2018. Journal of Arachnology 46:373–375

SHORT COMMUNICATION

A method for accurately estimating social spider numbers without colony damage

Bharat Parthasarathy and **Hema Somanathan**: IISER-TVM Centre for Research and Education in Ecology and Evolution (ICREEE), School of Biology, Indian Institute of Science Education and Research, Thiruvananthapuram, Kerala 695011, India. E-mail: bharat@iisertvm.ac.in

Abstract. Ecologists are often required to accurately estimate the number of individuals residing in groups of variable sizes inside opaque shelters. Here, we have used x-rays as a non-destructive solution to this problem in social spiders which reside within collectively built opaque, silken, nest-like retreats. Social spiders are model systems for understanding social organization, collective behaviours and population genetics of inbred populations. Such studies often require an accurate determination of the number of individuals, developmental stages and orientation of individuals within the colony which is difficult without compromising the integrity of their retreat or affecting colony behaviour. We demonstrate the effectiveness of x-rays in accurately estimating colony size in the social spider *Stegodyphus sarasinorum* Karsch, 1892 (Eresidae). This method can also be applied to evaluate body sizes, developmental stages and individual orientation within the colony. We show that this technique does not alter spider prey-capture behaviour or short-term survival compared to control colonies.

Keywords: Animal-built shelters, colony size, social animals, X-rays

Studies in diverse fields such as ecology, behaviour, animal architecture and biocontrol often require screening opaque material for the presence of macroscopic animals without inducing damage to individuals within them. In the recent past, several technological advances such as near-infrared spectroscopy or three dimensional x-ray technologies have been used in studying infestation of cereal kernel (Maghirang et al. 2003; Aldrich et al. 2007) or in studying bee behaviour inside natural colonies, respectively (Greco et al. 2005). X-rays have been used in the past to detect hidden insects, largely wood-boring pest species (Fischer & Tasker 1940; Berryman & Stark 1962) by the pest management industry. However its application in ecology and behavioural investigations is rare. Here, we describe an inexpensive and efficient method that we have used to accurately quantify social spider numbers. Our method can be used to quantify developmental stages, measure body sizes and spatial orientation within colonies of social spiders or shelters of other animal groups.

Social spiders are rare and an interesting model organism for the following reasons. Unlike eusocial insects, social spiders lack morphological castes, but show personality types in certain behaviours (Pruitt et al. 2008, 2010; Pruitt & Reichert 2009), at least in certain contexts (Beleyur et al. 2015; Lichtenstein et al. 2016). Their colonies have highly female-biased sex ratios and inbreed with natal kin, which is believed to have led to a dramatic reduction in diversification rates (Lubin & Bilde 2007). Studies of social spiders often require a reliable, quick and inexpensive method to determine the numbers of individuals without undesirable effects on the colony. Because social spiders live in dense silken colonies, it is impossible to visualize their numbers, developmental stages and spatial orientation inside the colony without severely damaging the retreat. In the past, indirect methods such as estimating retreat/nest volume to infer colony size has been employed (Powers & Avilés 2007), however, this requires standardisation for every species being studied and may not give accurate estimates due to dispersal or immigration. In this study, we examine whether x-rays can be reliably used to determine social spider numbers and whether colonies subjected to x-rays have altered survival and behavioural responses.

We collected twenty colonies of *Stegodyphus sarasinorum* Karsch, 1892 of varying sizes (numbers of resident spiders) in and around Kuppam (12.75° N, 78.37° E), Andhra Pradesh, India in January 2014. Whole colonies were removed by carefully cutting the branch of the plant which contained their retreats. The colonies were immediately transferred into well-ventilated rectangular plastic containers (25 × 18 × 10 cm). The spiders remained within the retreat during this process. We subjected twenty colonies to x-rays (hereafter referred to as x-ray colonies) at 60 kV and 100 mA for 0.4 *s* (Allengers 325/525) at a local medical x-ray facility close to the field site and obtained digital images of the radiogram (Fig. 1). The cost of each x-ray was approximately 7.5 USD (approximately 500 Indian rupees). The colony was placed at an angle of 90^0 from the incident x-ray beam. From these images, we later counted spider numbers. To verify the degree of accuracy of our estimates, we also did manual counts by opening up the retreat structure. Intra-class correlation (ICC) between the spiders counted from the x-rays film and from the manual counts were very similar (ICC = 0.975, $P = 0.0003$, $n = 20$ colonies each; Mean ± SD from x-ray films = 30.8 ± 20.30 and manual count = 33.3 ± 20.94). The means from the two counts were comparable (paired sample *t*-test after 5000 bootstrap runs: t = 1.83, $P = 0.110$, $n = 10$ colonies each; Fig. 2).

