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A New Technique for the Production of Large Numbers of Clay Models for Field Studies of Predation

One of the cornerstones of ecology is the study of interspecific interactions, such as predation, herbivory, and mutualism. Studies of predation have proven to be difficult since actual field observations are often limited. Synthetic prey models have proven to be useful tools for measuring predation attempts, as identical stimuli can be created and used without harming the test organism in question. In addition, replication of a particular prey phenotype is more tenable with models than with real organisms, and allows for careful manipulation of phenotypic traits of interest, such as color and size.

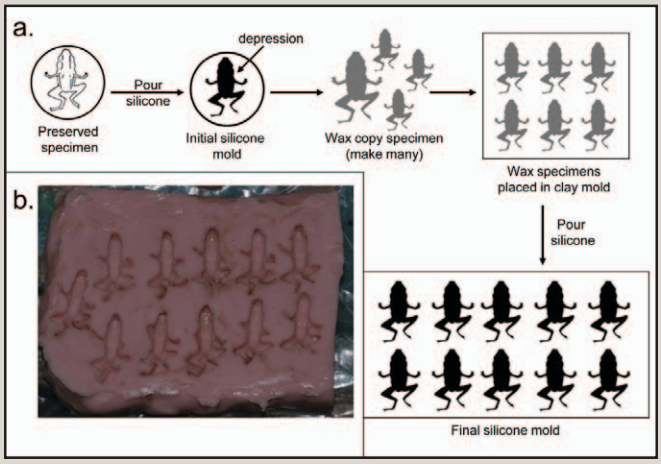

FIG. 1. A) Sequential procedure for making master silicone molds, B) Final silicone master molds.

FIG. 2. Clay well surrounding the preserved specimen.

Although predation can be studied relatively easily in captive settings (e.g., Lindström et al. 2006), studies of predation are more problematic, as they require models that withstand variable habitats and unpredictable weather conditions. Predation on several reptile and amphibian taxa [including snakes (Brodie 1993; Harper and Pfennig 2007), salamanders (Kuchta 2005), and frogs (Saporito 2007; Noonan and Comeault 2009) has been subject to field research using clay or plasticine models. Similarly, for clay models of prey species that are highly variable in coloration and patterning, high attention to detail is warranted, but the development of highly realistic models is often prohibitively time-consuming or costly. Moreover, recent experiments (Noonan and Comeault 2009) have demonstrated that predation rates are often low, necessitating a large quantity of models to elucidate predation patterns.

We present a novel technique to generate large numbers of life-like models using customizable silicone master molds to produce clay models. Master molds allow for quick development of clay models of varying size and shape, which can subsequently be painted for patterning and coloration studies. Due to the nature of the clay used, marks left by predators can be scored for presence or absence of predation attempts, and in some cases used to identify predator species.

Materials and Methods.—Construction of the models began by taking an initial mold of a preserved specimen of a poison frog, *Ranitomeya imitator*, available from previous research. A small layer of modeling clay (Sculpey) was smoothed onto a small wood board and heated with an alcohol burner to soften it. A preserved frog was then placed into a life-like resting pose and lightly pressed into the clay in a posture similar to a sitting stance (Fig. 1). Although it is possible to make a 3-D mold that includes ventral characteristics, it would involve additional steps unnecessary for our purposes, so the ventral surface of the frog was flattened against the clay.

A well was then constructed around the frog approximately 2 cm high using the same modeling clay (Fig. 2). The clay and specimen were then coated with a Krylon Matte Finish Spray® (Krylon Products Group) to protect the skin of the specimen as well as seal in chemicals that could inhibit the curing process of the silicone. A mold release (Mann Ease Release 200™, Mann Formulated Products) was sprayed over the entire frog and clay bed/wall to allow for easy release of the silicone. The silicone and catalyst (Smooth-Sil 940®, Smooth-on Corp.) were mixed in a 10:1 ratio by weight. This mixture was then poured, from a

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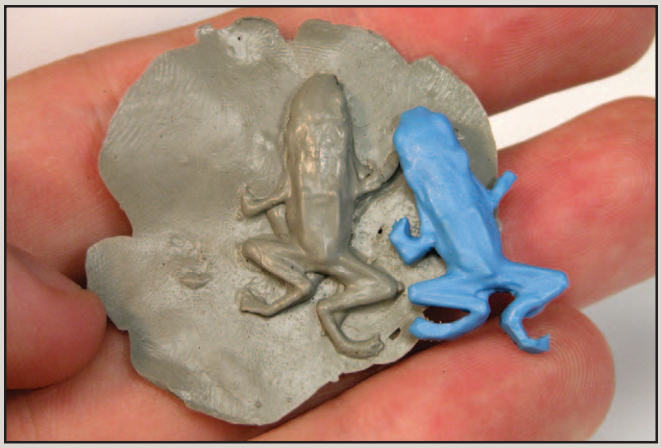

FIG. 3. Wax model (left) with resin model (right).

