Dynamics of a cooperative lock-and-key model in genital matching of *Sitona* Germar (Coleoptera: Curculionidae)

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**ABSTRACT.** It has been argued that the copulatory structures of the weevil genus *Sitona* Germar have their equivalents in the opposite sex. Hence, the male intromittent organ represents a species-specific key to unlock the corresponding genital tract of its mate, and the female internal genitalia displays several positions that match the functional armature and surface prominences of the endophallic internal sac from the conspecific male. However, this claim can lead to a major challenge due to the perceived lack of variation in the females copulatory organs across the species of a large genus, *Sitona*. Therefore, a special case can be very helpful for modeling an elaborate correspondence of male and female genital traits in this group. One of the most intriguing and unusual forms of the genitalia in the tribe Sitonini that is assumed to be the representative of an advanced type of the “lock-and-key” relationship belongs to *S. fairmairei*. A pair of peculiar “bursal plugs” along with the two slender accessory pouches were revealed inside the female bursal lumen of this species that were considered to operate in concert with the unusual ejaculatory pump and anchoring device of the male transfer apparatus respectively, in the process of insemination. The Modification of the pumping device and the way that the endophallic armature threads along the female spermathecal duct in *S. fairmairei* were compared to another species, *S. cylindricollis*. Our results provide evidence on how the biomechanics of copulation can drive the evolution of a “cooperative lock-and-key” isolating mechanism in the genital traits of the weevil genus *Sitona*, as well as the significance of a clear understanding about the reciprocal morphologies of male and female copulatory organs.

**Key words:** Bio-syringe, Evolutionary trend, Genital coupling, Lock-and-key, Sitonini, Sperm pump; Transfer apparatus

**Introduction**

Much of the evidence for the sexual selection hypothesis comes from studies of insects (Simmons, 2014). Less than 180 years ago, Dufour (1844) proposed the so-called mechanical or “structural lock-and-key” concept, and argued that differences in genital morphology between dipteran species would prevent or reduce the success of matings, as well as post-copulatory reproductive fitnesses. Kullenberg (1947), was among the first authors who...
followed this idea to examine the genital matching of certain Heteroptera. Subsequently, De Wilde (1964) discussed that variations in the genital traits of both sexes provide behavioral or physiological responses that result in reproductive fitness problems. This idea shaped the second mechanism of reproductive isolation as “sensory lock-and-key”, that was later improved by Eberhard (1985). The history of lock-and-key mechanisms has been discussed thereafter, with an emphasis on insects (Shapiro & Porter, 1989). In parallel, several studies also revealed that in some insects, the shape of male spermatophore and female bursa copulatrix or even the external specific structures are often the reason for unsuccessful matings (Rentz, 1972; Mikkola, 2008). However, non of the earlier hypotheses addressed a cooperative lock-and-key model that might have been formed, at least partly, by the dynamic interaction of both sexes at the time of copulation. In this perspective, the locks and keys are not mechanically exclusive in a vast array of life, and there are several interactive parts that would function in concert to give rise to the successful operation during coitus. This approach innovates a challenge for incorporating female genital diversity in future studies, and provides a sound framework for the cooperative processes that draws from the contributions of both sexes during copulation.

