

On the function of cornuti, sclerotized structures of the endophallus of Lepidoptera

Carlos Cordero

Received: 6 January 2009 / Accepted: 6 April 2009 / Published online: 24 April 2009
© Springer Science+Business Media B.V. 2009

Abstract The genitalia of many male insects include structures whose functions are unknown or poorly understood. The endophallus of many Lepidoptera bears sclerotized structures known as cornuti, which in some species break off during copulation and remain within the female genital tract (“deciduous” cornuti). I describe previous and original hypotheses on the role of cornuti, identify the selective pressures invoked by these hypotheses, propose different ways of testing them and briefly review pertinent evidence. I describe ten functional hypotheses for non-deciduous cornuti and four for deciduous cornuti; six hypotheses invoke natural selection and eight involve sexual selection. In some cases more than one of the proposed functions could be performed by cornuti; evolutionary change from one function to another is also possible. I suggest that the wide morphological variation observed in non-deciduous cornuti across taxa supports hypotheses invoking sexual selection. I propose that the function and evolution of cornuti can be revealed with a combination of descriptive studies, cornuti removal experiments and comparative tests.

Keywords Genitalia · Sexual coevolution · Sexual selection

Introduction

The genitalia of male insects are complex organs composed of several structures (Tuxen 1970; Eberhard 1985) whose positions, movements and morphologies suggest that they play different roles in sexual interactions (Eberhard 1985). Unfortunately, in many cases, such roles are unknown or poorly understood (Eberhard 1985; Powell 2003; Galicia et al. 2008; Sihvonen 2007; Werner and Simmons 2008). The high rates of evolutionary diversification commonly observed in male genital structures are consistent with the general hypothesis that they are a product of sexual selection (reviews in Eberhard 1985; Hosken and Stockley 2004; cf. Mikkola 2008). However, comprehension of the forces responsible for the evolution of male genitalia requires understanding the functions fulfilled by their different parts.

The copulatory organ of male Lepidoptera is composed of a sclerotized tubular structure called the phallus or aedeagus (Powell 2003) that contains a membranous tube (in some cases with one or more diverticula) known as the endophallus or vesica (Klots 1970), which is connected to the ductus ejaculatorius, along which spermatozoa move from the testes (Klots 1970). The endophallus is everted during copulation and in many species bears on its external surface one or more sclerotized structures generally known as cornuti (Fig. 1; Klots 1970; Drummond 1984; Powell 2003). The endophallus is everted into the ductus bursae and the corpus bursa (a sac-like female structure where the male deposits his spermatophore). In some species the cornuti break off and remain in the bursa, in which case they are called deciduous cornuti (Tuxen 1970; Powell 2003). There are species with deciduous and non-deciduous cornuti (e.g. *Eremonidia mirifica*, Notodontidae; Rawlins and Miller 2008). Other terms have been used to

C. Cordero (✉)
Departamento de Ecología Evolutiva, Instituto de Ecología,
Universidad Nacional Autónoma de México, Circuito Exterior
s/n, Ciudad Universitaria, Apdo. Post 70-275, 04510 Coyoacán,
DF, México
e-mail: signa_cornuti@yahoo.com.mx

