

<table>
<thead>
<tr>
<th></th>
<th>MALES</th>
<th>FEMALES</th>
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<tbody>
<tr>
<td>Frontal yellowness</td>
<td>0.085</td>
<td>0.035</td>
</tr>
<tr>
<td>Head yellow</td>
<td>0.142</td>
<td>0.117</td>
</tr>
<tr>
<td>Dorso yellow</td>
<td>0.141</td>
<td>0.127</td>
</tr>
<tr>
<td>Rear yellow</td>
<td>0.125</td>
<td>0.134</td>
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</table>

Table 3.2. Parametric correlations for SUL and proportion of yellow in different regions of the body for males (N=62) and females (N=57). The values provided are Pearson correlation coefficients \(r\). No significant relations were found.

It has been argued that aposmatic polymorphisms might originate and be maintained as a result of the interplay between natural and sexual selection (Maan & Cummings 2009; Nokelainen et al. 2012). Wood tiger moths, for example, are aposmatic and males of the species can be either white or yellow. Yellow males seem to be better protected against predators, whereas white males have a higher mating success (Nokelainen et al. 2012); the mating advantage of being white is balanced out by the advantage of being yellow in order to avoid predation. I have shown an advantage of being a yellower female in *D. tinctorius* but there is no evidence that yellow individuals pay a higher cost in terms of higher predation attempts, except when they have been

Figure 3.8 Differences in frontal yellowness among females
tested as a novel form in a population where all individuals have yellow stripes on a black background and blue limbs (Comeault & Noonan 2011).

One interesting aspect of these findings is that it is females, and not males, who have a 'yellow advantage'. A possible explanation could be that males, and not females, choose their mates in this species, therefore exhibiting some degree of sex role reversal. The occurrence of sex-role reversal is widespread in the animal kingdom with well-known examples in spiders, insects, fish and birds (Trivers 1972; Andersson 1994; Eens & Pinxten 2000). Summers (1989) studied the possibility of a reversal of sex roles in D. auratus, a species closely related to D. tinctorius, in which males provide all parental care and females compete with other females for access to mates. His study failed at finding evidence of a sex role reversal in mating because even though male D. auratus provide parental care, females were choosier than males about mates, and female-female competition was as common as male-male competition. However, Summers' study did not take into account intrinsic characteristics of the individuals; as opposed to D. tinctorius, D. auratus' colouration is rather homogeneous at the population that he studied, with at least no visible sex dimorphism in colouration (pers. observ.). In D. tinctorius males provide parental care in the form of clutch attendance and tadpole transport, female-female aggression occurs in the wild, and I have found that there is sexual dimorphism in yellowness. This combination of life-history, behavioural and phenotypical characteristics seems well suited for the occurrence of a sex role reversal. On-going molecular analyses linked with studies about the spatial distribution of males and females in the field will provide valuable insights about the mating system in this species.

On the other hand, the yellow advantage might just be the result of better visibility. A male that enters the territory of a female might be more likely to find and approach her if she is more visible. Preliminary observations suggest that females can
spend a considerable amount of time staying motionless on a spot (MEAN ± SD= 79.12±15.73 % of the time during 2-hour observation periods, N=10; Rojas and Devillechabrolle, unpubl. data). In these cases, their posture is head up and arms stretched. In other words, their ‘frontal view’ is very conspicuous, especially when it has a high yellow coverage. These females could be easier to find by males and as a consequence be more frequently engaged in courtship than darker, less conspicuous females. However, it is not known whether males generally look for females rather than the other way around, or whether there is mutual search, and I found no indication that males differ from females in movement patterns (Chapter 4).

In species with known (or potential) aerial predators dorsal coloration might be subject to natural selection. Limb and ventral coloration, on the other hand, might be subject to selection due to predation by ground predators such as crabs or spiders. Ventral or limb coloration, could also be subject to sexual selection (Siddiqi et al. 2004; Maan & Cummings 2008) especially if during the process of pair formation the colouration of these regions is more visible than the dorsal one or if the use of visual signals is more important than auditory or other sensory modes, as in D. tinctorius (pers. observ.). However, since the individuals follow each other for a considerable amount of time (up to seven hours, pers. observ.) while courting and searching for a suitable place for oviposition, the dorsal colour patterns may also be important in courtship because they are visible to the frog which follows. Females in courtship often have a dark patch surrounded by yellow in their back, which is in turn confined mostly to a single, uninterrupted yellow patch. When courting individuals follow each other, they need to have a way to find and be found easily. If the dark patch in the surrounding yellow is located in the point of rotation of the frog’s body as it moves, it may be difficult to be tracked in the forest. Interestingly, the analyses of videos of eight pairs in courtship (Rojas, unpubl. data) revealed that in 75% of the cases (between 11% and 100% of the
filming time) it was the female who predominantly followed the male (MEAN±SD=76.4%±34.9). Although the sample size is too small for tests, it is interesting that females, which would be potentially more difficult to track by males than the other way around, spend more time following than being followed. This is consistent with my finding that males in general seem to be yellower in the rear part of their back, which is visible to females while following them. This region of the body can also be seen by potential aerial predators and, given that more conspicuous signals enhance predator learning (Ruxton et al. 2004), males could benefit from having a yellower rear back by deterring attacks while transporting tadpoles to suitable bodies of water, a task that implies long displacements and prolonged exposure. Future research should aim at evaluating the trackability of different patterns by both conspecifics and potential predators in a complex environment such as the habitat of dyeing poison frogs.

These results do not provide any evidence for a role of sexual selection via assortative mating on the maintenance of the great phenotypic variation in the population studied. The lack of assortative mating in a polymorphic aposematic species has been previously reported for soldier beetles, *Chauliognathus fallax* (Machado & de Araujo 2003), leaf beetles, *Chrysophtharta agricola* (Nahrung & Allen 2005), and ladybirds, *Harmonia axyridis* (Ueno et al. 1998). Interestingly, in the latter case the authors also found that despite of the randomness of matings, non-melanic males engaged in mating were significantly larger than solitary ones (Ueno et al. 1998). In contrast, my study does not support previous findings about the positive assortative mating by colour in the strawberry poison frog *Oophaga pumilio* (Summers et al. 1999a; Reynolds & Fitzpatrick 2007; Maan & Cummings 2008). However, those studies are not directly comparable with mine for three reasons. Firstly, because they have been carried out in different conditions (laboratory vs. field). Secondly, because they have been mostly focused on the phenotypic divergence among different allopatric or parapatric populations spread
over a small geographic scale in a group of islands (the Bocas del Toro archipelago in Panama) that separated as recently as 1000-10000 years ago (Wang & Shaffer 2008; Brown et al. 2010a); sympatry of colour forms in Bocas del Toro is limited in the number of morphs as well as being in a very small part of the geographic range. Finally, while positive assortative mating might aid in the maintenance of intra-specific geographic variants and even lead to speciation in the presence of genetic flow (Kondrashov & Shpak 1998; Maan et al. 2004; Puebla et al. 2007; Terai & Okada 2011; Puebla et al. 2012), negative assortative mating can maintain different phenotypes within a population (Roulin 2004; Follett et al. 2007; Takahashi & Hori 2008; Hancox et al. 2010). Furthermore, *O. pumilio* and *D. tinctorius* species differ in their ecology, parental behaviour and habitat use (Brust 1987; Pröhl & Hödl 1999; Prohl & Berke 2001; Pröhl & Berke 2001; Born et al. 2010), and these differences may well underlie differences in the way in which sexual selection affects their aposematic signals (Chapter 2, Chapter 4, Chapter 5 and Chapter 6).

