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Egg toxicity in diverse spider taxa

Justin O. Schmidt[1], **Richard S. Vetter**[2] and **Amanda K. Howe**[3†]: [1]Southwestern Biological Institute, 1961 W. Brichta Dr., Tucson, AZ, USA; E-mail: ponerine@dakotacom.net; [2]Department of Entomology, University of California, Riverside, CA, USA; [3]25606 SE 400th St., Enumclaw, WA, USA; [†]Deceased

Abstract. Eggs of black widow spiders in the genus *Latrodectus* Walckenaer, 1805 (Theridiidae) are known to be toxic when injected into mammals. We surveyed eggs from 39 species of spiders in 21 families to determine if spider egg toxicity is a unique property of widow spiders, or if spider egg toxicity is associated with other spider taxa. Eggs from 13 species of spiders in three families were determined to be highly lethal to mice, eggs from four species in four families were moderately lethal, and eggs from 22 species in 17 families lacked detectable lethality. Egg lethality appears to have evolved multiple times in spiders within the Araneidae, Theridiidae, and Agelenidae, and possibly also within the Tetragnathidae, Pimoidae, and Linyphiidae. These toxins in the various spiders may have differing chemical structures and could represent new sources of toxins that may be of future research interest.

Keywords: *Latrodectus, Steatoda, Araneus, Neoscona, Loxosceles,* toxin

Spiders are well known for their venomous bites and for the properties of their associated venoms. In contrast, potential toxins in non-venomous parts of spiders have received scant attention, much of it many years ago. In the late 1800s and early 1900s, R. Kobert, H. Sachs, V. Kellogg, R. Levy and others investigated the toxic properties of spider body tissues and eggs in several species of *Latrodectus* Walckenaer, 1805 for their ability to cause hemolysis and lethality in various animals including rabbits, guinea pigs, cats, dogs, and horses (Kellogg 1915; Bettini & Maroli 1978). D'Amour et al. (1936) extensively investigated the venom and egg toxicity of *Latrodectus* and reported that the precipitates of homogenized eggs, but not of young spiderlings, were toxic when injected intraperitoneally into rats. The authors also demonstrated that the heat-labile material in *Latrodectus* eggs was highly hemolytic to suspended rat erythrocytes.

Russell and associates revisited the properties of black widow (*L. hesperus* Chamberlin & Ivie, 1935) egg toxins and reported that eggs were highly lethal when injected intravenously into mice, and were hemorrhagic when injected intradermally into rabbits (Buffkin & Russell 1971, 1973). The effects from the injected black widow egg toxins were "dramatically different" than those elicited from *Latrodectus* venom toxins (Buffkin et al. 1971). Empty egg shells were not lethal (Buffkin & Russell 1971; Buffkin et al. 1971). They later partially purified several egg protein fractions and reported the main toxin to be a protein of 90–97 kDa (Buffkin et al. 1978). Several active toxins from eggs of *L. tredecimguttatus* (Rossi, 1790), a congeneric of *L. hesperus*, were subsequently isolated and shown to be unique and different from spider venom proteins or other known proteins (Li et al. 2013, 2014; Yan et al. 2014; Lei et al. 2015). One protein of molecular weight 23.8 kDa was highly toxic to mice. In contrast, the main venom component active against vertebrates is α-latrotoxin, which has a molecular weight of about 130 kDa (Rash & Hodgson 2002). Newly emerged widow spiderlings also exhibited high toxicity to mice (Peng et al. 2014).

Debated within the literature is the question of whether egg toxicity is limited to the genus *Latrodectus*, or if spider egg toxins are present in other taxa. Sachs (reported in Kellogg 1915) recorded a toxic principle in the body fluid of *Araneus diadematus* Clerck, 1757 (Araneidae), whereas the bite of that spider caused only locally irritating results. McKeown et al. (2014) also reported a clinical study involving five bites by *A. diadematus*, none of which resulted in serious injury or dermonecrotic lesions, though the bites sometimes caused minor to moderate reactions. D'Amour et al. (1936), working in Denver, Colorado, USA found that the eggs of "the common brown house spider" (species not given, though only one species in Denver, *Parasteatoda tepidariorum* (C.L. Koch, 1841) (Theridiidae), was referred to at that time as the "common house spider" (Paula Cushing, pers. comm.)) were toxic, though less so than those of *Latrodectus*. The authors felt that this poisonous material is found in the tissue fluids of all spiders, especially in their eggs. Buffkin et al. (1971) found no toxicity of eggs from the Arizona recluse spider, *Loxosceles arizonica* Gertsch & Mulaik, 1940 (Sicariidae), a medically important species, and *Araneus* sp. (Araneidae) when tested against mice. They did not explain their reasoning for choosing these two species and the eggs of "several other spider species" for their survey.