Next, we randomly transferred 30 spiders from each of 10 out of the 20 colonies subjected to x-rays into separate well-ventilated plastic containers. For controls, we transferred another 30 spiders from 10 colonies collected from the same area and not subjected to x-radiation into identical plastic containers. Controls and x-ray colonies built dense capture webs inside the box after two days. From the third day onwards, we performed prey capture experiments for six consecutive days as follows: A live honey bee, *Apis cerana*, (maintained inside hive boxes in the field) was added as prey to each box containing spiders. The time taken for the first attack and the number of attackers were recorded for five minutes. The mean number of attackers was similar in controls and x-ray colonies (Mean ± SD = 3.08 ± 2.53 and 3.04 ± 3.10 respectively, Mann Whitney $U = 1039.50$, $P = 0.506$, $n = 10$ colonies each). The mean time to initiate prey capture was also similar between the control and x-ray colonies (34.23 ± 71.04 *s* for control and 25.81 ± 48.73 *s* for x-ray colonies; U = 1060.50, $P = 0.614$, $n = 10$ colonies each). We built separate general linear mixed models (GLMM) in R (version 3.0.2, R Core Team 2013) with number of attackers or prey capture time as response variables and treatment (x-rays or control) as binary dependent variable, colony number and trial number as random effects, and compared these against the null

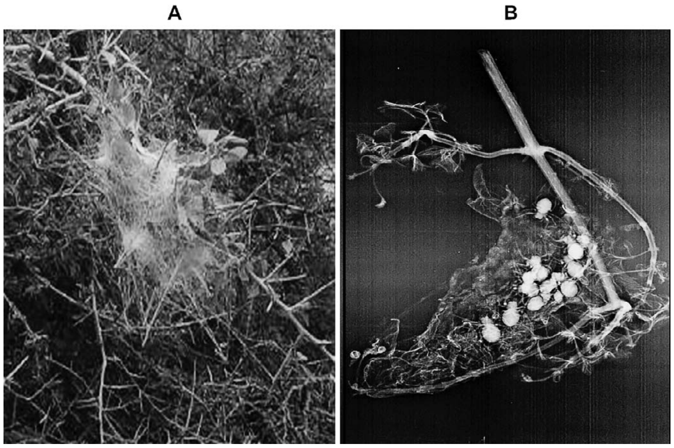

Figure 1.—Retreat of *Stegodyphus sarasinorum*. A. Picture of a spider retreat in the field. Spiders live within the dense opaque silken retreat. B. X-ray image of a spider retreat showing spiders. Twenty colonies were subjected to x-radiation at 60 kV and 100 mA for 0.4 *s*. Social spiders which reside within the dense silken retreat are clearly visible here and the numbers were estimated by counting individuals in the image. The count from the x-ray image was 10 while the actual numbers (from manual count) were 11.

