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COMMENTARY

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The future of clay model studies

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Abstract

Background: Many intriguing questions about predator-prey interactions can be addressed by using clay models of prey animals. These are placed in the field to test predators' avoidances or preferences (testing e.g. color or shape) or to gain insights into predator identity. Modeling clay allows teeth, beak and jaw marks to remain on the model for identification. First used 30 years ago, clay models are now widely deployed. Ever since, the complexity of hypotheses, modeled species as well as the number of clay models used per study has increased. Although clay models are a valuable research tool, the method has limitations. Some questions cannot be addressed with these experiments, yet there is potential for improvement.

Main body: We focus on the following aspects that need attention for clay model studies (CMS) in the future: (1) Use of proper clay materials, (2) how to standardize attack identification, (3) limitations of clay model studies, (4) use of clay models beyond predation experiments and (5) the next generation of clay model studies.

Conclusion: We conclude that certain aspects of the clay model paradigm urgently need greater standardization. We advocate the use of harmless clay products and non-toxic inks, as well as having a neutral person to evaluate the marks left in the clay against pre-defined inclusion criteria. Further we suggest to use experimental data more cautiously in respect to evolutionary explanations, to use clay studies in detection experiments and to develop methods for attacker identification based on predator salivary DNA.

Keywords: Ecological studies, Predator-prey interactions, Predator salivary DNA

Background

Many intriguing questions concerning predator-prey interactions can be addressed by using clay models of prey animals; the models are placed in the field to test predators' avoidances or preferences, e.g. of different traits, or to gain information on predator identity. The first clay model studies were conducted 30 years ago [1, 2] and have become more popular ever since with increasing complexity of hypotheses, numbers of clay models per study as well as modeled species. Clay allows tooth, beak and jaw marks to remain on the model for identification. A comprehensive review by Bateman et al. [3] on the use of clay model experiments across various vertebrate taxa as well as in nest predation studies is already available. The authors discuss potential ecological questions that can be addressed by using clay models as a research tool. They also discuss the potential of clay models in studies on interspecific sociality and competition and review problems and limitations of the method. Having conducted clay model experiments ourselves we have come across additional aspects that need attention and may affect future studies.

Main body

Use of proper clay materials

Bateman et al. [3] address the issue of insufficient labeling of clay products in many studies. Labeling, if present at all, often is merely a material name, lacking specific product or brand details. Among other materials, plasticine (often used synonymously with 'modeling clay') and oven-hardening clay materials are most commonly used [3]. Both materials are tested and labeled "non-toxic" as toys. However, oven-hardening clays are based on polyvinyl chloride (PVC) mixed with plasticizers, ingredients criticized for potential health hazards. This is an overlooked risk that urgently needs addressing for future studies. Often models are not only left with a mark, but instead are found destroyed, partly or even completely missing ([4, 5]; own study). With numbers of up to 9600 models used in one study [6], it is worth having a closer

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look at the material that is being used and lost in the habitats. Researchers do not adequately address the potential ingestion of clay by predatory animals, which might not be harmless. Oven-hardening clays are not food-safe and not recommended for ingestion by humans (according to written statements of two leading brands received by us) and consequently not by small animals. Nonetheless, these materials are widely used throughout studies [7–9]. Although tests on effects are lacking, we think it is time to question the use of PVC-based clays and to go "green" by considering them unfit for the described purposes, especially as with plasticine, a non-hazardous, ethical alternative is available.

