

## A Test of Aposematism in the Dendrobatid Poison Frog *Oophaga pumilio*: The Importance of Movement in Clay Model Experiments

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**ABSTRACT.**—Brightly colored frogs of Dendrobatidae contain alkaloid-based chemical defenses which appear to be utilized as a deterrent to predators. The conspicuous coloration of these frogs is generally considered to be an aposematic signal to color-visioned predators. A previous field-based experiment tested this hypothesis in the dendrobatid frog *Oophaga pumilio* (Strawberry Poison Frog) from the La Selva Biological Station using a stationary clay model experiment to assess natural predation. Avian predation rates on brown frog models were almost twice that of red frog models, supporting the hypothesis that coloration in *O. pumilio* is aposematic. A criticism of clay model experiments has been that they do not accurately represent natural organisms, especially with regard to movement. Many predators utilize movement in prey selection and, therefore, may not perceive motionless clay models as prey. In an attempt to understand the importance of movement better in assessing natural predation rates using clay model experiments, we conducted a similar field-based clay model experiment but instead used moving models of *O. pumilio* and of brown control frogs. Moving brown models were attacked more than nine times the rate of moving red models, supporting the finding of the previous study and providing further evidence that color is aposematic in *O. pumilio*. When compared directly to the previous study using stationary clay models, birds attacked moving brown models significantly more whereas red moving models were attacked significantly less. Our findings suggest that moving models may provide a better estimate of natural predator responses to an aposematic frog.

Aposematism is defined commonly as the use of conspicuous coloration or patterning (or both) by chemically defended organisms as a signal to deter potential predators (Poulton, 1890; Cott, 1940). This warning signal is considered an advertisement of the organism's unpalatability or noxiousness and is effective as a defense mechanism only if predators are able to recognize and avoid the signal (Servedio, 2000; Ruxton et al., 2004). Previous research suggests that some predators avoid brightly colored, unpalatable prey through learned responses from prior experiences (e.g., Ruxton et al., 2004; Mappes et al., 2005); however, there is also evidence that prey avoidance can be an innate response (e.g., Smith, 1975; Pough, 1988; Endler and Mappes, 2004; Ruxton et al., 2004). Aposematism is a relatively widespread defensive adaptation and is found in a diversity of animal taxa including invertebrates (Guilford and Cuthill, 1991), birds (Baker and Parker, 1979), mammals (Sutton and Patterson, 2000), reptiles (Brodie, 1993), and amphibians (Kuchta, 2005).

Dendrobatidae contains several species of frogs that possess defensive alkaloids and are conspicuously colored (Daly et al., 2005; Saporito et al., 2012). The alkaloids present in dendrobatids are considered unpalatable (Saporito et al., 2007a) to several invertebrate and vertebrate predators (Brodie and Tumbarello, 1978; Fritz et al., 1981; Szelistowski, 1985), and it is generally believed that the conspicuous coloration associated with these frogs functions as an aposematic signal (Myers and Daly, 1983; Pough et al., 2001; Summers and Clough, 2001), although frog color is also involved in sexual selection for certain species (Maan and Cummings, 2008, 2009; Brown et al., 2010). Aposematism in dendrobatids has been tested experimentally using two different, but complementary, field-based approaches (e.g., Darst and Cummings, 2006; Saporito et al., 2007b; Comeault and Noonan, 2011). Darst et al. (2006) and Darst and Cummings (2006) used live dendrobatid frogs in experimental predation trials with domestic chicken predators whereas Saporito et al. (2007b), Noonan and Comeault (2009), Comeault and Noonan (2011), and Hegna et al. (2011, 2012)

used clay replicas of frogs in field-based experiments with natural bird predators.

Studies by Darst et al. (2006) and Darst and Cummings (2006) experimentally demonstrated that naïve domestic chickens were able to learn and associate the conspicuous colors of the dendrobatid species *Ameerega* (= *Epipedobates*) *parvula* (Common Name) (Grant et al., 2006), *Ameerega bilinguis* (Common Name) (Grant et al., 2006), and *Ameerega hahneli* (Common Name) (Grant et al., 2006) from Ecuador with unpalatability and ultimately avoid preying upon these frogs. These predator-prey experiments provide evidence that bright coloration in dendrobatids functions as an aposematic signal to potential predators. To date, the documented natural predators of poison frogs include an ant (*Paraponera clavata*; Common Name), fish (*Brycon guatemalensis*; Common Name), amphibian (*Pristimantis crenunguis*; Common Name), and bird (*Baryphthengus martii*; Rufous Motmot), and several spiders and snakes (reviewed in Santos and Cannatella, 2011; Alvarado et al., 2013). However, the evolution of bright colors in dendrobatid frogs appears to be directed largely towards birds (Siddiqi et al., 2004; Saporito et al., 2007b; Maan and Cummings, 2012), which are visual predators that utilize color vision (Hart, 2001).

