



HOKKAIDO UNIVERSITY

Title	Traumatic copulation and counterpart female traits are widespread in fritillary butterflies (Lepidoptera: Nymphalidae : Argynnini)
Author(s)	Matoba, Tatsuki; Kamimura, Yoshitaka; Yoshizawa, Kazunori
Citation	Entomological science, 27(2) https://doi.org/10.1111/ens.12570
Issue Date	2024-04-03
Doc URL	https://hdl.handle.net/2115/94565
Rights	This is the peer reviewed version of the following article: MATOBA, T., KAMIMURA, Y. and YOSHIZAWA, K. (2024), Traumatic copulation and counterpart female traits are widespread in fritillary butterflies (Lepidoptera: Nymphalidae: Argynnini). Entomological Science, 27: e12570, which has been published in final form at https://doi.org/10.1111/ens.12570 . This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions. This article may not be enhanced, enriched or otherwise transformed into a derivative work, without express permission from Wiley or by statutory rights under applicable legislation. Copyright notices must not be removed, obscured or modified. The article must be linked to Wiley's version of record on Wiley Online Library and any embedding, framing or otherwise making available the article or pages thereof by third parties from platforms, services and websites other than Wiley Online Library must be prohibited.
Type	journal article
File Information	2024_ENS.pdf



Traumatic copulation and counterpart female traits are widespread in fritillary butterflies (Lepidoptera: Nymphalidae: Argynnini)

Tatsuki Matoba 1, Yoshitaka Kamimura 2, Kazunori Yoshizawa 1

1 Systematic Entomology, School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

2 Department of Biology, Keio University, Yokohama 223-8521, Japan

Correspondence: K. Yoshizawa (psocid@agr.hokudai.ac.jp)

Abstract

Female fritillary butterflies (Lepidoptera: Nymphalidae: Argynnini) are known to have diversified genital structures, especially at the entrance of the bursa copulatrix. However, the origin and function of such elaborated structures are poorly known to date. We examined the male and female genital structures of six Japanese species selected from five genera/subgenera (*Argynnis* (*Argynnis*), *Argynnis* (*Damora*), *Argynnis* (*Argyronome*), *Fabriciana*, and *Speyeria*) of the Argynnini tribe and found that traumatic copulation is widespread in Argynnini. Various male genital structures, such as the uncus, valva, and phallus, can cause wounds to the female genitalia. The 8th venter of the female, where the highest morphological diversity in female genitalia was detected, is hooked and held by the male uncus during copulation. It is very likely that the diversified female genital structure coevolved with the male uncus as a counterpart for traumatic coupling.

Key words: genitalia, coevolution, sexual conflict, function, morphology

Introduction

Although it has generally been thought that the genitalia of female animals, including insects, are less variable than male genitalia, recent studies have shown that female genitalia are just as or even more diversified than male genitalia (e.g., Simonsen, 2008; Sloan & Simmons, 2019; Simmons & Fitzpatrick, 2019; Roth et al., 2024). The female genital structures of many animals also function as organs for oviposition or parturition. Thus, changes in female oviposition sites and behaviors can cause diversification of female genitals in insects, requiring changes concerted with conspecific male genitals (e.g., Muto et al., 2018). Alternatively, in many cases, diversification of female genitalia is thought to be achieved as a consequence of coevolution with male genital structures driven by conflict of reproductive interests between the sexes (sexual conflict: Arnqvist & Rowe, 2005; Kokko & Jennions, 2014). For example, male genital structures of many animals inflict wounds on the female genitalia while securely holding a female during copulation (reviewed in Lange et al., 2013). In insects, females usually develop membranous pouches where conspecific males inflict wounds (e.g., Onuma et al. 2023).

