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The use of bleach to dissolve spider silk

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Summary

Commercially-available chlorine bleach dissolves spider silk. This application was tested for utility in the identification of prey caught in spider webs, the collection of spider eggs from eggsacs and the cleaning of webs from vials that had housed spiders.

Introduction

The importance of silk to a spider is undeniable. However, for the scientist studying spiders, the silk can be an annoying hindrance to the performance of simple tasks, such as collecting prey remains and eggs or cleaning housing vials. A survey of spider literature revealed 50 studies (list available from RSV upon request) that investigated the natural, field-caught prey composition of spiders that snare prey with webs and cover them at least in part with silk. In these life history studies, prey were removed from webs, sometimes preserved in alcohol and then identified to some taxonomic level (most often family, genus or species but sometimes only to order).

Many lepidopteran larvae use a proteinaceous silk in construction of their cocoon (Chapman, 1982) when metamorphosing to the adult stage. This silk is easily dissolved with commercially-available bleach which is used routinely in the mass-rearing of moths to collect pupae (e.g., Strong *et al.,* 1968). Dilute bleach is also used to dissolve the egg attachment adhesive when procuring lepidopteran eggs laid on paper substrates (Stewart, 1984).

No mention of bleach use to dissolve silk is made in any of the basic araneological texts we examined (Comstock, 1948; Savory, 1977; Kaston, 1978, 1981) although bleach is used to clear epigyna (Griswold, 1993). None of the prey studies we examined employed bleach to aid prey determination.

Herein, we tested (1) the use of bleach to dissolve silk in prey determination studies, (2) collecting spider eggs with bleach and its effects on egg viability, and (3) cleaning maintenance vials with bleach and its subsequent residual toxicity.

Materials and methods

Prey composition of web-spinning spiders: Silk-enswathed prey were removed from the webs of snare-building spiders from August through October 1994 in Riverside, California. The prey consisted of both fresh, recently killed animals being fed upon by spiders and dried carcasses, covered by silk, near a living spider. The latter were assumed to be more difficult to identify properly.

Spiders taken as prey ($n=38$) were soaked in bleach (5% sodium hypochlorite (undiluted Clorox®) was used throughout the study) until the silk shroud dissolved. For these spiders, it was recorded whether the male palp or female epigynum was clearly visible before and after bleach treatment. Prey were identified to the most precise taxonomic level possible. If females were undeterminable from external genitalia, they were dissected.

Enswathed insects removed from spider webs ($n=43$) were identified using external morphological features only. Insects were recorded before and after bleach treatment to the lowest taxon attainable and data were tallied in the following categories: species, genus, family, order, unknown/undeterminable.

Identification of prey items was influenced by our familiarity with the local fauna. Pre- and post-treatment data were tested with a χ^2 test of independence to ascertain if bleach treatment affected the precision of taxonomic determinations.

Two predator spiders predominated in the study: (1) a non-native European pholcid, *Holocnemus pluchei* (Scopoli), which is extremely plentiful in urban southern California, completely enshrouds many of its prey with silk and readily feeds day and night, and (2) the western black widow spider, *Latrodectus hesperus* Chamberlin & Ivie, which is also common, enshrouds its prey lightly but with very strong silk threads and is nocturnal. A preference was biased toward medium-sized prey as our collective taxonomic expertise encompasses spiders, beetles and moths.

To determine if either prey-capture and/or bleach treatment would deleteriously affect the taxonomic characteristics necessary for proper identification of moths, specimens of known species were fed to spiders. Ten moths each of the greater wax moth, *Galleria mellonella* (Linn.) and the tobacco budworm, *Heliothis virescens* (Fabr.) were fed to western black widow spiders. Within several days, the moth carcasses were retrieved, soaked in bleach for approx. 10 s and examined to determine if the diagnostic features were still present. Similarly, moth carcasses of the cabbage looper, *Trichoplusia ni* (Hübner) ($n=3$) and the tobacco budworm ($n=4$) were removed from a cage containing a female bolas spider, *Mastophora cornigera* (Hentz). As above, moths were determined to the most definitive level possible, pre- and post-treatment. Bolas spider prey were soaked in bleach for 2 s and the softened silk was removed with forceps (since 10 s had proved too long in the previous experiment).

