

# Appendix from N. Pinter-Wollman et al., “The Effect of Keystone Individuals on Collective Outcomes Can Be Mediated through Interactions or Behavioral Persistence” (Am. Nat., vol. 188, no. 2, p. 240)

## Empirical System

To obtain empirical data on naturally occurring boldness distributions and values for behavioral persistence, we examined the boldness of *Stegodyphus dumicola* spiders.

## Study Site and Animal Maintenance

Colonies of *Stegodyphus dumicola* were collected in February 2014 along the southern Kalahari Desert near Upington, Northern Cape, South Africa (28°27'11.8"S, 21°22'51.8"E). Colonies ranged in size from 232 to 689 individuals. To determine boldness distribution, all spiders were brought to our field lab in Griekwastad, Northern Cape, and their boldness was determined, as detailed below. To determine behavioral persistence, a subset of spiders were transported back to the University of Pittsburgh and kept in isolation for up to 90 days, and their boldness was tested at multiple time points.

## Boldness Assays

We assessed individuals' boldness using an established aversive stimulus assay (Grinsted et al. 2013; Keiser et al. 2014). After 30 s of acclimation in a clean container, we administered two rapid puffs of air to the anterior prosoma of the spider using an infant nose-cleaning bulb. Like many spiders (Jones et al. 2011; Watts et al. 2014), *Stegodyphus* spiders respond to this stimulus by drawing in their legs and becoming still. We then recorded spiders' latency to resume a normal posture and move one complete body length. Trials were terminated after 10 min (600 s). Shorter latencies to resume movement correspond to greater boldness; consequently, we subtract individuals' latency from 600 s to generate a more intuitive “boldness index,” where larger numbers correspond to greater boldness.

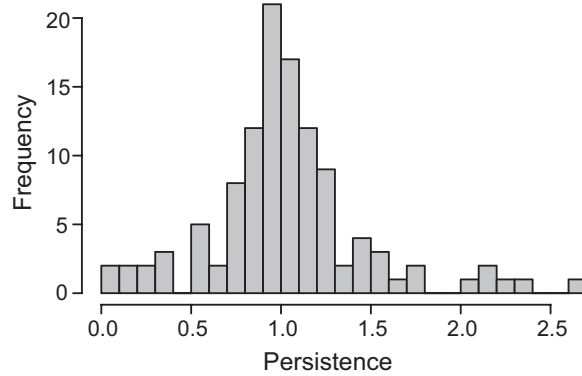
## Persistence of Boldness

Past work on *Stegodyphus dumicola* showed that individuals' boldness is highly repeatable (i.e., persistent) over several weeks when spiders are kept in groups (Pruitt et al. 2013; Laskowski and Pruitt 2014; Modlmeier et al. 2014). However, when in a group, boldness might be influenced by the behavior of other individuals. Therefore, to obtain behavioral persistence values that are equivalent to the persistence parameter of our simulation, we repeatedly examined the boldness of isolated spiders. We followed two protocols; in one, the boldness of 21 isolated spiders was assayed every day for 24 days. Most of these spiders died before the end of the 24-day period, resulting in two to 24 (average 11.2) measurements per spider for a total of 236 measurements. Because daily exposure to an aversive stimulus during the boldness assay may change a spider's response (Pruitt et al. 2016), we also examined changes to boldness in 250 spiders that were isolated for 90 days and whose boldness was assayed once at the beginning of the isolation and once at the end, after 90 days.

To obtain empirical values for behavioral persistence (parameter  $P$  in our model, which is the proportion of boldness kept between two successive time points), we calculated proportion change in boldness (i.e., the boldness of one measurement divided by the boldness of the previous measurement). When more than 1 day elapsed between the two measurements (e.g., 90 days), we raised this value to the power of  $1/(\text{number of days elapsed})$  to obtain the proportion of boldness preserved per day.

Spiders both decreased and increased their boldness over time. When boldness was assayed only twice with 90 days between measurements, per-day change in spider boldness was small. Mean persistence was 1, ranging from 0.97 to 1.05. When spiders were assayed every day for 24 days, persistence varied greatly, ranging from 0.03 to 17 (average 1.31). However, when removing the three outliers that produced persistence greater than 2.6, average persistence went down to

1.03. Most persistence values were between 0.5 and 1.5, with a peak around 1 (fig. A1). Our parameter sensitivity analysis (see supplementary material) predicted that persistence values that lead to a reduction in boldness over time between 0.5 and 1 are most likely to have the greatest impact on collective outcomes. This model implements persistence only in the context of boldness decay over time. Future work is needed to examine what happens when individuals that are not persistent increase their boldness over time. All empirical data are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.5mm01> (Pinter-Wollman et al. 2016).



**Figure A1:** Distribution of empirical persistence values.