In the absence of negative assortative mating for colour patterns and the existence of an advantage of yellower females over duller ones, the mechanism(s) that maintain the diversity in the aposematic signals of *D. tinctorius* is still unclear. One possibility is that they are subject to frequency-dependent predation, i.e. the yellower forms are also differentially preyed upon. Recent studies with different populations of *D. tinctorius* have shown that novel forms in a population are selected against by more frequent predator attacks (Noonan & Comeault 2009) and that stabilising selection aids in the maintenance of a local signal in sites where density of individuals displaying that signal is high, but not when the density is low (Comeault & Noonan 2011). This supports the idea that predators’ ability to learn the association between a warning signal and prey unpalatability is maintained when prey densities are above a certain threshold (Endler & Mappes 2004; Endler & Rojas 2009), but further research is needed to prove the
hypothesis of frequency-dependent predation of yellower individuals at the study site, where the signals are naturally variable.

It is unlikely that the variation in colour patterns in the population studied is the product of genetic drift given the mating advantage of yellower females over duller ones, and the fact that recent molecular analyses using a neutral marker suggest that the colour patterns in *Dendrobates tinctorius* are a composite trait in which some components (dorsal and flanks colouration) have evolved under selection (Wollenberg et al. 2006). Environmental factors like differences in light environment may also promote diversity in the warning signals, leading to differential microhabitat use by the different morphs. This hypothesis remains to be tested. Finally, I cannot rule out the possibility that, as suggested by Mallet (2010), the diversity in aposematic signals may be explained by their recent origin and rapid evolution so that they have not had the time to achieve the equilibrium where there is only one single aposematic form as predicted by theory.
Chapter 4

COLOUR PATTERN GEOMETRY IS ASSOCIATED WITH DIFFERENCES IN MOVEMENT IN AN APOSEMATIC FROG

Abstract. Aposematism is an anti-predator strategy involving the use of conspicuous colour patterns to advertise unprofitability. Aposematic colour patterns are expected to have low variation in order to enhance predator learning, but variable aposematic signals are found in many species. Previous studies have revealed a link between types of movement and colour pattern geometry in non-aposematic snakes. Striped patterns generate a visual illusion of reduced speed making it difficult for predators to follow their trajectory, but this has not been examined in aposematic species. Moreover, neither this nor any other questions aimed at understanding the maintenance of aposematic signal variability have been addressed in natural populations. Here I make use of the extensive colour pattern variation in the aposematic poison frog *Dendrobates tinctorius* to test in the wild whether the variation in the geometry of its aposematic signals is associated with differences in movement in a natural population. Individuals that move most often continuously in one direction have more elongated colour patterns than individuals that move randomly, suggesting that polymorphic aposematic species may also benefit from differences in behaviour as an additional strategy to avoid potential visual predators. These results highlight the importance of considering the interaction between behaviour and aposematic signal variation in future attempts to understand the origin and maintenance of aposematic polymorphisms.

Introduction

Aposematism is an anti-predator strategy by which animals use warning colour patterns to inform predators about their unprofitability (Poulton 1890; Ruxton et al. 2004). Because the success of this strategy depends on predator learning and remembering the relationship, selection should favour animals whose colour patterns are simple and have little variation (Endler 1988; Joron & Mallet 1996; Endler & Mappes 2004; Darst & Cummings 2006) because they consequently are easy to learn (Endler & Mappes 2004; Ruxton et al. 2004; Mappes et al. 2005) and more memorable (Speed 2000). However, variation in aposematic signals is present in many taxa, both at the
inter- and intra-population levels (Myers & Daly 1983; O’Donnell & Majerus 1984; Ueno et al. 1998; Mallet & Joron 1999; Wollenberg et al. 2008; Nokelainen et al. 2012). Despite recent studies suggesting an interaction between natural and sexual selection in the origin and maintenance of geographically variable aposmatic signals (Maan & Cummings 2009; Nokelainen et al. 2012), the mechanisms by which this variation is maintained, and its ecological and behavioural correlates, are still poorly understood.

Previous studies on non-aposmatic snakes have shown that certain colour patterns seem to be more effective when accompanied by matched escape behaviours (Jackson et al. 1976; Brodie 1989; Brodie 1992). Individuals with striped patterns benefit from fleeing as their anti-predator defence because the pattern appears to remain stationary or move more slowly than the escaping animal (Jackson et al. 1976; Brodie 1992), whereas individuals with banded, broken or spotted patterns tend to stay motionless and change direction during flight (Jackson et al. 1976; Brodie 1989).

Aposmatic species rely on their unprofitability to deter predators once colour patterns have been learned. However, learning and remembering becomes difficult if aposmatic colour patterns vary within populations. In variable aposmatic species, individuals with certain colour patterns may benefit from adopting pattern-specific behaviour, which enhances the signal under pattern- and behaviour-specific conditions. I tested this hypothesis in a natural population by extensive mapping the movement trajectories of 39 individuals of the aposmatic frog *Dendrobates tinctorius*, a species with an extensive inter (Wollenberg et al. 2008)- and intra-populational colour pattern variation (pers. observ.;

![Figure 4.1 Colour pattern variation in *Dendrobates tinctorius*. A typical elongated pattern (a) and an interrupted one (b).](image)
Methods

Study species and study site

*Dendrobates tinctorius* is a diurnal, large frog of the family Dendrobatidae that occurs associated to canopy gaps of primary forests in the Eastern Guiana Shield (Noonan & Gaucher 2006). It exhibits striking variation in colour patterns (Wollenberg et al. 2008), has skin toxins (Summers & Clough 2001), and field experiments with plasticine models suggest that birds are among its major predators (Noonan & Comeault 2009; Comeault & Noonan 2011). This study was carried out at Camp Pararé, Les Nouragues Reserve, French Guiana (3°59'N, 52°35'W, approximately 120 m.a.s.l.), between February and July 2011.

Frog trajectories

Thirty-nine individuals, 25 females and 14 males, were followed, each for two continuous hours. I chose this duration based on preliminary observations that individuals can remain motionless on the same spot for almost one hour. I marked with a flag every place at which each frog stopped after moving, and counted the number of foraging attempts during the whole session. After the 2-hour period of observation, I measured the distance and angle between pairs of flags (segments), and estimated the total linear distance travelled (distance between starting and end points), the linear speed, and the length of the path travelled by each individual by adding all segment lengths. I calculated the mean angle and angular distance of displacement, and ran Rayleigh tests (Zar 1996) for each individual in order to know whether their movement was random or directional. With this information I created a new categorical variable,
'directionality'. A frog was classified as 'directional' if the Rayleigh test was significant, and 'random' if not. Individuals that moved uniformly/randomly according to the Rayleigh test were assigned 0, whereas the individuals that moved directionally according to the Rayleigh test were assigned 1.

**Colour pattern analysis**

Each frog was photographed against graph paper for size calibration, and its colour patterns were analysed by means of a new method (Endler In press) that uses transects across colour patterns, and allows the estimation of different geometric parameters based on the distribution of the numbers of transitions between adjacent colours (in this case yellow and black). The parameters I used were pattern simplicity (calculated as the mean distance between colour transitions both longitudinally and transversally), pattern elongation (estimated from the ratio of transition densities along and perpendicular to the body axis) and proportion of yellow. All pattern analyses were done with MATLAB. I also recorded for each frog the number of interruptions of yellow in their back.

**Statistical analyses**

Both colour pattern and trajectory variables were tested for normality (Shapiro-Wilks tests with p>0.05). Variables that did not meet this assumption were log-transformed. One individual was excluded from the analyses because it was identified as an extreme outlier for the number of segments. Colour pattern geometry variables (pattern elongation, pattern simplicity and proportion of yellow) were compared between the two groups of directionality by means of a Manova, or Mann-Whitney tests when the variables violated the normality assumption even after transformation. The same
statistical analyses were performed to compare the characteristics of individual trajectories (number of segments, mean and maximum segment length, and linear speed) between the two groups. All tests were two-tailed, and the alpha level was 0.05 in all cases. Statistical analyses were done with the software SPSS 19.0 for Mac.