Fritillary butterflies (Lepidoptera: Nymphalidae: Argynnini) are known for their diversity in female genital structures (Simonsen 2006). As a conspicuous example, the females of silver-washed fritillary butterflies (*Argynnis paphia* L.) possesses a characteristic projection called the cornucopia (Bayard, 1944; Jolvet, 2008). The cornucopia was once thought to be an example of an inverted genitalia structure, i.e., a female's intromittent organ for pumping up semen from males. However, our recent study showed that the cornucopia does not function as an intromittent organ (Matoba et al., 2023). Instead, it is strongly suggested from the study that the cornucopia has evolved as a counter-adaptive structure to mitigate the traumatic copulation that occurs due to the serrated male uncus.

Structures similar to the cornucopia are also known among some species related to silver-washed fritillary (e.g., Simonsen, 2006). However, the presence/absence of traumatic copulation, as well as the presence/absence of counterpart functions, in other fritillary butterflies are completely unknown to date. To understand the evolutionary factors underlying the high diversity of female genital structures in fritillary butterflies, elucidation of the morphology and function of the male and female fritillary genitalia is needed.

In this study, we observed the female and male genital structures of some Japanese species selected from the tribe Argynnini. By examining the presence/absence and distribution of copulatory wounds, we discussed the function of the male and female genital structures in the tribe. We also discussed the evolutionary changes in the female counterpart traits by referring to the phylogenetic hypothesis proposed previously.

Materials and Methods

The terminology used in this study for the lepidopteran genital structures follows that of Klots (1970). See Matoba et al. (2023) for an overview of male and female genitalia in Argynnini and the relevant terminology. The generic and subgeneric classification in this study followed that of de Moya (2016).

The following six Japanese species representing five genera or subgenera of Argynnini were collected at several localities in Hokkaido (Iwamizawa, Mashike, Sapporo, and Yubari) and were examined for this study: *Argynnis* (*Argynnis*) *paphia* (L.) (see Matoba et al., 2023), *A. (Damora) sagana* Doubleday (15 males, 13 females), *A. (Argyronome) ruslana* Motschulsky (13 males, 5 females), *A. (Am.) laodice* (Pallas) (17 males, 2 females), *Fabriciana adippe* (Denis & Schiffermüller) (22 males, 13 females) and *Speyeria aglaja* (L.) (5 males, 1 female). All specimens were preserved in the Hokkaido University Insect Collection. The abdomen was removed from each male and female specimen and soaked in 10% KOH solution at 40 °C for 24 hours to clear the tissue. Then, these specimens were dissected and observed in 75% ethanol under a SZX12 stereomicroscope (Olympus Co., Tokyo, Japan).

A copulating pair of *A. (D.) sagana* was also observed. The larvae were reared to obtain virgin males and females. At 5–6 days after emergence, a virgin female and a virgin male were placed into a net cage (20 cm diameter, 110 cm height). Fifteen minutes after the initiation of copulation, a mating pair was killed with freezing insecticide spray (Earth Corporation, Tokyo, Japan), placed into cold 75% ethanol for fixation, and then stored at –20 °C for 3 weeks. For microscopy observations, the coupling abdomens were removed and placed in BABB (benzyl alcohol/benzyl benzoate = 1:2) at 40 °C for one month to render them transparent following the methods of Kamimura & Mitsumoto (2011). After this treatment, we observed the samples in BABB under the SZX12 stereomicroscope.

Female genitalia of three species, *A. (D.) sagana*, *A. (Am.) ruslana* and *F. adippe*, were also subjected to μ CT analyses, as were the female genitalis of *A. paphia* examined previously (Matoba et al., 2023). The samples were dehydrated in a gradient series of 80–100% ethanol before critical-point drying (EM CPD300, Leica, Wetzlar, Germany) to remove water without extensive organ shrinkage. The sample was then scanned using synchrotron μ CT at the BL20XU beamline (Uesugi et al., 2012) of the Super Photon ring-8 GeV facility (SPring-8; Hyogo, Japan) using a stable beam energy of 15 keV in absorption-contrast mode. The tomography system consisted of a full-field X-ray microscope with Fresnel zone plate optics (Uesugi et al., 2017).