The purpose of the present report is to provide a clearer picture of the possible occurrence of egg toxins within a diversity of spider taxa via a broad survey of species. We sought to determine if egg toxins were restricted to *Latrodectus*, were present in close relatives to that genus, or were widespread throughout the Theridiidae, the large family that includes *Latrodectus*. Our goal was to determine if the evolution of spider egg toxins was a single event, or independently occurred several times within spider lineages. As a by-product of this research, we hope that these results might initiate investigations of spider eggs as a source of new toxins of potential use as pharmaceutical tools.

METHODS

Spiders.—All spiders were collected from natural environments in the states of California, Washington, Arizona and Kansas, all in the USA. The spiders were brought into the lab and fed *ad libitum* until they produced egg sacs. Egg sacs, or in some cases live pregnant spiders, were mailed to Tucson,

Table 1.—Taxa, lethality to mice of spider egg extracts, and collection locations of spiders used in this study. Taxa arranged alphabetically by family, then genus and species.

Spider species	Family	LD_{50} (*i.v.*, mg/kg)	Collection location
Agelenopsis potteri (Blackwall, 1846)	Agelenidae	>227.2	USA: WA: King Co.: Enumclaw
Eratigena agrestis (Walckenaer, 1802)	Agelenidae	9.3	USA: WA: King Co.: Enumclaw
E. atrica (C.L. Koch, 1843)	Agelenidae	9.3	USA: WA: King Co.: Enumclaw
Hololena nedra Chamberlin & Ivie, 1942	Agelenidae	>227.2	USA: CA: Fresno Co.: Fowler
Tegenaria domestica (Clerck, 1757)	Agelenidae	40.2	USA: WA: King Co.: Enumclaw
Callobius severus (Simon, 1884)	Amaurobiidae	>227.2	USA: WA: King Co.: Enumclaw
Metaltella simoni (Keyserling, 1878)	Amphinectidae	>227.2	USA: CA: Riverside Co.: Riverside
Araneus diadematus Clerck, 1757	Araneidae	2.5	USA: WA: King Co.: Enumclaw
Neoscona oaxacensis (Keyserling, 1863)	Araneidae	1.3	USA: CA: Fresno Co.: Fresno
*Zygiella atrica (*C.L. Koch, 1845)	Araneidae	5.0	USA: WA: King Co.: Enumclaw
Cicurina pusilla (Simon, 1886)	Dictynidae	>227.2	USA: WA: King Co.: Enumclaw
Cheiracanthium inclusum (Hentz, 1847)	Eutichuridae	>227.2	USA: CA: Riverside Co.: Riverside
Herpyllus propinquus (Keyserling, 1887)	Gnaphosidae	>227.2	USA: CA: Riverside Co.: Riverside
Urozelotes rusticus (L. Koch, 1872)	Gnaphosidae	>227.2	USA: CA: Riverside Co.: Riverside
Bathyphantes brevipes (Emerton, 1917)	Linyphiidae	80.3	USA: WA: King Co.: Enumclaw
Hogna carolinensis (Walckenaer, 1805)	Lycosidae	>227.2	USA: AZ: Cochise Co.: Willcox
Pardosa californica Keyserling, 1887	Lycosidae	>227.2	USA: CA: Riverside Co.: Riverside
Reo eutypus Chamberlin & Ivie, 1935	Mimetidae	>227.2	USA: CA: Riverside Co.: Riverside
Philodromus dispar Walckenaer, 1826	Philodromidae	>227.2	USA: WA: King Co.: Enumclaw