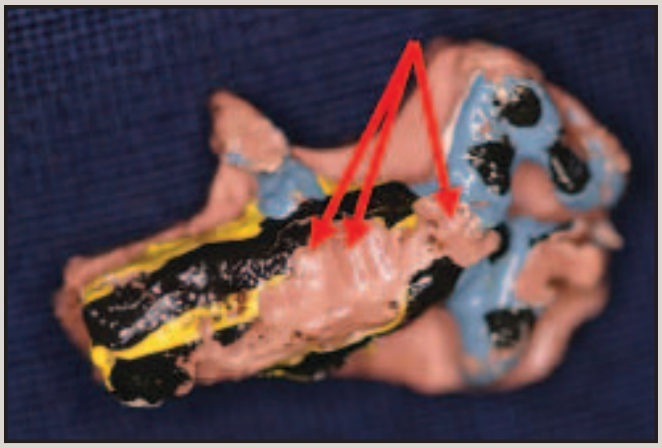

FIG. 4. Model *Ranitomeya imitator* with mammalian incisor marks (arrows depict attacked regions).

height of approximately 1 m, into a thin stream in one corner of the well, allowing it to spread evenly over the specimen. Pouring in a thin stream away from the specimen allows for the spread of the silicone to cover all surfaces and avoids development of air bubbles. The silicone master mold cured in approximately 18 h at which point the preserved frog was extracted and returned to alcohol. Although specimens remain largely intact during creation of molds, damage to skin is likely as a result of the matte finish spray.

Following production of the mold, resin (plastic) forms were poured that were then used for the production of the final silicone production molds, in which the clay models are made (Fig. 3). The resin (Smooth-cast 300®, Smooth-on Corp.) was mixed according to the manufacturer's indications (1:1 by volume) and poured into the silicone voids using the pouring technique described above for the pouring of silicone. Curing time for this step is approximately 30 minutes. Two methods were tested to cure the resin, both curing them in ambient temperature and pressure conditions versus under high pressure. Both yielded similar results. There may be a possible benefit of using increased pressure to avoid air bubble imprints, although these benefits were minimal in our testing. The resin frog models were then spaced on the modeling clay (the same as originally used to make the initial silicone mold), in groups of 10–12 models per mold and the final silicone was mixed and poured. When the resin frogs were removed, the voids were used to pour melted plasteline clay.

To heat the clay we used a small crock-pot on the highest setting to melt no more than half of a two-pound block. The clay was heated in the crock-pot for approximately one hour, or until it was liquefied. Release agents can be added to the silicone prior to pouring clay to facilitate clay extraction, though this is not necessary. While release agents will extend the life of molds, it can also serve as an olfactory attractant to predators.

The clay was spooned onto the molds, tapping it in each form to eliminate air bubbles. An extra 1/4-inch of clay was added and smoothed over the molds to allow for easy separation from the silicone, as well as to strengthen the very thin legs. The tablets of clay then were painted using non-toxic acrylic paints. Alternatively, colored clays could for species with simple color patterns, avoiding the need to paint.

For our uses, we replicated the silicone master mold step 15 times to be able to make roughly 150 models with each heating of the clay block. While it is possible to do much larger master molds, extracting the models from smaller molds is easier as smaller molds allow for release of the clay out of the mold by gentle bending. This allows for finer detail of the models, such as small toes or tail tips, to be retained, with a reduced risk of breaking fragile body parts. When the molds are carefully handled and used in combination with a non-toxic release agent, they can be expected to last several years. We produced nearly 2,000 models with no noticeable detrimental effects to the master molds (Fig. 4).

Discussion.—We provide a novel method for the rapid production of large quantities of detailed models. This method is preferable to other production methods for clay models for several reasons. First, due to the ease of model preparation from master molds, models can be made in even the most remote locations, provided there is a way to melt the clay. Once the resin forms are made they can be used unlimited times to produce silicone master molds, potentially allowing production of thousands of models in a short time. Lastly, for predation studies, accurate models allow for the testing of specific hypotheses of color or specific traits, while partially alleviating concerns that predators will not recognize unrealistic forms. However, we note that investigators should conduct field trials of paint and model formulation prior to deploying models—as an example, we found that some paint formulations were subject to attack by ants in the Neotropics.

The future of clay models is promising with higher attention to detailed realism and faster production times allowing for more rigorous hypothesis testing. Our method provides a way to generate large amounts of accurate clay models with minimal effort.

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Literature Cited

Brodie, E. D., III. 1993. Differential avoidance of coral snake banded patterns by free-ranging avian predators in Costa Rica. Evolution 47:227–235.

