

# Female Penis, Male Vagina, and Their Correlated Evolution in a Cave Insect

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## Summary

Sex-specific elaborations are common in animals and have attracted the attention of many biologists, including Darwin [1]. It is accepted that sexual selection promotes the evolution of sex-specific elaborations. Due to the faster replenishment rate of gametes, males generally have higher potential reproductive and optimal mating rates than females. Therefore, sexual selection acts strongly on males [2], leading to the rapid evolution and diversification of male genitalia [3]. Male genitalia are sometimes used as devices for coercive holding of females as a result of sexual conflict over mating [4, 5]. In contrast, female genitalia are usually simple. Here we report the reversal of intromittent organs in the insect genus *Neotrogla* (Psocodea: Prionoglarididae) from Brazilian caves. Females have a highly elaborate, penis-like structure, the gynosome, while males lack an intromittent organ. The gynosome has species-specific elaborations, such as numerous spines that fit species-specific pouches in the simple male genital chamber. During prolonged copulation (~40–70 hr), a large and potentially nutritious ejaculate is transferred from the male via the gynosome. The correlated genital evolution in *Neotrogla* is probably driven by reversed sexual selection with females competing for seminal gifts. Nothing similar is known among sex-role reversed animals.

## Results and Discussion

The genus *Neotrogla* (Figure 1A) contains four named species (adult body length 2.7–3.7 mm) [6, 7]. Its most striking feature is the presence of a large penis-like structure in the female, termed a gynosome (Figures 1, 2, and 3; Figures S1, S2, and S3 available online). We show here that the gynosome is erectile, basally membranous, and apically sclerotized. Its sclerotized part consists of a proximal rod-like extension and a penis-like distal prominence. The latter encloses a duct leading to the sperm storage organ (spermatheca), and is interpreted as a novel structure differentiated from the opening region of the spermathecal duct (Figure 1, light blue) [6]. In contrast, the male genitalia (phallosome) consist of a simple, thin arc lacking an intromittent organ (Figures 1G and 3E). In related insects, the spermathecal duct has a simple opening and the phallosome is well developed (Figure 1).

We observed coupling in all *Neotrogla* species and found that the gynosome acts as an intromittent organ to receive voluminous spermatophores from the male. As in most related taxa, including those having well-developed male genitalia (Figure 1C) [8], the male is positioned under the female during copulation (Figure 1A). The apical sclerotized part of the gynosome, bearing the opening of the spermathecal duct, deeply penetrates the male (Figures 2, 3, S2, and S3), and its tip fits the opening of the seminal duct (Figures 2D and 3C). The membranous part inflates within the male genital chamber, and numerous spines on the membrane internally anchor the female to the male (Figures 2B, 2E, 3A, 3D, and S2). In this position, the male sternum is gripped between the female paraprocts and inflated gynosome (Figures 2B and 2C). Only the connection of the abdominal tips holds pairs fixed in copula together. Furthermore, pulling apart coupled specimens (*N. curvata*:  $n = 1$ ) led to separation of the male abdomen from the thorax without breaking the genital coupling, showing that the female can hold the male tightly using the gynosome and paraprocts.

The gynosomal structures are species specific. The distal sclerotized part is strongly curved in *N. curvata* (Figures 2A–2D), but is straight or only slightly curved in other species (Figures 3 and S2). The membranous region of *N. curvata* has a smooth dorsal lobe (yellow) and five areas bearing sclerotized spines: a dorsal (red), a pair of lateral (green), and a pair of ventrolateral (purple) spiny areas (Figure 2). In *N. aurora* and *N. brasiliensis*, the dorsal and lateral spiny areas are present (Figure S2), but the dorsal lobe and ventrolateral spiny areas are absent. The gynosome of *N. truncata* lacks all elaborations, but its membranous part is densely covered by tiny bristled spines (Figure 3).

