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SHORT COMMUNICATION

Making the invisible visible: methods to enhance features of tiny spider webs

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Abstract. The characteristics and designs of webs provide valuable information on ecology, behavior and phylogenetic relationships. Characters are often obtained from detailed analyses of web photographs. We describe new methods to enhance web visibility; they consisted of painting the inner surfaces of Petri dishes with an opaque black spray paint that produced a rough surface, and then applying a salt spray from a nebulizer or fine white powder to tiny webs constructed in there. Using these methods on webs of *Oecobius concinnus* Simon, 1893, we discerned several unknown details of the refuge and cribellar threads that had previously gone undetected.

Keywords: Nebulizer method, tiny webs, *Oecobius concinnus*

Web designs and web building behavior have been useful to support and clarify phylogenetic relationships among higher taxonomic groups (e.g., families) and to delineate evolutionary patterns in web construction (Eberhard 1990; Agnarsson 2004; Lopardo et al. 2004; Lopardo & Ramírez 2007, Eberhard et al. 2008a; Kuntner & Agnarsson 2009; Eberhard & Barrantes 2015). Much of this information was derived from analyses of high-contrast web photographs that revealed web features and web construction behavior (Lopardo & Ramírez 2007; Barrantes et al. 2017).

Several techniques have been used previously to enhance the visibility of silk threads in spider webs (Comstock 1940; Langer & Eberhard 1969; Eberhard 1976; Carico 1977; Ramírez et al. 2013). Coating webs with cornstarch or talcum powder has been a useful technique to enhance the visibility of webs to allow taking photographs with enough contrast to measure fine details under both field (Eberhard 1976) and lab conditions (Barrantes & Eberhard 2012). However, detailed information on design and features of tiny webs (about 2 cm diameter), such as those of oecobiids, remains elusive, because particles of talcum powder or cornstarch are too large to allow resolution of many of the details.

The purposes of this paper are (1) to describe a new method of enhancing the visibility of fine features of the tiny webs, using *Oecobius concinnus* Simon, 1893 (Oecobiidae) as an example; and (2) to compare it with the talcum powder coating method (Eberhard et al. 2008b). *Oecobius* are known to construct tiny, oval to circular webs that include a tent (Hingston 1925), and an inner layer (carpet) that is closer to the ground and serves as a floor; the spider inhabits the space between the tent and the carpet. The tent is attached at several points to the ground, and spaces between them serve the spider as exits and entrances to the space covered by the tent. In addition, the web has long radial threads that extend outside of the carpet, and cribellate silk threads surrounding it (Glatz 1967), both nearly invisible to the naked eye.

We placed each of 15 adult females in Petri dishes that were previously painted with black paint (BBQ Black Ultra High Heat Spray, Harris Paints). This spray paint produced a fine-grained rough, irregular surface similar to sites where spiders build webs in the field. Each of five finished webs were placed inside a plastic box (length = 30 cm, width = 15 cm, height = 5 cm). We then applied a nebulizer (OMRON model NE-C801LA) twice for 3 min, with a pause of 10 min between applications, covering the box immediately after each application. The nebulizer contained a 0.2 M saline solution that produce a mist of 3 μm crystals in the box that gradually accumulated on the web, making its threads easier to see, measure, and photograph. The 10-min pause between applications was enough to allow the mist to deposit on silk threads and dry out, but we did test shorter intervals.

We applied talcum powder to another five webs (Eberhard et al. 2008b), then turned each Petri dish upside down and gently tapped its bottom repeatedly. Tapping the dish removed most of the talcum powder from the dish and from non-adhesive threads, but talcum adhered tightly to sticky threads. To coat webs with talcum powder, we put talcum inside a cloth bag made from a pair of socks (one inside the other), and then patted the bag gently from a distance of about 10 cm, as described by Eberhard (1976) for cornstarch. For the last five webs, we applied both methods in sequence; first the web was nebulized and then talcum coated. In this case, we also removed the talcum from the dish and non-adhesive threads as described above. We photographed each of the 15 webs with a Nikon Coolpix P100 and a Canon EOS Rebel T2i.

