

Spiderling emergence in the tarantula *Grammostola mollicoma* (Ausserer 1875): an experimental approach (Araneae, Theraphosidae)

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Abstract. The ability of *Grammostola mollicoma* (Ausserer 1875) spiderlings (Araneae, Theraphosidae) to emerge from the cocoon without the assistance of their mother was tested experimentally. We created two experimental groups with 23 cocoons in each group. In one of the groups we cut the cocoon wall creating an opening; in the other group, the cocoon remained untouched. We found no differences between the groups in either the number or instar composition of the spiderlings that emerged. The spiderlings were able to emerge without the assistance of their mother. The emerging instars in both groups were precocious compared to previous suggestions in the literature.

Resumen. Ponemos a prueba experimentalmente la capacidad de *Grammostola mollicoma* (Ausserer 1875) (Araneae, Theraphosidae) para salir de la ooteca sin asistencia de su madre. Creamos dos grupos experimentales cada uno con 23 ootecas. En uno de los grupos cortamos la pared de la ooteca mientras que el otro permaneció intocado (grupo control). No encontramos diferencias en el número de arañitas emergidas ni en los estadios de emergencia entre los grupos. Los hijos pudieron emerger sin la asistencia de su madre. Los estadios en que las arañitas emergieron en ambos grupos fueron levemente más precoces que lo indicado previamente en la literatura. Se discuten posibles explicaciones para estos resultados.

Keywords: Cocoon-opening, mother assistance, emergence-instars

Once spiders hatch from their eggs, they usually molt one or more times inside the cocoon or egg sac before they emerge. Although cocoon care and postembryonic development in spiders have been thoroughly studied, spiderling emergence is only known from a few observations, mainly in Araneomorphae (Eason 1964; Engelhard 1964; Fujii 1978; Vannini et al. 1986; Riechert & Jones 2001; Kürpick 2000; Viera et al. 2007 a & b). The mother's assistance is indispensable for the emergence of juveniles from the cocoon in lycosids (Fujii 1978; Higashi & Rovner 1975), in the subsocial theridiid *Anelosimus cf. studiosus* (Viera et al. 2007b), and in the eresid *Stegodyphus lineatus* (Latreille 1817) (Schneider and Lubin 1997). In these species, spiderlings are unable to open and exit the egg sac by themselves and die if the mother does not open it. In contrast, in other Araneomorphae like orb weavers, where the mother dies a few days after building the cocoon, the spiderlings are capable of emerging even in her absence (Foelix 1996).

In mygalomorphs, Coyle & Icenogle (1994) suggested from indirect evidence that spiderlings of the antrodiaetid *Aliatypus* spp. may need the mother's assistance to emerge, while Marechal (1994) observed that spiderlings of the diplurid *Ischnotele guianensis* (Walckenaer 1837) are able to hatch by themselves from the cocoon without external help. No other reports about spiderling emergence are available for most mygalomorph families.

Members of the family Theraphosidae are usually large and long-lived and in the last decades they have become popular as pets in many countries. Although several papers report different aspects of their biology (Petrunkévitch 1911, 1934; Baerg 1928, 1958; Gerhardt 1929, 1933; Bucherl 1952; Melchers 1964; Galiano 1969, 1973a & b, 1984, 1992; Stradling 1978, 1994; Minch 1979; Celerier 1981; Kotzman 1990; Costa & Pérez Miles 1992, 2002; Pérez-Miles & Costa 1992; Marshall & Uetz 1993; Schillington & Verrell 1997; Huber 1998; Janowsky-Bell & Horner 1999; Loch et al. 1999; Punzo & Henderson 1999; Yañez et al. 1999), none describes

spiderling emergence in detail. Although we observe mother care of cocoons and spiderlings in several theraphosids, including *Grammostola mollicoma* (Ausserer 1875), spiderling emergence remains obscure.

Usually the eclosion of the spider from the egg determines the transition from the embryonic to postembryonic life (Foelix 1996). However, the characteristics in which the spiders hatch varies in different species (Holm 1940; Vachon 1957; Peck & Whitcomb 1970; Ramousse & Wurdak 1984), contributing to a confusion in the terms and descriptions of developmental instars in spiders (Foelix 1996). As Galiano has provided a comprehensive study of theraphosid development (1969, 1973a & b, 1984), we followed her terms and concepts. Using her terms, instar A is the 1st intrachorionic state and consequently instar B is the first instar out of the egg. Although in instar B the spider is completely free of the egg, the body is bent and the legs are extended but do not contact with substratum and are not functional for locomotion. In addition, the eyes and several kinds of setae are absent. These instars as well as instars C and D are completed within the cocoon in *Grammostola pulchripes* (Simon 1891). More intra-cocoon instars and less development in the early instars was considered as a derived characteristic for Theraphosidae in comparison with other mygalomorphs (Galiano 1969).