Results

I identified two groups among the individuals studied: 36% of the individuals showed consistent directional movement, whereas 64% moved randomly. There were no differences between males and females in movement patterns (X² = 0.986, df = 1, two-tailed p > 0.05). Individuals that move directionally have trajectories with longer segments and a higher linear speed, while individuals that tend to move randomly move over shorter segments at a slower speed (MANOVA on directional /random movement Pillai’s trace = 0.819, F₁,₁₉₅ = 37.445, p < 0.001, Wilks’ lambda = 0.181, F₁,₁₉₅ = 37.445, p < 0.001; number of segments F₁,₁₉₅ = 1.793, p > 0.05; log mean segment length F₁,₁₉₅ = 24.720, p < 0.001; log maximum segment length F₁,₁₉₅ = 22.001, p < 0.001; linear speed F₁,₁₉₅ = 84.125, p < 0.001; Fig. 4.2).

There are significant differences in colour pattern geometry between the two movement groups. Individuals that move directionally have more elongated patterns (MANOVA on directionality: Pillai’s trace = 0.187, F₂,₂₆₆ = 4.144, p = 0.024, Wilks’ lambda =
0.813, F(2,36)=4.144, p=0.024; pattern elongation F(1,36)=4.876, p=0.034; pattern simplicity F(1,36)=1.268, p>0.05; Fig. 4.1a, 4.3a), while individuals that tend to move randomly have more interruptions in their yellow patches (Fig. 4.1b; Mann-Whitney U=68.0, two-tailed p=0.002, N=39; Fig. 4.3b). Pattern elongation is significantly negatively correlated with the number of interruptions of yellow (Spearman’s rho= -0.489, two-tailed p=0.002, N=39).

Discussion

These results suggest that polymorphic aposematic species could use distinct alternative behavioural strategies to mitigate the disadvantages of variable colour patterns. Within a single population, frogs that move continuously in a given direction rather than randomly have more elongated patterns. Such patterns can create a visual illusion of a static pattern or a pattern with a greatly reduced speed that affects predators’ abilities to track the trajectory of moving individuals in order to predict their attack angle (Jackson et al. 1976; Stevens 2007). This effect might be more pronounced when movements occur at a higher speed (Stevens et al. 2008) and over longer segments (Sherratt et al. 2004). Frogs that move randomly, with unpredictable changes of direction with every movement, have more interrupted patterns and move at a lower speed over shorter segments. Interrupted patterns may be visually disruptive.
(Jackson et al. 1976), and the combination of disruptive patterns and slower movements might be advantageous for the avoidance of motion-oriented predators (Hatle et al. 2002), and related to a more cryptic anti-predator behaviour (Wells 2007). The use of computer games with human ‘predators’ has yielded similar results, showing that moving targets with stripes running along the movement axis (elongated) are missed more often than targets with other types of patterns or pattern-less (Stevens et al. 2011), and that simulated prey items that move over longer segments are more difficult to catch (Sherratt et al. 2004).

We need to discard the assumption that aposematism works in the same way for monomorphic and polymorphic species, or even different morphs of the same species. Polymorphic species face predator learning and memory disadvantages that might be compensated with behavioural and morphological mechanisms. It is possible that the evolutionary forces that gave rise to aposematic polymorphisms are more intricate than it has been acknowledged to date, and that individual escape strategies may be as important as predation and sexual selection as a factor allowing for the simultaneous occurrence of different aposematic signals within populations. This study reveals that behaviour can interact with aposematic colour pattern in similar ways that they do in non-aposematic species, and highlights the importance of considering behaviour-pattern interactions in future attempts to understand the great diversity in the aposematic colour patterns in nature.
Chapter 5

JUMPING IN THE SPOTLIGHT: TREE-FALL GAP INVASIONS ARE LED BY INDIVIDUALS WITH SIMPLER APOSEMATIC COLOUR PATTERNS

Abstract. Many species use conspicuous coloration to warn potential predators that they are toxic or unprofitable. In spite of many theoretical and laboratory studies showing that such coloration must be simple enough to be easily learned and remembered the direct consequences of simplicity in the wild are unknown. Here I take advantage of the extensive colour pattern variation in a natural population of an aposematic poison frog, Dendrobates tinctorius, to investigate the effect of simplicity in relation to tree-fall gap colonisation. These frogs invade canopy gaps as soon as the trees fall to exploit foraging and reproductive opportunities, thus exposing themselves to naïve predators also drawn to the newly created habitat by feeding opportunities. I show that the first frogs that invade fresh treefalls have simpler colour patterns than frogs from a random sample of the population, reinforcing the idea that simpler patterns work better at training naïve predators or that individuals with certain patterns are more willing to exploit new resources. This also highlights the importance of considering anti-predator defences in processes of biological invasions.

Introduction

Some animal species advertise their unpalatability with conspicuous colour patterns (Ruxton et al. 2004; Mappes et al. 2005). The success of this aposematic strategy relies on predators learning and remembering the association between warning colours and distastefulness, so that the frequency of attacks to aposematic prey rapidly decreases (Endler 1991a; Speed 2000; Ruxton et al. 2004). Simpler colour patterns enhance predator learning and delay forgetting (Endler & Mappes 2004; Ruxton et al. 2004), and therefore individuals exhibiting them are expected to be favoured by selection. Although demonstrated in the laboratory, the consequences of simplicity have never been verified in a natural ecological context (Stevens & Ruxton 2012).
Habitat disturbance can favour new adaptations (Sousa 1984), and the resulting new environments often attract species which benefit from the changes (Sousa 1984; White & Jentsch 2001). Invaders can have different properties than their source populations (Shine et al. 2011), and may affect community structure and ecosystem functioning in the long term (Strayer 2012). Invasion and colonization of forest tree-fall gaps by plant and animal species have been well documented, especially in gaps older than six months and in terms of species richness. However, the phenotypic, ecological and behavioural characteristics, as well as the reasons behind the differential arrival of invaders, have been neglected, particularly in the context of anti-predator defences.

Contrary to what is expected for most aposematic species, the poison frog *Dendrobates tinctorius* displays significant colour pattern variation both within (Fig. 5.1) and between populations (Wollenberg et al. 2008). This species is known to invade tree-fall gaps (Born et al. 2010) and field experiments with plasticine models indicate bird predation (Noonan & Comeault 2009; Comeault & Noonan 2011). The opening of new gaps soon attracts birds and other predators, which are likely to be canopy species that have not been exposed to the terrestrial *D. tinctorius*. As individuals attempting to invade new niches can be more susceptible to predation (Mayr 1963), the initial predation rate on the invading frogs is likely to be higher than it would be after a few weeks. Therefore selection should favour immediate gap invasion by individuals whose patterns educate predators faster and are more memorable than those of later arrivals, in a form of spatial sorting (Shine et al. 2011). This could be achieved by
displaying colour patterns of lower complexity (i.e. simpler) (Endler & Mappes 2004; Ruxton et al. 2004). I tested this prediction by monitoring nine natural tree-fall gaps and their invasion by adult *D. tinctorius* within three days of their formation, and comparing three characteristics of the colour patterns of individuals (simplicity, elongation and yellowness; Fig. 5.2) from a random sample of the population with those of early invaders of tree-fall gaps.

**Methods**

*D. tinctorius* (Dendrobatidae) is a neotropical frog with remarkable within (Fig. 5.1)- and among (Wollenberg et al. 2008)- population variation in colour patterns. It occurs in primary forests in the Eastern Guiana Shield (Noonan & Gaucher 2006; Wollenberg et al. 2008; Born et al. 2010), has skin toxins (Summers & Clough 2001) and its colours signal unpalatability to potential predators (Noonan & Comeault 2009; Comeault & Noonan 2011). This study was done at Camp Pararé, Les Nouragues, French Guiana (3°59′N, 52°35′W), where *D. tinctorius* is abundant (4.3 individuals/100sq-m (Devillechabrolle 2011)), over two field seasons (February 2009 and January-June 2011).