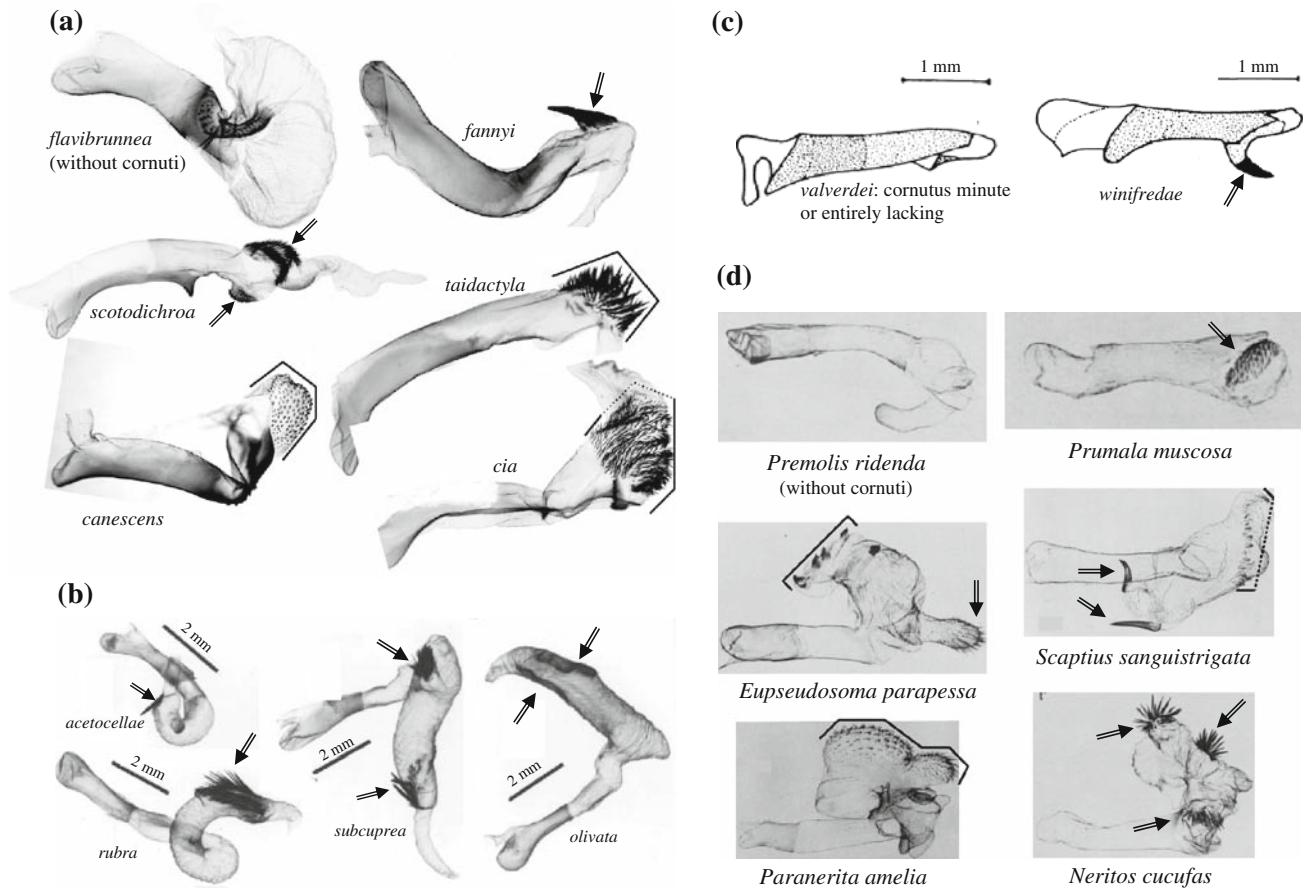


Fig. 1 Examples of within and between genera variation in the shape, number and location on the endophallus (=vesica) of the sclerotized cornuti. **a** *Diarsia* (Noctuidae) (Varga and Ronkay 2007). **b** *Mesogona* (Noctuidae) (Crabo and Hammond 1995/1997). **c** *Paradirphia* (Saturniidae) (Lemaire and Wolf 1988/1989). **d** *Neotropical Arctiinae* (Arctiidae) (Watson 1971). Arrows and lines indicate the location of cornuti on the expanded endophallus. Note the

wide intra-generic variation, including species without cornuti (e.g. *D. flavibrunnea* and *P. valverdei*), and the existence of more than one type of cornuti in some species (e.g. *E. parapessa* and *S. sanguistrigata*). In all cases the proximal part of the male copulatory organ (i.e. the sclerotized phallus or aedeagus) is located to the left of the figure

refer to cornuti or to specific arrangements of cornuti (see Tuxen 1970), and sometimes light sclerotizations of the endophallus are not considered cornuti. Some authors have argued that the cornuti of different taxa are not homologous (e.g. Powell 1973). Here, I will use the term cornuti to refer to all sclerotized structures present on the surface of the everted endophallus, independently of their ontogenetic origin or specific characteristics.

Although some functional hypotheses have been proposed (see references in Table 1), the role of the cornuti in reproduction is unknown in most cases and, therefore, the forces responsible for their evolutionary origin, maintenance and diversification remain virtually unknown. In this paper I review previous and original hypotheses on the role of cornuti, identify the selective pressures invoked by these hypotheses and describe ways of testing them. Lizeth Abundis (Instituto de Ecología, UNAM, Mexico) and I are beginning a comparative and experimental study of some

of these hypotheses and most of the examples that I present in the following sections are derived from a literature review in progress and from preliminary observations made in my laboratory. The examples discussed in the text and illustrated in Fig. 1 were chosen arbitrarily in the sense that we know of other cases that also could be appropriated. It is our aim to present in the near future a broad, systematic assessment of the different hypotheses based in our literature review.

Hypotheses on the function of non-deciduous cornuti

Ten hypotheses regarding the function of non-deciduous cornuti are described in Table 1. The first four hypotheses (H1–H4) propose functions that are important for a successful copulation, independently of competition with other males and of pre-copulatory female choice (because they

Table 1 Hypotheses on the function of non-deciduous sclerotized structures (=cornuti) of the endophallus (=vesica) of male Lepidoptera and the types of selection they imply

Hypothesis	Type of selection
H1: Organs that assist in the insertion of the endophallus into the female's corpus bursae	Natural
H2: Organs that assist in the transfer of the spermatophore to the female bursa (Callahan 1958; Callahan and Chapin 1960)	Natural
H3: Organs "for stirring and fragmentation of spermatophore" (Tuxen 1970)	Natural
H4: Organs for holding the female genitalia for maintaining a position that allows an adequate transfer of the spermatophores	Natural
H5: Organs for "locking" with complementary structures of the genital tract (e.g. the signa of the corpus bursa) of conspecific females	Natural
H6: Organs for holding the female genitalia for avoiding being displaced from the female by other males before completing a successful copulation	Intrasexual
H7: Organs for holding the female genitalia for avoiding interruption of the copula by the female	Intersexual
H8: Organs for stimulating females (Tuxen 1970; i.e. they are instruments for copulatory courtship or antagonistic seduction)	Intersexual
H9: Organ for piercing or tearing the female genital tract (i.e. they are instruments for copulatory courtship, antagonistic seduction or physical coercion)	Intersexual
H10: Organ for protecting the endophallus from being damaged by the signa (=sclerotized structures located on the inner wall of the female's corpus bursa; C. Macías, personal communication)	Intersexual

refer to processes happening during copulation). For this reason natural selection is the most likely force responsible for their evolutionary origin (although, once they have evolved, intersexual selection—i.e. female choice or antagonistic coevolution—could act subsequently on them; see "Multiple Functions" below). H5 proposes that cornuti are organs for "locking" with complementary structures of the genital tract (e.g. sclerotized portions of the ductus bursa) of conspecific females thus contributing to reproductive isolation (i.e. they are part of a "lock and key" mechanism; Mikkola 2008) and, therefore, also invokes natural selection. The other hypothesised functions (H6–H10) involve sexual selection. One hypothesis proposes that cornuti help males to prevent displacement from the female by competitor males during copulation (H6), whereas the others propose that cornuti are used by males to resist female attempts to interrupt copulation (H7), or to

induce or coerce females responses (physiological and/or behavioural) that favour male's reproduction (H8 and H9). Finally, considering that in some species females have sclerotized structures in the form of sharp spines, teeth or blades on the inner wall of the corpus bursae (called signa; Cordero 2005; Galicia et al. 2008) and that these structures could damage the endophallus (for possible reasons why females would be selected to touch the endophallus with their signa see Galicia et al. 2008), H10 proposes that cornuti are used to protect the endophallus from being damaged by signa (C. Macías, personal communication).