Pholcus phalangioides (Fuesslin, 1775)	Pholcidae	>227.2	USA: CA: Riverside Co.: Riverside
Pimoa altioculata (Keyserling, 1886)	Pimoidae	80.3	USA: WA: King Co.: Enumclaw
Salticus scenicus (Clerck, 1757)	Salticidae	>227.2	USA: WA: King Co.: Enumclaw
Scytodes globula Nicolet, 1849	Scytodidae	>227.2	USA: CA: Riverside Co.: Riverside
Loxosceles reclusa Gertsch & Mulaik, 1940	Sicariidae	>227.2	USA: KS: Johnson Co.: Lenexa
Olios giganteus Keyserling, 1884	Sparassidae	>227.2	USA: AZ: Pima Co.: Tucson
Metellina segmentata (Clerck, 1757)	Tetragnathidae	40.2	USA: WA: King Co.: Enumclaw
Enoplognatha ovata (Clerck, 1757)	Theridiidae	5.0	USA: WA: King Co.: Enumclaw
E. thoracica (Hahn, 1833)	Theridiidae	5.1	USA: WA: King Co.: Enumclaw
Latrodectus geometricus C.L. Koch, 1841	Theridiidae	10.0	USA: CA: Riverside Co.: Riverside
L. hesperus Chamberlin & Ivie, 1935	Theridiidae	10.0	USA: CA: Riverside Co.: Riverside
Parasteatoda tepidariorum (C.L. Koch, 1841)	Theridiidae	>227.2	USA: CA: Riverside Co.: Riverside
Steatoda albomaculata (De Geer, 1778)	Theridiidae	14.2	USA: WA: King Co.: Enumclaw
S. bipunctata (Linnaeus, 1758)	Theridiidae	10.0	USA: WA: King Co.: Enumclaw
S. grossa (C.L. Koch, 1838)	Theridiidae	10.0	USA: CA: Riverside Co.: Riverside
S. triangulosa (Walckenaer, 1802)	Theridiidae	5.0	USA: CA: Riverside Co.: Riverside
Theridion melanurum Hahn, 1831	Theridiidae	>227.2	USA: CA: Riverside Co.: Riverside
Tidarren sisyphoides (Walckenaer, 1841)	Theridiidae	>227.2	USA: CA: Riverside Co.: Riverside
Trachelas mexicanus Banks, 1898	Trachelidae	>227.2	USA: AZ: Maricopa Co.: Phoenix
Uloborus diversus Marks, 1898	Uloboridae	>227.2	USA: CA: San Bernardino Co.: Redlands

Arizona, USA in insulated containers with cool packs. Intentional efforts were made to collect as broad a diversity of spiders from different taxa as possible. In total, 39 species in 33 genera and 21 families were analyzed (Table 1).

Processing of eggs.—The eggs were removed from their sacs using forceps and a dissecting microscope, weighed fresh, and frozen at –25° C until used. Eggs that were discolored, shrunken, or contained unhatched spiderlings were discarded.

Lethality determinations.—White ICR mice of mixed sex and weighing 20–25 g were used for lethality tests. Whole spider eggs were homogenized in saline (0.9% NaCl) and injected intravenously (*i.v.*) in volumes of 100 µl or less into tail veins of mice. Cohorts of four mice per dose were used and doses were doubled until an LD_{50} value was reached, or the highest dose equaled 227.2 mg/kg. In some instances, only enough egg extract was available to test 2 or 3 mice at a dose level of 227.2 mg/kg, in which case, lethality was recorded as >227.2 mg/kg. Lethality was determined at 24 h as described in Schmidt (1995).