The reference for the phylogenetic relationships of Argynnini was that of de Moya (2016), which is a molecular phylogeny estimated based on the mitochondrial *COI* and nuclear *Wingless*, *GAPDH*, *RPS5* and *EF1a* genes.

Results

Morphology of the female genitalia

Females of *A. (A.) paphia*, *A. (D.) sagana* and *F. adippe* (Fig. 1A, B, E blue; Matoba et al., 2023) have a well-developed projection, which is called the cornucopia, arising from the ceiling of the bursa copulatrix entrance (wrinkled region of the 8th venter), cylindrical in shape and bent in the middle. In the former two species, the cornucopia is bellows-like in structure (i.e., an extendable structure), whereas that of *F. adippe* is evenly sclerotized except for its membranous upper surface and thus is not extendable. The opening of the ductus seminalis is located at the anterior margin of the base of the cornucopia in all three species (Figs 1A, B, E, 2A, C; Matoba et al., 2023). The cornucopia of *A. paphia* is largest among the cornucopia observed, and that of *A. (D.) sagana* is smallest but had an elongated tube-like structure at the apex.

In contrast, such a cylindrical projection is absent in *A. (Am.) laodice*, *A. (Am.) ruslana* and *S. aglaja*. However, the wrinkled 8th venter is broadly expanded ventrally in *A. (Am.) laodice* and *A. (Am.) ruslana* (Fig. 1C–D), whereas only a small membranous tubercle (or mini-cornucopia) was detected in *S. aglaja* (Fig. 1F). In *A. (Am.) ruslana*, which was subjected to μ CT analyses, the opening of the ductus seminalis is located just anterior to the expanded membrane (Figs 1C, 2B).

Morphology of the male genitalia

The shape of the male genitalia (Fig. 3) is species-specific and highly variable, as reported previously (Shirôzu & Saigusa, 1973; Simonsen, 2006). The uncus is long and apically hooked in all the Argynnini species but is variable in many respects among the species (Fig. 3 middle). In *A. paphia*, it is narrow, strongly bent, and bears several long posterodorsal spines (Fig. 3A). In *A. (D.) sagana*, it is broad, strongly bent, and bears several dorsal spines and a pair of dorsolateral lamella (Fig. 3B). In *A. (Am.) ruslana* and *A. (Am.) laodice*, it is nearly straight, without dorsal spines or serrations but with long hairs, and is very short in *A. (Am.) ruslana* (Fig. 3C, D). In *F. adippe*, it is strongly bent and bears a triangular projection with its posterior margin serrated (Fig. 3E). In *S. aglaja*, it is broad and strongly bent, with a serrated posterodorsal margin (Fig. 3F).

The ampulla of the valva (Fig. 3 left) is also variable in size and shape: large, with

dense, strong spines in *A. paphia* and *A. (D.) sagana* (Fig. 3A, B); relatively small, but the valva having pointed stout spines anterior to the ampulla in *A. (Am.) ruslana* and *A. (Am.) laodice* (Fig. 3C, D); and small and denticulated in *F. adippe* and *S. aglaja* (Fig. 3E, F), with *F. adippe* having a long sinuated projection arising from the anterodorsal margin of the valva.

The phallus (Fig. 3 right) is more or less asymmetrical in all the species and is especially strongly skewed in *A. (Am.) laodice* (Fig. 3D). The phallus has an area dorsally bearing dense, strong spines (= cornuti) (Fig. 3 right). The cornuto spines are variable in length and shape: long right-side spines and small, conical left-side spines in *A. paphia* (Fig. 3A); short, conical right-side spines and a dense, tiny, denticulated left-side area in *A. (D.) sagana* (Fig. 3B); very strong and long spines on the right side in *A. (Am.) ruslana* and *A. (Am.) laodice* and, in *A. (Am.) ruslana*, the left-side region having dense long spines (but smaller than the right-side ones), whereas *A. (Am.) laodice* lacked left-side spines (only having some tiny denticles) (Fig. 3C, D); small numbers of very thick and short spines in *F. adippe* (Fig. 3D); and densely arranged tiny spines in both areas in *S. aglaja* (Fig. 3F).