The external genitalia of male weevils, as in most Coleoptera, comprise a double-folded tube (Lawrence & Britton, 1991). Firstly, there is a sclerotized invagination into the abdomen, the median lobe or phallus, and again a membranous and usually armed internal sac inside the median lobe (Fig. 1). At the junction between the ejaculatory duct and the internal sac, there is a heavily sclerotized armature called the sperm transfer apparatus, which bears the functional orifice of the sac and together with it forms the endophallus (Sharp & Muir, 1912; Tuxen, 1970). Upon eversion, the internal sac is inflated into the female vagina and usually affixes the tip of its exposed armature exactly over the opening of the spermathecal duct inside the bursa copulatrix (Alichi, 2021). Most investigators have considered the parts of the external male genitalia of weevil species that are involved in the support and movement of endophillic structures, while the functional morphology of the male intromittent organs has been hitherto neglected. References to the female genitalia of curculionids are somewhat scarce as well, except for the external morphology and the physiology of egg production (Aslam, 1961; Garthe, 1969; Gültekin et al., 2019), or other associated sclerotized parts such as the spermathecae (Kevan, 1960; Velazquez de Castro et al., 2007; Sert, 2006). According to Gaiger & Vanin (2008), the female characters (genitalia and related tergite and sternite VIII) proved to be informative for establishing the phylogenetic relationships among genera of Entimini. The first work that brought attention to the subject of genital correspondence in curculionids dates back to Sharp (1920) who wrote a paper on the sexes of *Contrachelus brevisetus* Champion. He was really excited about some genitalic correlation between males and females in this species, and believed that this will prove to be the case to a considerable extent. However, the functional morphology of the copulatory system in weevils has received only scant attention thereafter, despite the importance of the family Curculionidae (Goldson & Emberson, 1981). Just recently, the concept of lock-and-key hypothesis has been suggested for mechanical footing in the weevil genus *Sitona* Germar, 1817 (Alichi, 2021). In the present work, the various modifications which have been found in the genital traits of two species of the same genus: *S. fairmairei* (Allard, 1869) and *S. cylindricollis* Fahraeus, 1840 are discussed, and a cooperative lock-and-key interaction is proposed for interpreting the evolutionary trend of such isolating mechanisms in the tribe Sitonini Gistel, 1848.
Material and methods

The protocol for preparing mounts of the male and female genitalia and the terms used for the endophallic structures are those that were established earlier by Alichi (2021). To avoid confusion that can result from the use of directions for the everted male endophallus, we followed Lindroth & Palmen (1970) and the parts were determined so that the internal sac and its armature (sperm transfer apparatus) are in the position of activity. Successful eversion of the internal sac in *S. cylindricollis* was conducted by a standard method. A hooked tool was made by bending the point of a fine needle and then blunting it so that it could catch the wall of the internal sac and enable a pull to tease it out little by little through the median orifice of the aedeagal tube, without tearing the sac. Then the endophallus was forced out by inserting the fine tip of a microsyringe into the median foramen and gently applying pressure. The difficulty was to hold the specimen with a pincer, without compressing it. Interpretation for the functional armature of the endophallus in *S. fairmairei* was bounded on the expanded distal lobe (anterior projection of the internal sac from the median foramen, in repose). For this purpose, the dissected aedeagal tube of this species was transferred to a cavity slide with a drop of 30% lactic acid and covered with a coverslip. The sample was left at least overnight to allow the distal lobe to become fully distended, and approximating the functional position of the transfer apparatus. Images were prepared using an Olympus BX51 phase-contrast microscope with a DP27 digital camera and cellSens software.

Results

*Sitona cylindricollis* Fahraeus, 1840

The internal sac of *S. cylindricollis* resembles that of the *S. humeralis* group of species by having three pairs of the lobes situated at anterior, posterior and lateral sides; a rough papilla (dorsal lobe); and a single distal lobe enveloping the transfer apparatus (Fig. 1). It seems likely that the endophallic armature in this species differentiated from a probable primitive type through some ecological and/or genetic changes resulted in a considerable reduction in the length of the supporting sclerites (“pinnae” of Velazquez de Castro, 1997), as well as the modification of the sperm pump (Fig. 1). In the latter case, the plunger sclerites are entirely lost and a chitinous pumping chamber that forms at the junction between the ejaculatory duct and the widened basal tip of the portal sclerites (Fig. 1) is therefore available to function as a pulsatile organ in the genital traits of this species.

The female genital chamber of *S. cylindricollis* shows the fundamental structures that were discussed earlier in *S. concavirostris* and *S. puncticollis* (Alichi, 2021). Seemingly, several pouch-like infoldings (Fig. 2) that match the surface prominences of the internal sac from conspecific male (Fig. 1) were determined inside the vaginal lumen of the female. A pad-like “bursal valve” occludes the anterior end of the vagina and separates it from the bursa copulatrix. The presence of such structure has been confirmed in some other curculionids, as well as several chrysomelid beetles (Kasap & Crowson, 1980; Goldson & Emberson, 1981). The bursal valve actually serves for suppressing the further penetration of the male internal sac, and probably to prevent diversion of the eggs in oviposition into the bursa copulatrix. The female genitalia of *S. cylindricollis* also comprises a hooked spermatheca (Fig. 2) which possesses a sclerotized wall and opens into the bursa copulatrix via a short and thin spermathecal duct.
Sitona fairmairei (Allard, 1869)