All webs were built at the junction between the floor and the wall of the Petri dish. The tent of *O. concinnus* had a length of 19.92 mm (SD = 2.54 mm), and a width of 8.25 mm (SD = 2.09 mm). Except for the tent, other parts of the web are nearly impossible to see in untreated webs, particularly the section of the tent connected to the substrate and threads laid around the margins of the tent.

The salt crystals made silk threads of the tent and carpet, as well as some radial and cribellate threads visible (Figs. 1 & 2). The threads attaching the tent to the substrate and the carpet, which was seen as a dense layer underneath the tent, could be discerned. Many threads of the tent and the carpet itself could also be distinguished using this technique (Fig. 1a, b). The coating of talcum powder made the cribellate threads around and on the carpet visible, but the talcum powder particles are too big to allow the individual resolution of these threads and had less resolution on the other web details that were visible with the saline solution (Fig. 1c). However, some details of threads, particularly of the carpet, were more difficult to discern in photographs taking after applying both methods in sequence (i.e., talcum powder after nebulizer), though radial threads appeared more evident (Fig. 1d).

For small webs like those of oecobiids, hahniids, erigonine linyphiids, and Mysmeninae (Hormiga et al. 2007), we suggest using each method (nebulizer and talcum powder) on different groups of webs. The application of saline solution will make all threads visible in fine detail, and the talcum powder will allow an easier discrimination of sticky threads. The threads of some small webs may also be further enhanced if both methods are applied in sequence (Fig. 1d). The nebulizer method has at least four advantages over the spray-painting method used by Richter (1970), when applied on tiny webs (GB unpubl. data). With the spray-painting method, threads are