Treefalls (one in 2009, eight in 2011) were discovered rapidly because they occurred in a 1.5 Km transect surveyed daily. I inspected treefalls within their first 24 hours and caught as many individuals as possible (N=140 from nine treefalls), moving fallen branches until no frogs were seen (after 3-4 hours). During the next two consecutive days I carefully looked for new frogs over a similar period of time (2-4h); most of the individuals seen then had been found on the first day. When frogs were seen
but not caught, I photographed them from a distance to record their colour pattern (which is individually unique and unchanging) for further identification upon capture. Frogs representing a random sample of the population (never seen in treefalls; \(N=130\)) were found during thorough surveys along our study transect.

Each captured frog was photographed and its colour patterns analysed with a new procedure (Endler In press) in which transects are taken across the frog’s surface, and the number of colour transitions per transect are recorded. Pattern simplicity is estimated by the mean distance between colour transitions, and pattern elongation by the ratio of transition densities along and perpendicular to the body axis (Endler In press). The effects of ‘presence in treefall’, sex, and the interaction between the two on colour patterns were tested by means of a MANOVA with SPSS 19.0 for Mac. All data were normal after suitable transformations.

Results and Discussion

Males were as likely as females to arrive in the nine fresh gaps studied within three days of a treefall (\(X^2=10.70, \text{df}=8, p > 0.05, N=140\)). The mechanism by which these frogs detect treefalls is unknown, but the vibration produced as the tree hits the ground may be a cue because some frogs detect vibrational signals from conspecifics (Lewis & Narins 1985), heterospecifics (Warkentin et al. 2007) and abiotic factors like rain (Caldwell et al. 2010b), and those three kinds of signals are much weaker than those produced by a tree when it falls. Early invaders of treefalls have simpler patterns than a random sample of individuals of the same study population that were never found in a tree-fall gap (MANOVA on ‘presence in treefall’: Pillai’s trace = 0.035, \(F_{(3,269)}=3.190, p=0.024\); pattern elongation \(F_{(1,269)}=0.510, p>0.05\); pattern simplicity \(F_{(1,269)}=14.960, p<0.01\); yellowness \(F_{(1,269)}=0.008, p>0.05\); Fig. 5.3). These results suggest that
aposematic pattern design could play a role in the individual willingness to exploit new resources (Speed et al. 2010) or that pattern simplicity may facilitate predator learning (Endler & Mappes 2004; Ruxton et al. 2004). Both factors may be operating in this predator-mediated spatial sorting (Shine et al. 2011).

The difference in pattern simplicity between invaders and the general population was greater in females (MANOVA on ‘presence in treefall’sex’: Pillai’s trace = 0.024, \( F_{(0,209)}=2.161, p=0.093 \); pattern elongation \( F_{(1,209)}=0.198, p>0.05 \); pattern simplicity \( F_{(1,209)}=5.338, p=0.012 \); yellowness \( F_{(1,209)}=-0.347, p>0.05 \); Fig 3). A possible explanation for this trend is that females and males had different reasons for invading tree-fall gaps, so that the costs of having slightly more complex patterns for one sex are compensated by other type of benefits. Females might be only attracted to a gap for food. Male \( D. \ tinctorius \), in contrast, transport their newly hatched tadpoles to phytotelmata (water pockets in plants; Rojas in prep.), which are a limited resource in the forest (Donnelly 1989a; Fincke 1992); failure to do so would result in death of their offspring. Therefore, the cost/benefit ratios might differ between the sexes.

Tadpoles, seen from above on a male’s back, might appear as black spots with an elongated end and have the potential to alter the male’s appearance. However, I did not find differences in pattern simplicity in a sub sample of 14 males for which I had photos with and without tadpoles (One-way Anova: \( F_{(1,28)}=2.903, p >0.05 \)). Therefore, it seems that the benefits of securing a place for their offspring to complete their development might outweigh the risks of males exposing themselves to inexperienced predators in fresh gaps, even if their colour patterns are slightly more complex than desirable for initial encounters with predators. This finding supports the idea that animals are able to make decisions that entail trade-offs between their own risk of predation and the benefits of carrying out a particular activity (Lima & Dill 1990), i.e. invading a ‘new’ environment in search of suitable places for their offspring’s development and survival.
A possible explanation for the match between behaviour and colour pattern could be correlational selection, a type of selection which favours specific combinations of two traits expressed simultaneously in a given individual without necessarily altering the distribution of each trait on its own (Endler 1986; Brodie 1992). In this case of tree-fall gap invasion, correlational selection could favour genotypes with the appropriate link between colour pattern and behavior and, thus, generate linkage disequilibrium between them (Endler 1986; Sinervo & Svensson 2002). Most often correlational selection results from frequency-dependent interactions like those between predators and prey or parasites and hosts (Sinervo & Svensson 2002). Predation, for example, has been suggested as the force generating correlational selection between the colour patterns and escape behaviour of some non-aposematic snakes (Brodie 1992). I consider that predation is also likely to play a role in the differential invasion of treefalls by D. tinctorius because very first predatory gap invaders are likely to be canopy species. Gap specialists, which feed mostly on fruits and nectar, usually arrive at much later successional stages, when fruits and flowers first become available (Levey 1988).

I provide unique empirical evidence from a wild population that the nature of aposematism might be affected by the ecological opportunities that arise from being able to move freely around a habitat or exploit new resources (Speed et al. 2010). Two other co-occurring inconspicuous poison frog species (Allobates femoralis and
*Amerega hahnelli* that would benefit from the resources that become available in fresh
tree-fall gaps were never seen or heard in the first days or even weeks after the
occurrence of a tree-fall (pers. observ.). Lacking the advantages of conspicuous
colouration may constrain their opportunities of exploiting new resources because they
may be at much higher risk than aposematic *Dendrobates tinctorius*. This is likely to
apply not only for cryptic vs. aposematic species, but also for the less favoured
individuals (i.e. with more complex patterns) of species with a great intra-populational
variation in colour patterns. Our results highlight the importance of considering anti-
predator defences, and their ecological and behavioural correlates, in processes of
spatial sorting and biological invasions in order to understand the reasons behind
sequential arrivals and community structure at later successional stages.
Chapter 6

IMMEDIATE INVASION OF TREE-FALL GAPS BY THE POISON FROG

DENDROBATES TINCTORIUS: A CASE OF RESPONSIBLE PARENTHOOD

Abstract. Habitat disturbances trigger adaptations in the life histories of the organisms affected. Tree-fall gaps are small-scale disturbances that offer resources previously unavailable to different plant and animal species. Their formation, colonisation and role in forest dynamics is well documented, whereas little is known about their effects on animal ecology apart from studies of gaps 6+ months old, focusing on their species richness compared to closed forest. I studied the immediate invasion of six natural tree-fall gaps by the frog Dendrobates tinctorius to test experimentally the hypothesis that males arrive in search of new tree-holes for tadpole deposition. I found that the latency to occupation of new pools and the rate of tadpole deposition in fresh gaps were shorter and higher, respectively, than in the closed forest, thus proving the importance of rearing sites in tree-fall invasion and offering novel insights about the role of tree-hole breeders in structuring gap communities.

Introduction

The habitat of a particular species can be disturbed in many ways, and these have the potential to act as an evolutionary force triggering adaptations in the life histories of the organisms affected (White & Jentsch 2001). Apart from man-caused phenomena, there are several natural disturbances at a large scale such as fires, floods, landslides and windstorms. However, habitat disturbance may also occur at a smaller scale, but have still a great impact in a particular ecosystem (Sousa 1984; White & Jentsch 2001). Such is the case of the gaps formed in forests when a tree falls or is snapped, or when its larger branches fall (van der Meer & Bongers 1996; Chao et al. 2009; Schlemann & Bockheim 2011). The new light environment favours the germination of buried seeds and the colonisation by pioneer plant species (Connell 1989; Schupp et al. 1989), and the trees or tree parts that fall on the ground (coarse wood debris), offer a whole new range of food items, shelter, perches for display and other
kinds of resources that were previously unavailable to different animal species (Blake & Hoppes 1986; Bouget & Duelli 2004). Plants in gaps, for example, tend to produce more fruit and more nutritious leaves than conspecifics in closed forest, which in turn explains why gaps are often invaded by fruit-eating birds and leaf or bark eating insects (Sousa 1984; Levey 1988). Reptiles take advantage of the increased temperatures to improve their physiological performance (Vitt et al. 1998; Sartorius et al. 1999; Greenberg 2001) and some species of ants profit from the low foraging costs of defoliating pioneer plant species (Peñaloza & Farji-Brener 2003). For these and other reasons, many species have evolved adaptations to invading disturbed or early successional habitats (Hill et al. 2001; White & Jentsch 2001).

