RESEARCH NOTE

WHICH SPERMATHECA IS INSEMINATED BY EACH PALP IN THERAPHOSIDAE SPIDERS?: A STUDY OF OLIGOXYSTRE ARGENTINENSIS (ISCHNOCOLINAE)

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Which female receptacle is reached by a particular (right or left) palpal organ and how deep the embolus is inserted are unresolved problems in mygalomorph spiders. Despite evidence for the sperm storage function of spermathecae in some haplogyne spiders (including Mygalomorphae) (Coyle et al. 1983), literature dealing with these questions is scarce. The complementarity between male and female genital structures has been, until now, the only useful evidence regarding the lateral correspondence and the depth of insertion in Mygalomorphae (Coyle et al. 1983; Costa & Pérez-Miles 1998) studied both issues using copulations by mono-palpectomized males and consequent histological identification and location of sperm masses in the two spermathecal receptacles.

Oligoxystre argentinensis (Mello-Leitão 1941) is a medium-sized theraphosid from temperate South America. Male palpal organs have a very long embolus (Fig. 1). The adult females have, attached to the bursa copulatrix, two separated spermathecal receptacles, each one consisting of a long stalk with a spherical fundus (Fig. 2). Three males and six females of this species were collected in Sierra de las Animas (34°45'S, 55°21'W), Maldonado, Uruguay. They were reared in petri dishes containing moist cotton, and fed mainly with larvae of *Tenebrio* sp. (Coleoptera, Tenebrionidae). Females molted in the laboratory, thus they had empty spermathecae.

The right palpal organ of each male was covered with a drop of paraffin to prevent its use, but the covered palps were autotomized one or two days after manipulation. After a week each male was placed together with a "virgin" female for the first series of copulations. At least a week after these encounters, each male was placed with another "virgin" female for a second series of copulations. As soon as copulations were finished females were mechanically sacrificed by a cephalothorax puncture, and their spermathecae were immediately removed by dissection. Spermathecae were fixed in paraformaldehyde, impregnated with osmium tetroxide and embedded in araldite. Longitudinal sections were stained with toluidine blue and examined with an optical microscope. Voucher specimens were deposited in the arachnid collection of the Facultad de Ciencias, Montevideo.

In the first series of copulations each male inserted his only palp (the left) 2, 3 and 4 times, respectively. In the second series each of the three males performed two insertions. In the first series two females each had both spermathecal receptacles completely filled with sperm (Fig. 3) (the third specimen, corresponding to the two-insertion copulation, was damaged). In the second series, one female had her left spermathecal receptacle filled with sperm and the right one empty; while in the other two females, both spermathecal receptacles were empty. The only male which had inseminated a female in the second series had performed only two insertions in the first series.

The availability of both filled and empty spermathecal receptacles made it possible for us to study and compare them. We observed in the spermathecal wall the presence of orifices and features that resemble pores and glands as described by De Carlo (1973) in species of *Grammostola* and *Acanthoscurria*. Sections of four sperm-filled receptacles and sections of five empty receptacles were measured (in mm), with an accuracy of 0.01 mm. Mean total width (measured in the middle of

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Figures 1, 2.—*Oligoxystre argentinensis.* 1, Left male palpal organ (ventral view); 2, Female spermathecae (ventral view). Scale = 1 mm.

their length) of filled receptacles was 0.30 (± 0.00 SD), while empty receptacles measured 0.274 (± 0.046 SD). The Student's *t*-test showed significant differences between them (t = 6.14, P < 0.001). Mean wall width (including inner cuticle and epithelial layer, following De Carlo 1973) showed no significant differences between filled and empty receptacles (0.125 ± 0.17 SD and 0.126 ± 0.17, respectively). The mean width of the lumen was 0.063 (± 0.010 SD) in sperm-filled receptacles, and 0.023 (± 0.022 SD) in empty ones. The Student's *t*-test showed significant differences between them (t = 3.63, P < 0.01).

Results indicate that a given palp is able to inseminate either or both spermathecal receptacles in O. argentinensis. Unexpectedly, there is no evidence of morphological or ethological constraints which prevent a palp from delivering sperm to either receptacle. Our findings also suggest that sperm are directly deposited by the embolus deep into the spermathecal receptacles, since females were sacrificed soon after mating and no immediate sperm transfer mechanisms along the spermathecae are known. The increased receptacle lumen width in filled spermathecae resulted from the stretching (expansion) of the spermathecal wall rather than the reduction of wall thickness. Finally, the low insemination level observed in the second series lead us to suspect that these males had difficulties recharging the palpal organs after their first copulations. One



Figure 3.—Longitudinal section of a spermathecal receptacle of *Oligoxystre argentinensis* filled with sperm mass.

possible explanation could be that the absence of one palp disturbed the sperm induction behavior of these males.

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