Male genitalia are simple, but also species specific, corresponding to the gynosomal structures (Table S1). In *N. curvata*, the seminal duct is strongly curved, as is the gynosomal apical sclerite (Figures 2B–2D), whereas these are straight or only slightly curved in the others (Figure 3 and S2). The male genital chamber of *N. aurora*, *N. brasiliensis*, and *N. curvata* has lateral pouches corresponding to the lateral spiny areas of the gynosome (Figures 1G, 2, S1B, and S2, green arrowheads), while ventrolateral spiny areas and corresponding male pouches are only present in *N. curvata* (Figures 2B, 2E, and S1B, purple arrowheads). During copulation, the spiny areas fit into the corresponding pouches and anchor the female (Figures 2 and S2). The gynosome of *N. truncata* lacks strong spines (Figure 3A and 3D), and the female of this species anchors itself using the entire surface of the bristled gynosomal membrane (Figure 3A and 3D). The male genital chamber of this species lacks any pouches (Figure 3E, open green arrowheads).

Spiny genitalia are present in many male animals [3, 5, 9]. These may be used as stimulatory devices [10, 11] or may result from sexual conflict [12, 13], in addition to having an anchoring function to grasp and hold mates. Species-specific membranous pouches in female genitalia are reported in some insects to accommodate the male genitalia [5, 9] and thereby reduce the cost of mating imposed by the corresponding male genital spines. The relative function and pattern of elaboration of male and female genitalia in *Neotrogla* are completely reversed relative to that generally observed.

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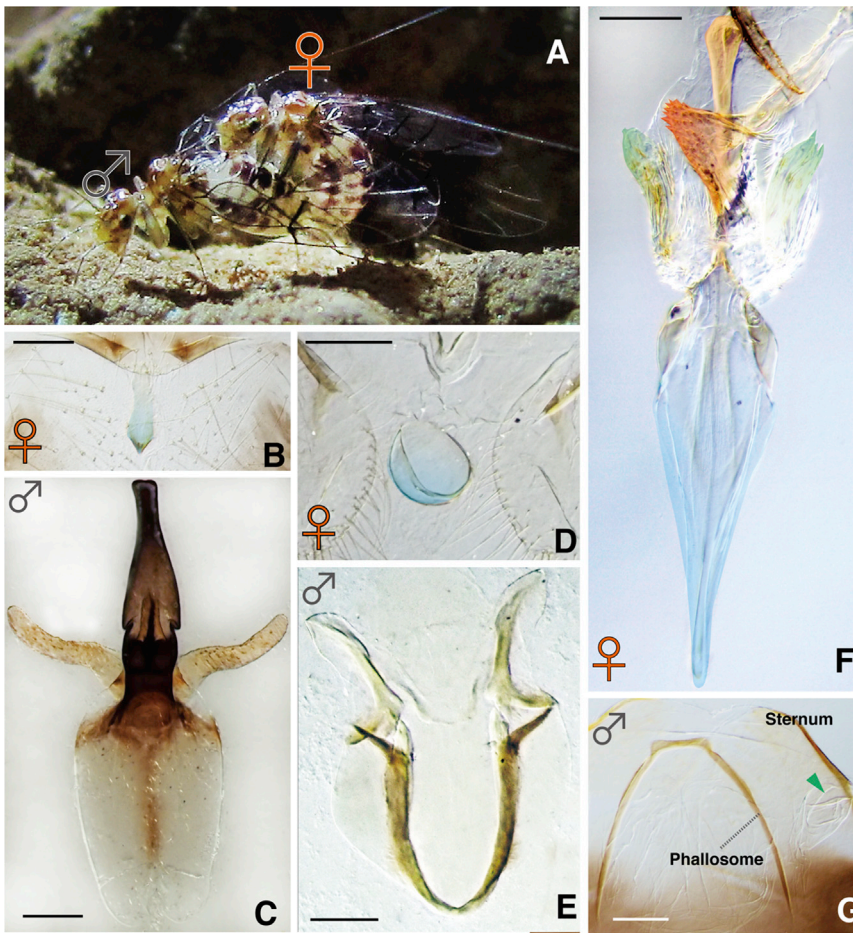