Hypotheses on the function of deciduous cornuti

The hypotheses regarding the function of deciduous cornuti are described in Table 2. The first (HA) proposes that these

Table 2 Hypotheses on the function of deciduous sclerotized structures (=cornuti decidui) of the endophallus (=vesica) of male Lepidoptera and the types of selection responsible for the evolution of such functions

Hypothesis	Type of selection
HA: Organ that assists females in the breaking of the spermatophore after the end of copulation	Natural
HB: Organ for reducing the success of males mating subsequently with the same female, by preventing the proper positioning of their spermatophores within the corpus bursae or by breaking off their spermatophores before sperm migration to the spermatheca	Intrasexual
HC: Organ for stimulating females after the end of copulation ^a	Intersexual
HD: Organ for reducing the probability of female remating ^b	Intersexual

^a In this case, it is possible that females inadvertently increase the stimulus when contracting their corpus bursae (e.g. when breaking off the spermatophore and/or when extracting its nutritious contents)

^b In this case, females might be reluctant to remate if the spermatophore of a subsequent mate exerts pressure on the cornuti decidui leaved by the previous male, forcing them to pierce or tear the wall of the corpus bursa and, probably, adjacent organs

structures are used by males to “help” females in the breaking of the spermatophore after the end of copulation: females could contract their corpus bursae and use cornuti as “tools” for breaking open the spermatophore. According to HB, a male uses deciduous cornuti to interfere with the proper positioning of spermatophores within the corpus bursa by subsequent males or to break off the spermatophores transferred by subsequent males before the optimal time for sperm migration to the spermatheca, thus obtaining an advantage in sperm competition. HC and HD propose that deciduous cornuti evolved to induce or coerce females to favour male reproduction, either via post-copulatory stimulation (HC) or post-copulatory damaging of the female reproductive tract (HD).

Multiple functions

In some cases cornuti could accomplish more than one function, either simultaneously (e.g. H6 + H8 or HB + HC) or sequentially (e.g. HA → HC or H1 → H8) and, therefore, different types of selection could be responsible for shaping the evolution of these genital structures. The cornuti-removal experiment proposed below could help uncover these multiple functions. Species such as *E. mirifica* (Notodontidae) (Rawlins and Miller 2008) that have both deciduous and non-deciduous cornuti are probably good models to study multiple functions of cornuti within a species.

However, the experimental approach can only help us to understand the current functions of these structures, and these could be different from the functions performed by cornuti when they first originated in particular taxa (i.e. the selective forces responsible for the origin of cornuti could be different from those currently acting on them; Shapiro and Porter 1989). For example, it is possible that the cornuti originally evolved to perform one of the functions proposed by H1–H4, but that subsequently sexual selection acting on females had favoured the evolution of mechanisms to discriminate between males on the basis of their ability to perform such functions (H8). An approach to try to distinguish the selective pressures responsible for the origin and the current maintenance of cornuti would be to perform functional studies in a taxonomically wide sample of species, followed by phylogenetic mapping of the cornuti functions uncovered and reconstruction of the ancestral functions with phylogenetic methods (Avisé 2006).

Testing the hypotheses: observational studies

Testing the hypotheses proposed requires measuring the effects of cornuti on behavioural and physiological traits of

females, as well as on male and female fitness, and this can be accomplished only with an experimental approach combined with appropriate comparative studies. However, detailed descriptions of mating behaviour in captivity and in the field, careful observations and measurements of inflated endophalli (Mikkola 2007), studies on the spatial relations between cornuti and the genital tract of females, and understanding of the patterns of spermatophore transfer and digestion, could help to shed light on the plausibility of different hypotheses.

An excellent method for understanding many of the process occurring within the female during copulation is “serial morphology” (Callahan 1958), that consists in the dissection of pairs killed in copula at different times after the beginning of copulation; this method can be complemented by histological sectioning of other mating pairs. Obtaining pairs in copula might be difficult in some cases and the endophallus could be retracted during handling or killing. If this is the case, two indirect alternative methods can be attempted. First, dissect, photograph and measure the inflated endophalli and the genital tract of females, followed by careful comparison of the superimposed preparations or images of the male and female structures (e.g. Mikkola 1992, 1993, 2008). This type of preliminary assessment is possible for some taxa because there are published studies that provide excellent drawings and photographs of inflated endophalli and of the corpus bursae of females. Second, dissect the female genital tract and superimpose the dissected male genitalia (with inflated endophallus) in the position that it adopts during copulation (this was the technique used for Fig. 2). Here I will discuss some illustrative cases of the utility of this type of data.

The hypothesis that non-deciduous cornuti are used by males to help in the proper transfer of the spermatophore to the female bursa (H2) was demonstrated for two species of the family Noctuidae (*Heliotis zea* and *Pseudaletia unipuncta*) in two classic studies by Callahan (Callahan 1958; Callahan and Chapin 1960). Spermatophore transfer in these species is very complex and involves deposition of the spermatophore in the corpus bursa and of part of the collum (the “filament” of the spermatophore where the aperture through which sperm migrates to the spermatheca is located) in the appendix bursa, where the aperture of the ductus leading to the spermatheca (the ductus seminalis) is located. Since in many Lepidoptera the appendix bursa is absent (Klots 1970; Scoble 1992) and spermatophore transfer seems much simpler, H2 is unlikely to be a general explanation.

