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Rapid Divergent Evolution of Genitalia

Theory and Data Updated

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INTRODUCTION: WHY THE INTEREST?

The evolutionary forces responsible for the evolution of animal genitalia have a long history of controversy. Why the special interest in genitalia? In addition to the intrinsic interest of organs that are so intimately related to reproduction and fitness, it is because of a classic property of genital evolution: the morphological forms of genitalia are often species-specific, and these forms are often more divergent among closely related species than other traits such as legs, antennae, eyes, etc. In addition, male genitalia often show exuberantly complex forms that seem inexplicable in terms of their sperm transfer function (figure 4.1). This trend toward greater diversity in genitalia than in other structures occurs in at least some subgroups of all major taxonomic taxa with internal fertilization (reviewed in Eberhard 1985).

This widespread, relatively consistent usefulness of genital morphology in distinguishing species can be translated into a statement about evolutionary processes (unless the data are severely biased—see below): genitalia tend to show an evolutionary pattern of sustained, relatively rapid and divergent morphological change (Eberhard 1985). “Rapid” in this sense is in relative terms, with respect to changes in other traits. Genitalia are often much more elaborate than seems necessary for the simple

function of gamete transfer to the female. What could be responsible for such an evolutionary pattern? The objective of this chapter is to review new data and ideas that have appeared since my 1985 book that can help answer this question.

As a result of the sustained exploitation by taxonomists of genital morphology to discriminate closely related species, we surely know more about the evolution of species-level divergence in the morphology of genitalia than any other set of structures in the animal kingdom. For more than 100 years this huge mass of data on genitalia accumulated in nearly complete isolation from the study of sexual selection. The isolation was explicit in the original description of sexual selection by Darwin (1871), in which he specifically excluded genitalia from his discussion of sexual selection: “There are, however, other sexual differences quite unconnected with the primary reproductive organs, and it is with these that we are especially concerned” (p. 567). It ended abruptly, with Waage’s path breaking paper (1979) demonstrating that male genitalia are used in sperm competition in damselflies. During this long period of isolation the study of genitalia was the nearly exclusive province of taxonomists, and was largely descriptive. For their part, students of sexual selection did not even begin to recognize the possibility of post-copulatory competition among males until another crucial paper, that of Parker (1970) on