Figure 1. Male and Female Genitalia of Prionoglarididae

(A) *Neotrogla curvata* in copula.  
(B and C) *Prionoglaris dactyloides* spermathecal duct opening (B; light blue) and phallosome (C).  
(D and E) *Speleketor irwini*, same as in (B) and (C).  
(F and G) *Neotrogla aurora* gynosome (F; parts highlighted as in Figures 2 and 3) and phallosome (G). The green arrowhead in (G) indicates the left lateral pouch in the male genital chamber.  
Scale bars represent 0.1 mm. See also Figure S1 and Table S2.

selection is reversed (without reversal of the intromittent organ). Males of this species transmit specialized spermatophores (seminal capsules formed during copulation within the female's spermatheca), which are produced by the enlarged seminal duct [19]. Males are choosier about mates than females, indicating the costs of spermatophore production, while females compete for the nutritious seminal gift [19, 20]. In *Neotrogla*, similarly shaped spermatophores (Figures 3F, S3C, and S3D) and an enlarged seminal duct producing voluminous spermatophore material (Figures 2B, S3A, and S3B) are present, suggesting that *Neotrogla* males also donate a nutritious seminal gift to females. All known *Neotrogla* species inhabit extremely dry oligotrophic caves and feed on bat guano and bat carcasses, which are relatively scarce

In certain astigmatan mites [14] and scirtid beetles [15], male genitalia are reduced and females possess an elongated intromittent tube or an eversible genital duct, respectively. Although these organs are used to obtain sperm or spermatophores, no anchoring mechanism has been observed. Female seahorses have an intromittent ovipositor to deposit eggs in the male brood pouch [16], but this is not a penis. The evolution of a female penis is likely to be strongly constrained because, in internally fertilizing animals, the ancestral condition is exclusively that of an inseminating male requiring an intromittent organ and a receiving female, so that integrated modifications in male and female structures and behaviors are required [17]. Therefore very few animals have reversed intromittent copulatory organs.

It is known that reversed sexual selection has sometimes caused the evolution of masculine characteristics or of secondary genitalia in females. For example, female ground weta (Orthoptera: Anostostomatidae: *Hemiandrus pallitarsis*) benefit from seminal gifts and compete intensely for them [18], using an elbow-shaped structure located in the middle of the abdomen to obtain them from males. An anchoring intromittent organ, such as the gynosome, might be even more effective at taking up seminal gifts from males. Nevertheless, reversed intromittent organs were previously unknown among animals with reversed sexual selection.

Insects related to *Neotrogla* suggest the potential of nuptial gifts favoring the evolution and diversification of the gynosome. In *Lepinotus patruelis* (Trogidae), the direction of sexual

resources [6, 7]. Under such circumstances, nutritious seminal gifts cause a strong selection pressure for increased female mating rate [21]. During their life, *Neotrogla* females may acquire several spermatophores (up to 11 have been observed in *N. brasiliensis*) (Figures 3F and S3C) [6]; they are evidently polyandrous. We also observed that females consumed the contents of the spermatophores after their first mating before producing mature eggs ( $n = 5$ ; Figure S3D), suggesting that the contents of the spermatophores are probably used for nutrition as well as fertilization.

This interpretation may explain the following unique characteristics of the female internal genitalia and of the coupling behavior of *Neotrogla*. The spermathecal duct of *Neotrogla* is divided by a spermathecal plate, such that the female can simultaneously maintain two filled spermatophores (Figures 3F and S3C), something unknown in related taxa [19, 22]. The duration of copulation in *N. curvata* is exceptionally long ( $52.5 \pm 11.2$  hr, mean  $\pm$  SD, range 41–73 hr,  $n = 12$ ; Table S2) in comparison with related taxa: approximately 40 min in *Prionoglaris stygia* [8], approximately 2 hr in *Trogium pulsatorium* [22], and a maximum of 4 hr in the genus *Lepinotus* [8, 19]. In *Neotrogla*, females have structures that can coercively hold males. The very long copulation, as well as polyandry, is probably controlled by females to obtain more seminal gifts from males.