In clay model experiments, replicas of prey items (e.g., frogs, snakes, millipedes) are made of soft clay, placed in the field, and predation by natural predators is assessed by examining signs of physical attacks (i.e., bite marks or other signs of handling). Clay model experiments have also been utilized successfully to study aposematism and mimicry in millipedes (Brodie and Moore, 1995; Marek et al., 2011), snakes (Brodie, 1993; Brodie and Janzen, 1995; Hinman et al., 1997; Buasso et al., 2006), and salamanders (Kuchta, 2005; Mochida, 2011). Recently, clay model experiments have become a popular approach for experimental testing of hypotheses surrounding aposematism in dendrobatid frogs. Saporito et al. (2007b) performed a clay model experiment to test if bright coloration in *Oophaga* (= *Dendrobates*) *pumilio* (Strawberry Poison Frog) (Grant et al., 2006) functions as an aposematic signal to natural predators in northeastern Costa Rica. This study demonstrated that bird predation on brown control frog models was almost twice that of conspicuously colored red frog models, supporting the

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hypothesis that coloration of these frogs acts as an aposematic signal to natural bird predators. Noonan and Comeault (2009) and Comeault and Noonan (2011) used clay model experiments to study how phenotypic color variation influences natural predation on *Dendrobates tinctorius* (Common Name) (Grant et al., 2006) from French Guiana. Results of these studies found that clay frog models that are novel in color are preyed upon more often than models that are similar in color to the local frog morph, suggesting that local aposematic phenotypes may have a selective advantage in deterring predators over novel forms. In a similar study, Hegna et al. (2012) found that the local yellow/green color morph of *O. pumilio* on Isla Colón in Bocas del Toro, Panamá was preyed upon more frequently when compared to a red color morph from the neighboring mainland, suggesting that in certain scenarios novel color morphs may provide a more effective warning signal to predators. Hegna et al. (2011) utilized clay models to examine the role of dorsal spot patterning in *O. pumilio* and found that it did not influence the effectiveness of the aposematic signal. Although clay model experiments have been widely used, they are all based on the use of stationary clay models that lack the movement associated with natural frogs.

Stationary clay models have been criticized for not representing natural organisms with respect to traits such as odor, anti-predator strategies (e.g., crouching or ceasing movement), chemical defenses, social context, and movement (Rangen et al., 2000; Thompson and Burhans, 2004; Cooper et al., 2008; Santos and Canatella, 2011). Prey movement is important to predation (Tordoff, 1980; Morey, 1990; Schwarzkopf and Shine, 1992) and, in particular, visual predators have been shown to use movement as a criterion for selecting prey items and are more likely to attack moving prey than stationary prey (Heinen and Hammond, 1997). Furthermore, aposematic organisms are known to advertise their warning signals by exhibiting slow movements and making no attempt at concealment (Poulton, 1890; Ruxton et al., 2004; Cooper et al., 2008a). Given the importance of movement in prey selection and in effective advertising of aposematic signals, experiments with moving clay models may represent a more natural assessment of predator responses to prey.

To examine the importance of prey movement in clay model experiments, we performed a study similar to that of Saporito et al. (2007b) by using moving clay model replicas of *O. pumilio* and brown leaf-litter frogs resembling members of the genus *Craugastor* (Frost, 2013). Given the results of Saporito et al. (2007b), we expected that moving brown frogs would be attacked more than stationary frogs and that moving red frogs would be attacked less than stationary frogs. The goals of our study were to assess how movement affected predator selection of prey in clay model experiments and to provide a potentially more realistic measure of predation in the aposematic frog *O. pumilio*.