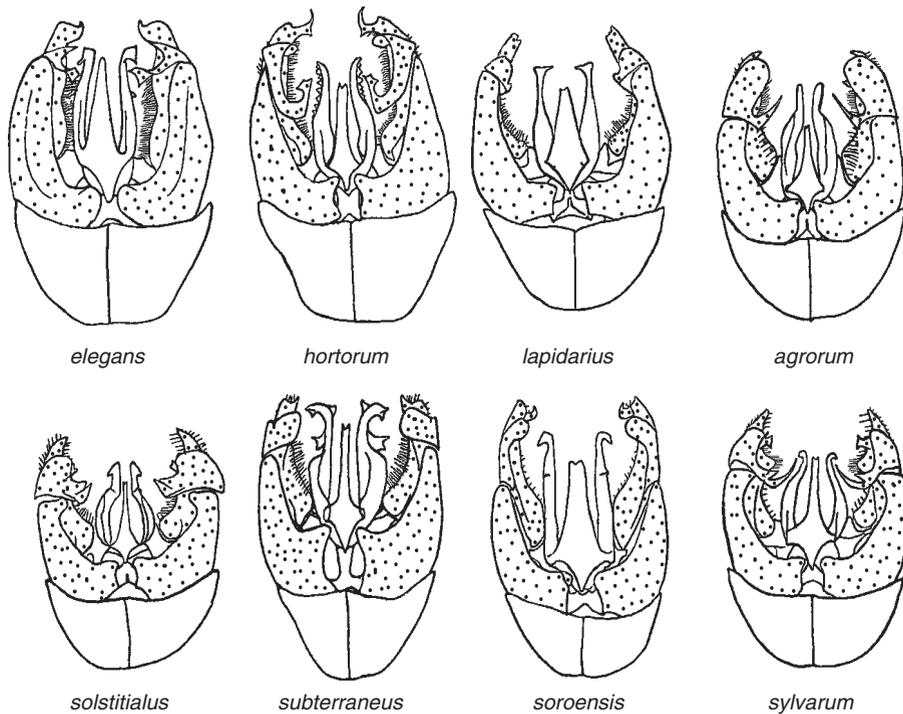
Bombus

FIGURE 4.1 Complex morphology of the male genitalia of different species of bumble bees in the genus *Bombus*, illustrating the pattern of diverse forms among closely related species that is very common in male genitalia. Much of this chapter is dedicated to evaluating hypotheses that attempt to explain why such relatively rapid divergent evolution should typify male structures that are specialized to contact females in sexual contexts. The stippled portions of these male genitalia are thought to contact only the external surface of the female's abdomen, and not to enter her reproductive tract during copulation. The area of the female that they contact is relatively featureless and differs little if at all between species, illustrating a common pattern in genitalia of more rapid morphological divergence in males than females. *Bombus* is especially interesting because it appears not to fit any of the currently popular hypotheses (see "Frontiers" below; drawings after Richards 1927).

sperm competition. Lagging even farther behind was recognition of the possible importance of active female roles in this competition. As interest in female choice surged in the early 1980s, the possibility that it might act on genitalia through cryptic female choice (CFC) (Thornhill 1982) was proposed (Eberhard 1985). More recently genitalia have been mentioned as targets of another type of sexual selection, sexually antagonistic coevolution (SAC) between males and females (Arnqvist & Rowe 2005; Gilligan & Wenzel 2008) (below).

Recent developments in several fields facilitated the linking of genital evolution and sexual selection (Birkhead 1996). The most important advances were: (1) the discovery that the doubts about whether females could gain payoffs from choosing among males, which were based on the theoretical "proof" that no genetic variance could exist among males for traits under selection by female choice, were unfounded; empirical data showed that variance is quite common (summary Andersson 1994); (2) the discovery that multiple mating by females (a prerequisite for

post-copulatory sexual selection to occur) is much more common in nature than previously thought; (3) the rediscovery of the importance of sexual selection by female choice; and (4) a gradual disillusionment (for several reasons) with previous, “species isolation” arguments to explain species-specific traits in general (e.g., Paterson 1982), and genital traits in particular (Scudder 1971; Eberhard 1985; Shapiro & Porter 1989). The recent increased emphasis on male–female conflicts during copulation (Parker 1984, 2005; Arnqvist & Rowe 2005) has led to further hypotheses regarding genital evolution on the basis of male–female coevolutionary conflicts.

In this chapter, I will update the search for a unitary explanation for sustained rapid divergent evolution of genitalia. Because of the great generality of the trend, which extends even to non-genital structures that are specialized to contact females in sexual contexts (below), there is probably a very general explanation. The reader should keep in mind, however, that because literally millions of species are involved, it is likely that there may be exceptions to most if not all generalizations. The male genitalia in different groups perform a wide variety of functions, ranging from fighting other individuals (“penis fencing” — Michiels 1998), visual displays (Wickler 1966; Bohme 1983), hooking and holding onto struggling females prior to copulation (Bertin & Fairbairn 2005), plugging the female’s reproductive tract (Koeniger 1983; Abalos & Baez 1966; Nessler et al. 2007), prying or squeezing open female ducts and valves (Fennah 1945; Eberhard 1993a; Schulmeister 2001; Sirot 2003; Moreno-Garcia & Cordero 2008), holding on with powerful suction cups (Schulmeister 2001), removing copulatory plugs (Aisenberg & Eberhard 2009), cleaning off detritus from previous copulations (Kumashiro et al. 2006), forming a reserve intromittent structure in case the other is damaged (Kamimura & Matsuo 2001), injecting prostate gland secretion through one aperture and sperm through another in bifid or trifid structures (Merrett 1989; Anthes & Michiels 2007), and rubbing or tapping the female before or after copulation (Otronen 1990; Eberhard 1990, 1994). Whether the structures that perform these different functions all show the same trend toward rapid divergent evolution is not known (the answer might be interesting). Perhaps no single explanation for diversity in form will be correct for all cases.