The hypothesis that non-deciduous cornuti are used by males to stir and fragment the spermatophores of other males (H3) can be rejected as a general explanation if the spermatophore envelope is broken after the end of copulation, as it seems to be the case for most Lepidoptera (Drummond 1984). On the other hand, the function of

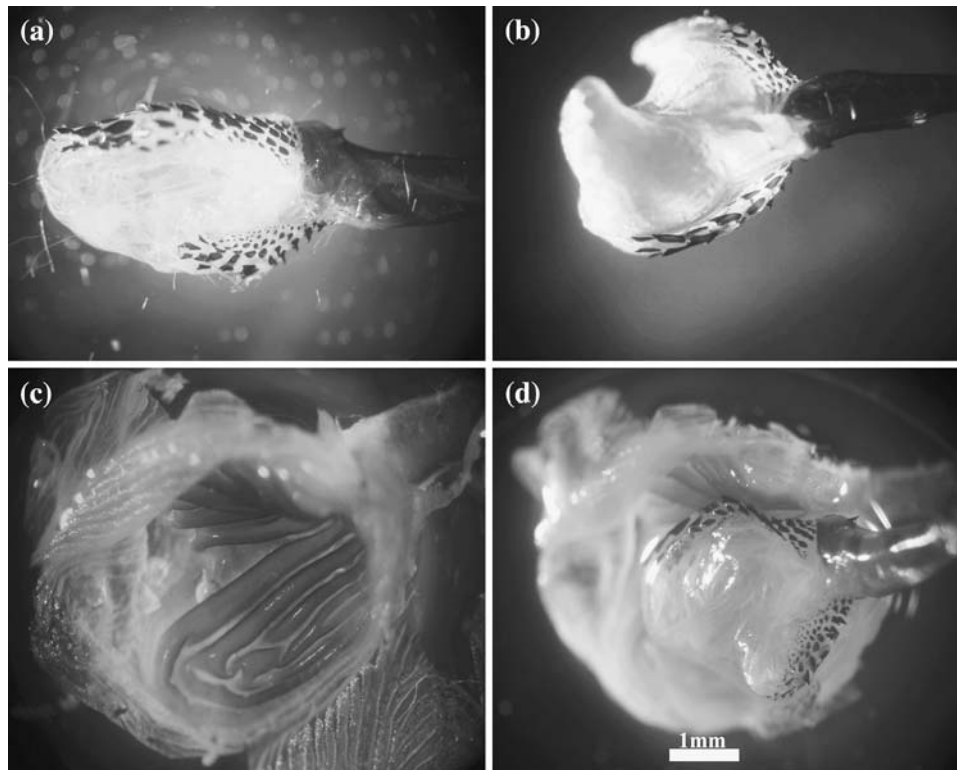


Fig. 2 Inferred spatial relationships between the male endophallus and the female corpus bursae in *Ascalapha odorata* (Noctuidae). **a** and **b** Two angles of the vesica showing its shape and the distribution of strongly sclerotized thorn-like cornuti. **c** Opened corpus bursa showing the sclerotized ridges that line most of its inner surface. **d** Male aedeagus with inflated endophallus superimposed on the opened

corpus bursae in the position that could adopt during copulation. In **a**, **b** and **d** the proximal part of the male copulatory organ (i.e. the sclerotized phallus or aedeagus) is located to the *right*; in **c** and **d** the ductus bursae of the female, through which the male copulatory organ is introduced, is oriented to the *right* of the photograph. (Dissections and photographs by Lizeth Abundis)

cornuti proposed by H3 is not necessary in species with signa, because females break open the spermatophore envelope with these genital structures (Hinton 1964; Cordero 2005; Galicia et al. 2008). Furthermore, for males of polyandrous species (the most common case in Lepidoptera; Drummond 1984; Eberhard 1985; Torres-Vila et al. 2004) it is adaptive to delay spermatophore rupture (Drummond 1984; Cordero 2005).

The hypothesis that cornuti are part of a “lock and key” system to avoid hybridization between related species (H5) can be rejected if such sympatric and related species do not exist or have not existed. In fact, the evolution of species-specificity of genitalia in isolation from close relatives in many animal taxa is one of the reasons why Eberhard (1985) considers that the lock and key is not a general explanation for genitalic divergence (for thorough discussions of arguments and evidence against the “lock and key” hypothesis for insect genitalia in general, including several examples from Lepidoptera, see Eberhard 1985; Shapiro and Porter 1989; Gilligan and Wenzel 2008; cf. Mikkola 2008).

The hypothesis that cornuti are used by males to hold on to the female genitalia to avoid being displaced from the female by competing males (H6) or to avoid interruption of the copula by the mating female (H7) can be discarded if males do not attempt to interrupt copulating pairs by force or if females do not try to finish copulations prematurely by force. The testing of hypotheses H5–H7 illustrates the importance of field studies of mating behaviour.

The hypothesis proposing that cornuti are used by males for tearing the female genital tract (H9) can be discarded if males never damage the female genital tract with their cornuti. The only evidence in favour of this idea that I am aware of are the observations made by Dr. Aletta Bakker and collaborators who found that in two species of *Yponomeuta* (Yponomeutidae) moths the spine-like cornuti, which are displayed as the “skeleton of an umbrella” when the endophallus is everted, actually pierce the corpus bursae of females near the ductus bursae (A. Bakker, personal communication). In some species the morphology of cornuti does not seem to be compatible with H9 (e.g. *Mesogona olivata* in Fig. 1; Crabo and Hammond 1995/1997).