Sexual conflict over the donation of a nutritious seminal gift is thus the most likely factor favoring the evolution of the gynosome. This organ may have a premating function



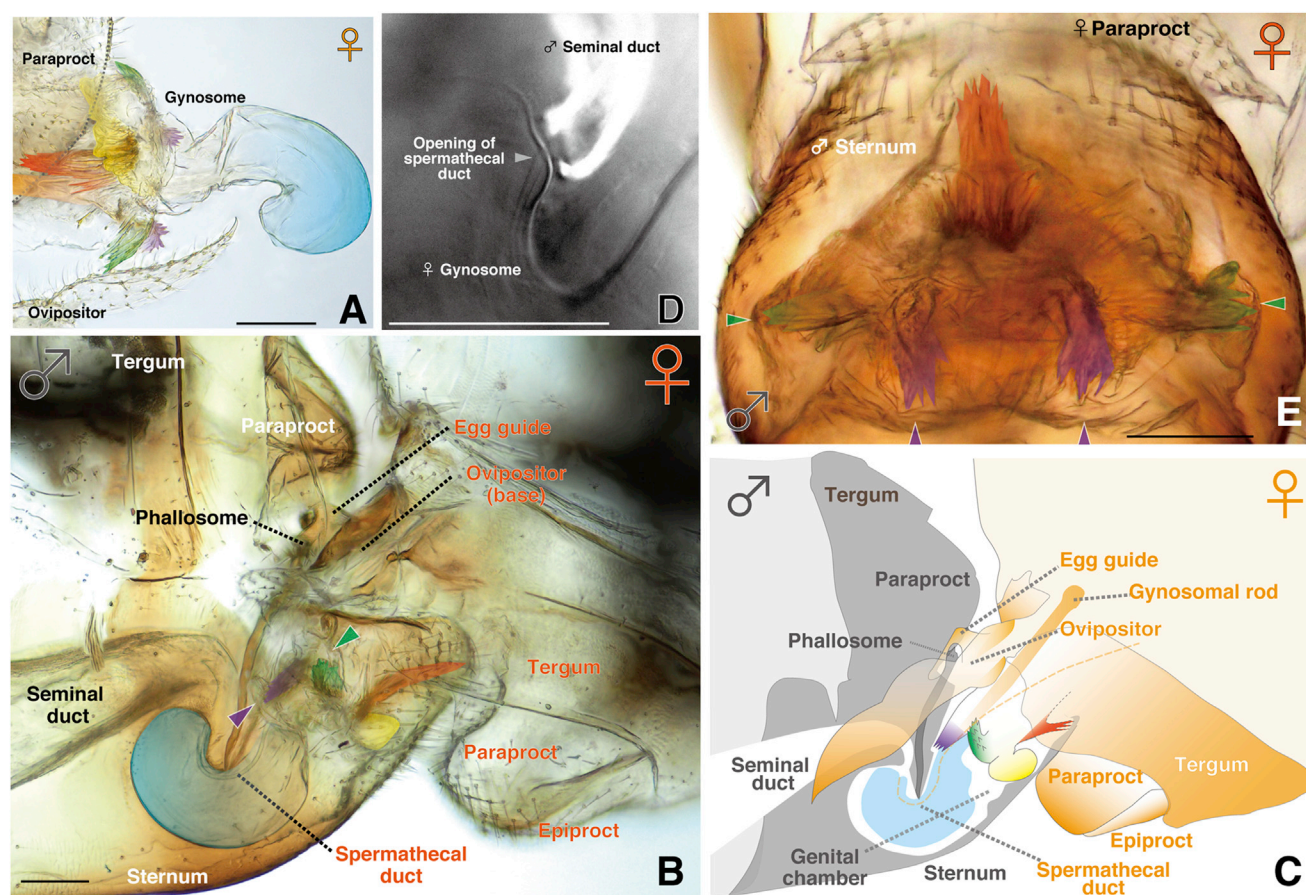


Figure 2. Terminal Structures of *Neotrogla curvata*

The following parts of the gynosome are highlighted: distal sclerite (light blue); basal rod (orange); and membranous region with dorsal lobe (yellow) and lateral (green), dorsal (red), and ventrolateral (purple) spiny areas. The corresponding male genital pouches are indicated by arrowheads of the same color.