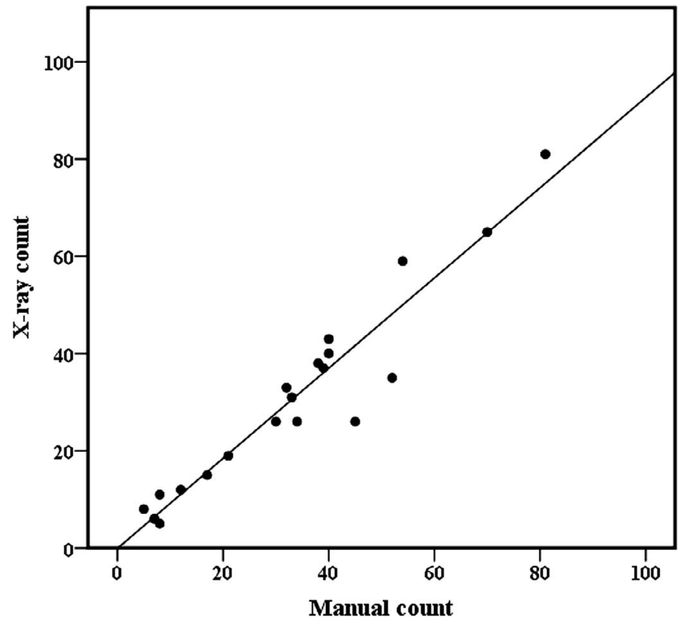

Figure 2.—Plots comparing social spider numbers determined from x-ray films (y-axis) and manual counts (x-axis). Mean ± SD for manual and x-rays count were 33.3 ± 20.94 and 30.8 ± 20.30 respectively (n = 20, ICC = 0.975, P = 0.0003). The regression equation was 0.927 ± 0.067.

model (without the dependent variable). The number of spiders attacking prey was similar in controls and in x-ray colonies as evidenced from non-significant χ^2 values after model comparisons ($\chi^2 = 0.04$, $P = 0.836$). So was the latency to attack between the two groups ($\chi^2 = 0.53$, $P = 0.466$). Survival in x-ray and control colonies was followed over a 10-day period and no mortality was recorded over this duration. Moreover, during this 10-day period, spiders laid four egg sacs in x-ray colonies and three egg sacs in control colonies. In a long-term study on dispersal, we found that x-ray colonies showed similar frequency and timing of dispersal as normal colonies (Parthasarathy and Somanathan 2018), and also produced viable eggs sacs as in normal colonies (unpublished data).

Thus, we demonstrate a reliable and accurate method using x-rays to ascertain social spider colony sizes which does not require opening of retreats, and minimizes disturbance that can trigger dispersal from retreats when colonies are counted manually. Branches containing the retreat, when carefully cut can be returned to the field for further studies after x-rays are taken. If experiments require intact capture webs then portable x-ray machines may be used. As long as the x-rays penetrate the entire retreat, the angle of x-ray beams is not of consequence to count the number of individuals or to examine their spatial orientation within the retreat. We also show that spiders subjected to x-rays behave similar to control colonies in performing collective prey capture, suggesting that x-rays do not alter behavioural responses. Moreover, colonies did not suffer mortality over the short term (10 days) due to the x-ray treatment; they built normal capture webs as in control colonies within two days and also reproduced. Only continuous exposure to x-rays may cause mutagenesis as shown by numerous studies in invertebrates (e.g., Muller 1928; Herskowitz 1950). It is essential to note that it would suffice to subject social spider colonies (or other insects) to x-radiation only once in order to ascertain their numbers, without suffering such consequences. The cost of an x-ray was approximately 7.5 USD in our field site in southern India, however the costs are likely to vary a lot across countries/sites where social spiders are found. Researchers often collect entire retreats containing spiders in boxes and transport them over long distances. In such cases the x-ray can be taken later (through the box), at a convenient time and facility, thus avoiding the use of more invasive methods.

In summary, we suggest that x-radiation may be used to census insects and other invertebrates living within opaque shelters. Thus, this method can also be extended to examine spatial organisation of individuals within communal shelters, developmental stages, presence or absence of kleptoparasites and so on without compromising the integrity of the shelter structure.

ACKNOWLEDGMENTS

We thank Sheheer Ali and Chinmay Joshi for help with experiments. We also thank Dr. Srikanta Dani and two anonymous reviewers for their useful comments on the manuscript. BP thanks IISER TVM for the PhD fellowship and the partial support from the American Arachnology Society. This study was supported by a grant to HS from Council for Industrial and Scientific Research (CSIR), India and intramural funds from IISER TVM.

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Manuscript received 17 September 2017, revised 30 October 2017.