Copulatory mechanics in A. (D.) sagana (Fig. 4)

In *A. (D.) sagana*, the male dorsal spines of the uncus penetrated the upper side of the female cornucopia during copulation, as also observed in *A. paphia* (Matoba et al., 2023). A pair of lamella on the dorsal uncus held the base of the elongated apical tube of the cornucopia. Different from the phallus of *A. paphia*, that of *A. (D.) sagana* was not directly associated with the cornucopia, but the cornuti made tight contact with the wrinkled area of the female's 8th venter anterior to the cornucopia. The valva held the female terminalia laterally, and the ampulla made tight contact with the anterolateral part of the 8th abdominal segment.

Copulatory wounds

We detected copulatory wounds as repaired, melanized patches on the genital tract in all mated females (i.e., females with spermatophores in the corpus bursae) of all the species observed (Fig. 1). We also confirmed in *A. paphia*, *A. (D.) sagana* and *A. hyperbius* (although not the main target of this study, we observed a female of this species) that virgin females did not have melanized patches on their genital structures (Fig. 1B'; Matoba et al., 2023). The size and detected location of the copulatory wounds differed among the species. In the species with (mini-)cornucopia (*A. paphia*, *A. (D.) sagana*, *F. adippe*, and *S. aglaja*), the wounds were observed at the tip of the (mini-)cornucopia in all mated individuals (Fig. 1A, B, E). In *A. paphia*, several wounds were also seen on the upper membranous side of the cornucopia, and some females had wounds on the lower sclerotized fold portion of the

cornucopia (Fig. 1A; Matoba et al., 2023). In *A. (Am.) ruslana* and *A. (Am.) laodice*, the species without cornucopia, wounds were observed in the membranous pocket located posterior to the surface of the expanded membrane of the 8th venter (Fig. 1C, D). They also had several wounds in the wrinkled area of the 8th venter.

In addition, except for in *S. aglaja* (only one female could be examined), melanized patches were detected on both sides of the 8th abdominal segment of the females of all the species observed (Fig. 1A–E). In particular, the melanized patches on the lateral 8th segment were large in *A. (Am.) ruslana* and *A. (Am.) laodice* (Fig. 1C, D). Superficially, no significant ornamentations were detected around this area, but μ CT scanning of the female terminal segments showed that the membrane around the lateral 8th segment is thickened, at least in *A. (Am.) ruslana* (Fig. 5).

Discussion

The present examinations revealed that traumatic copulation is widespread in fritillary butterflies (Argynnnini). The copulatory wounds were detected in various parts of the female genitalia but were observed most frequently on the wrinkled area of the female 8th venter (including on the (mini-)cornucopia arising from the wrinkled area: Fig. 1). Most likely associated with this, the structure of the female 8th venter is more or less elaborated and diversified in Argynnnini. As far as the four species subjected to μ CT scanning are concerned, such ornamentations (cornucopia or expanded membrane with a small pocket) are located just posterior to the opening of the ductus seminalis (Fig. 2; Matoba et al., 2023). Therefore, these ornamentations, which probably correspond to characters 132 and 133 used in the phylogenetic analysis by Simonsen (2006), are considered to occur at homologous positions in all species.

In *A. (D.) sagana*, as well as in *A. paphia* (Matoba et al., 2023), the male uncus has long dorsal spines (Fig. 3A, B). We confirmed that the spines penetrate and cause copulatory wounds on the female cornucopia (Figs 1, 4; Matoba et al., 2023). *F. adippe* females also have a well-developed and sclerotized cornucopia (Fig. 1E), and *S. aglaja* females have a membranous tubercle (or mini-cornucopia) at the part homologous with the cornucopia (Fig. 1F). In both species, copulatory wounds were found on the upper and/or apical, less sclerotized part of the (mini-)cornucopia (Fig. 1E,F), and the males of these two species had a bent uncus with dorsal spines (Fig. 3E,F middle). Therefore, in these species, the uncus probably functions to hook and hold the (mini-)cornucopia during copulation, by which copulatory wounds are formed. Females of *A. (Am.) ruslana* and *A. (Am.) laodice* do not have developed cornucopia but have an expanded membrane with a posterior pocket on the