The first studies by Madsen as well as Brodie [1, 2, 10] used PVC-based materials due to their property of lacking UV reflection and the availability of a broad color range. Depending on where the study takes place, certain properties of the clay material are required. Whether in moist tropical or hot desert-like conditions, clay must remain soft enough to allow predation marks to stay on the model. Clay that might remain in perfect shape and consistency in a tropical forest might melt or dry out in the desert sun. Every brand has its own recipe having a direct effect on the clay properties (P. W. Bateman, pers. comm.). Nonetheless we find studies successfully using plasticine clay in both humid and arid study areas [11, 12], thus this factor should not be a criterion to use PVC-based clays. In terms of color availability we admit a drawback, but suggest highly pigmented, fluid acrylic inks, to be deployed on the clay. The ink is water resistant, non-toxic, all colors can be mixed and the ink is easily applicable to the clay. Further, these inks do not form a hard (gum-like) surface and have no effect on the quality of imprints. We want to point out that most pigments used in acrylic inks are also not meant for ingestion. Yet, so far, alternatives for conspicuous colors are lacking, whereas for certain tones, natural earth color pigments are available. We further want to underline the importance to use colors that match hue and reflectance of actual prey as close as possible. Spectrometric measurements of both prey skin and ink (on clay) are suggested [6, 7, 11].

How to standardize attack identification

An aspect that Bateman et al. [3] already discussed in their review is the deployment of controls in clay model studies. Controls can be lumps of clay simultaneously put in the habitat to ensure that models were attacked because they have been identified as prey items and not due to clay odor, color or novelty of the item alone. The design of clay controls has to be made with care to ensure that they are not attacked simply because they have been confused with the prey item. Especially the shape must be standardized and should not look anything like the modeled prey (e.g. a flat square or circle). Although also novelty effects might lead to attacks on controls, they are important to show prey recognition has taken place. Checking the attack spots on the body of the models can also add proof to this question. Attacks directed towards the head or the body center are more likely to indicate prey recognition.

The basis of all clay model studies is the identification of attacks and allocation of imprints to a predator group. However, the analysis of attacks is also the most deficient part of clay model studies as bias is highly likely to interfere in the decision making of the researcher when evaluating attacks. To counteract this bias and to standardize analyses we suggest two things: i) developing a priori inclusion criteria, and ii) using a neutral person to evaluate any marks left on the clay.

First, we suggest for researchers to develop clear guidelines prior to each study defining a frame stating which attacks are counted or neglected and why. Although not universal and varying depending on the study this would help standardizing analyses. For example, often clay models show imprints inflicted by insects or other animals which can be excluded as actual predators of the focal prey item. Unless predation has been observed e.g. in bullet ants and banana spiders on frogs [13], such incidents should never be included in the analyses. Caution should also be taken when studying e.g. effects of coloration of diurnal prey, and attacks are inflicted by nocturnal predators. Such attacks are interesting and worth reporting. Although there recently has been evidence for effective color vision under poor light conditions for various species [14], conclusions on chromaticity effects are difficult. There might still be effects of the studied signal that could be explained by twilight-activity of predators and the potential achromatic appearance of clay models. This kind of data needs to be handled carefully and is best analyzed separately. A general issue that can be drawn from this example is the placement of models at times or locations where the model species would not be present or available to certain predators, thus potentially leading to effects that are not necessarily valid. Another issue are missing models. Although it is possible that a missing model was attacked, carried away or even ingested by an actual predator there rarely is proof of predation. Yet, again, it is worth checking that kind of data separately in case there are significant differences between studied groups. Despite lack of proof, a significantly higher number of missing models of one model type compared to another can still yield information on predation. To counteract losing models we encourage any means of attaching the model to the substrate (e.g. toothpick) and to install camera traps when models keep disappearing at certain positions.

Second, we advocate consulting a neutral second rater, who is unaware of the hypotheses of the experiment, to double check each model (photo) to evaluate attacks. Observer bias is common in behavioral studies and ambiguous attacks likely to be counted or neglected depending on which confirms best the hypotheses being tested [15]. Additionally, field notes are an important tool for the interpretation of imprints and should be made available to the second rater as the first observer might have noted incidents (e.g. fallen branch next to the model), that explain the nature of the impression.