Effect of bleach on spider eggs: Four black widow spider eggsacs (1–4 days old) were individually grasped with forceps and held under bleach in a vial. After the silk dissolved, the eggs were rinsed on a 40 mesh brass screen and excess water was blotted off with paper towels. The eggs were placed in a filter-paper-lined, 60 mm glass petri dish and counted. Two control eggsacs were ripped apart and the eggs placed in dishes with no further manipulation. Dishes were taped shut and kept at 28°C at a 14:10 light:dark cycle until 2nd instars were seen hanging in webs inside the dish and then counted. Data from eggsacs in each category were pooled and compared with a χ^2 test of independence.

Cleaning of silk from vials: Vials that had housed black widow spiders were filled with bleach for 10 min, then soaked for 30 min in tap water and air-dried overnight. Pholcid spiders, *H. pluchei* adults and near-adults, were housed individually in either new or bleach-cleaned vials ($n=10$ for each group). Each spider was fed 1 house fly, *Musca domestica* Linn., per week for 4 weeks and the survivorship noted weekly.

Results

Prey composition of web-spinners: When prey items were placed in bleach, the surface of their bodies became effervescent as the bleach dissolved the silk. Usually they were cleared of the enshrouding webs within 10–60 s (Fig. 1). Of the 38 spiders taken as prey, 4 were easily identified before treatment by characteristic somatic features protruding from the silk wrapping. Of the remaining 34 spiders, only in 7 could the diagnostic genitalia be seen while bound, even though the silk swathing of some was very light. After the 38 spiders were treated with bleach, 35 were then identifiable to species. Bleach significantly aided spider species identification in this study ($\chi^2=31.72$, df=1, $p<0.001$). Two unidentifiable spiders were immatures requiring genitalia for generic determination. The remaining spider was a female salticid identifiable only to the genus *Habronattus* with the available keys.

With desiccated, depredated spider specimens, the bleach left a pile of disarticulated body parts which were usually sufficient to allow familial or generic identification despite the disarray of pieces. With fresh spider prey specimens, the bleach treatment made palpi, epigyna and other features accessible for study and had minimal effect on the spider's condition such that they could be subsequently stored in 70% alcohol as museum specimens. Re-examination of these specimens showed no morphological degradation 6 months after treatment.

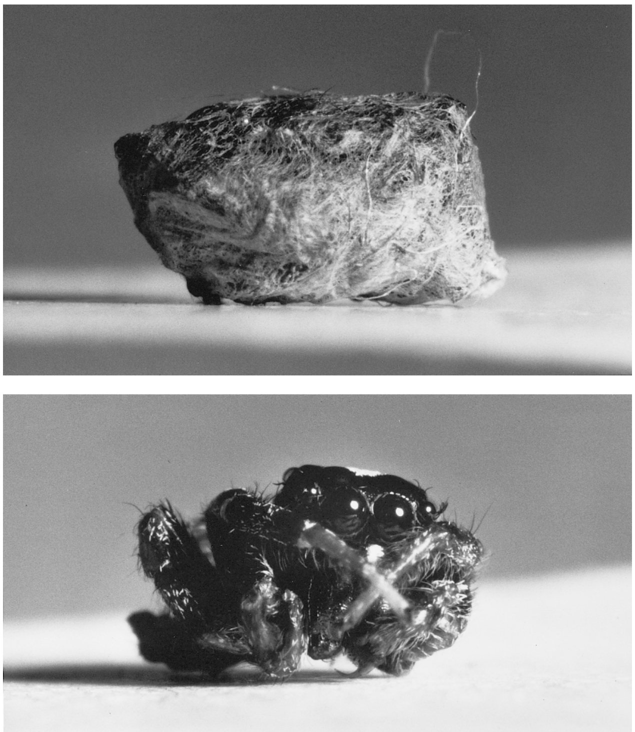

Fig. 1: A prey item before (top) and after (bottom) 30–60 s exposure to bleach treatment. This spider, *Thiodina* sp., was removed from the web of *Holocnemus pluchei.*

Of 43 insect prey items examined before treatment, 0 were identifiable to species, 20 to genus, 19 to family, 2 to order and 2 were unknown/undeterminable. After bleach, these numbers were 12, 22, 6, 3 and 0, respectively. Bleach significantly enhanced the taxonomic determination of insect prey taken from spider webs ($\chi^2=21.06$, df=4, $p<0.001$).