Despite the broad documentation of natural tree-fall gaps’ formation and colonisation as a relevant phenomenon in forest dynamics (Hartshorn 1980; Sousa 1984; van der Meer & Bongers 1996, 2001), little is known about their direct effects on animal assemblages (Sousa 1984; Shelly 1988). Moreover, with only a few exceptions, most studies on ‘young’ tree-fall gaps up to date have been carried out in gaps between six months and three years old (Blake & Hoppes 1986; Popma & Bongers 1988; Feener & Schupp 1998; Peñaloza & Farji-Brener 2003; Champlin et al. 2009; Fukui et al. 2011), with no information on the immediate (within hours and days) or short-term (within weeks) effects of treefalls. This is presumably due to the unpredictability of their occurrence and to the difficulty of quantifying their effects on mobile versus sessile organisms (Sousa 1984). Among the successful approaches to this question, numerous studies have focused on the differences in species richness and composition in gaps compared to closed forest (Schemske & Brokaw 1981; Shelly 1988; Greenberg 2001; Greenberg & Lanham 2001; Hill et al. 2001; Gorham et al. 2002; Stojny & Hunter 2010), but little or nothing is known about animal breeding adaptations associated with the invasion of fresh tree-fall gaps.
Frogs of the Neotropical family Dendrobatidae (commonly referred to as dart-poison frogs) are diurnal rainforest dwellers with elaborated parental care that consists of clutch attendance and the transport of newly hatched tadpoles to bodies of water in plants (phytotelmata) such as tree-holes, palm bracts, or bromeliad axils where they complete their development until metamorphosis (Weygoldt 1987; Summers 1990; Crump 1996; Pröh & Hödl 1999; Summers & Erm 1999; Summers & McKeon 2004; Brown et al. 2010b). Phytotelmata are a limited resource for animals that breed in them (Donnelly 1999a, b; Fincke 1992). Individuals of the dart-poison frog *Dendrobates tinctorius* are known anecdotally to invade the gaps formed by fallen trees in the forests of French Guiana (Fig. 6.1). According to sporadic observations, up to 40 frogs have been found in a newly formed gap within a couple of days of its formation (Born et al. 2010), some of which are tadpole-carrying males (pers. observ.). I examined eight natural treefall gaps in a healthy, undisturbed forest at Les Nouragues Reserve, French Guiana, within 0.5 and 24 hours of their formation, and monitored six of them for at least one month in order to test the hypothesis that males might go immediately to fresh treefall gaps in search for new phytotelmata for tadpole deposition. If this is the case, then (1) the latency to occupation of new bodies of water in fresh gaps should be considerably shorter than that in the closed forest, and (2) the rate at which tadpoles are deposited in water bodies (i.e., tadpoles deposited per pool per day) within a fresh gap will be considerably higher than that in water bodies in the closed forest. These results
may provide new insights to our understanding of the biology of invasions, the processes related to the colonisation of unoccupied habitats, and the effect of early invaders in the structure of gap communities.

Material and methods

Study species and study site

*D. tinctorius* is a diurnal, large (37-53mm; Rojas unpubl. data) frog of the neotropical family Dendrobatidae which inhabits canopy gaps in primary forests in the Eastern Guiana Shield from sea level up to 600 m (Noonan & Gaucher 2006; Wollenberg et al. 2006; Born et al. 2010). In the field, pairs lay clutches of 4-5 eggs that are looked after by the male and hatch after approximately two weeks (pers. observ.). Tadpoles are then carried by the male, one or two at a time, to phytotelmata such as tree holes or palm bracts at variable heights (P. Gaucher, pers. comm.; Rojas, unpubl. data) where they remain unattended until metamorphosis. This study was carried out at Camp Pararé, Les Nouragues Reserve, French Guiana (3°59’N, 52°35’W), in a lowland forest where there are approximately 4.3 individuals of *Dendrobates tinctorius* per 100m² (Devillechabrolle 2011). Data collection took place between 2 February and 2 June 2011, during the breeding season of the species.

Tree-fall gaps

Treefalls were easily identified as fresh because they occurred over or next to a 1.5 Km longitudinal transect that was surveyed daily. I found eight tree-falls during the study period, two of which were not included in the study because they occurred at the end of the field season and there was no time to collect data from them. Following a tree fall, I inspected it within the first 24 hours and placed either 2 or 3 plastic bowls with 350 ml of
water (N=14 in total) within the newly formed gap, on the fallen tree trunk, limbs, or tangled branches. This is a conservative sampling number because a single fallen tree may have up to 13 visible water-filled tree-holes (pers. observ.). The same kind of bowls had been used for a different experiment involving tadpoles and their use did not show any negative effects on survival (Rojas, unpubl. data). I compared the tadpole deposition activity in tree-fall gaps with places far from treefalls by setting 15 bowls in the closed forest. Because I put bowls in places similar to those that frogs actually used, this test was conservative. The bowls were checked at least five times a week from the moment they were placed and for the duration of the study period (between 3 weeks and 1.5 months) for the presence/absence and number of *D. tinctorius* tadpoles. Seventeen phytotelmata (15 tree-holes and 2 palm bracts; hereon referred altogether to as tree-holes), in the closed forest were also monitored for tadpole deposition as a reference to validate tadpole deposition activity in bowls far from fresh gaps. ‘Latency to occupancy' was defined as the number of days from the day when bowls were placed in the forest until the day when the first tadpole was deposited. ‘Tadpole deposition rate (TDR)’ was defined as the number of tadpoles deposited per bowl, per day.

Data analysis

The validity of the closed canopy bowl locations was tested by comparing their patterns of tadpole deposition (TDR, and maximum and average number of tadpoles)
with those of natural treeholes. I then tested for differences in TDR, maximum (MNT) and average number of tadpoles (ANT), and latency to occupancy between bowls placed in fresh treefall gaps and bowls placed in the closed forest. Comparisons were done by means of Anovas or the equivalent non-parametric tests when data distribution deviated from normal.

Because not all of the tree-falls occurred at the same time, the number of days that each of them was monitored was different. To account for this differences and their effect on the maximum number of tadpoles recorded for each bowl, I re-expressed these numbers as values between 0 (no tadpoles) and 1 (the maximum number of tadpoles recorded in each bowl), and calculated the time it took for each bowl to reach the maximum number of tadpoles. Later I divided the dataset for each bowl in 2 groups: one from the moment of the first tadpole deposition until the maximum number of tadpoles was reached (A), and another from the moment when the maximum until the next minimum number of tadpoles was recorded during the study (B). These two groups give larval number growth and decline rate, respectively. For each group of data per bowl I ran a growth regression ($\ln(Y) = a + bX$) and used the unstandardized coefficient $B$ as an estimation of growth rate ($A$; how fast the maximum number of tadpoles was reached) or decline ($B$; how fast the number of tadpoles reached its minimum) rates. In this way, for each bowl I obtained a value of growth and decline rates. These values were compared between
bowls in the closed forest and bowls in fresh gaps by means of Mann-Whitney tests, as variables did not fit the assumptions of normality even after being transformed. All statistical analyses were done with the software SPSS 19.0 for Mac.

