Hidden morphological novelty enabling the evolution of female penis in the sex-role reversed cave insects

The discovery of a female penis in the Brazilian cave insect genus *Neotrogla* (order Psocodea, family Prionoglarididae, tribe Sensitibillini) received considerable attention by the scientific community and the public (Fig. 1) [1]. Similar female penis is also known from the African cave insect genus *Afrotrogla* (also a member of Sensitibillini) [2]. During copulation, the females of *Neotrogla* (and probably *Afrotrogla*) receive nutritious semen from the males using the female penis, and the semen forms a capsule within the female's sperm storage organ (spermatheca) (Fig. 1(d)). The female *Neotrogla* consume the contents of this seminal capsule not only for fertilization but also as nutrition. In the majority of animals, males are more active for mating (conventional sex-role). In contrast, to compete for this nutritious semen, the female *Neotrogla* evolved a more active role during mating than males (sex-role reversal), which is considered to be the major evolutionary pressure (reversed sexual selection) facilitating the evolution of female penis.

Although there are other more examples of sex-role reversed animals, the genera *Neotrogla* and *Afrotrogla* are the only ones known to date in which a female penis has evolved [3]. Due to the systematic position of these two genera (Fig. 3), an independent origin of the female penis has to be assumed (since the genus *Sensitibilla*, the sister group of *Afrotrogla*, lacks female penis) [2]. Therefore, in addition to the reversed sexual selection caused by sex-role reversal, there must be at least an additional key factor which facilitated the evolution of a female penis. In search of such a potential factor, we focused on a specialized structure (spermathecal plate) located at the entrance of the spermatheca.

In order to examine the detailed structure of this minute spermathecal plate, we used synchrotron μCT (SR-μCT) imaging, as well as light microscopes and confocal scanning laser microscopy. High resolution SR-μCT was carried out at SPring-8 **BL47XU** using a beam energy of 8 keV in absorption-contrast mode. The tomography system consists of a full field X-ray microscope with Fresnel zone plate optics. The field of view and the effective pixel size are 0.11 mm×0.11 mm and 0.0826 μm×0.0826 μm, respectively.

As a result, we identified the existence of a tiny switching valve (about 0.3 mm wide) at the entrance of the spermatheca (Fig. 2) [4]. The valve system is composed of an inlet duct, a closure element, actuator muscles to incline the closure element, resilin patches (a rubber-like protein) supporting the closure element, and two plates forming two insemination slots. This valve system serves to direct the seminal flow provided from the male into one of two slots

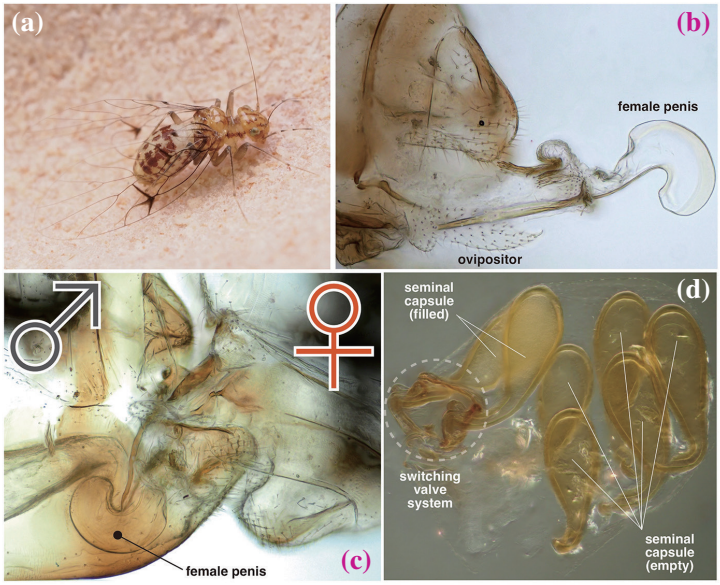

Fig. 1. **(a)** *Neotrogla brasiliensis* photographs in a cave in Brazil. **(b)** Female penis of *N. curvata*. **(c)** Male and female terminal abdomens of *N. curvata* in copula. **(d)** Light microscopy photograph of the spermatheca, including the switching valve system and seminal capsules.

