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Elaborate gonopods in the myriapod genus *Chersastus* (Diplopoda: Trigiulidae)

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Abstract

Two-paired gonopods of *Chersastus* were examined under light and scanning microscopy. Species-specific structures with complexities are described. These gonopods are test models for elaborate genitalia hypotheses: lock-and-key, pleiotropism, genitalic recognition, mechanical conflict of interests, cryptic female choice. Structures of potential sperm displacement are described.

Keywords: arthropod, function, progoneate, spirobolid.

1. Introduction

Millipedes display sexual dimorphism in primary sexual characters^[1]. External openings of reproductive tracts are anteriorly sited *i.e.* progoneate, stemming from penes on third diplo-segment or vulvae on the second diplo-segment, of males or females respectively. The ejaculate is indirectly transferred from the male penes to the female vulvae^[2]. This is achieved by paired accessory intromittent gonopods, modified legs of the seventh diplo-segment. The gonopods are species-specific characters that may supersede the primary male genitalia in taxonomy^[3, 6]. The non-inclusive functional morphology of gonopods is noted^[7, 15].

The genital structures of odonates and diplopods have evolved rapidly and divergently if considering indirect sperm transfer^[16, 17]. The structures have dual functions in transferring ejaculate and displacing rival ejaculate from females^[7, 8, 11]. *Chersastus* gonopods do not develop as gradual modifications of functional walking legs but pass through unique progressive growth differentiation^[18], and both pairs of legs on diplo-segment VII are modified.

Copulating millipedes exemplify how hypotheses for evolution of genitalia are testable in millipedes^[7, 14, 19, 20]. The hypotheses: ^[1] lock-and-key^[21, 22], ^[2] genitalic recognition^[23, 24], ^[3] mechanical conflict of interests^[25, 26], ^[4] pleiotropism^[22], and ^[5] female control^[20, 26].

2. Materials & Methods

Four species of *Chersastus* were delimited based on morphology. The gonopods were dissected from males of each species and prepared for scanning electron microscopy (SEM). Specimens were fixed, first in 2.5% glutaraldehyde (pH 7.4 phosphate-buffered saline) at 4 °C for 24 hours, then in osmium tetroxide (2%). Dehydration through graded alcohol (50% through 100% ethanol) and critical point drying followed. Specimens were mounted on stubs and sputter-coated with gold-palladium. Gonopods were viewed under a Cambridge S200. SEM micrographs were examined and the individual components of the gonopods were identified according to the available species descriptions. Two sets of linear measurements were made from the SEM micrographs: ^[1] the prominence of the sternite, which has been estimated as a ratio of how far it extends from the basal region up to the top of the coleopods; ^[2] the length of the spines present on the distal knob were estimated from measurements taken from SEM images.

3. Results

The gonopods of *Chersastus* are complex unlike walking legs. SEM revealed all structures with possible functions in lock-and-keys, mechanical and sensory recognition, conflict of interests and copulatory courtship. Each gonopod consists of an anterior and posterior sex leg. The phallopods, being the posterior legs, are hidden behind the anterior legs. The coleopods, being the anterior legs, are visible without magnification when males are viewed ventrally. Potentially informative regions seen and labelled on species:

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(i) *Chersastus anulatus*

Coleopods: The basal joint is angular on the inner side. The distal joint bears a lappet near the terminal thickening, the latter beset with little conical points that will be termed the "spines". Phallopods: The distal lamellae, termed the lappet for obvious and also potentially functional reasons, are broadly rounded and low, not reaching the margin ^[27].

(ii) *Chersastus inscriptus*

Coleopods: The sternite is triangular and prominent up to the middle of the gonopod. The basal joint is broad and rounded at the tip while the second joint is somewhat narrow with two lateral lobes. The summit of the telopodite is covered with little knobs and spines seated in pits. Phallopods are not immediately visible: the description of a broad tibial process that projects a little ^[30]. The lappet differs from the previous species in that it extends beyond the margin ^[28].

(iii) *Chersastus fulgidus*

Coleopods: The distal joint has an elongated lower lobe produced laterally plus three ridges medially. The distal region of the coleopods are not as broad in the other three.

The species descriptions given here are congruent with those originally described and accompanied by line drawings made through light microscopes and hand lenses by ^[27, 31]. The original abbreviations have been retained wherever possible. The position of the phallopods is such that they are seated behind, and protected by the coleopods. The static examination of the gonopod ultrastructures did not reveal the phallopods but the entire structural complexity of the phallopod is revealed from an examination of their mechanics in copula.

(iv) *C. ruber*

Coleopods: The basal joint has a straight internal margin and oblique terminal margin, connected by a wide arch with the outer margin. The tip of the second joint has a thick black knob, bearing a little lobe. The lappet of the distal joint is close to the terminal thickening. The sternite is pointed and relatively short. Phallopods: The tibial process is broad, projecting but little, with a smooth margin; between the tibial process and the tip is a pointed lobe, the distal lappet is longer than wide ^[28].