(A) Erect gynosome, dorsolateral view.

(B–E) Terminal abdomens in copula, lateral (B–D), and ventral (E) views. The gynosome tip and seminal duct opening are magnified in (D). In the schematic drawing (C), female structures, except for the distal part of the gynosome, are indicated in orange and male structures in gray.

Scale bars represent 0.1 mm. See also [Figure S2](#) and [Table S1](#).

grasping reluctant mates or a postmating function holding mates to ensure prolonged copulation, although these functions are not mutually exclusive. Because other paternal investments, such as parental care, are not observed in *Neotrogla*, the correlated evolution of morphologically and functionally novel genital organs has probably been driven by reversed sexual selection on copulatory function. Sex-role-reversed animals are valuable for testing the generality of theories of sexual selection [23]. Because sex-role-reversed females usually cannot coercively hold males, they may be constrained in terms of evolving adaptations that relate to sexual conflict. The female genital anchoring mechanism of *Neotrogla*, correlating morphologically with specialized reduced male genitalia, is unique, and nothing similar is known among other sex-role-reversed animals. However, in addition to anchoring, the gynosomal spines may have other functions, such as genital stimulation or inflicting harm. The evidence for reversed sexual selection and sexual conflict provided here is mostly circumstantial, but further controlled studies of the mating system of *Neotrogla* species, together with an exploration of their phylogeny, would provide an extremely rare opportunity to test the generality and relative importance of some hypotheses about sexual selection

[1–5, 9–13]. *Neotrogla* also offers a significant opportunity to study evolutionary novelty, an area of central interest in contemporary evolutionary biology [17, 24].

#### Experimental Procedures

See the [Supplemental Information](#) for additional details.

#### Sampling

*Neotrogla* specimens in copula were killed with hot water (~80°C) and fixed with 80% ethanol in caves. We observed three pairs of *N. aurora*, four pairs of *N. brasiliensis*, 11 pairs of *N. curvata*, and six pairs of *N. truncata*.

#### Morphological Observations

We used BABB (1:2 benzyl alcohol:benzyl benzoate) to make muscles and sclerites transparent for examination [25]. This method does not dissolve soft tissues, and specimens in copula can be observed in situ (Figures 2D and 3C). However, BABB could not make fat bodies transparent, and we used 1% KOH to dissolve soft internal tissues after embedding abdomens in 1% agarose. Observations were performed in glycerol (unmounted) or after slide mounting (dissected female and male genitalia). An Olympus SZX16 stereo microscope and a Zeiss Axiophot compound light microscope were used for examination. Photographs were taken with an Olympus E-330 or OM-D E-M5 digital camera attached to the microscopes. Partially focused photographs were combined using Helicon Focus (Helicon Soft) to obtain images with high field depth.