The endophallic armature of *S. fairmairei* is unique among the examined *Sitona* species from Iran (Alichi & Ahmadi, 1993; Alichi, 2021). However, the surface morphology of its internal sac (not everted) seems to be typical of the genus as a whole, based on the corresponding positions for the male endophallic lobes in the female genital chamber (Fig. 2). Formerly, Sert (2006) and Velazquez de Castro et al. (2010) illustrated the endophallic armature of *S. fairmairei*, however, the pump assembly and the anchoring device (“accessory rods”) attached to the sperm transfer apparatus in this species (Figs. 3–4) are described herein for the first time. The so-called bio-syringe (Alichi, 2021) of *S. fairmairei* is comprised of three functional structures that all together form an integrated transfer apparatus. Firstly, a fusion zone that consists of two portal sclerites (deeply bifurcated basally); a curved lamina (“stand”); as well as a pair of lateral processes (“flanges”), one on each side ending in a small apical flap (Fig. 3). During copulation, these flaps can be affixed on the surface of a “bursal disc” which is attached over the opening of the spermathecal duct inside the female bursal lumen (Figs. 2, 4). A pumping device forms the second zone of the bio-syringe. It is composed of two plunger sclerites which are articulated at the base of the portal, a paired connecting rods, and aligned plugging sclerites (“hamuli” of Velazquez de Castro, 1997) (Fig. 3). Each of the elongated connecting rods has a wider first end which connects to a plunger sclerite by the adductor and abductor muscle fibres, and a narrower distal part that penetrates into a plugging sclerite and holds it by tiny denticles. Another striking feature of the male bio-syringe in *S. fairmairei* is the loss of supporting sclerites which are replaced by a paired “accessory rods” articulated at the tip of the portal branches (Figs. 3–4), however,
determining the homology of these structures remains a challenge. The accessory rods can be considered as the third zone of the bio-syringe in this species, such that the whole transfer apparatus is presumably anchored posteriorly by these rods to the female bursal lumen during copulation (see discussion).

*Sitona fairmairei*, as well as other examined species in this genus, possesses a tubular type of female genital chamber with double openings separated by a bursal valve. The spermatheca of this species shows significant differences compared with *S. cylindricollis*, by having a tube-like “corpus”, as well as the presence of a “ramus” (Fig. 2). *Sitona fairmairei* is also featured for a pair of large bursal plugs embedded in the thickened wall of the female bursal lumen (Figs. 2, 4), which probably take part in the pumping operation during coitus, through interaction with the corresponding plugging sclerites of the conspecific male. A pair of slender accessory pouches (Fig. 4) in the female genital chamber of this species were recognized as the actual positions for anchoring the accessory rods of the male bio-syringe, as well. These materials have made possible the formation of a cooperative type of the lock-and-key model in genital matching of *S. fairmairei*.

![Figure 2. Dorsal views of the female genital chambers in *Sitona cylindricollis* Fahraeus (left) and *Sitona fairmairei* (Allard) (right) (Coleoptera: Curculionidae). ap: anterior pouch, bv: bursal valve, dop: dorsal pouch, lp: lateral pouch, pp: posterior pouch.](image)
Figure 3. Dorsal (left) and ventral (right) views of the male transfer apparatus in *Sitona fairmairei* (Allard) (Coleoptera: Curculionidae) in repose.

Figure 4. Corresponding structures of female (left) and male (right) genitalia in *Sitona fairmairei* (Allard) (Coleoptera: Curculionidae). Left: ventral view of the female genital chamber; top-right: dorsal view of the reposing transfer apparatus; bottom-right: lateral view of the functional transfer apparatus.
Discussion