RESULTS AND DISCUSSION

Eggs from three spider families were highly lethal to mice with LD_{50} values of 1.3–14.2 mg/kg (Table 1). Four spider families contained members whose eggs were moderately toxic in the range of 15–81 mg/kg, and 17 spider families possessed at least some members with eggs having no detectable lethality at 227.2 mg/kg, the highest dose found practical to test. Overall, 17 of the 39 species, just under half of the tested species, produced toxic eggs. The most toxic eggs, with a lethality of 1.3 mg/kg of whole eggs, were produced by the orb-web-building spider *Neoscona oaxacensis*, a species whose bite produces only minor local reactions and is not considered toxic to humans. Three spider genera, including the widows (*Latrodectus*), false widows (*Steatoda* Sundevall, 1833), and

Enoplognatha Pavesi, 1880, all in the comb-footed spider family Theridiidae, contain species with toxic eggs, though none were as toxic as the eggs of *Neoscona oaxacensis*. Two or more species from within a genus were tested in four genera, three within the Theridiidae and one within the Agelenidae. In all cases, the lethalities of eggs from species within a single genus were either the same, or similar, in being very toxic.

Theridiidae.—Particular emphasis was placed on the eggs of *Latrodectus* species because they were the first species to be noted to have toxic eggs. Eggs of the two species analyzed here, plus of the Old World species *L. tredecimguttatus* (Li et al. 2013) are all toxic, an indication that egg toxicity within the widow genus is widespread, if not universal. The sister genus to *Latrodectus* is *Steatoda*, the false widows (Agnarsson 2004; Arnedo et al. 2004), also known to possess at least mildly toxic venoms similar to those of the true widows (Isbister & Gray 2003). For this reason, we tested four species of *Steatoda*, all of which have egg toxicities similar to those of *Latrodectus*. The Theridiidae is an enormously diverse family of over 120 genera and 2400 species, the fourth largest of the spider families worldwide, and a family whose phylogeny is well-established (Agnarsson 2004; Arnedo et al. 2004). We tested members of six theridiid genera and found three genera to have toxic eggs, and three genera with non-toxic eggs. The third toxic genus, *Enoplognatha*, is not closely related to the subfamily Latrodectinae, in which *Latrodectus* and *Steatoda* reside. The other three genera, *Theridion* Walckenaer, 1805, *Parasteatoda* Archer, 1946 and *Tidarren* Chamberlin & Ivie, 1934, all lack egg toxicity and are in the distant subfamily Theridiinae. From these data, we conclude that egg toxicity evolved independently at least once, possibly twice, in the Theridiidae, but is not universal within the family. Because we were not able to analyze eggs from any genus between the latrodectines and *Enoplognatha*, we cannot determine if toxicity of eggs evolved once or twice. Likewise, we cannot determine if an ancestor of the latrodectines and *Enoplognatha* had toxic eggs and whether that toxicity was lost before the Theridiinae lineage, or if it evolved independently twice, once in the latrodectines and again in a lineage leading to *Enoplognatha*.

Araneidae.—Three species in different genera were evaluated. The eggs of *Neoscona oaxacensis*, a species whose bite is not considered toxic to humans, were the most lethal of all spider eggs. The two other orb-web builders, *Araneus diadematus* and *Zygiella atrica*, also produced highly lethal eggs, though somewhat less lethal than those of *N. oaxacensis*.

Agelenidae.—Five species in four genera in the Agelenidae were analyzed, with both species in the genus *Eratigena* Bolzern, Burckhardt & Hänggi, 2013, exhibiting high egg toxicity. In contrast, eggs of *Tegenaria domestica* exhibited moderate toxicity, and eggs of both *Agelenopsis potteri* and *Hololena nedra*, were non-toxic (Table 1). Recently, the two agelenid species with highly toxic eggs, the hobo spider *Eratigena agrestis* and its congener *E. atrica*, have been transferred to a newly erected genus from the closely related genus *Tegenaria* Latreille, 1804. That eggs of *T. domestica* were only moderately toxic compared to *Eratigena* corroborates the taxonomic transfer by Bolzern et al. (2013).

Other spider families.—Based on the phylogeny of Nentwig (2013), the families Pholcidae, Sicariidae and Scytodidae are nearer the base of the spider tree than are the toxic species. The members of these families that we tested all had non-toxic eggs. Within the Orbiculariae, *Uloborus diversus* eggs were also non-toxic. Moving up the Orbiculariae phylogeny is the Araneoidea, which contains the families Theridiidae, Mimetidae, Tetragnathidae, Araneidae, Pimoidae, and Linyphiidae. We tested members of all six of these families and all, except the Mimetidae, contained species with toxic eggs.