wrinkled region of the 8th venter. In these species, copulatory wounds were found in the pocket (Fig. 1C, D). Judging from its positional congruence (the wrinkled 8th venter just posterior to the opening of the ductus seminalis: Fig. 1C), the expanded membrane with a pocket can be regarded as a structure corresponding to the cornucopia (Fig. 1A, B), and the male uncus likely hooks the pocket during copulation in these two species. The male uncus lacks dorsal spines and is simple and relatively short in these species (Fig. 3C, D), which may be related to the lack of a cornucopia in these species.

Because of positional congruence, elaborations of the female 8th venter are considered to occur at homologous positions in all species. However, referring to the phylogeny of Argynnini, the evolution of the elaborated structures does not seem to be straightforward (Fig. 6). Two species with well-developed and bellows-type cornucopia (*A. paphia* and *A. (D.) sagana*) are considered to form a monophyletic group (de Moya, 2016), such that their cornucopia can be regarded as having a common origin. In contrast, their closest relatives among the species examined here, *A. (Am.) ruslana* and *A. (Am.) laodice*, both lack cornucopia. Notably, the other species having well-developed and evenly sclerotized cornucopia (*F. adippe*) is most closely related to the species having membranous tubercles (or mini-cornucopia: *S. aglaja*), and both of these species are phylogenetically distant from the species having bellows-type cornucopia (Fig. 6). Therefore, the emergence of the well-developed cornucopia seems to have occurred independently in these two clades of Argynnini. In addition, the morphology of well-developed cornucopia is also quite different between the two clades (likely formed from multiple wrinkles in *A. paphia* and *A. (D.) sagana* but from a single wrinkle in *F. adippe* and *S. aglaja*: Fig. 6), which also provides additional support for the independent origins of the cornucopia. According to Simonsen (2006), modifications in the female 8th venter also occur in fritillary butterflies that were not included in the present study (e.g., *A. (Pandoriana) pandora*). To discuss the origins and evolution of these structures, broader sampling and observation are needed.

As mentioned above, the phylogenetic distribution of the strongly bent uncus with dorsal spines or serrations is also in alignment with the distribution of (mini-)cornucopia. These results suggest that the elaboration and diversification of the female 8th venter, as reflected in the expanded membrane, pocket, tubercle and two types of the cornucopia, coevolved as counterpart structures to the penetrating male uncus. Our previous observation showed that the upper membranous side of the cornucopia contains resilin, a rubber-like protein often found in insect cuticles (Matoba et al., 2023). In bed bugs (Hemiptera: Cimicidae), this protein in the female cuticle is known to reduce copulatory damage by sealing the puncture immediately after male puncturing for traumatic insemination (Michels

et al., 2015). Although the presence of resilin was not examined for the other species, the nonsclerotized cornucopia and expanded membrane as its precursor seem to represent a similar trait for female “tolerance” of mating that is damaging. In *Drosophila* fruitflies, females develop another type of counter-adaptation, sclerotized shields, where spined or serrated male genitals make contact during copulation (Kamimura 2016; Onuma et al., in press). The sclerotized cornucopia of *F. adippe* may also represent this type of female “resistance” to possible copulatory wounding. In contrast, there is no straightforward correlation between the development of (mini-)cornucopia and oviposition sites: all six species examined here are known in Japan to lay eggs on various objects (dead/fallen leaves, mosses, tree bark, etc.) near the host plants (Fukuda et al., 1983), while on-host oviposition has also been reported for *A. (Am.) laodice*, *F. adippe* and *S. aglaja* (Simonsen, 2006). The female genitals of Argynnini are entirely concealed in the hatch-like, well-developed ostium bursae + 7th sternite (Hannemann, 1954; Simonsen 2006; Matoba et al., 2023). Theoretical and empirical studies demonstrate that female genitals can diversify through sexual conflict, potentially leading to coevolution of male morphological and/or behavioral traits (Gavrilets & Waxman, 2002; Roth et al., 2024). In lowland Japan, *A. paphia* and *A. (D.) sagana* mate even after aestivation, whereas females of the other studied species primarily copulate just after emergence (Fukuda et al., 1983). This suggests a prolonged period of male-male competition in the former two species. Sexual conflict over access to concealed female genitals, modulated by species-specific differences in the intensity of male-male competition, may underlie the diversification of male and female genital morphologies in this group of butterflies.