The evolutionary origin of the various sclerites incorporated in the transfer apparatuses of the *Sitona* weevils has been remained enigmatic so far. However, two characters may be more likely to explain the spectrum of existing variation: the sperm pump, and the way that the endophallic armature threads along the female spermathecal duct. In this respect, the transfer apparatus of *S. fairmairei* provides a different strategy for its lateral processes (flanges) to being involved in affixing the gonopore over the opening of the bursal disc, in place of participating in the pumping operation. Alichi (2021) has presented in detail, the functions of these structures in *S. concavirostris* and *S. puncticollis*. A similar interpretation can be applied to *S. cylindricollis* as well. Moreover, the position of the bursal plugs relative to the bursal disc is another case in point. Bursal plugs in the majority of the studied species (such as *S. cylindricollis*, Fig. 2), are normally embedded in the thickened wall of the female genital lumen just above a bursal disc. However, in *S. fairmairei* these pouches are located distant to the bursal disc (Fig. 4), which is in correspondence with the presence of a paired connecting rods to achieve precise assembly between the plugging sclerites and the plungers.

Although it is probably impossible to reconstruct in high resolution the interaction between the male and female genital organs during copulation, yet it is assumed that these traits might have been evolved under correlational selection in *S. fairmairei*. Males of this species display a peculiar sperm pump assembly by incorporation of the plugging sclerites (as the contraction absorbing pedals) into it. These slipper-shaped pedals inflate into the corresponding pouches (bursal plugs) of the female genital chamber during copulation. A paired connecting rods are required to transmit the compressive and tensile forces conducted to the plugging sclerites from the female bursal lumen, and convert them into the pulsatile motion of the plunger sclerites. This phenomenon could not be interpreted unless the cooperative role of the female neuromuscular complex comes into play. The approach of genitalic neuroanatomy was also noticed by Shapiro & Porter (1989) who considered it as a new frontier of hypothesis testing for the lock-and-key concept. The physiological responses induced by the cryptic female choice (Eberhard, 1985) and the morphological plasticity of the male endophallic structures during interaction with female genital tissues (Kelly & Moore, 2016) support this idea, as well.

As Johnson et al. (2012) pointed out, trait loss or reduction can be discussed in the context of an evolutionary adaptation. According to Velazquez de Castro (pers. comm.), the loss of pinnae (supporting sclerites) in some species of the genera *Sitona* Germar (such as *S. fairmairei*) and *Charagnus* Schonherr might be considered as an apomorphic condition. This structure displays a transitional state in *S. cylindricollis*, regarding a normal type of the supporting sclerites observed among the *Sitona* fauna of Fars Province (Alichi & Ahmadi, 1993), while such a device has been disappeared in *S. fairmairei*. The loss of plunger sclerites in *S. cylindricollis* and the further gaining of this character by *S. fairmairei*, reflect another evolutionary innovation in these taxa. Such fitness trade-offs refer to the category of phenotypic regression, which is the most commonly studied form of evolution in reverse (Porter & Crandall, 2003). Currently, we can not estimate that how many transitional or even more primitive/advanced types of the bio-syringes have been remained to be discovered in this group of organisms. However, future attempts are necessary to address the lost pieces of this chain, and give rise to improvements in our understanding from the lock-and-key isolating mechanisms.
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Conflict of Interests
The authors declare that there is no conflict of interest regarding the publication of this paper.

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چکیده: وجود بخشی یا قابل تطبع در انداز جفت‌گیری سرخرطومی‌های نر و ماده Sitona Germar (Coleoptera: Curculionidae) از جنس Sitona مورد نیاز قرار گرفته است. بر این اساس انداز تناسلی حشره نر نقش کلیدی را برای پرورش قفل جنیتیالی در ماده‌ها یا هم گونه خود داشته. در حالی که در محفظه جنسی حشرات ماده نیز جنبه‌هایی مشابه داشته که حضور آن‌ها به درمان مشکلات در انتقال اسپرم در جنسیت‌های سرخرطومی‌های نر می‌باشد. در عملیات انتقال اسپرم حشرات نر و ماده به‌مثابه یک مانند S. fairmairei و S. cylindricollis - با همکاری گونه S. fairmairei - به کلید همیار در انداز جفت‌گیری سرخرطومی‌های جنس Sitona Germar نیز اهمیت یک درک صحیح از ریخت شناسی متقابل نر و ماده را ارائه می‌نماید.

واژگان کلیدی: پوسرگ، روند تکاملی، تطبع جنیتیالی، قفل و کلید، پمپ اسپرم نر، انداز انتقال اسپرم

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