The sister clade to the Orbiculariae spiders is the RTA clade. We tested members of 11 families within this clade and found only those in the Agelenidae contained members with toxic eggs. The Agelenidae reside midway within the RTA clade. These findings within the Agelenidae suggest that egg toxicity evolved independently at least once within the RTA branch and not in the ancestor of that clade.

Comparisons with other studies.—Direct comparisons of our lethality results to those in the literature are difficult. Kellogg (1915) did not provide quantitative data based upon toxin weight and D'Amour et al. (1936) used number of eggs tested. Buffkin et al. (1971) and Li et al. (2013) used either lyophilized egg extracts, or partially purified extracts of eggs. In our tests, we used fresh frozen whole eggs without drying or processing beyond homogenization to minimize possibilities of toxin degradation. These techniques also mean that the actual egg toxins were diluted and are considerably more toxic than reported in our data. Our results differ from those of the other investigators in the taxonomic scope of the spider eggs investigated. D'Amour et al. (1936) felt that body and egg toxins likely were present throughout spiders. In contrast, Buffkin et al. (1971) indicated a lack of egg toxicity in the several species tested. Our results reveal about half of our tested spiders produced toxic eggs, thereby indicating egg toxicity is not limited to *Latrodectus*. The cross spider, *Araneus diadematus*, represents an interesting case study. As reported in Kellogg (1915), both Sachs and Koch found non-venom tissues of *A. diadematus* to contain toxins whereas Buffkin et al. (1971) reported eggs of *Araneus* sp. (presumably *A. diadematus* based on their later work (Russell & Maretić 1979)) to be non-lethal. We found eggs of *A. diadematus* to be highly lethal, four times more lethal than *Latrodectus* eggs.

Biological role of egg toxins.—Our lack of understanding of the biological function of toxins in spider eggs is a source of personal frustration to the authors. This, however, is not without precedent among spider toxins. For example, humans and primates are highly susceptible to the effects of venom toxins from the Sydney funnel web spider, *Atrax robustus* Pickard-Cambridge, 1877 (Hexathelidae), whereas non-primates, including rodents and domestic animals such as dogs are little affected (Sutherland & Tibballs 2001). Since humans and primates were absent until geologically recent times, the evolution of Sydney funnel web spider toxins was unlikely in response to humans or primates.

The fact that egg toxicity is widely distributed within spiders, likely having independently evolved multiple times begs that these toxins have some important biological function. Protection from predators and parasites is one possible role. Arguing against a defensive role are the observations that *Latrodectus* eggs fed to mice produced no symptoms of toxicity (Buffkin et al. 1971) and that *Latrodectus, Steatoda*, and *Araneus* Clerck, 1757 have numerous specialized wasp parasitoids and a chloropid fly egg predator that attack their eggs (Austin 1985; Vetter et al. 2012). In addition, one of us (R.S.V.) fed large gravid female western black widows, *Latrodectus hesperus*, to several potential predators including mice, rats, scorpions, tarantulas, centipedes, and alligator lizards. These animals readily ate the spiders without deleterious effects. An interesting exception was a shrew, *Sorex* sp., that exhibited a negative reaction upon piercing an egg sac with its teeth and immediately retreated and rubbed its snout in the soil of the cage. Arguing in favor of egg toxins having predator protective potential are the observations of Russell & Maretić (1979) that showed lethality and altered behavior in *A. diadematus* when they were fed *Latrodectus* egg extracts, and the shrew behavior described above. The role of spider egg toxins might turn out to be selective protection against some predators or parasitoids, but not others.

Conclusions.—Spider egg toxicity appears to have evolved multiple times: once or twice within the Theridiidae, once in the Tetragnathidae, at least once in the clade containing Araneidae, Pimoidae, Linyphiidae (plus others), and at least once in the Agelenidae. Egg toxicity is not correlated with venom toxicity, as only *Latrodectus* has both eggs and venom that are toxic to mammals. The fact that many different spider taxa produce toxic eggs suggests that spider egg toxins constitute a diverse group of new toxins, or even possibly new classes of toxins, that might be potentially useful in various areas of toxicology or pharmacology.

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