#### MATERIALS AND METHODS

This study took place at the La Selva Biological Research Station in northeastern Costa Rica. *Oophaga pumilio* is a common leaf-litter frog at this location and is characterized by its alkaloid defense and conspicuous, reddish-orange dorsal coloration and blue-black appendages (Guyer and Donnelly, 2005). Many brown colored leaf-litter frogs (e.g., members of genus *Craugastor*) are also found in the same microhabitat as *O. pumilio* and are of similar size (Saporito et al., 2007b), yet they

lack defensive chemicals and are considered palatable to potential predators.

**Model Design.**—Frog models were constructed using a rubber mold following the methods of Hegna et al. (2011), and legs were constructed and attached to the body by hand. To make the frogs more realistic, black eyes were drawn on the frog models with a permanent marker (Sharpie Fine Tip®). Precolored, nontoxic, plasticine modeling clay (Sculpey III®) was used to make models. This soft clay has been shown to preserve impressions of predation attempts (e.g., Madsen, 1987; Brodie, 1993; Saporito et al., 2007b) and has no UV reflectance (Saporito et al., 2007b); Summers et al. (2003) found that *O. pumilio* has no UV reflectance, suggesting that avian predators are not utilizing reflected light in the UV range to detect these poison frogs. Two model types were constructed: red-bodied models with blue appendages (representing *O. pumilio*) and completely brown models (representing common leaf-litter frogs in the genus *Craugastor*). Following Saporito et al. (2007b), Red Hot Red (Sculpey III color 583) and Blue (Sculpey III color 063) clay was used to construct red models, and a mixture of 1109 Suede Brown (Sculpey III color 1109) and Chocolate Brown (Sculpey III color 053) clay was used to construct brown models.

**Experimental Design.**—Six-hundred moving frog models (300 red and 300 brown) were placed along 30, 100-m transects, and 150 stationary frog models (75 red and 75 brown) were placed along six additional 100-m transects as a control for frog model movement. Models for both experiments were placed throughout La Selva Biological Station, Costa Rica between 20 June and 23 July 2012. Transects were placed at least 100 m away from one another and were evenly distributed in old-growth, secondary, and agro-forestry sites to represent the different habitats at La Selva. Each transect contained an equal number of red and brown models, and every model was placed 5 m apart. Placement of models was randomized with a coin toss, but no more than two of either model type were placed consecutively along a single transect. Moving models were constructed by placing clay frogs on a 3.5-inch sweep second hand attached to an ISI Continuous Sweep High Torque Clock Movement (Innovation Specialties, Inc.) to rotate the frog model in a continuous, circular motion. The base of each clock was placed under leaf litter, 1 m away from the transect line, and only the shaft and second hand containing the clay frog model were visible (Fig. 1). Every clock movement was sprayed with Coleman® Pro-Tech™ Water Repellent and placed in a 16.5 × 14.9 cm Ziploc® sandwich bag to prevent damage from rain, humidity, and moisture. Stationary models were placed directly on leaf-litter 1 m away from the transect line (see Saporito et al., 2007b). All frog models were collected after 48 h in the field. Models were examined for predation attempts, and clocks were allowed 48 h to dry before being reused.

**Statistical Analysis.**—Each model was assessed for predation attempts upon collection, and all marks were recorded using a digital camera. On the basis of Saporito et al. (2007b) and Hegna et al. (2011), each mark was assigned to one of three predator types; bird, mammal, or arthropod. If multiple marks were found on a single model, they were counted as a single predation attempt. If consecutive attacks occurred within the same transect, they were assumed to have occurred by the same predator (see Brodie, 1993 and Saporito et al., 2007b). If models could not be found after a search time of 5 min, they were recorded as missing. Binary logistic regression was used to determine if model color was a significant predictor of predation rate for both experiments. Statistical tests were run including and excluding consecutive



FIG. 1. Plasticine model of *Oophaga pumilio* on a clock mechanism.

attacks and missing models. All statistical analyses were performed using SPSS v. 18 for Mac.