In this study we experimentally tested if spiderlings of the theraphosine *G. mollicoma* are able to emerge from cocoons without the assistance of their mothers. We additionally compared the instars of emergence with predictions from previous postembryonic studies (Galiano 1969, 1973), discussing their possible adaptive value.

METHODS

We used 46 cocoons of *G. mollicoma* (northern form) obtained through the Uruguayan mail and confiscated from illegal trade. All were presumably collected at a site near Achar, Tacuarembó, Uruguay [32°23'60"S, 56°04'57"W] con-

Table 1.—Number of cocoons and distribution of instars in which spiderlings emerged in treatment and control groups. See methods for explanation of abbreviations for different instars (C–E).

Instars of emerged spiderlings	C	C + D	D	E	D + E	Not registered
No. of Cocoons Treatment group	2	0	6	2	8	4
No. of Cocoons Control group	0	1	3	1	12	3

sidering police evidence, habitat description and known distribution of this species. In addition, 800 adults of *G. mollicoma* were simultaneously confiscated in another mailing by the same person from the same locality.

Cocoons were maintained in plastic containers (83 mm diam. $\times$ 105 mm high). Observations took place from 24 January to 7 March 2007. We divided the cocoons into two experimental groups with 23 cocoons in each one. In one of these groups (treatment group) we made a cut 5 mm long with scissors in the cocoon wall on the first day of observation (this cut was approximately of the same size as natural orifices made by spiderlings); in the other group (control), the cocoons remained closed. During the experimental period the room temperature varied between $26.6 \pm 1.4^\circ\text{C}$ and $24.2 \pm 1.4^\circ\text{C}$, and photoperiod was natural (approximately 14 h day:10 h night).

We examined the cocoons daily to monitor the spiderling's emergence. When the spiderlings emerged, we counted them and determined the postembryonic instars (following Galiano 1969). Instar A is the last intracorial instar and B is the first extracorial instar; we did not observe these instars in our study. Instar C characteristics include: bent body, absence of body pigments, absence of eyes (only maculas), legs not completely functional. Instar D has cephalothorax and abdomen in the same plane, pigments present, eyes present, absence of tarsal scopulae and claw tufts, slow locomotion. Instar E shows body densely hirsute, scopula and claw tufts present and normal locomotion. When more than 50 spiderlings emerged, the remaining progeny was preserved in ethanol and examined, the cocoon was measured (major and minor axis) and its natural openings were counted. At the end of the experiment, the cocoons that remained closed were opened and examined. Voucher spiderlings and opened cocoons were deposited in the arachnology collection of Facultad de Ciencias, Montevideo, Uruguay.

RESULTS

Spiderlings successfully emerged from 22 cocoons in the treatment group and 19 in the control group (non-treatment). No significant differences were found in the frequency of spiderling emergence between groups ($X^2 = 2.02$, $P = 0.15$). The mean number of spiderlings born alive per cocoon in the treatment group was (mean $\pm$ SD) 91.5 ± 46.8 and in the control 102.0 ± 38.4 , again with no significant differences between groups ($t = 0.78$, $P = 0.45$).

The spiderlings emerged from their cocoons in instars C, D, and E, both in the treatment and control groups. Very few spiderlings emerged in instar C: one and six from each of two cocoons of the treatment group and one from a cocoon of the control group. Curiously, in some cocoons spiderlings emerged simultaneously in two different instars (Table 1). No additional teeth on the chelicerae nor bifurcated cheliceral tips or other structures related to cocoon opening were found

in the emerged spiderlings. Table 1 shows how the pattern of instar emergence was distributed among the cocoons. The distribution of mean numbers of spiderlings emerged in instars D and E by cocoon group are shown in Fig. 1. All the spiderlings that emerged in instar D molted to instar E within 24 h after emergence.