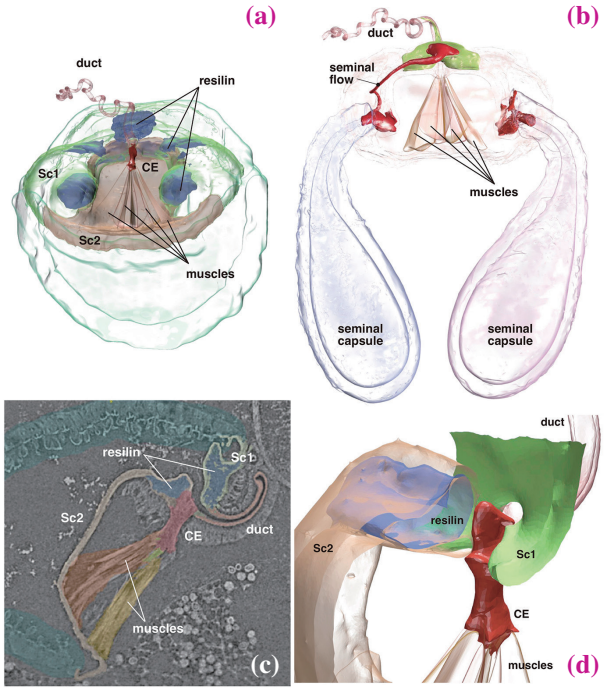

Fig. 2. Morphology of the spermatheca and spermathecal plate of *Neotrogla*. **(a)** 3D segmentation of the spermathecal plate with no seminal capsule. Abbreviations. CE: closure element; Sc1 and 2: body sclerite 1 and 2. **(b)** 3D segmentation of the spermathecal plate with two seminal capsules and showing seminal flow. **(c)** Muscle attachment to CE. **(d)** Detail of CE.

of insemination (Fig. 2(b)). The existence of similar spermathecal plate is also confirmed in *Afrotrogla* and *Sensitibilla* (i.e., all genera of Sensitibillini), although its detailed structure and function have not been examined. This represents the first discovery of a functional biological switching valve in the animal kingdom.

The females of close relatives of Sensitibillini can only receive one seminal capsule at a time. In contrast, by using the switching valve, the females of Sensitibillini can hold two seminal capsules at a time (Fig. 1(d) and Fig. 2(b)). Therefore, the switching valve makes it possible for female Sensitibillini to receive more nutrition from the males, rendering the female-female competition for the nutritious semen more intense in comparison to other sex-role reversed animals. In addition, independent origins of the female penis, which is an extremely rare evolutionary event, were identified in Sensitibillini, even though this is a small group of cave insects containing three genera and eleven named species, only (Fig. 3) [2]. It is very likely that the evolution of this switching valve was a prerequisite for the reversal of the genital organs in Sensitibillini.

Many mechanical solutions engineered by humans do not exist in nature. The switching valve discovered in *Neotrogla* is constructed in a fundamentally different manner than engineered switching valves (Fig. 2) such as those in the oil and gas industry or for changing the water flows in the shower. This micrometer-sized structure could inspire alternative valve technologies on a micrometer/nanometer scale.

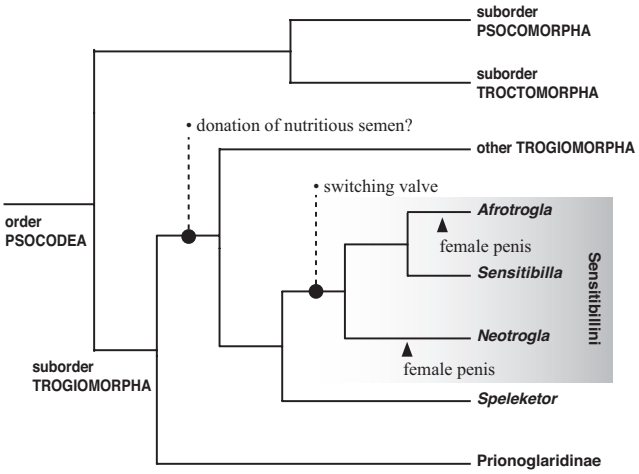

Fig. 3. Phylogeny of Psocodea and assumed history of character evolution related to the origin of female penis.

Kazunori Yoshizawa[a,*] and Alexander Blanke[b]

[a] Systematic Entomology, School of Agriculture, Hokkaido University
[b] Institute for Zoology, University of Cologne

*Email: psocid@res.agr.hokudai.ac.jp

References

[1] K. Yoshizawa *et al.*: Curr. Biol. **24** (2014) 1006.
[2] K. Yoshizawa *et al.*: Biol. Lett. **14** (2018) 20180533.
[3] Y. Kamimura and K. Yoshizawa: Sex Role Reversal in Encyclopedia of Animal Cognition and Behavior (Springer, 2007).
[4] *K. Yoshizawa, Y. Kamimura, C. Lienhard, R.L. Ferreira and A. Blanke: eLife 7 (2018) e39563.*