The line between general and local explanations, and in particular the number and scope of

refutations that are needed to reject a hypothesis as a general explanation, is difficult to determine (Coddington 1987; Shapiro & Porter 1989). I have no magic answers, but believe it is useful to explore the limits of generality of different hypotheses that attempt to explain a widespread phenomenon like this. In keeping with the general focus of this book (and also with the much larger accumulation of data), I will concentrate on the evolution of the morphology of genitalia, rather than that of sperm and other seminal products, even though these also show signs of being under sexual selection (Miller & Pitnick 2002; Holman & Snook 2006; Markow & O’Grady 2005). They are probably crucial for understanding some aspects of the reproductive morphology and physiology, especially of females, as illustrated by the coevolution between the length and the form of sperm cells and female storage organs in *Drosophila* (Miller & Pitnick 2002), diopsid flies (Kotrba 1995, 2006), scathophagid flies (Minder et al. 2005), and featherwing beetles (Dybas & Dybas 1981). Before I begin, I need to make two preliminary points: one concerns non-genital “genitalia”; the other the possibility that the pattern of accentuated diversity in genitalia is an illusion that has arisen from biases in how taxonomists work.

NON-GENITAL CONTACT STRUCTURES

I will discuss in this chapter not only primary genitalia (structures associated with the gonopore), but also secondary genitalia (which receive sperm from the male’s gonopore and introduce them into the female), and also non-genital male structures that are specialized for contact with the female (usually in a non-genital part of her body) prior to or during copulation. All three clearly show the same evolutionary pattern of common species-specificity and frequent overly-elaborate form for their relatively simple functions, and thus probably require a similar explanation (Robson & Richards 1936; Eberhard 1985). This pattern in secondary genitalia and non-genital contact structures was discovered long ago by taxonomists in many groups. In fact, entomologists have repeatedly included as “genitalia” some structures which are not associated with the segment on which the genital opening occurs), such as cerci and sternites near the “true” genitalia, in groups in which these structures also

show a pattern of rapid divergent evolution and elaborate forms that is typical of more strictly genital structures (e.g., Tuxen 1970; Wood 1991).

Other structures that are even farther from the genitalia and that are specialized to contact non-genital parts of the female in sexual contexts also show the same pattern (Eberhard 1985, 2004b; also Darwin 1871; Robson & Richards 1936). Almost any part of the male can be modified in this way, from the sucker-like “bursa” of male nematodes to the cephalothorax, the chelicerae and anterior legs of spiders, the antennae and telson of crustaceans, and the head, mandibles, antennae, pronotum, cerci, legs, and wings of insects (figure 4.2). As pointed

out by Robson and Richards (1936), the mechanical function of many (though not all) of these structures is to grasp the female during copulation; this is the same function that is performed by a large fraction of the male genital structures that are species-specific in form (summaries in Scudder 1971; Eberhard 1985, 2004a). In the end, the line between “true” genital claspers and non-genital claspers is arbitrary (Darwin 1871; Chapman 1969; Eberhard 1985; see also chapters by Leonard & Cordoba-Aguilar and Ghiselin in this book).

Inclusion of non-genital contact organs is especially useful for understanding this evolutionary pattern of rapid divergence because they have two