Harper, G. R., and D. W. Pfennig. 2007. Mimicry on the edge: why do mimics vary in resemblance to their model in different parts of their geographical range? Proc. R. Soc. B. 264:1955–1961.

Kuchta, S. R. 2005. Experimental support for aposematic coloration in the salamander *Ensatina eschscholtzii xanthoptica*: implications for mimicry of Pacific newts. Copeia 2005: 265–271.

Lindström, L., A. Lyytinen, J. Mappes, and K. Ojala. 2006. Relative importance of taste and visual appearance for predator education in Müllerian mimicry. Anim. Behav. 72:323–333.

Noonan, B., and A. Comeault. 2009. The role of predator selection on polymorphic aposematic poison frogs. Biol. Lett. 5:51–54.

Saporito, R. 2007. Experimental evidence for aposematism in the dendrobatid poison frog *Oophaga pumilio*. Copeia 2007:1006–1011.

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Toes versus Swabs? Evaluation of The Best Tissue Source For Detection of *Batrachochytrium dendrobatidis* in Field-Caught Amphibians

The pathogenic fungus *Batrachochytrium dendrobatidis (Bd;* Phylum Chytridiomycota, Class Chytridiomycetes, Order Chytridiales) has been associated with amphibian extinctions and declines throughout the planet (Berger et al. 1998; Crawford et al. 2010; Lips et al. 2006; Mendelson et al. 2006; Stuart et al. 2004). Since its discovery in 1998, researchers have documented the distribution of *Bd* (Fisher et al. 2009; Ron 2005; *Bd* maps: http://www.spatialepidemiology.net/bd-maps/), potential dispersal patterns and vectors (Johnson and Speare 2005; Lips et al. 2009), the effect of epidemics on amphibian communities (Brem and Lips 2008; Briggs et al. 2010; Lips et al. 2006; Vredenburg et al. 2010), and the life history traits and environmental factors that might contribute to pathogen virulence and/or host vulnerability (Bielby et al. 2008; Burrowes et al. 2008; Garner et al. 2009; Lips et al. 2003; Longo et al. 2010; Pounds et al. 2006). Regardless of the type of question that we are addressing, effective detection of infected individuals and estimation of *Bd* load is key to further understanding the dynamics of this disease among affected populations. At present, this is done via molecular assays using end-point PCR (Annis et al. 2004) or TaqMan quantitative real-time PCR (Boyle et al. 2004). However, the kind of tissue source (toes versus ventral skin swabs) used for detection has been rarely tested (Hyatt et al. 2007). The aim of this study is to evaluate the reliability of these sample methods applied to the same animal, in detecting *Bd* and estimating the intensity of the infection in the field at a given sampling time.

Although sampling toes are considered unethical by some authors because they may cause pain or hinder survivorship in some species (Hyatt 2007; McCarthy and Parris 2004, Phillott et al. 2007), *Eleutherodactylus coqui* is not negatively affected by toe-clipping and can regenerate a toe within 4–5 months to a round (versus the usual T-shape) disk, thus allowing for future recognition and re-sampling (Joglar 1998, Ulmar et al. 2011). In addition, the usefulness of this tissue for scientific studies such as population genetics (Burrowes and Joglar 1999), systematics (Gonsner and Collura 1996), and disease diagnostics (Longo et al. 2010; St-Amour and Lesbarrères 2007) has been recognized. In a study of population-level responses to *Bd*, toe-clips may be very valuable to assess the temporal progression of the disease and survivorship of marked individuals (Longo and Burrowes 2010). This is suitable for amphibians that can re-grow their toes quickly, but may not work well with other species. On the other hand, swabs are easily taken, non-invasive, conveniently stored, and have shown high sensitivity for *Bd* diagnosis (Hyatt et al. 2007).

Pushendorf and Bolaños (2006) compared the probability of detecting *Bd* using tissues from different body parts with histology and found that sections from the pelvic patch and the innermost finger with a fungal-specific stain (periodic acid-Shiff-PAS), were generally more effective. Hyatt et al. (2007) compared the diagnostic sensitivity of swabs, toe clips and water baths in detecting *Bd* via TaqMan qPCR assays in frogs inoculated with *Bd* in the laboratory. They define diagnostic sensitivity as the proportion of known reference animals that are *Bd* positive, that test positive for *Bd*. Their work revealed that toe clips were more effective in detecting *Bd* only at early stages of infection, and they recommend the use of swabs as the standard sampling method (Hyatt et al. 2007). Our study is different in that we evaluate the probability of detecting *Bd* in the field (unknown *Bd* status) from the same animal at a particular time using two sampling protocols. In addition, we assess the difference between swabs and toes at estimating the level of infection (*Bd* zoospore load) of individuals within a population. We expect that the results presented herein, may help scientists decide the best sampling method for *Bd* diagnostics according to the type of question they wish to address in future field studies.

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