#### RESULTS

Of the 600 moving frog models (300 red and 300 brown) placed on transects, 107 models (18%) were attacked and five models were missing (0.8%). A total of 33 red frog models and 74 brown frog models were attacked. Birds accounted for 50% of all predation attempts but only attacked 9% of all models. Bird attacks were recognizable by distinctive U- or V-shaped marks, large indentations on the models, or both (Fig. 2A; Brodie, 1993; Saporito et al., 2007b). In many cases multiple attacks by the same bird were apparent on a single model. Avian predators attacked six (2%) red frog models and 48 (16%) brown frog models (Fig. 3). "Frog model color" was a significant predictor of avian predation, and moving brown models were attacked at more than nine times the rate of moving red models ( $P \leq 0.001$ ;  $\text{Exp}(B) = 9.3$ ;  $\text{CI}_{0.95} = 3.9, 22.2$ ; Fig. 3). These data were analyzed assuming missing models were not preyed upon; however, the results are unchanged when missing models are included as

predation attempts (data not shown). No differences in predation rates were found when consecutive model attacks were included and excluded from the data analysis (data not shown). Brown frog models were attacked by Motmots (Motmotidae) more frequently than were red frog models; however, frog model color was not a predictor of Motmot predation ( $P = 0.175$ ). Motmot predation attempts were identifiable by distinctive serrated edges left by the serrated bill of Motmots (Fig. 2B). Mammals accounted for 10% of all attacks (attacking 2% of all models) and were characterized by teeth marks (Fig. 2C). Arthropods accounted for 41% of all attacks (attacking 7% of all models) and were characterized by a series of small indentations (Fig. 2D).

Of the 150 stationary frog models placed on transects, 19 models were attacked (13%) and six models were missing (4%). Of these stationary models, a total of nine red frog models and 10 brown frog models were attacked. Birds accounted for 32% of all predation attempts (attacking 4% of all models) and attacked three red models and three brown models. Frog model color was not a significant predictor of avian predation for stationary models ( $P = 0.999$ ). Mammals accounted for 11% of all attacks (attacking 1% of all models) and arthropods accounted for 58% of all attacks (attacking 7% of all models).

#### DISCUSSION

The results of our study support the hypothesis that movement is an important factor to consider when using clay model experiments to evaluate natural predation rates for mobile organisms such as frogs. Our study found that avian predation on moving models was significantly higher for brown frogs when compared to red frogs (Fig. 3). These findings are similar to those of Saporito et al. (2007b) and provide additional support for the hypothesis that bright red coloration in *O. pumilio* functions as an aposematic signal to bird predators. Avian predation on frog models was identical (9%) between the moving models of the present study and the stationary models of Saporito et al. (2007b); however, moving brown models were more than nine times as likely to be attacked by birds as were red models, whereas in Saporito et al. (2007b) stationary brown

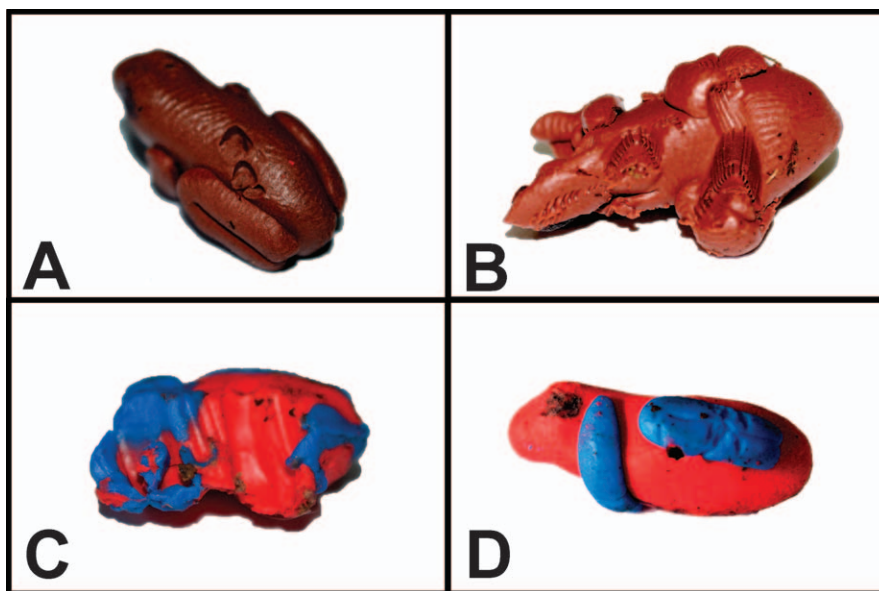


FIG. 2. Examples of clay models attacked by a (A) bird, (B) Motmot, (C) mammal, and (D) arthropod.