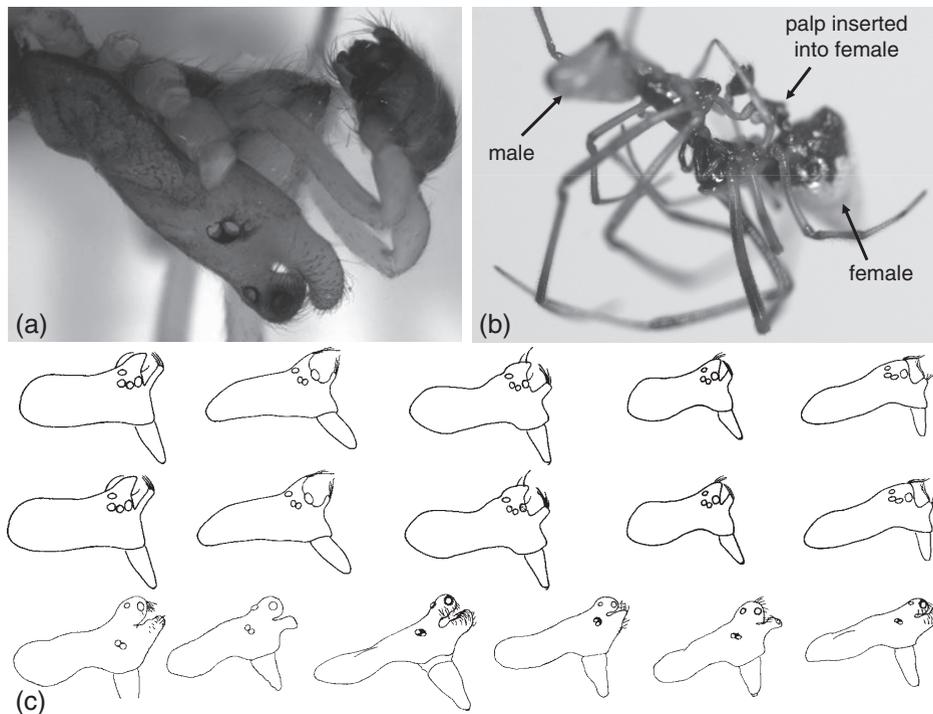


FIGURE 4.2 The elaborate anterior portion of the male cephalothorax of *Argyrodes elevatus* (a) is specialized to contact the female during copulation (b). As with many other non-genital contact structures, the forms are elaborate and species-specific (each drawing in (c) is of the male of a different species of *Argyrodes*). SAC explanations for male cephalothorax form based on species-specific female defensive behavior or morphology to avoid damage are unconvincing. The female’s mouth area, which contacts the modified area of the male’s cephalothorax during copulation (b), does not show any modifications. The female is not physically coerced, as she is free to pull her mouth away from the male at any time during copulation, and thus avoid possible tactile and chemical stimulation; in fact the female pulls away when the gland openings on the male’s modified cephalothorax are covered (G. Uhl, personal communication). (a) and (b) courtesy of Gabriele Uhl; (c) from Exline & Levi 1962).

advantages over “true” genitalia: the details of their physical interactions with the female and their possible functions (often grasping the female) are generally better understood; and the female structures that they contact are often more easily studied, because the male organs contact the female’s outer surface rather than internal genital structures. I will use the phrase “non-genital contact structures” below to indicate species-specific male structures that are not near his genitalia and that are specialized to contact females in sexual contexts.

IS THE PATTERN OF RAPID DIVERGENT EVOLUTION AN ARTIFACT?

The historical isolation of taxonomic research on genitalia has both advantages and disadvantages in studies of sexual selection. It makes the data more trustworthy in some respects, because they are independent of observer bias with respect to hypotheses about sexual selection. The data are also, however, subject to other possible biases that could result in over-estimating the relative rapidity of genital evolution and divergence (Coddington 1987; Tanabe et al. 2001; Huber 2003, 2004; Mutanen 2005; Mutanen & Kaitala 2006; Song 2006). The trend toward rapid divergent evolution discussed above might be an artifact if taxonomists rely too heavily on genital differences in deciding which groups of individuals should be recognized as species: they might fail to recognize species which differ with respect to other traits but not their genitalia; and they might over-split species if they find genital differences among different populations of the same species, especially in well-studied faunas where discovery of truly new species is rare (Mutanen 2005). Such over-reliance on genitalia could lead to overestimates of the relative rapidity of divergence of genitalia (Shapiro & Porter 1989; Huber 2003; Song 2006). The splitting problem could be particularly important if the amount of intra-specific variation in genitalia is underestimated, and some previous over-splitting mistakes have been documented (Mutanen 2005). Genitalia do vary intraspecifically among geographically distinct populations (Ware & Opell 1999; Siro 2003; Polihranakis 2006; Song 2006; Gilligan & Wenzel 2008), during the ontogeny of a given individual (Song 2006), between different seasons (Kunze 1959; Vitalievna 1995), and even within a single