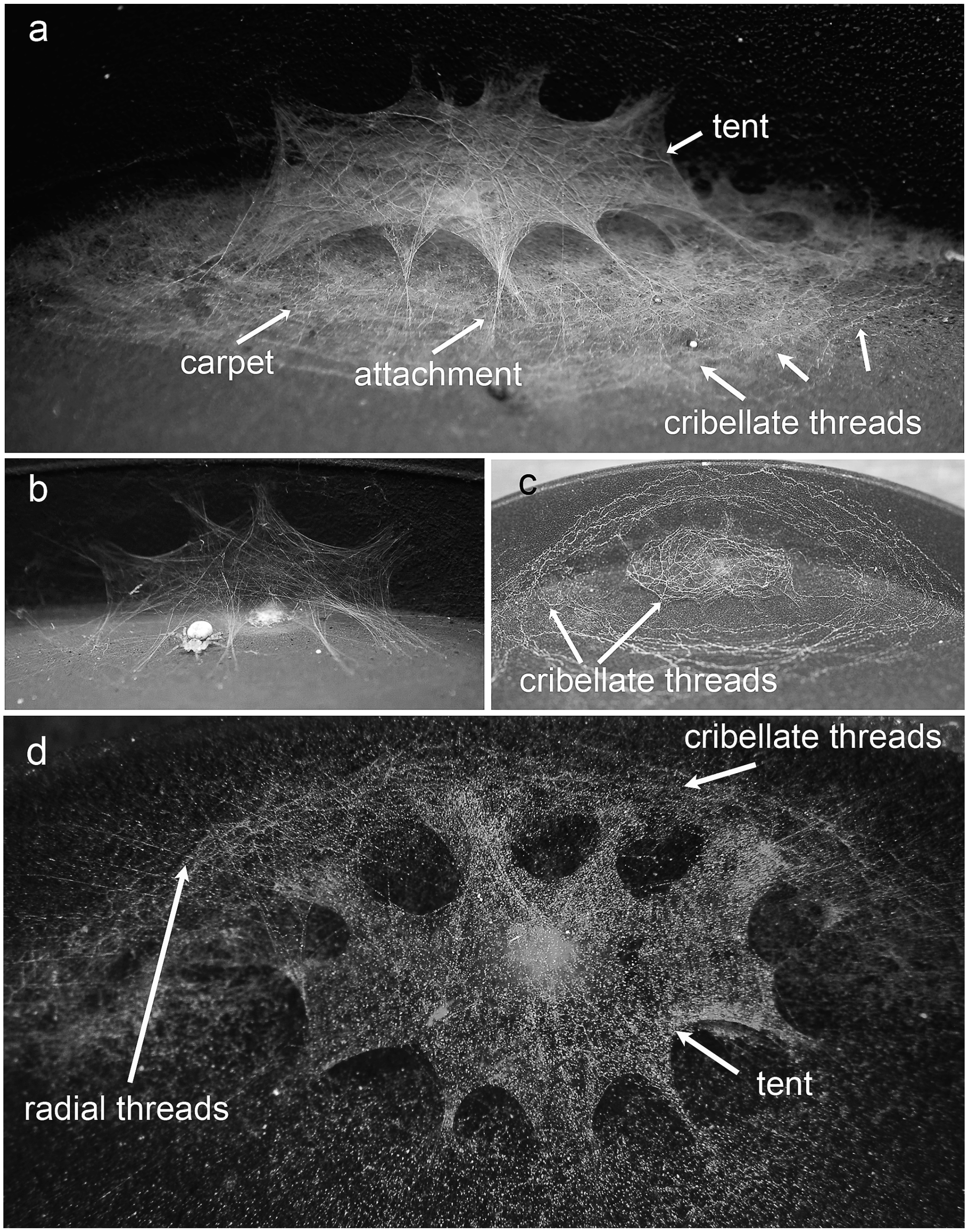

Figure 1.—Characteristics of the web of *Oecobius concinnus*. a- Different components of the web. b- Closeup of the web tent showing individual threads of the tent web. c- Band of cribellate threads around the tent and on the carpet. d- Features of web after applying the nebulizer and the talcum powder methods in sequence. We used the nebulizer method for a and b, talcum powder for c, and both methods for d.

more difficult to discern as they tend to stick to each other; the substrate is also painted, making contrast of threads more difficult; delicate webs could be damaged if spray is applied close to them; and spiders are often affected by paint. The nebulizer method is ecologically friendly and could potentially be applied in the field, but there are two possible limitations. Firstly, webs need to be enclosed right after each application, and this could be difficult in the field, particularly for larger webs. Secondly, the nebulizer has to be connected to an electric source, though this could be solved using a cordless nebulizer.

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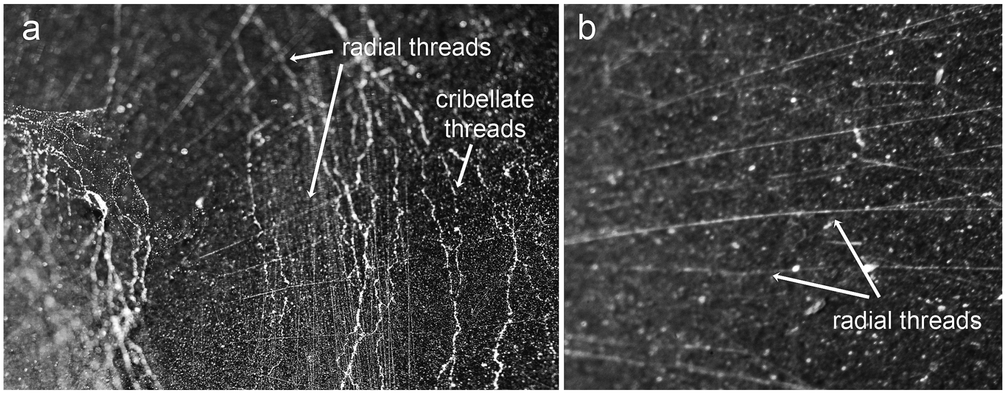

Figure 2.—Detail of radial threads seen with the salt crystal technique. a- Radial and cribellate threads near the tent. b- Detail of radial threads beyond the outer edge of the carpet.

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