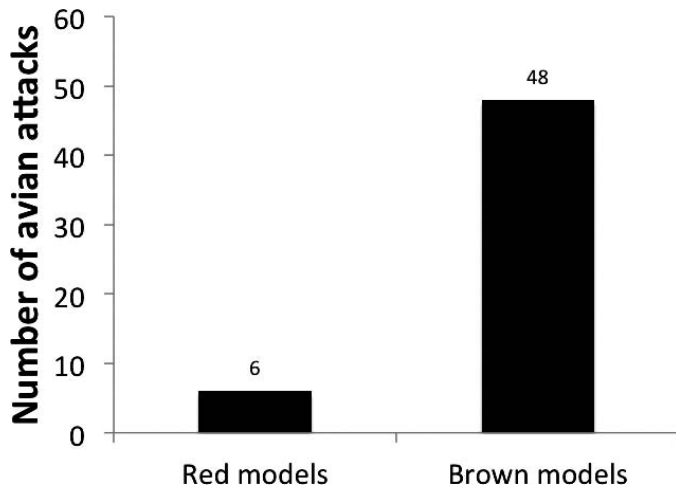


FIG. 3. Total number of avian predation attempts on red and brown moving models.

models were only twice as likely to be attacked as were red models. Furthermore, moving brown models experienced an increase in predation whereas moving red models experienced a decrease in predation when compared to Saporito et al. (2007b), suggesting that birds respond differently to moving and non-moving models.

Movement is an important factor in prey selection for birds (e.g., Tordof, 1980; Schwarzkopf and Shine, 1992), and it is likely that stationary clay models are not always recognized as prey. In Saporito et al. (2007b), birds accounted for 11% of the attacks on stationary brown models whereas in the present study, birds attacked 16% of moving brown models (Table 1). Stationary brown models placed on leaf-litter are relatively inconspicuous, cryptic, or both and in some cases may be overlooked by birds, resulting in lower predation. However, the increased predation on moving brown models is likely because of their increased visibility to birds and better recognition of these models as potential prey. In Saporito et al. (2007b), birds accounted for 7% of the attacks on red stationary models whereas in the present study birds attacked only 2% of the red moving models (Table 1). It is likely that some birds do not recognize stationary red models as frogs but, instead, consider them as edible seeds or fruits. There are a number of red fruits found at La Selva that could possibly be confused with a red model and eaten by birds including *Anthurium flexile* (Common Name), *Chamaedorea pinnatifrons* (Common Name), *Chione venosa* (Common Name), *Marcgravia mexicana* (Common Name), *Miconia barbinervis* (Common Name), and *Pouteria reticulate* (Common Name), resulting in higher attacks on red stationary models. Moving red models are less likely to be mistaken as a seed (or fruit) by birds

but are likely perceived as brightly colored frogs and thus avoided more often than stationary models.

Interestingly, we found no difference in avian predation between the brown and red stationary models examined in the present study, which is in contrast to Saporito et al. (2007b) and the present moving model experiment. Although this difference may be the result of our small sample size (150 models vs. 800 models in Saporito et al., 2007b), other stationary clay model experiments have not detected a difference in predation between control and experimental frogs (see Table 1; Saporito et al., unpubl. data), making it difficult to fully interpret the results of these studies. The low bird predation on brightly colored stationary models in these studies is likely the result of predator avoidance (i.e., aposematism) whereas low predation on brown models is probably because of their cryptic nature. Given the increased visibility of moving models, coupled with greater bird predation on brown models and decreased predation on red models (Table 1), model movement appears to be an important aspect to consider when designing frog clay model experiments.

Movement is an important criterion used by natural predators in locating and capturing prey (Tordoff, 1980; Morey, 1990; Schwarzkopf and Shine, 1992), which suggests that moving clay models may provide a better estimate of natural predation. Aposematic prey (including *O. pumilio*) typically exhibit slow movement, reduced predator escape behavior, and no attempt at concealment (Poulton, 1890; Ruxton et al., 2004; Cooper et al., 2008a), all of which are behaviors that are crudely represented by our moving models. Conversely, cryptic prey (including members of the genus *Craugastor*), which are often used as controls in clay model studies, generally rely on camouflage and immobility (Cooper et al., 2008b). Although predators are more likely to attack moving prey (Heinen and Hammond, 1997), as evidenced by the increased attacks on moving brown models (Table 1), continuously moving models may not best represent the behavior of cryptic frogs. Incorporating movement into clay model experiments may provide a more life-like appearance to the models; however, neither continuously moving nor stationary models provide an accurate representation of the differences in behaviors associated with both aposematic and cryptic animals.