The hypothesis that cornuti are used by males to protect their endophallus from being damaged by the spines of the female signa (H10) could apply to species such as *Calycopis sullivanii* (Lycaenidae) where the two “fan-shaped” signa appear to have several sharp projections that could damage the endophallus and the long and thin spine-shaped cornuti could help to avoid contact with the nearest signa. This interpretation is based in the assumption that the endophallus depicted in Figs. 10 and 12 of Robbins and Duarte (1999–2005) is fully everted, in which case the scale provided in the figures suggests that cornuti and signa come into contact during copula. On the other hand, H10 can be discarded if cornuti are never in contact with the endophallus during copulation.

Understanding how deciduous cornuti function requires more information. We need to clarify a number of facts. (a) Is there individual variation in the number of cornuti? At least in some species there is variation. In *Larisa subsolana* (Olethreutidae) the number of deciduous cornuti varied between 8 and 17 in a sample of 12 individuals (Miller 1978). Wright (2008) reports variation in the number of deciduous cornuti in four species of *Pelochrista* (Tortricidae): *P. palpana*, 8–14 ($n = 6$); *P. fuscostriata*, 12–24 ($n = 4$); *P. fuscosparsa*, 12–27 ($n = 12$); and *P. medio-striata*, 18–42 ($n = 13$). (b) Do females participate in the breaking of the cornuti or their rupture depends completely on the male? (c) Do all cornuti or only some of them break off in one copula? Illustrations and descriptions of some groups suggest that at least in some cases the cornuti are gradually shed in several copulations. For example, in their description of the genus *Mesogona* (Noctuidae), Crabo and Hammond (1997) said “the cornuti are fragile and entire cornuti or fragments are often left in the female corpus bursae following copulation”. (d) In case only some cornuti break off during a copula, do they break off in every copula or only in some of them? (e) At what time during copulation do cornuti break off (before, during or after spermatophore transfer, during the separation of the couple, etc.)? (f) Where and in what position within the corpus bursae do cornuti remain? (g) What is the relationship between the cornuti remaining in the corpus bursae and the spermatophores deposited in it (by the male shedding his cornuti and by previous and subsequent males); in this case, we need to know if cornuti puncture or tear the spermatophores. (h) If a male loses all his cornuti in one copula or gradually in several copulations, how do the results of his different copulations (copula duration, amount of ejaculate transferred, number of offspring when there is sperm competition, etc.) compare to each other. This information will help us to test and refine the proposed hypotheses. For example, if cornuti never puncture or tear spermatophores we can discard hypothesis HA, and if they never puncture the female genital tract we can discard HD.

Testing the hypotheses: experimental manipulation of cornuti

One way of testing the functional hypotheses for both types of cornuti is by removing cornuti or altering their size, shape or texture in experimental males (hereafter, EM) and comparing the performance of these males with that of control males with their cornuti intact (hereafter, CM) under conditions that will depend on the particular hypothesis tested. To my knowledge, nobody has tried to remove or modify cornuti in live lepidopterans and it is clear that such experimental manipulations will be technically challenging, particularly the access to fully inflated endophalli on which to perform cornuti manipulations. For non-deciduous cornuti at least two approaches could be attempted. First, copulating pairs can be anesthetized and then the inflated endophallus could be extracted out of the female tract by gently pulling the male and/or the female. This technique requires previous knowledge of when the endophallus is fully deployed within the female tract and it will be difficult or even impossible to employ with certain types of cornuti (for example, if cornuti are long spines oriented to the anterior part of the male body (e.g. *Diarsia fanny* and *Mesogona rubra*; Fig. 1a, b) the endophallus could be stuck in the bursa or ductus bursa and torn when attempting the extraction). Second, a copulating pair can be anesthetized and then the female killed and dissected to expose the endophallus for manipulation. In the case of deciduous cornuti experimentation will be easier because of the “natural removal” of cornuti during copulation, creating experimental males lacking some or all of their cornuti. Knowledge about the natural patterns of cornuti deposition in subsequent females will help to design these experiments. Although it will be difficult to control the number of cornuti deposited within experimental females, subsequent dissections of the male and of the females with which he mated can reveal how many cornuti were lost in each copulation.

Predictions for non-deciduous cornuti

In general, it is expected that the reproductive success of EM will be less than that of CM for reasons that are specific to each hypothesis. If H1 is correct, EM would fail to insert their copulatory organs in a suitable way resulting in failure in the transfer of spermatophores or in the deposition of spermatophores in inappropriate sites inside the female. If H2 is correct, EM would be unable to properly transfer their spermatophores, even though they properly inserted their copulatory organs. According to H3, EM would fail to rupture the spermatophore or rupture would be delayed. In the case of H4, EM would fail to maintain their copulatory organs in a position that permits an

adequate transfer of the spermatophore, which would result in failure to deposit the spermatophore in a suitable location. H5 predicts that EM will be unable to lock with complementary structures of the female and copulation will fail somehow (e.g. no transfer of spermatophore). Testing H6 would require comparing EM and CM in the presence of other sexually receptive males during copulation and predicts a higher rate of displacement of EM from the female by competing males before the end of copulation. In the case of H7 a higher rate of female-produced copula interruptions is expected for EM than for CM. Interrupted copulations predicted by hypotheses H6 and H7 also would result in shorter copula durations and probably more failures to transfer complete ejaculates by EM. Hypotheses H8 and H9 propose that cornuti are designed for the internal stimulation of females and in both cases a reduced ability to induce behavioural and physiological responses in females (decreased sexual receptivity post-copula, increased rate of egg maturation and oviposition, increased sperm transport to the spermatheca, larger egg size, etc.) is expected in EM compared with CM. If H9 is correct, females mated to EM would not suffer damage (or would suffer reduced damage, depending on the way cornuti are experimentally manipulated) in their genital tracts and would not, for example, delay remating with another male as much as CM. Finally, if H10 is correct, EM would suffer more damage to their endophalli than CM. The specific type of intersexual selection involved in the evolution of the functions proposed by hypotheses H7–H10 depends on the effect of cornuti on female fitness: female choice would be responsible if females use cornuti characteristics to discriminate adaptively between males, whereas the use of cornuti for antagonistic seduction or physical coercion would result in a decrease in female fitness and selection for female resistance adaptations (Holland and Rice 1998; Cordero and Eberhard 2003).