Results

All of the bowls placed in fresh treefall gaps were occupied at least once, whereas two out of 15 (13.3 %) bowls placed in the closed forest were never used by the study species (one of them was used on a regular basis by another species of poison frog, *Dendrobates ventrimaculatus*), and were therefore excluded from the analyses. Nine bowls (five in fresh treefall gaps and two in the closed forest) had tadpoles of other poison frog species: *Allobates femoralis* (N=5), *Ameerega hahneli* (N=1) and *D. ventrimaculatus* (N=3). Among the phytotelmata used by Dendrobates tinctorius, 13 out of 17 (76.5%) had tadpoles of *Allobates femoralis* at some point, whereas two (11.8 %) had tadpoles of *Ameerega hahneli* and two had both eggs and tadpoles of *Rhinella castaneotica*. Both the eggs and tadpoles of *R. castaneotica*, as well as the tadpoles of the other dendrobatid species were readily consumed by the carnivorous tadpoles of *D. tinctorius* (pers. observ.).

The patterns of tadpole deposition in bowls placed in the closed canopy did not differ significantly from those in treeholes within the closed canopy, suggesting that the bowls were placed in locations that the frogs recognised as as equally suitable (Mann-Whitney tests: TDR: U= 167, N=32; Growth rate: U=57.5, N=23; Decline rate: U=50.5, N=18; ANOVAs: MNT: F(1, 32)= 0.04; ANT: F(1, 32)= 0.12; p >0.05 in all cases). Therefore, all subsequent analyses included only comparisons between bowls in tree-falls and bowls in the closed forest. Bowls placed within fresh treefall gaps were occupied significantly sooner (within three days) than bowls in the closed forest (ANOVA: F(1,27)=44.97,
Table 6.1. Descriptive statistics for the parameters measured in bowls placed in the closed forest (CF), bowls placed in fresh treefall gaps (FG), and natural tree-holes (TH) used by *Dendrobates tinctorius*. * See ‘Methods’ section for details on the calculation of these parameters.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pool type</th>
<th>Range</th>
<th>Mean ± SE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to occupancy (d)</td>
<td>CF</td>
<td>5 – 57</td>
<td>24.30 ± 4.90</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>1 – 20</td>
<td>3.57 ± 1.32</td>
<td>14</td>
</tr>
<tr>
<td>Max. number of tadpoles</td>
<td>TH</td>
<td>1 – 10</td>
<td>3.67 ± 0.65</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>0 – 10</td>
<td>3.60 ± 0.82</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>2 – 31</td>
<td>13.71 ± 2.54</td>
<td>14</td>
</tr>
<tr>
<td>Average number of tadpoles</td>
<td>TH</td>
<td>0.10 – 5.30</td>
<td>1.56 ± 0.40</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>0.00 – 5.60</td>
<td>1.36 ± 0.39</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>1.20 – 18.20</td>
<td>6.46 ± 1.32</td>
<td>14</td>
</tr>
<tr>
<td>Tadpole deposition rate</td>
<td>TH</td>
<td>-0.22 – 0.67</td>
<td>0.03 ± 0.07</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>0.00 – 0.22</td>
<td>0.03 ± 0.02</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>0.00 – 2.44</td>
<td>0.81 ± 0.21</td>
<td>12</td>
</tr>
<tr>
<td>Estimated growth rate*</td>
<td>TH</td>
<td>0.00 – 0.11</td>
<td>0.03 ± 0.01</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>0.00 – 0.08</td>
<td>0.03 ± 0.01</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>0.04 – 0.18</td>
<td>0.10 ± 0.01</td>
<td>14</td>
</tr>
<tr>
<td>Estimated decline rate*</td>
<td>TH</td>
<td>-0.33 – 0.00</td>
<td>-0.06 ± 0.03</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>-0.09 – 0.00</td>
<td>-0.03 ± 0.01</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>-0.14 – 0.01</td>
<td>-0.06 ± 0.01</td>
<td>12</td>
</tr>
</tbody>
</table>
tadpoles were deposited at a significantly higher rate in bowls located within fresh gaps than in those located in the closed forest (Mann-Whitney U=171.0, p<0.001, N=27; Table 1; Fig. 6.3). Bowls within fresh gaps also reached a higher maximum (ANOVA: F[1,27] = 19.61, p<0.001) and average (ANOVA: F[1,27] = 27.67, p<0.001) number of tadpoles than bowls in the closed forest (Fig. 6.4).

There was much higher variation in the number of tadpoles over time in the bowls placed in tree-falls than in those in the closed forest (Fig. 6.5). The estimated values of both growth (Mann-Whitney U=153.5, N= 26, p<0.001) and decline rates (Mann-Whitney U= 18.0, N= 20, p=0.02) were significantly higher in bowls placed in tree-falls than in bowls placed in the closed forest (Table 6.1; Fig. 6.6).

Discussion

With a combination of observational and experimental approaches, this study provides evidence on the importance of the availability of rearing sites for the invasion of
fresh treefall gaps by the dart-poison frog *Dendrobates tinctorius*. I found that the latency to occupy new bodies of water placed in fresh gaps is significantly shorter than those in the closed forest, the tadpole deposition rate within a fresh gap is considerably higher than that in water bodies in the closed forest, and both the growth and decline rates of tadpole populations in each pool are significantly higher in fresh gaps than in the closed forest. This experiment has internal validity, as the parameters measured in the bowls placed in the closed forest did not differ from those measured in the tree-holes used by the study species.

In support of my first prediction, most of the bowls placed in fresh gaps were occupied within three days after they were placed (in some cases even on the same day). This is consistent with the fact that some males carrying tadpoles can be seen in the tree fall gap only a few hours after a tree falls (Fig. 6.7). In at least two cases males were found with tadpoles on their back less than three hours after the event were known to live (i.e. had been recaptured in the same area over at least the previous two months) more than 100 m away from the new tree-fall (pers. observ.), which makes it unlikely that they were nearby before the treefall. Also, at least four males were seen next to or going inside the bowls without tadpoles,
and depositing tadpoles a few days later (Fig. 6.8). Unfortunately, nothing is known about the distances that males of this species travel with their tadpoles away from their core or nesting areas before they deposit them in a pool. How frogs are able to find fresh treefall gaps is also unknown, although it has been speculated without any experimental support that the vibration produced by the tree as it hits the ground may play a role (P. Gaucher, pers. comm.). This could be plausible given that frogs are known to be able to detect conspecific seismic signals (Lewis & Narins 1985; Narins 1990; Caldwell et al. 2010a) which are much weaker than the ones that might be produced by a falling tree.

In favour of my second prediction, the number of tadpoles deposited per bowl per day (TDR) in fresh tree-fall gaps was over 20 times that of bowls in the closed forest. The same pattern was found for the estimated value of tadpole population growth. This could be seen both as cause and consequence, since the over use of bowls in tree falls can be a by-product of individuals being more concentrated around treefalls. Higher rates of tadpole deposition have been found for *D. auratus* in locations of higher adult density in Panama (Summers 1990). In the case of *D. tinctorius*, in turn, the possibility exists that adult density is high in treefalls because they are aggregating around a relevant resource, i.e. new tadpole deposition sites. With the exception of two palm bracts on the ground, all of the natural tadpole deposition sites used by *D. tinctorius* were on fallen trees that had between one and 13 visible water-filled holes. This means that the tree-holes in tree-fall gaps keep being used in later successional stages, not only at the beginning, and this might be further shaping the patterns of patchy distribution of this species (Noonan & Gaucher 2006).
There are several reasons why treeholes in a fresh treefall gap could be a valuable resource. Studies comparing the growth rate and development of larval amphibians in ponds with varying canopy cover have demonstrated that primary productivity, quality of detritus and the amount of dissolved oxygen are higher in open canopy ponds, and all these factors contribute to higher growth rates of the tadpoles living in them (Skelly et al. 2002; Schiesari 2006). It is possible that variations in canopy cover might also affect smaller aquatic ecosystems as are found in phytotelmata, rendering higher growth rates in tadpoles reared in treeholes in treefall gaps as opposed to treeholes in the closed forest. Similar trends have been found in the moth *Zunacetha annulata* in which females are adapted to choose placing their eggs in gaps over shrubs in the understory. The growth rates of this moth’s larvae are 37% lower in the understory than in gaps, probably due to the fact that the leaves of host plants in gaps are thicker, and therefore more nutritious, than their counterparts in the understory (Harrison 1987).