In all the species except for *S. aglaja* (for which only one female was examined), wounds were also observed on both sides of the female 8th abdominal segments. We confirmed that in *A. paphia* and *A. (D.) sagana*, males hold this region using the valva, and the spines on the internal surface of the valva (including the ampulla) cause wounds (Fig. 4; Matoba et al., 2023). Judging from their positions, the wounds observed in the other species were also likely caused by the ampulla and valval spines of the males. Superficially, no significant elaborations, as observed on the wrinkled 8th venter, were detected on the lateral 8th segment in females. However, μ CT images showed that the membrane of the lateral 8th abdomen is thickened at least in *A. (Am.) ruslana* (Fig. 5), the species with notably stout valval spines. The wounds observed on the lateral part of the 8th segment were especially large in *A. (Am.) ruslana* and *A. (Am.) laodice* (Fig. 1C, D), both of which have stout spines. Therefore, the occurrence of a counterpart structure (as thickened lateral 8th segment) against traumatic coupling is also likely between the male valva and the lateral region of female

genitalia. The uncus of *A. (Am.) ruslana* and *A. (Am.) laodice* is simple (Fig. 3C, D middle). Thus, these valval spines may play a more important role in holding a female during copulation than uncus spines in *A. (Am.) ruslana* and *A. (Am.) laodice*. Similar compensatory changes in male mate-holding organs have been suggested for a sibling pair of *Drosophila* species (Muto et al., 2018). Flight during copulation, during which either the male carries the female or vice versa, has been reported for all six fritillary species examined in this study (Fukuda et al., 1983). Irrespective of the responsible male organs, secure holding of the mates would be equally important for these species.

Our previous study showed that the cornuto spines on the phallus also cause wounds on the lower part of the cornucopia in *A. paphia* (Matoba et al., 2023). In *A. (Am.) ruslana* and *A. (Am.) laodice*, wounds were also found on the wrinkled 8th venter anterior to the pocket for the uncus (Fig. 1C, D). The cornuti of *A. (D.) sagana* tightly associated with this region during copulation (Fig. 4). Judging from the positional congruence of the wounds, these were possibly caused by the male cornuti. The wrinkled genital tract itself may also be a counterpart trait for the cornuti on the phallus.

In conclusion, our present observations showed that traumatic copulation is widespread in Argynnini. Various male genital structures, such as the uncus, valva, and phallus, can cause wounds to the female genitalia, and counterpart female traits were also evident corresponding to each structure. The highest morphological diversity was observed on the female 8th venter, and the region was hooked and held by the male uncus during copulation. It is very likely that this highly diversified female genital structure coevolved with the male uncus as a counterpart for traumatic coupling.

Acknowledgments

We thank Akihisa Takeuchi and Masayuki Uesugi for their support at SPring-8. The research at SPring-8 was approved through project number 2022A1201 to KY. This study was partly supported by JSPS grant numbers 15H04409 and 19H03278 to KY and 15K07133 to YK. This study was conducted to fulfill the master's degree of TM, who thanks Shin-ichi Akimoto and Masahiro Ohara for their continuous guidance.

Author contribution

YK and KY conceived the project. TM designed the experiments, collected specimens, analyzed morphology, and performed experiments. KY performed μ CT analysis. All authors wrote and approved the final manuscript.