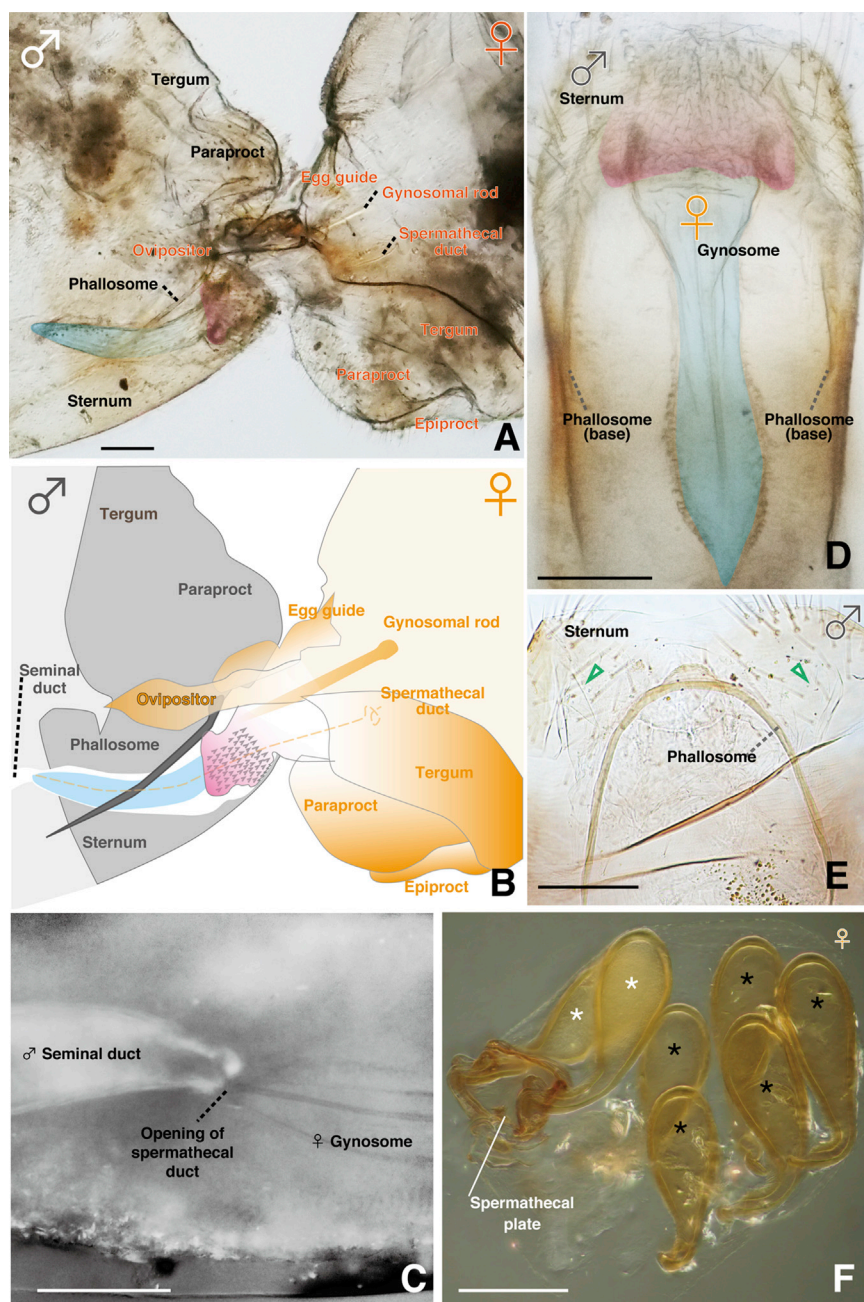


Figure 3. Terminal Structures of *Neotroglia truncata*

Color scheme as in Figure 2, plus pink color indicating the basal gynosomal membrane.

(A–D) Terminal abdomens in copula, lateral (A–C) and ventral (D) views. (B) shows a schematic drawing. The gynosome tip and seminal duct opening are magnified in (C).

(E) Slide-mounted male genitalia. Green open arrowheads indicate a lack of membranous pouches. See Figure 1G for comparison.

(F) Spermatheca fixed during copulation. Seven spermatophores are present, of which two attached to the spermathecal plate are filled (indicated by white asterisks); the others are separated from the plate and are empty (black asterisks).

Scale bars represent 0.1 mm. See also Figure S3 and Table S1.

### Behavioral Observations

A total of 12 couplings of *N. curvata* were observed (Table S2). Specimens were kept in Styrofoam boxes during observation. Adults were placed together, and when a couple formed, it was placed in a separate vial for observation. Copulations were observed at 30 min intervals. After copulation, some pairs were kept for observation until they died ( $n = 2$ ), sometimes in the presence of their F1 nymphs.

### Supplemental Information

Supplemental Information includes three figures, two tables, and Supplemental Experimental Procedures and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2014.03.022>.

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