It is well known that gaps have warmer temperatures, more sunlight, are subject to a higher wind speed, and experience reduced relative humidity in spite of the high amounts of rain (Vitt et al. 1998; Bouget & Duelli 2004). Although favourable for heliothermic organisms like lizards (Vitt et al. 1998; Sartorius et al. 1999; Greenberg 2001), these conditions do not seem ideal for adult frogs, which may face the risk of increased water loss (Shoemaker 1992; Wells 2007). However, an increased temperature, within the limits imposed by physiology, has been proven to exert positive effects on larval growth rate (Ultsch et al. 1999). Therefore, it may be that male *Dendrobates tinctorius* invade and deposit their tadpoles in fresh gaps because treeholes in freshly fallen trees offer resources that are altogether of better quality than treeholes in the closed forest.

If phytotelmata in fresh gaps indeed are a high quality larval resource for *D. tinctorius*, it would make sense that parents always try to take their tadpoles to tree-
holes. However, a factor which may alter this opportunistic behaviour is that larval cannibalism gives a survival advantage to first arrivals. Tadpoles deposited earlier will be larger than the later arrivals and will be likely to be predators rather than prey. Therefore, selection should favour individuals that deposit their tadpoles there as soon as possible after the tree falls. There is evidence that *D. tinctorius* are cannibalistic (Fig 6.9; B. Rojas, unpubl. data) and that carnivorous diets in dendrobatids and other frog species speed up growth rates (Summers 1999; Alvarez & Nicieza 2002). A tadpole that grows faster not only minimizes the risks of being cannibalised but is also able to leave the tree-hole sooner, diminishing the risks of potential pool desiccation and predation by other organisms like odonate naiads (Fincke 1992). Further research on the actual advantages in terms of life history traits of tadpoles raised in gaps as opposed to tadpoles raised in the closed forest would contribute to refine the proximate and ultimate causes of treefall gap invasion in *D. tinctorius*.

It is also possible that adult frogs invade the gaps because they can find abundant prey and that males find new places for tadpole deposition just as a by-product of their search for food. A previous study showed that adult *D. tinctorius* captured in gaps had a greater variety of prey items in their stomach (Born et al. 2010) which could be a reflection of the greater variety of species in gaps versus closed forest. On the other hand a greater variety of food items may not occur until several months after the tree falls. The body condition of adult *D. tinctorius* in fresh gaps does not differ from that of individuals from a random sample of the population (pers. observ.), so maybe, as suggested by a previous study (Born et al. 2010), it is not the nutritional
value of the food what frogs are after, but other chemical compounds they could get from it, such as precursors of their toxins.

This study provides evidence that the availability of new tadpole rearing sites is a key factor explaining the invasion of tree-fall gaps by *D. tinctorius*. The immediate invasion of treeholes in treefalls is favoured by cannibalism, and by the pressure of competition from both intra- and inter-specific later arrivals. Parents of species that use tree-holes as breeding sites, either for oviposition (such as mosquitoes or damselflies) or larval deposition (such as frogs), face trade-offs between the benefits of a high-quality place for offspring development and the costs of its ‘popularity’ in terms of high predation risks and competition for food. Selection, therefore, should favour early arrivals to newly available breeding sites. Overall, these findings offer elements that broaden our understanding of the invasion processes that take place in disturbed habitats, and highlight the implications of tree-hole breeders in shaping the structure of gap communities at later successional stages.
Chapter 7

THE APPARENT PARADOX OF COLOUR VARIATION IN APOSEOMATIC POISON FROGS

The origin and maintenance of aposmotic polymorphisms is not completely understood (Joron & Mallet 1998). Since the success of aposmatism as an anti-predator strategy relies on predators’ ability to associate colour patterns with unprofitability (Ruxton et al. 2004), aposmotic species are supposed to have simple patterns with minimum variation (Mallet & Joron 1999; Endler & Mappes 2004; Mappes et al. 2005). These assumptions are problematic when signal variability occurs within populations because individuals with different colour patterns are exposed to the same predator communities (Endler & Rojas 2009) and this may reduce the effectiveness of aposmatism when the variation occurs within populations. Intraspecific variation in aposmatic colour patterns occurs in many species (Myers & Daly 1983; O’Donald & Majerus 1984; Ueno et al. 1998; Mallet & Joron 1999; Lötters et al. 2007; Nokelainen et al. 2012). In most cases, variation occurs in the form of geographic variants (Myers & Daly 1976, 1983; Siddiqi et al. 2004), but it has also been documented within populations (Osawa & Nishida 1992; Borer et al. 2010; Nokelainen et al. 2012). Within-population variation presents a real challenge for the assumptions of aposmatism.

It has been argued that a possible explanation for the maintenance of aposmatic polymorphisms is the interaction between predation and sexual selection by mate choice (Maan & Cummings 2008, 2009; Nokelainen et al. 2012). For example, some individuals might be more attractive for conspecifics but less protected from predator attacks and vice versa (Nokelainen et al. 2012). However, in some cases the interaction between natural and sexual selection can be synergistic (Maan & Cummings 2009).

I studied a natural population of an aposmatic frog species with extensive colour
pattern variation in order to investigate the mechanisms by which such variation is maintained in the wild. My work considered the components that have been suggested in previous studies as agents of selection for colour pattern variation, and I also considered other behavioural and ecological traits of the species that could be associated with variation in their aposematic signals. In particular, I examined the relationship between colour pattern variation and sexual dimorphism, microhabitat use, courtship success, movement style, activity and propensity to invade freshly disturbed habitats.

Aposematism works in different ways for males and females

The population studied has remarkable variation in colour patterns. Even though there seem to be discrete morphs given the little or no resemblance among some individuals, colour pattern variation in this population is continuous. In spite of this, there is sexual dimorphism in at least one characteristic of colour patterns: males are overall significantly yellower than females in their back and have also bluer arms (Chapter 2). This result could be explained in the light of what has been found previously for Oophaga pumilio, a species in which at least in some populations males are brighter than females and females prefer to mate with brighter males (Maan & Cummings 2009). However, I found no evidence that yellower males had a mating advantage over duller ones. In fact, I found a mating advantage for yellower females (Chapter 3), suggesting that in D. tinctorius the colouration of males and females could be subject to different selective pressures.

Additional support to the idea of different selective forces affecting males and females differently is provided by the propensity of individuals with simpler colour patterns to invade fresh tree-fall gaps earlier than individuals with complex patterns
(Chapter 5). Simple patterns enhance predator learning and, therefore, individuals with simpler patterns would educate the naïve predators that arrive to disturbed habitats more efficiently than frogs with more complex patterns which arrive later. However, I found that females that arrive early to fresh tree-fall gaps have even simpler colour patterns than males arriving at the same time. This suggests that males and females may have different reasons to go to these gaps, and that even though it would be risky for males with slightly more complex patterns to invade gaps early, they might compensate this risk with other type of benefits.

I showed how the availability of new tadpole deposition sites is a key factor in tree-fall gap invasion by males (Chapter 6). Since males are responsible for the transport of tadpoles to suitable places at which they will complete their development until metamorphosis, ensuring a good rearing site increases the probability of offspring survival, especially given that there are high rates of larval cannibalism. Males that arrive early to a tree-fall gap increase the likelihood of their offspring being predators rather than prey. This supports the idea that animals are able to make decisions involving trade-offs between their own risk of predation and the benefits of carrying out certain activities (Lima & Dill 1990), for example invading a ‘new’ environment that holds valuable reproductive resources.