Predictions for deciduous cornuti

If HA is correct, females mating with EM would have reduced or delayed rates of spermatophore rupture in comparison with females mated to CM. Testing HB requires comparing the effect of EM and CM on the reproductive success of subsequent males mating with the same female. HB predicts that subsequent males would have a higher reproductive success after mating with females previously mated to EM than to CM. Hypothesis HC proposes that cornuti are designed for stimulating females after copulation; if it is correct, EM would have a reduced ability to induce behavioural and physiological responses in females that are advantageous to the male (e.g. decreased sexual receptivity post-copula or increased rate of egg maturation and oviposition). Hypothesis HD

proposes that cornuti are designed for damaging the female genital tract during subsequent copulations thus coercing females to reject (or delay) remating with another male while healing the wounds inflicted by cornuti. Therefore, females mated to EM are expected to receive less damage and to remate more often or to remate sooner than females mated to CM.

Testing the hypotheses: comparative studies

In the section “Multiple Functions” I mentioned how mapping cornuti functions on a phylogeny could help us to infer ancestral functions and to disentangle the selective pressures responsible for the origin of cornuti from those currently acting on them. Comparative studies are also necessary for understanding other fundamental aspects of the evolution of cornuti.

All hypotheses involving sexual selection (H6–H10 and HB–HD) predict that cornuti will tend to show a pattern of divergent and relatively rapid evolution (Eberhard 1985, 1996; Hosken and Stockley 2004); such a pattern is not expected from most of the natural selection hypotheses (H1–H4 and HA). This prediction can be tested by the phylogenetic mapping (Avice 2006) of cornuti shapes, numbers, sizes and locations on the endophallus. A literature review study still in progress (Lizeth Abundis and Carlos Cordero, unpublished), strongly suggests that the expected pattern of divergent and relatively rapid evolution exists (Fig. 1). Cornuti vary in number, position and shape between species in many groups, and for this reason they are considered of “great taxonomic value” (Tuxen 1970). While in some species these structures are absent, others have one cornutus or a few cornuti, and there are species with many cornuti (Fig. 1). In some species cornuti are located in one or a few specific spots on the endophallus, whereas in others they cover great portions of its surface. “Spine-shaped” cornuti are very common, but their particular thickness, shape, orientation and relative size show great variation between species (Fig. 1). In different species they have been described as spinules, hairs, teeth, spines, spurs, thorns, blades, etc. (Tuxen 1970). To give just two examples, most of the variants above mentioned, including species without cornuti, are observed in the Holarctic species of the noctuid genus *Diarsia* (Varga and Ronkay 2007; some examples are shown in Fig. 1a), and in the Neotropical members of the subfamily Arctiinae (Watson 1971; some examples are shown in Fig. 1d). The cornuti also have other shapes, like rods, bands or plates (e.g. Penz and DeVries 1999; Choi 2001), and they have variously ornamented surfaces. In many species with several cornuti, the cornuti present in the same vesica have different shapes (Fig. 1; Duckworth 1971).

Hypothesis 5 is the only “natural selection hypothesis” that predicts rapid divergence in cornuti. Although there is much evidence against the “lock and key” hypothesis (Eberhard 1985; Shapiro and Porter 1989; Hosken and Stockley 2004), it is difficult to test because it predicts divergence not only for species in which there is a relatively high risk of interspecific mating, but also for species in which there was in the past such a risk (Shapiro and Porter 1989). The derivation and testing of specific predictions is particularly important because sexual coevolution resulting from one, or some combination, of the hypotheses H6–H10 can produce a specific “fit” between cornuti and female structures in the absence of risk of hybridization, giving the misleading impression of a lock and key mechanism (see below).

Since sexual selection is more intense in polyandrous than in monandrous species, the hypotheses involving sexual selection predict that cornuti will be present mainly in polyandrous species and will tend to be absent in monandrous species (unless the species is effectively monandrous due to antagonistic manipulation of females by males via the cornuti functioning as proposed by hypotheses H8 and H9 or HC and HD). This also can be tested with a formal comparative analysis, taking advantage of the unusual fact that female mating patterns can be reasonably estimated from counts of spermatophore remains in the corpus bursa (Drummond 1984; Eberhard 1985; Cordero 1999). For example, by mapping on a phylogenetic tree the female mating pattern (monandry/polyandry) and the presence/absence of cornuti, it is possible to look for correlated evolution between this pair of binary discrete traits by using the maximum-likelihood method proposed by Pagel (1994; included in the computer package *Bayes Traits*, developed by Pagel and Meade and available in the website: www.evolution.rdg.ac.uk/BayesTraits.html).