References

Arnqvist, G. & Rowe, L. (2005) Sexual conflict. Princeton University Press, Princeton

- Bayard, A. (1944) Observations sur l'accouplement de *Dryas paphia* (L.) [Lep, Nymphalidae]. *Bulletin de la Société entomologique de France* 42: 92–95.
- de Moya, R. S. (2016) Molecular phylogenetic analysis of *Argynnis* Fabricius (1807) including North American *Speyeria* Scudder (1872). Unpublished thesis available at https://scholarlycommons.pacific.edu/uop_etds/168/.
- Fukuda, H., Hama, E., Kuzuya T., Takahashi, A., Takahashi, M., Tanaka, B., Tanaka, H., Wakabayashi, M. & Watanabe, Y. (1982) The life histories of butterflies in Japan. Vol. II. Hoikusha, Osaka. Japan. (In Japanese)
- Gavrilets, S. & Waxman, D. (2002) Sympatric speciation by sexual conflict. *Proceedings of the National Academy of Sciences of the United States of America* 99: 10533–10538. DOI: 10.1073/pnas.152011499.
- Hannemann, H. J. (1954) Zur muskelfunktion der weiblichen genitalsegmente von *Argynnis paphia* (L.) (Lep.). *Zoologischer Anzeiger* 153: 149–154.
- Jolivet, P. (2008) Inverted Copulation. *Encyclopedia of Entomology* 55: 2041–2044.
- Kamimura, Y. (2016) Significance of constraints in genital coevolution: why does female *Drosophila* appear to cooperate males by accepting harmful traumatic matings? *Evolution* 70: 1674–1683.
- Kamimura, Y. & Mitsumoto, H. (2011) Comparative copulation anatomy of the *Drosophila melanogaster* species complex (Diptera: Drosophilidae). *Entomological Science* 14: 399–410.
- Klots, A.B. (1970) Lepidoptera. In: Tuxen SL ed. Taxonomist's glossary of genitalia in insects. Copenhagen: Munksgaard, pp. 115–130.
- Kokko, H. & Jennions, M.D. (2014) The relationship between sexual selection and sexual conflict. In: Rice, W. & Gavrilets, S. (eds) Additional perspectives on the genetics and biology of sexual conflict. Cold Spring Harb Perspect Biol, Woodbury 6(9):a017517
- Lange, R., Reinhardt, K., Michaels, N.K., & Anthes, N. (2013) Functions, diversity, and evolution of traumatic mating. *Biological Reviews* 88: 585–601.
- Matoba, T., Kamimura, Y. & Yoshizawa, K. (2023) Is the copulation of silver-washed fritillary inverted? Function of the female genital projection (Lepidoptera: Nymphalidae: Argynnini). *Organisms Diversity & Evolution* 23: 871–879.
- Michels, J., Gorb, S. N. & Reinhardt, K. (2015) Reduction of female copulatory damage by resilin represents evidence for tolerance in sexual conflict. *Journal of the Royal Society Interface*. 12: 20141107.
- Muto, L., Kamimura, Y., Tanaka, K. M., & Takahashi, A. (2018) An innovative ovipositor for niche exploitation impacts genital coevolution between sexes in a fruit-damaging *Drosophila*. *Proceedings of the Royal Society B* 285: 20181635. DOI:10.1098/rspb.2018.1635.
- Onuma, M., Kamimura, Y. & Sawamura, K. (in press) Genital coupling and copulatory wounding in

the interspecific mating of the *Drosophila auraria* species complex (Diptera: Drosophilidae). *Biological Journal of the Linnean Society*.

Roth, S., Siva-Jothy, M. T., Balvin, O., Morrow, E. H., Willassen, E. & Reinhardt, K. (2024) The evolution of female-biased genital diversity in bedbugs (Cimicidae). *Evolution* 78: 329–341.