population (Mutanen 2005). In addition, male genitalia are polymorphic in some species (Johnson 1995; Huber & Pérez Gonzalez 2001; Mutanen & Kaitala 2006), and there is reason to believe that polymorphism has been underestimated (Huber 2003). Over-reliance on genitalia could be especially damaging when sample sizes are small, an uncomfortably common circumstance in many taxonomic studies (Huber 2003). These problems could lead to overestimates of the rapidity with which genitalia diverge.

Are there reasons to doubt the importance of these taxonomists’ self doubt? I think the answer is yes. In the first place, there are data on genitalia that are independent of possible over-reliance on genitalia to distinguish species and that also indicate rapid divergent evolution. The variation in genital morphology at higher taxonomic levels, where uncomfortable questions about what is and what is not a species are not a problem, strongly imply especially rapid, sustained divergence in genitalia. Despite major long-term efforts, homologies have been much more difficult to establish among male genital structures than for other structures (Tuxen 1970; Coddington 1990; Wood 1991; Schulmeister 2001). For instance, Coddington (1990: p. 1) summarizes the situation for araneoid spiders: “On the whole, the century-long effort to homologize the palpal sclerites of male spiders across families and superfamilies seems to have been a rather dismal failure.” Similar pessimism characterizes attempts to find homologies within insect orders (Tuxen 1970), and even within a single spider family (Agnarsson et al. 2007). These difficulties testify to divergence that is so rapid and substantial that even highly trained eyes and minds are unable to find and agree upon commonalities.

A second striking aspect of genital evolution is the extraordinarily wide range of groups in which genitalia are thought to constitute especially useful traits for distinguishing species (Eberhard 1985). Perhaps no major group of animals with internal insemination is an exception. Taxonomists working on many different groups have apparently convergently realized that genitalia are especially useful traits in distinguishing otherwise difficult to distinguish taxa. This convergence was not simultaneous; even within the insects, genitalia were used very early in some groups of flies (Dufour 1844 in Shapiro & Porter 1989), and only began to be used later in others, such as papilionid butterflies and sphingid moths (Jordan 1896, 1905), tortricid

moths (Dampf 1908 in Gilligan & Wenzel 2008), and certain Hymenoptera (Perez 1894 in Shapiro & Porter 1989), and even later in others such as *Culicoides* flies (Carter et al. 1920 in Jamnbeck 1965).

Could it be that use of genitalia in studies of fly taxonomy induced beetle, snake, rodent, nematode and earthworm taxonomists to concentrate excessively on aedeagi, hemipenes, bacula, spicula and penile spines to distinguish species? Such cross-group imitation is imaginable, but I expect it is relatively unimportant, because I have confidence in the hard-headed independence of taxonomists. Take for example, the likely result of communication among workers on different groups of animals. A worker on group X might begin to examine genital traits after learning that workers in group Y found genitalia to be useful in distinguishing species. But only if the genitalia in group X worked as well or better than the other traits that were previously used to distinguish species in this group, and if the groupings were in at least general agreement with those indicated by other traits, would the taxonomist working on X be likely to adopt them.