Of the four cocoons from which spiderlings did not emerge, we found eggs infected with fungus in two of them, dry eggs in another, and nine spiderlings alive (three of them molting), seven dead, and several unhatched eggs in the last cocoon.

The period from the beginning of the observation to the emergence of spiderlings from the cocoons took 12.9 ± 8.2 days in the treatment group and 10.1 ± 5.9 days in the control group, with no significant difference between these periods ($t = 1.25$, $P = 0.23$).

Cocoons averaged 43.3 ± 6.8 mm and 39.6 ± 7.4 mm (major and minimum axes) in the treatment group and 43.8 ± 5.8 mm and 41.6 ± 5.8 mm in the control group, showing no significant differences in cocoon size between the groups (major axis: $t = 0.28$, $P = 0.78$; minimum axis: $t = 0.96$, $P = 0.34$). The number of natural perforations, if we do not consider the experimental cut, was 0.55 ± 0.60 in the treatment group and 1.53 ± 0.61 in the control group. Significant differences were found between groups ($t = 5.19$; $P < 0.0001$).

DISCUSSION

Our results clearly showed that spiderlings of *G. mollicoma* are able to emerge from the cocoon without the assistance of their mother as was reported by Marechal (1994) for the diplurid *I. guianensis*. Coyle & Icenogle (1994) did not observe

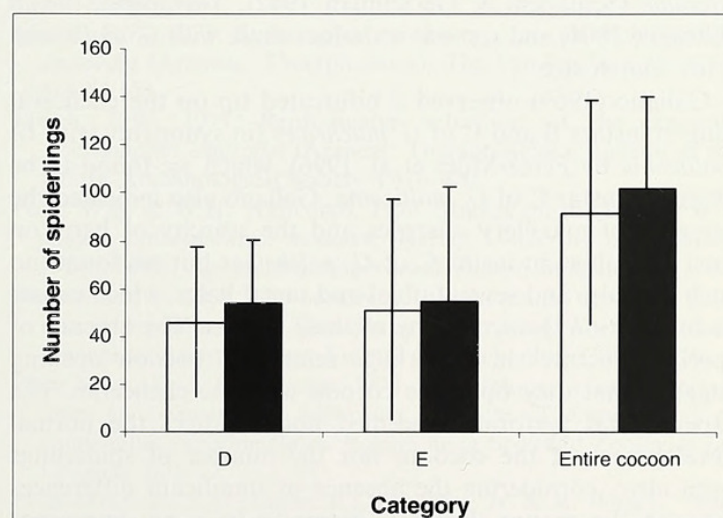


Figure 1.—Means and standard deviation of spiderlings emerged in instars D, E and of total occurrence of spiderlings alive per cocoon (including those that remained inside the cocoon). The treatment group is represented in white and the control group in black.

Table 2.—Reports of number of spiderlings* or eggs per cocoon and cocoon size in Theraphosidae taken from the literature.

Species	Number of spiderlings* or eggs	Cocoon Size (major axis)	Author
<i>Avicularia avicularia</i>	103–145 *	-	Stradling 1994
<i>Avicularia metallica</i>	178–182	-	Charpentier 1992
<i>Avicularia versicolor</i>	12–221	-	Charpentier 1992
<i>Pachistopelma rufonigrum</i>	30, 30 *	-	Dias & Brescovit 2003
<i>Acanthoscurria gigantea</i>	600	-	Ibarra Grasso 1961
<i>Aphonopelma chalcodes</i>	454–555	-	Minch, 1978
<i>Aphonopelma hentzi</i>	206–911	-	Punzo, 1999
<i>Aphonopelma joshua</i>	51 *	12 mm	Prentice 1997
<i>Ceropelma longisternale</i>	16–111	-	Costa et al. 1992
<i>Dugesiella crinita</i>	800–1000	-	Baerg 1958
<i>Dugesiella hentzi</i>	500–1000	-	Baerg 1958
<i>Euathlus smithii</i>	> 700	-	Clarke 1991
<i>Eurypelma californica</i>	621–1018	-	Baerg 1938
<i>Grammostola burzaquensis</i>	100–120	30 mm	Ibarra Grasso 1961
<i>Grammostola mollicoma</i>		43.5 mm	this study
<i>Phamphobetus roseus</i>	1200	-	Ibarra Grasso 1961
<i>Theraphosa blondi</i>	36, 44 *	60 mm	Lambert & Dupre 1992
<i>Theraphosa blondi</i>	78 ± 5.57	-	Marshall & Uetz 1993

direct evidence for the mother's assistance in the antrodiaetid *Aliatypus* spp. Their indirect observations provided weak support for the hypothesis that the mother's help is required. The small number of natural perforations found in the cocoons of the treatment group suggests that spiderlings could utilize pre-existent holes.