Shirôzu, T. & Saigusa, T. (1973) A genetic classification of the genus *Argynnis* and its allied genera (Lepidoptera: Nymphalidae). *Sieboldia* 4: 99-104.

Simmons, L. W. & Fitzpatrick, J. L. (2019) Female genitalia can evolve more rapidly and divergently than male genitalia. *Nature Communications* 10: 1312.

Simonsen, T. J. (2006) Fritillary phylogeny, classification, and larval host plants: reconstructed mainly on the basis of male and female genitalic morphology (Lepidoptera: Nymphalidae: Argynnnini). *Biological Journal of the Linnean Society* 89: 627–673.

Simonsen, T. J. (2008) Phylogeny of the cactus-feeding phycitines and their relatives (Lepidoptera, Pyralidae) based on adult morphology: Evaluation of adult character systems in phycitine systematics and evidence for a single origin of Cactaceae-feeding larvae. *Insect Systematics & Evolution* 39: 303–325.

Sloan, N. S. & Simmons, L. W. (2019) The evolution of female genitalia. *Journal of Evolutionary Biology* 32: 882–899.

Uesugi, K., Hoshino, M. & Takeuchi, A. (2017) Introducing high efficiency image detector to X-ray imaging tomography. *Journal of Physics: Conference Series* 849: 012051.

Figure captions

- Fig. 1. Terminalia of mated female (7th sternum removed), lateral view (head comes to left), photograph (left) and line drawing (right). A. *Argynnis* (*Argynnis*) *paphia*. B. *A. (Damora) sagana* (B'. cornucopia of virgin female, ventral view: head comes to bottom). C. *A. (Argyronome) ruslana*. D. *A. (Am.) laodice*. E. *Fabriciana adippe*. F. *Speyeria aglaja*. Red: copulatory wounds; blue: (mini-)cornucopia; green: position of ductus seminalis opening. Abbreviations: cor: cornucopia; exp.m.: expanded membrane; tub: tubercule (or mini-cornucopia). Scale = 1.0 mm (0.5 mm only for B').
- Fig. 2. Longitudinal section μ CT images of cornucopia and neighboring region (the head is on the left). A. *A. (Damora) sagana*. B. *Fabriciana adippe*. C. *A. (Argyronome) ruslana*. Abbreviations: cor: cornucopia; ds: ductus seminalis; exp.m.: expanded membrane. Arrow heads indicate the opening of ds. Scale = 1.0 mm.
- Fig. 3. Male genital structures: left valva (internal view: right); uncus (right lateral view: middle) and phallus (dorsal view: left). The head is on the right. A. *Argynnis* (*Argynnis*) *paphia*. B. *A. (Damora) sagana*. C. *A. (Argyronome) ruslana*. D. *A. (Am.) laodice*. E. *Fabriciana adippe*. F. *Speyeria aglaja*. Abbreviations: amp: ampulla; cot: cornuti. Scale = 1.0 mm.
- Fig. 4. Male and female terminalia of *A. (Damora) sagana* in copula. A and B show photographs of the copulated condition (B with traces of key structures). C. Line drawing of the copulated condition. Red: female; blue: male. Abbreviations: amp: ampulla; cor: cornucopia; ph: phallus; un: uncus; val: valva. Scale = 1.5 mm.
- Fig. 5. Cross section μ CT images of 8th female abdominal segments, showing the thickness (arrow heads) of the lateral membranous region. A. *A. (Damora) sagana*. B. *A. (Argyronome) ruslana*. Abbreviations: cor: cornucopia; exp.m.: expanded membrane. Scale = 1.0 mm.
- Fig. 6. Phylogenetic relationships of six Argynnini species examined here (according to de Moya, 2016), with schematic illustrations of assumed evolutionary scenarios of the female counterpart structures occurring around the ventral region of the 8th abdominal segment (from wrinkled membrane through expanded membrane or tubercule to the cornucopia). Abbreviations: cor: cornucopia; exp.m.: expanded membrane; tub: tubercule (or mini-cornucopia).