Finally, the comparative method can be used to test more specific hypotheses on the evolution of particular types of cornuti. For example, the hypothesis that propose that cornuti pierce the female genital tract to induce or coerce female responses beneficial for males (H9) predicts that females could evolve thickenings, sclerotizations or structures that diminish or prevent the damage provoked by cornuti. In fact, in several species the signa have shapes that suggest a protective function (Galicia et al. 2008), and in others some portions of the female genital tract (e.g. the ductus bursa) are strongly sclerotized. For example, in *Scopula immorata* (Geometridae) the inner wall of the area of the ductus bursa that is in contact with the cornutus during copulation is sclerotized, whereas in *Scopula frigidaria* the ductus bursa is unsclerotized and the male endophallus lacks cornuti (Sihvonen 2007). Interpretation of such “protective” female traits is not simple, however, because they could

represent female mechanisms to screen males on the basis of their cornuti, rather than female defenses against all cornuti (Cordero and Eberhard 2003, 2005).

Another example is the hypothesis that cornuti are used to protect the vesica from damaging structures present in the genital tract of females (H10). Potentially damaging structures include signa in the shape of long spines (such as those present in *Argyrotaenia* spp.; Brown and Cramer 1999), serrated plates (such as those present in several Pieridae) or relatively large structures covered by spines (such as that of *Zamagiria* sp.; Iruegas et al. 2002). A comparative phylogenetic study of this hypothesis would test for correlated evolution of potentially damaging signa and cornuti whose structure is appropriate for protecting the vesica. The hypothesis predicts the order of appearance of male and female traits: male defenses against particular female traits should not appear until those female traits have evolved.

Acknowledgments I thank Lizeth Abundis, Constantino Macías and two anonymous reviewers for thoughtful comments on a first version of the manuscript. Tino Macías outlined hypothesis H10 and kindly allowed me to include it here. Raúl Martínez provided technical help. For permission to reproduce photographs and drawings I thank Drs. Lars Crabo (Fig. 1b), Kirby Wolfe (Fig. 1c), and Zoltan Varga, Lázló Ronkay and the *Acta Zoologica Academiae Scientiarum Hungaricae* (Fig. 1a). My research is supported by a grant from PAPIIT-UNAM (IN223508). I dedicate this paper to my friend and mentor William G. Eberhard.

References

- Avice JC (2006) Evolutionary pathways in nature. A phylogenetic approach. Cambridge University Press, Cambridge
- Brown JW, Cramer A (1999) Five new species of *Argyrotaenia* (Tortricidae: Archipini) from Mexico and the southwestern United States. *J Lepid. Soc* 53:114–125
- Callahan PS (1958) Serial morphology as a technique for determination of reproductive patterns in the corn earworm, *Heliothis zea* (Boddie). *Ann Entomol Soc Am* 51:413–428
- Callahan PS, Chapin JB (1960) Morphology of the reproductive systems and mating in two representative members of the family Noctuidae, *Pseudaletia unipuncta* and *Peridroma margaritosa*, with comparison to *Heliothis zea*. *Ann Entomol Soc Am* 53:763–782
- Choi S-W (2001) Phylogeny of *Eulithis* Hübner and related genera (Lepidoptera: Geometridae), with an implication of wing pattern evolution. *Am Mus Novit* 3318:1–37. doi:10.1206/0003-0082(2001)318<0001:POEHBA>2.0.CO;2
- Cordero C (1999) Is spermatophore number a good measure of mating frequency in female *Callophrys xami* (Lycanidae)? *J Lepid. Soc* 53:170–171
- Cordero C (2005) The evolutionary origin of signa in female Lepidoptera: natural and sexual selection hypotheses. *J Theor Biol* 232:443–449. doi:10.1016/j.jtbi.2004.08.031
- Cordero C, Eberhard WG (2003) Female choice of sexually antagonistic male adaptations: a critical review of some recent research. *J Evol Biol* 16:1–6. doi:10.1046/j.1420-9101.2003.00506.x