When 10 moths each of the tobacco budworm and the greater wax moth were fed to black widows, initially only 1 wax moth was identifiable to species owing to a wing being extended and visible; all the other moths were identifiable only to family. After a 10 s bleach treatment, the silk was removed from the moths; however, the bleach also removed scales from the wings and all moths could then only be identified to family.

When analysing bolas spider prey, the moths were soaked for only 2 s and the softened strands were easily removed. Before treatment, 2 of 4 tobacco budworms were identifiable to genus and 2 of 3 cabbage loopers to species. After bleaching, all 7 moths were identified to species.

Effect of bleach on eggs: When bleach was used to collect eggs by dissolving eggsacs, the black widow eggsacs dissolved in 15–25 s. The rinsing process added another 15–30 s to the procedure. Average egg hatch for the bleach-collected eggs was 39.6% for the 4 sacs (number hatched/total eggs: 21/41, 16/69, 13/56, 62/117) compared with 100% of the control eggs (147/147, 166/166). Bleach had a highly deleterious effect on black widow egg hatch ($\chi^2=265$, df=1, $p\ll 0.001$) but nonetheless many eggs did hatch.

Cleaning silk from vials: Bleach dissolved all black widow spider silk and loosened debris/faecal material in the vials within 10 min so that the vials could be used again with minimal cleaning effort. Subsequent housing of pholcid spiders in these vials showed no deleterious effects. All spiders in both the control and the experimental group were alive after 4 weeks with several having moulted.

We observed that bleach loses effectiveness with age: bleach that had been in the lab for 6+ months was virtually inert; it softened the silk but did not dissolve it, in contrast to freshly purchased bleach, which was rapidly effective. We also discovered that dipping enshrouded prey in alcohol before bleach treatment reduced the hydrophobic nature of the silk and the silk pall dissolved more quickly.

Discussion

The use of bleach to dissolve spider silk proved to be helpful for applications in arachnology. Prey were identifiable to more refined taxonomic levels than when they were wrapped, eggs could be collected from eggsacs (albeit with greatly reduced viability) and maintenance vials were easily cleaned with no residual toxicity.

Bleach made the task of prey composition identification much easier by allowing us to expose diagnostic features. However, a familiarity with the local fauna was beneficial since some of the somatic features in the depredated specimens were beyond recognition in the digested abdomen or as the parts became disarticulated from the body. The short exposure to bleach does not seem to have sufficiently adverse effects on freshly-captured spiders to preclude their use as museum specimens. Bleach treatment can destroy taxonomically important characters, such as scales on lepidopteran wings, so a balance must be struck between ease of morphological examination and hierarchic level of identification desired.

The use of bleach for collecting eggs reduced the hatching success of black widow spiderlings. Possibly, this might be improved with dilution of the bleach (in moth rearing, a 1% solution is used to dislodge the eggs from paper oviposition substrates (Stewart, 1984)). Also, bleach would be of use if only egg number or volume is desired (e.g., female fecundity) and the success rate of hatched spiderlings is irrelevant.

In its most mundane application, bleach was useful to clean vials used to house spiders. There appears to be no residual effect on spider survival upon subsequent use.

Two results of this study bear on other araneological topics. Identification of bolas spider prey to species level might aid the study of the chemical ecology of these spiders which use sex pheromones of female moths to attract prey (Stowe, 1986). We also caught 8 specimens of *Metaltella simoni* (Keyserling), a South American spider previously unknown in the western United States (Vetter & Visscher, 1995), highlighting the taxonomic significance of spider prey studies facilitated by the bleach techniques reported here.

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