Overall, these results insinuate that parental care is an important factor when considering how aposematism works differently for males and females. Brighter colours enhance predator learning (Prudic et al. 2007) and brightness can be related to hue (Maan & Cummins 2009), so it is possible that by being yellower males are better protected during their parental duties, which require a prolonged exposure to predators. This is the first study to consider the role of parental care as a selective force affecting the way in which aposematism works in a polymorphic species.
Differences in behavioural strategies and ecological traits reflect variation in aposmatic colour patterns

Just as aposatism should not necessarily be expected to work equally in males and females, it must not be assumed to work in the same way for individuals with different colour patterns. I found that variation in colour patterns is reflected in concomitant behavioural and ecological differences. Individuals with simpler patterns seem to be bolder or more willing to exploit new resources (Chapter 5), and are more often associated to tree roots than to other microhabitats (Chapter 2). Individuals with more elongated patterns, on the other hand, are more active and exhibit directional movement, whereas individuals with interrupted patterns move randomly (Chapter 4).

*Colour pattern variation might explain differential invasion of new niches*

A recent approach to alternative explanations about the origin of aposatism argues that it may have evolved in response to the ecological opportunities that result from the possibility of moving freely around a habitat or exploit new resources thanks to protective colouration (Speed et al. 2010). This could explain why individuals with simpler patterns invade fresh tree-fall gaps earlier, and is congruent with the fact that other co-occurring inconspicuous poison frog species that would benefit from invading tree-fall gaps were not seen or heard soon after their occurrence. Without the advantages granted by conspicuous colouration, the opportunities for such species of exploiting new resources may be limited. Such limitations are likely to apply not only for cryptic species when compared to aposmatic ones, but also for the less protected individuals (in this case, those with more complex patterns) of species with an extensive within-population colour pattern variation, like *D. tinctorius*.

The propensity of *D. tinctorius* to invade fresh tree-fall gaps is a very efficient
strategy to educate predators because frogs can be in densities far above the threshold required for predator learning. For aposematism to work, predators must have enough encounters with the aposematic prey in order to retain the association between conspicuousness and unprofitability (Endler & Rojas 2009). Similarly, predator learning is facilitated when aposematic prey are aggregated (Gamberale & Tullberg 1996, 1998; Riihipi et al. 2001).

*Differential microhabitat use according to colour patterns*

Differences in environmental factors such as light environment or background structure may also favour variation in warning signals, allowing differential microhabitat use by the different morphs. There are two possibilities for differential microhabitat use in a polymorphic aposematic species: either individuals of each morph choose the microhabitat that maximises their conspicuousness (Pröhl & Ostrowski 2011; Rudh et al. 2011), or dull, less protected individuals choose microhabitats that confer extra protection, for example hiding places. I found that individuals with simpler patterns seem to be more often associated to tree roots. This is unexpected considering that individuals with simpler patterns might be better protected by aposematism (simple patterns are easier to learn by predators) and that tree roots seem to be convenient hiding places. It is possible that the conspicuousness of individuals with the simplest patterns is maximised in that particular microhabitat, hence the choice, but such hypothesis requires further testing.

*Activity and movement: the influence of colour pattern variation*

Dart poison frogs are considered active foragers in the spectrum of foraging modes (Pough & Taigen 1990; Pough et al. 1992). They have short limbs, hop or walk,
are chemically defended (have skin toxins), have higher aerobic capacities, are diet specialists and are aposematically coloured (Santos & Cannatella 2011). Just as different species occupy a particular place in this continuum, a similar spectrum of foraging modes with their behavioural, ecological, morphological and physiological correlates can be found within species. Thus, some individuals of a species of active foragers can be more active than others. This seems to be the case of Dendrobates tinctorius. Individuals that covered longer distances and moved over a higher number of segments had the higher number of foraging attempts. Most interestingly, these differences in activity are associated to differences in colour patterns. Individuals with more elongated patterns moved over longer segments and covered longer distances at a higher speed.

Previous studies on cryptic snakes have revealed differences both between and within populations in their escape behaviour (Brodie 1989). Moreover, such differences were coupled to differences in colour patterns (Jackson et al. 1976; Brodie 1992), so that individuals with striped (elongated) patterns flee in a nearly constant direction whereas individuals with a blotchy pattern move randomly in order to confuse predators. These differences have never before been explored in aposematic species. In D. tinctorius I found two distinct groups of individuals in terms of movement style. As in the case of the cryptic snakes, these two groups differ in certain characteristics of their colour patterns: individuals that move in a given direction have more elongated patterns, and move over longer segments at a higher speed; individuals that move randomly, on the other hand, have interrupted patterns and move over shorter segments and more slowly. Striped patterns in movement generate a visual illusion of immobility of reduced speed that alters predators’ ability to track their trajectories (Jackson et al. 1976; Brodie 1992). Hence, when moving along the elongated body axis, such patterns are more difficult to track and capture (Stevens 2007; Stevens et al. 2011), especially if they do so at a high speed (Stevens et al. 2008). Blotchy patterns, in contrast, may appear
disruptive (Jackson et al. 1976) and combined with lower speeds over shorter segments may be an efficient way to distract motion oriented predators (Hatle et al. 2002).

On the maintenance of variability in aposematic colour patterns

Polymorphisms can be maintained through negative assortative mating. Positive assortative mating can contribute to the maintenance of geographic variants of the same species, as has been shown for butterflies of the genus Heliconius (Melo et al. 2009) and suggested for the geographic differences among populations of the poison frog _Oophaga pumilio_ in the Bocas del Toro archipelago in Panama (Summers et al. 1999a; Reynolds & Fitzpatrick 2007; Maan & Cummings 2008). Negative assortative mating, on the other hand, can help maintaining variation within populations (Takahashi & Hori 2008). Therefore, I would have expected to find evidence of negative assortative mating for colour patterns in the population studied. However, I found that individuals seem to be mating randomly (Chapter 3). This means, that sexual selection via assortative mating is unlikely to be responsible for the extensive variation of colour patterns found in _D. tinctorius._

Colour pattern variation in _D. tinctorius_ does not seem to be an example of a trade-off between natural and sexual selection as has been suggested for _O. pumilio_ (Maan & Cummings 2009) or the wood tiger moth (Nokelainen et al. 2012), because although yellower females seem to have a mating advantage there is no evidence at present that yellower females are preyed upon more intensely (neither is there evidence against this). I suggest, however, that variation in colour patterns between the sexes can be explained by a synergy between natural selection in the form of enhanced aposematism and sexual selection in the form of parental care, and variation within the
population, regardless of sex, can be at least partially explained through alternative behaviour-colour pattern combination strategies.

An alternative explanation is that the variation in colour patterns in the population studied is the product of genetic drift. This is unlikely given the mating advantage of yellower females over duller ones, the large effective population size (about 4.1 frogs per 100m²; Devillechabrolle 2012, and the fact that recent molecular analyses using a neutral marker suggest that the colour patterns in Dendrobates tinctorius are a composite trait in which some components (dorsal and flanks colouration) have evolved under selection (Wollenberg et al. 2008).

Finally, the possibility remains that aposematic signal variation could be explained by their recent origin and rapid evolution (Mallet 2010). If that were the case, it would be only a matter of time until aposematic signals reached the equilibrium that represents the uniformity predicted by theory.

General conclusions

Aposematism does not work in the same way in monomorphic and polymorphic species because polymorphism reduces the rate of learning and increases the rate of forgetting associations between colour patterns and profitability. Polymorphic species face disadvantages that need to be compensated, for example, with behavioural and ecological adaptations to local environments. These adaptations have been largely overlooked.

This study suggests that we must consider other selective pressures besides mate choice and predation in order to understand how variation in aposematic signals is maintained. Aspects of the behaviour and ecology of polymorphic aposematic species such as colour-pattern-specific escape behaviour, microhabitat selection, activity
patterns, alternative colour pattern behaviour strategies, and the willingness to take advantage of ecological opportunities might enable the simultaneous existence of such variability in colour patterns while being exposed to the same predators.

The evolutionary forces leading to aposmatic polymorphisms might represent processes more complex than acknowledged to date. Future research would benefit from exploring more aspects of the biology of aposmatic species if it aims at understanding the paradox of the great diversity in aposmatic color patterns that exists in nature today.
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