- Cordero C, Eberhard WG (2005) Interaction between sexually antagonistic selection and mate choice in the evolution of female responses to male traits. *Evol Ecol* 19:111–122. doi: [10.1007/s10682-004-7918-2](https://doi.org/10.1007/s10682-004-7918-2)
- Crabo L, Hammond PC (1995/1997) A revision of *Mesogona* Boisduval (Lepidoptera: Noctuidae) for North America with descriptions of two new species. *J Res Lepid* 34:83–98
- Drummond BAIII (1984) Multiple mating and sperm competition in the Lepidoptera. In: Smith RL (ed) Sperm competition and the evolution of animal mating systems. Academic Press, Orlando, FL, pp 291–371
- Duckworth WD (1971) Neotropical microlepidoptera XX: revision of the genus *Setiostoma* (Lepidoptera: Stetiomyidae). *Smithson Contrib Zool* 106:1–45
- Eberhard WG (1985) Sexual selection and animal genitalia. Harvard University Press, Cambridge, MA
- Eberhard WG (1996) Female control: sexual selection by cryptic female choice. Princeton University Press, Princeton, NJ
- Galicia I, Sánchez V, Cordero C (2008) On the function of signa, a genital trait of female Lepidoptera. *Ann Entomol Soc Am* 101:786–793. doi: [10.1603/0013-8746\(2008\)101\[786:OTFOSA\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2008)101[786:OTFOSA]2.0.CO;2)
- Gilligan TM, Wenzel JW (2008) Extreme intraspecific variation in *Hystriochophora* (Lepidoptera: Tortricidae) genitalia—questioning the lock-and-key hypothesis. *Ann Zool Fenn* 45:465–477
- Hinton HE (1964) Sperm transfer in insects and the evolution of haemocelic insemination. In: Highnam KC (ed) Insect reproduction. London, UK: Symposium of the Royal Entomological Society of London, pp 95–107
- Holland B, Rice WR (1998) Chase-away sexual selection: antagonistic seduction versus resistance. *Evol Int J Org Evol* 52:1–7. doi: [10.2307/2410914](https://doi.org/10.2307/2410914)
- Hosken DJ, Stockley P (2004) Sexual selection and genital evolution. *Trends Ecol Evol* 19:87–93. doi: [10.1016/j.tree.2003.11.012](https://doi.org/10.1016/j.tree.2003.11.012)
- Iruegas R, Gómez B, Cruz-López L, Malo EA, Rojas JC (2002) A new record of a moth attacking sapodilla, with descriptions of female genitalia and the last instar larva. *Fla Entomol* 85:394–397. doi: [10.1653/0015-4040\(2002\)085\[0394:ANROAM\]2.0.CO;2](https://doi.org/10.1653/0015-4040(2002)085[0394:ANROAM]2.0.CO;2)
- Klots AB (1970) Lepidoptera. In: Tuxen SL (ed) Taxonomist' glossary of genitalia in insects, 2nd edn. Munksgaard, Copenhagen, pp 115–130
- Lemaire C, Wolfe KL (1988/1989) Three new species of *Paradirphia* (Saturniidae: Hemiluecinae) from Mexico and Central America with notes on the immature stages. *J Res Lepid* 27:197–212
- Mikkola K (1992) Evidence for lock-and-key mechanisms in the internal genitalia of the *Apamaea* moths (Lepidoptera, Noctuidae). *Syst Entomol* 17:145–153. doi: [10.1111/j.1365-3113.1992.tb00327.x](https://doi.org/10.1111/j.1365-3113.1992.tb00327.x)
- Mikkola K (1993) The lock-and-key mechanisms of the internal genitalia of the noctuid and geometrid moths (Lepidoptera) in relation to the speciation concepts. *Folia Baeriana* 6:149–157
- Mikkola K (2007) The rise of eversion techniques in lepidopteran taxonomy (Insecta: Lepidoptera). *SHILAP Rev Lepid* 35:335–345
- Mikkola K (2008) The lock-and-key mechanisms of the internal genitalia of the Noctuidae (Lepidoptera): how are they selected for? *Eur J Entomol* 105:13–25
- Miller WE (1978) *Larisa subsolana*, a new genus and species from Eastern North America (Olethreutidae). *J Lepid. Soc* 32:256–260
- Pagel M (1994) Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc R Soc Lond B Biol Sci* 255:37–45. doi: [10.1098/rspb.1994.0006](https://doi.org/10.1098/rspb.1994.0006)
- Penz CM, DeVries PJ (1999) Preliminary assessment of the tribe Lemoniini (Lepidoptera: Lycaenidae) based on adult morphology. *Am Mus Novit* 3284:1–32
- Powell JA (1973) A systematic monograph of New World Ethmiid moths (Lepidoptera: Gelechioidea). *Smithson Contrib Zool* 120:1–302
- Powell JA (2003) Lepidoptera (moths and butterflies). In: Vincent VH, Cardé RT (eds) Encyclopedia of insects. Academic Press, New York, pp 631–664
- Rawlins JE, Miller JS (2008) Dioptine moths of the Caribbean region: description of two new genera with notes on biology and biogeography (Lepidoptera: Notodontidae: Dioptinae). *Ann Carnegie Mus* 76:203–225. doi: [10.2992/0097-4463\(2008\)76\[203:DMOTCR\]2.0.CO;2](https://doi.org/10.2992/0097-4463(2008)76[203:DMOTCR]2.0.CO;2)
- Robbins RK, Duarte M (1999/2005) Two phylogenetically significant new species of *Calycopis* (Lycaenidae: Theclinae: Eumeini). *J Res Lepid* 38:27–34
- Scoble MJ (1992) The Lepidoptera. Oxford University Press, Oxford
- Shapiro AM, Porter AH (1989) The lock-and-key hypothesis: evolutionary and biosystematic interpretation of insect genitalia. *Annu Rev Entomol* 34:231–245. doi: [10.1146/annurev.en.34.010189.001311](https://doi.org/10.1146/annurev.en.34.010189.001311)
- Sihvonen P (2007) Mating behaviour and copulation mechanisms in the genus *Scopula* (Geometridae: Sterrhynae). *Nota Lepid* 30:299–313
- Torres-Vila LM, Rodríguez-Molina MC, Jennions MD (2004) Polyandry and fecundity in the Lepidoptera: can methodological and conceptual approaches bias outcomes? *Behav Ecol Sociobiol* 55:315–324. doi: [10.1007/s00265-003-0712-2](https://doi.org/10.1007/s00265-003-0712-2)
- Tuxen SL (ed) (1970) Taxonomist' glossary of genitalia in insects, 2nd edn. Munksgaard, Copenhagen
- Varga Z, Ronkay L (2007) On the taxonomy of the genus *Diarsia* Hübner, [1821] 1816 (Lepidoptera: Noctuidae): the Holarctic species-groups of the genus. *Acta Zool Academi Sci Hung* 53(suppl. 1):141–209
- Watson A (1971) An illustrated catalog of the Neotropical Arctiinae types in the United States National Museum (Lepidoptera: Arctiidae). Part I. *Smithson Contrib Zool* 50:1–361
- Werner M, Simmons LW (2008) The evolution of male genitalia: functional integration of genital sclerites in the dung beetle *Onthophagus taurus*. *Biol J Linn Soc Lond* 93:257–262
- Wright DJ (2008) Nearctic Eucosmini (Tortricidae) associated with *Pelochrista occipitana* (Zeller) and *Eucosma biquadrana* (Walshingham): two new synonymies and four new species. *J Lepid. Soc* 62:216–231