Limitations of clay model studies

When talking about the evaluation of attacks we quickly reach limitations in terms of their interpretation. A model that has not been attacked is not equivalent to a model that has not been detected. Aspects such as odor, details of shape, reflectance, lack of movement etc. can have an effect on whether a predator attacks post-detection. Especially in aposematic contexts this must be considered carefully. Aposematic signals are mostly conspicuous and attract the attention of potential predators. Predators supposedly detect but do not attack such a prey. This detection, however, is nowhere to be found within clay model data. It is impossible to know whether a model in the field has been detected by a predator and subsequently was avoided, leaving the model unharmed, which is why we suggest additional detection experiments or laboratory experiments (see next passage). Although generally difficult, independent pre-studies using camera traps could also help in gathering evidence of detection events. Moreover, a bird detecting a conspicuous and defended prey animal, might peck on it during a first encounter, learn its unprofitability and subsequently not attack it further. Looking at imprints on a clay model, such a non-lethal incident would not be distinguishable from successful predation. These aspects pose two different issues. The key issue here, however, is that ideally attacks should be carried out by as many individual predators as possible in order to avoid learning effects due to repeated encounters to interfere with the results (cf. [16]). To potentially avoid learning by few predator individuals one can either try to ensure that predator density of the study site is generally high (via observations or pre-studies) or expand the study site (e.g. transects), thus decreasing prey density, to ensure a high number of individuals will encounter prey models (cf. [17]).

Many different questions can be addressed using clay models, including how differences in a particular trait affect predation risk, social interactions or how habitat variation may affect predation. These studies can also shed light on abundance or diversity of predators [3]. In literature we frequently find conclusions regarding selective mechanisms, for example linking the evolution of a particular prey trait to selection posed by a particular predator [18, 19]. Although generally valid, giving an insight on these mechanisms, caution should be taken as there is no proof of past predation or past predator communities, in the frame of evolutionary time. Further, there are predators that might impose selective pressures on prey, however are not represented in CMS, as they use different sensory modes in prey detection. For example snakes use chemo- and thermoreception as well as movement for prey detection and we assume that snakes are unlikely to attack non-moving clay models.

There are a number of factors that can influence the outcome of CMS which should be taken into account prior to creating the study design. We would like to mention the following aspects. 1. Seasonal change of predator abundance, e.g. migrating birds: if migrating birds are known potential or main predators of the study species, this must affect the planning of the study (season) and especially the interpretation of the results. Likewise, CMS can also be used to explore such effects [20]. 2. Disturbance by the researchers in the habitat when regularly checking the models: regularly checking the models is especially important when it comes to survival analyses and can increase the power of the study. However, depending on study site and predator species (e.g. shy, rare, with good olfactory senses), too much disturbance by humans might influence predator behavior. 3. Effects of novelty and neophobia: while interpreting results it is important to consider that models might be attacked in the beginning of the study simply because they are new in the environment. Likewise they can be avoided due to neophobia. Such effects could explain sudden shifts in the data (e.g. significantly higher or lower attack rates in the beginning of the experiment compared to the mean attack rate). 4. Avoidance of clay models by predators due to learning their unprofitability when models are placed in the wild for a long period of time: according to our knowledge, there are no studies investigating avoidance learning of clay items by potential predators. However, especially for birds, it is known that they avoid unpalatable prey. They might also learn about the unprofitability of the clay and therefore will avoid attacking the models after some time. Predator avoidance learning has to be taken into account when planning the period of the study (depending on expected predator types).