Birds commonly prey on frogs in the Neotropics (Stiles and Skutch, 1989; Poulin et al., 2001) and were the primary predator of frog models in this experiment. Birds have color vision and are able to detect the bright coloration of *O. pumilio* and other aposematic organisms (Hart, 2001; Siddiqi et al., 2004). Avian predators only attacked six moving red frog models but attacked 48 moving brown models. The low number of bird attacks on clay models of *O. pumilio* suggests that birds are able to discriminate between red and brown frogs, and ultimately

TABLE 1. Comparison of avian attacks on nonmoving and moving clay models.

	Brightly colored models	Brown models
	Avian attacks/total number of models (% of attacks)	Avian attacks/total number of models (% of attacks)
Saporito et al. (2007b)	27/400 (6.8%)	44/400 (11.0%)
Noonan and Comeault (2009) <sup>a</sup>	Local phenotype: 6/420 (1.4%)	4/420 (1.0%)
Comeault and Noonan (2011) <sup>a</sup>	Local phenotype 1: 4/385 (1.0%)	4/385 (1.0%)
	Local phenotype 2: 7/280 (2.5%)	6/280 (2.1%)
Hegna et al. (2012) <sup>a</sup>	Local phenotype: 14/406 (3.4%)	1/406 (0.2%)
Present study (stationary models)	3/75 (4.0%)	3/75 (4.0%)
Present study (moving models)	6/300 (2.0%)	48/300 (16.0%)

<sup>a</sup> Each of these studies included one or more clay model treatments of a novel color phenotype, but these data are not included in Table 1.

avoid attacking brightly colored frogs, presumably because they are chemically defended. However, avian predators attacked a small number of red models, suggesting that some birds are either naïve predators or are able to prey successfully upon *O. pumilio*. Two of the six red models were attacked by Motmots (Motmotidae), which were easily identified based on the distinctive serrated edges of their beaks left as imprints on the models (Fig. 2B; also Brodie, 1993). This is particularly interesting because there are two recorded observations of Motmots preying upon poison frogs. *Dendrobates auratus* (Common Name) was reportedly preyed upon by an adult Rufous Motmot at La Suerte Biological Station in northeastern Costa Rica (Master, 1998), and *O. pumilio* was recently observed being preyed upon by the same Motmot species at La Selva Biological Station (Alvarado et al., 2013). These observations suggest that Motmots likely represent natural predators of dendrobatids, although the extent to which these birds prey upon these frogs is not known. Furthermore, it is not known how Motmots are resistant to the chemical defenses of dendrobatid frogs. Motmots are known to prey upon a variety of small vertebrates such as frogs, lizards, and snakes (Stiles and Skutch, 1989; Remsen et al., 1993) and, in the present study, also attacked six moving brown frog models.

Mammals and arthropods accounted for the remaining imprints on the frog models in our study but were not considered as frog predators. Mammals attacked 11 moving models (2%) and 2 stationary models (1%), and arthropods attacked 44 moving models (7%) and 11 stationary models (7%). The number of imprints is very similar between moving and stationary models, suggesting that mammals and arthropods use signals other than vision while foraging for food, such as olfaction in mammals (Vander Wall, 1998) and chemoreception in arthropods (Bell and Cardé, 1984). Studies of bird predation using artificial nest experiments have demonstrated that certain mammals (e.g., mice) are attracted to the unnatural odor of plasticine clay (Rangen et al., 2000; Thompson and Burhans, 2004). Both mammals and arthropods left imprints on brown and red models at relatively the same rate, suggesting that they are unable to distinguish differences in color or that they were not deterred by coloration. Mammals and arthropods often attacked models consecutively, suggesting that these organisms were attracted to the odor/chemical cues of the clay and did not perceive the models as frogs.

**Conclusion.**—This study experimentally demonstrated that bright coloration in *O. pumilio* from northeastern Costa Rica functions as an aposematic signal to bird predators. Our results also demonstrate the importance of movement for prey selection by visual predators in clay model experiments. Avian predation rates on moving models were significantly different when compared to previous studies using stationary models. Not only did bird predation greatly increase on moving brown frog models, but predation was also greatly reduced on moving red frog models when compared to stationary models. Given the significance of movement in selection of prey, and the need to provide more realistic measures of natural predation on aposematic frogs, it will be important to study further the importance of movement in clay model experiments.

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