4. Discussion

The divergent evolution and complexity of gonopods is updated in *Chersastus*. Although taxonomic review alone reveals the gonopods as the primary taxonomic characters, this is insufficient evidence for proving rapid evolution and divergence. Reasonable deductions can be made concerning each hypothesis for the evolution of complex male genitalia from the static morphologies of *Chersastus* gonopods simply by examining the component structures to discover their potential functions in copulation.

4.1. Pleiotropism

Pleiotropism has been used to explain complex gonopods in the past ^[32], however it is not considered testable in animals with secondary genitalia ^[20]: "[T]hat the supposed pleiotropic effects...discredits the theory."

4.2. Lock-and-key

Chersastus has combinations of structures, spines and pits, distal and basal joints, and double paired gonopods as interlocking. Besides the circularities that the original lock-and-key hypotheses are fraught with ^[33], it is neither an alternative to a conflict of interests nor recognition hypotheses.

The actual keying process of the male genitalia into the female may be conflicting when the gonopods insert inside the female. Whether there is a recognition system involved or whether the movements performed by the male stimulate the female cannot be determined from male genital morphology alone. The earliest functional explanations for millipede gonopods in accord with the original lock-and-key hypothesis ^[33] are outdated. Interspecific mating experiments remain the way to falsify a lock-and-key.

4.3. Conflict of interests

Setae and spines that are associated with the male gonopore in earthworms hold the copulating pair together ^[23] as do similar structures found in some insects ^[34]. The spines discovered on the gonopods of *C. inscriptus* suggest mechanical wear. Whether this is the outcome of a conflict of interests or a by-product of recognition or stimulation remains to be tested. Further examination of the structures would be uninformative unless they are costly to produce. This is doubtful given their relative size (to body) and number (to legs). The last alternative is that they perform other functions during copulation such as stimulating female choice. If there is a conflict of interests between the sexes then ^[1] multiple mating by females; ^[2] incomplete sperm precedence; and ^[3] fertilization separate from insemination are predictions.

4.4. Specific mate recognition

Some females are able to discriminate males on the basis of their grip through tactile stimuli conducted in order to determine whether it is possible for the males of one species (*C. inscriptus*) to effect mechanical coupling of genitalia with a heterospecific (*C. anulatus*) ^[35]. This is a phenomenon that has only once been demonstrated in other animals that occur in geographical sympatry ^[40]. Together with consideration as to whether normal amounts of sperm get transferred to regions in the female where they may be used for fertilization, this is the evidence needed to clearly refute a lock-and-key hypothesis for gonopod evolution. I cannot envisage a female process that would have evolved 'for' the female to discriminate among males from different species while simultaneously discriminating individual males. The process of recognition, like the lock-and-key, is unlikely because reproductive "isolation" is more likely to occur during the precopulatory and postcopulatory stages of mating ^[41].

4.5. Cryptic female choice

The positive association of spine length with sternite prominence is worthy of note since it could mean directional evolution in gonopod form, and that those forms may be chosen by females. There is no direct evidence for a Fisherian runaway effect in gonopods but it is possible considering that these secondary genitalic structures are species-specific and have evolved at rapid rates ^[20]. The gonopod structures that are potential candidates for stimulation are those that rub back and forth against females in copula ^[36, 38]. Small structures of this kind that are exposed during copulation avoid the high costs that are associated with larger secondary sexual characters can stimulate cryptic female choice. These internal courtship devices may indirectly serve in paternity assurance. A prediction generated from Fisherian runaway is that promiscuous mating by females correlates with genitalic complexity and copulation behaviour ^[39]. The more elaborate spines and prominent sternite of *C. inscriptus* are a case. A distinction between sensory and mechanical functions of gonopods ^[14] generates testable hypotheses for cryptic female choice.

4.6. Gonopod function in sperm competition

Chersastus gonopods are equipped with structures suggestive of dual functions in sperm transfer and/or sperm displacement. They may not be dual functions^[16] but separate structures that function in transfer and displacement. The second joint of the anterior gonopods possess lateral lobes that may facilitate the movement of sperm. The distal lamellae of the phallopods are the primary suspects of sperm displacement. They fall into the range of morphological devices that actuate sperm displacement^[20, 41]. Scoops have also been recognised on the distal regions of odontopygid and spirostreptid gonopods^[8]. Spinescent hairs also occur on the distal regions of the gonopod coxites in the millipede *Orthoporus pyrhocephalus*^[9]. Spinescent structures of this sort can displace sperm in the libellulid dragonflies by performing a 'scouring' effect^[17]. If gonopod spines in *C. inscriptus* serve a sensory function, they may benefit males in seating the gonopods, detecting previously inseminated rival ejaculates, discovering the point of oviduct emergence and positioning ejaculate closer to fertilization site and enhancing fertilisation success^[11]. The structural differences between the gonopods provide secondary evidence for rapid divergence in already complex structures. The five hypotheses for gonopod evolution are not mutually exclusive. Thus comparative tests should be performed. In order to do this the female morphology needs to be examined and the copulatory mechanics needs to be reconstructed from functional morphology.

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