An even greater limitation and often criticized aspect of CMS is the lack of movement and the thereby relatively poor representation of actual prey items, especially for visually oriented predators. The effect of movement in clay models was tested by Paluh et al. [21] who showed that movement increased the attack rates significantly for cryptically colored prey and significantly decreased attacks on moving aposematically colored prey compared to stationary ones. Moving models do not only better represent real prey, but further add a whole modality (movement) to the perception process which might also change post-detection decision of predators whether or not to attack the model. This is especially and foremost important when studying aposematic prey as they move and forage more openly [22], whereas movement is less important in camouflaged prey, as they normally rely on staying hidden by being stationary. Whether or not to implement movement functions thus strongly depends on the study species and should appropriately represent its natural behavior. Moving aposematic clay models could potentially increase the validity of the method, however is challenging to be implemented in studies using large numbers of models. We are convinced that large numbers of stationary models still enable researchers to draw valid conclusions about predator-prey interactions for both aposematic as well as cryptically colored prey.

Use of clay models beyond predation experiments

Beyond questions addressing predator-prey interactions we advocate the use of clay models in visual detection experiments. Following Rojas et al. [23], who tested detection of models under different lighting conditions as well as different aposematic morphs, we deployed clay models to gain insights on detectability of prey with different morphological traits on different positions in the habitat using "human predators". In this kind of experiment the task is to spot models in the natural habitat to assess the importance of trait or habitat differences for human vision. We think it is very important to differentiate between conspicuousness and detectability of prey. Mostly conspicuousness is merely a measurement of color contrast between an animal and its background. One step further is to actually calculate this contrast on basis of the predator's visual ability by means of visual modeling [24]. However, even when being able to show that a predator is physiologically capable of perceiving said contrast, this does not mean that a detection is taking place in the habitat. Backgrounds in habitats can be complex (e.g. forest floor) and can even have changing light conditions. Thus, these analyses are lacking cognitive aspects. Human predators can be used as a proxy for natural predators as their visions share similarities [25], even in object recognition, as recently shown [26]. Detection experiments with humans are easy to conduct and are a reasonable approach to add a cognitive aspect to conspicuousness measurements and consequently to gain a more comprehensive understanding of conspicuousness and detectability of prey. Still, the drawbacks of this proxy should receive careful evaluation in the context of each study.

One possibility to gain information on cognitive aspects of predator species are laboratory experiments using clay models. Such experiments are especially feasible with various bird species and provide insights into the detection process, decision making and attack events in a controllable environment [27]. These complementary experiments improve the understanding of field-based CMS and lead to a more comprehensive understanding of predator-prey interactions and predator behavior.

The next generation of clay models

In times of eDNA (environmental DNA), where species can be identified from fragments of DNA that are filtered from water, soil and other substrate, it is eligible to ask why we are still trying to identify predators on basis of imprints on clay models or with camera traps. Predator identification on basis of salivary DNA left on clay models are an intriguing and realistic field for the future of clay model studies. A recent study successfully sequenced environmental DNA from residual saliva of brown bears on salmon carcasses [28]. This more forensic approach has a lot of potential in tracking down actual predators and beyond that would enable clay model experiments to be used to provide evidence for species abundance in unknown areas or help with inventories, especially with nocturnal or rare species that are difficult to observe or detect. As DNA degradation is problematic, especially depending on temperature and other environmental conditions, appropriate studies are needed to develop protocols for isolation of predator salivary DNA (psDNA) from clay models.

Conclusion

Clay models are an effective research tool in ecological studies that have led to a considerable amount of knowledge on predator-prey interactions, predator species, effects of trait differences etc. Like all tools, clay models have limitations. More standardization is needed to use clay models as a successful tool especially with regard to the interpretation of attacks, unclear attacks and missing models. Further it is necessary to address the hazard that predatory animals are exposed to, when hazardous clay materials are put in the habitat in large numbers. As alternatives are available that can be deployed in all environments and sufficiently fulfil material requirements, we strongly suggest to stop using PVC-based modeling clays in future clay model studies. Beyond predation experiments, we encourage the use of clay models in detection experiments as a valuable addition to assess to conspicuousness of prey. Lastly we would like to take clay model studies to the next level by encouraging the development of protocols to track usable salivary DNA of predators from clay models to not only identify predators to the species level but also to deploy clay models in the wild to collect evidence for species abundance.

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