Copulation during egg sac care was reported recently for this species (Postiglioni 2007). The ability of spiderlings to emerge without assistance might be an important trait that permits the mother to be receptive and exposed to the risks (i.e., predation) of courtship and copulation without jeopardizing the success of her spiderlings.

The mean number of spiderlings per cocoon (or clutch) for *G. mollicoma* was moderately low in comparison with most Theraphosidae (Table 2). This characteristic could be interpreted as plesiomorphic if compared with the sister family Barychelidae with 20–80 eggs (Raven 1994). Other theraphosids as *Aphonopelma joshua* Prentice 1997, *Plesiopelma longisternale* (Schiapelli & Gerschman 1942), *Theraphosa blondi* (Latreille 1804) and some avicularines share with *G. mollicoma* a low clutch size.

Galiano (1969) observed a bifurcated tip on the cheliceral fang in instars B and C of *G. pulchripes* (in synonymy with *G. mollicoma* by Pérez-Miles et al. 1996), which we found to be absent in instar C of *G. mollicoma*. Galiano also indicated the presence of maxillary cuspules and the scarcity of hairs on tarsi and tibiae in instar C of *G. pulchripes* but we found no such cuspules and several tibial and tarsal hairs, which causes us to question the synonymy of these species. The absence of special structures in spiderlings related to cocoon opening suggests that they open the cocoon with the chelicerae. The experimental perforation seemed not to affect the normal development of the cocoons nor the number of spiderlings born alive, considering the absence of significant differences between the groups. Instar C seems to be a pre-emergence instar because few individuals emerged in this instar, and only in the treatment group. The absence of eyes and pigments and the bent body, which impedes locomotion (Galiano 1969), also agree with a pre-emergence instar.

Galiano (1969) incubated isolated eggs of *G. pulchripes* outside the cocoon, and based on morphological evidence during development, proposed that spiderlings emerge in instar E. Our results partially agree with this author because we found that half of *G. mollicoma* spiderlings emerged in instars D, molting to E outside the cocoon within about 24 h. The emergence in two different instars also indicates a slight asynchrony of molting between instars D and E. However, instar E seems to be the first in which the spiderlings are independent enough and ready for their free life (Galiano 1969). Instar E is the first instar where urticating hairs develop (Pérez-Miles 2002) and the spiderlings are able to feed by themselves (occasional observations). It is not clear what the advantages are for the spiders to emerge in the precocious instar D. Probably in this instar spiderlings are not able to open the cocoon by themselves and instead use the openings made by spiderlings in instar E to emerge. Prentice (1997) reported that spiderlings of *Aphonopelma joshua* Prentice 1997 emerge in the fourth or fifth instar probably homologous with D and E, which is similar to our findings in *G. mollicoma*.

Galiano (1969), following Holm (1956), indicated that a low quantity of yolk and an increased degree of organization in early extracocoonal instars imply a few number of instars intra-cocoon and a plesiomorphic condition, as in *Telechoris striatipes* (Simon 1889) [(ex *Ischnothele karschi* (Bösenberg & Lenz 1895)]. Galiano (1969) has stressed the importance of having four instars intra-cocoon in *G. mollicoma* and consequently considered this characteristic as derived in comparison with most mygalomorphs and other Araneae. For example the Mesothelae *Heptathela kimurai* (Kishida 1920) and other mygalomorphs such as *Atypus karschi* Dönitz 1887 and *Telechoris striatipes*, have only two or three states intra-cocoon (Yoshikura 1952, 1958 and Holm 1956; cited by Galiano 1969). In *H. kimurai* and *T. striatipes*, spiderlings have two instars inside the cocoon while *A. karschi* has three. Stradling (1994) reported only one intra-cocoon postembryonic instar in *Avicularia avicularia* (Linnaeus 1758), which seems to be a record in Theraphosidae. Our findings in *G. mollicoma* with three instars intra-cocoon question the indirect

evidence of Galiano (1969) and consequently the evolutionary interpretation of Galiano.

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