There are also other reasons to think that taxonomists in different groups have not been slavishly dependent on others in choosing the traits on which they concentrate. In many subgroups of insects and arachnids, for instance, taxonomists have never used genitalia or have secondarily abandoned their use in particular groups, including most ichneumonid wasps (I. Gauld personal communication.), lampyrid beetles (Lloyd 1997), field crickets (Alexander et al. 1997), Jerusalem crickets (Tinkham & Renz 1969), tephritid fruit flies (Eberhard 1996), polycatenid bugs (Ferris & Usinger 1939), satyrid butterflies (Cardé et al. 1970), aleyrodid whiteflies (Ossiannilssen et al. 1970), and scorpions (Jacob et al. 2004a) (see Robson & Richards 1936 for others). In some taxa, species that were originally recognized on the basis of non-genital traits were subsequently found to also differ in genitalia (Shapiro & Porter 1989). These data indicate that taxonomists have not been so strongly tradition-bound in choosing characters as the arguments above suggest, and that genitalia do often tend to diverge relatively rapidly.

One further concern (Song 2006, Song & Bucheli 2009) is that the fact that genitalia often evolve slowly enough that their pattern of differences reflect higher-level groupings of different species implies a limited rapidity of genitalic divergence.

Song (2006) found that 94.7% of 89 papers presenting phylogenetic analyses in 19 different arthropod orders concluded that genital characters were phylogenetically informative, and was thus led to the unsurprising conclusion that “genitalia do not evolve chaotically.” This pattern does not weaken, however, the possibility that genitalia tend to diverge more rapidly than do other body traits. Rather they probably often evolve rapidly enough to be especially useful compared with other traits in distinguishing closely related species, but nevertheless slowly enough in at least some aspects to also retain a phylogenetic signal.

This is not to say that both improved methods of quantifying genital divergence (e.g., Tanabe et al. 2001; Mutanen & Pretorius 2007) and use of other, independent characters such as molecular differences have not corrected some errors that have resulted from previous over-reliance on genital morphology (e.g., Hedin 1997; Stoks et al. 2005) (such checks have also confirmed distinctions on the basis of genitalia in other taxa—Pizzo et al. 2006a, b). But the general message is that the trend for genitalia to diverge relatively rapidly does exist, although the evidence may not be as conclusive as some have thought. Calls to check species for possible genital polymorphism, and to test for correlations between molecular and genital differentiation (Huber 2003; Jacob et al. 2004a) represent healthy skepticism that promises to help determine the scope of the general trend in particular groups.

WHY RELATIVELY RAPID DIVERGENCE? HYPOTHESES IN DISFAVOR

Many explanations have been proposed to explain the tendency for genitalia to diverge rapidly. One major hypothesis that is generally judged to have failed is Mayr's pleiotropism hypothesis (Mayr 1963). He proposed that genes that are involved in adaptations to other factors such as different ecological conditions also have pleiotropic effects on genital morphology, and that divergent ecological adaptations incidentally resulted in genital divergence. This hypothesis does not explain, however, why such pleiotropic effects should be concentrated in genitalia rather than other structures, or why in groups with other male sperm-transferring structures besides the primary genitalia (e.g., the secondary genitalia of spiders, solfugids, pseudoscorpions,

and odonates) it is always the secondary genital structures that show the typical rapid divergent evolution while the primary genitalia do not. Nor does it explain why the genitalia of species with external fertilization show a complete lack of such pleiotropic effects (Eberhard 1985).

A second major explanation, the oldest of all, is the “lock and key” hypothesis. This holds that selection on females to avoid insemination by males of other species has resulted in the evolution of female genital structures that prevent entry or coupling by the male genitalia of other species. Males may also profit from not transferring sperm to heterospecific females, but probably to a lesser degree, given their less costly gametes. The lock and key hypothesis provides a clear explanation for rapid divergence and male species specificity, but it is nevertheless probably in the process of slow death under an accumulation of contrary evidence (Eberhard 1985; Shapiro & Porter 1989). Most notably, the females of many species simply do not have any structures that could act as a “lock” to exclude heterospecific males (summary in Shapiro and Porter 1989; subsequent data in Eberhard & Pereira 1996, Eberhard 2001a–d, 2003, 2004b, c, 2005; Peretti 2003; Ohno et al. 2003; Vanacker et al. 2003; Eberhard and Ramirez 2004; Jagadeeshan and Singh 2006; Briceño et al 2007; Ingram et al. 2008). The existence of mirror image genital dimorphism in one sex of a mantid (Howell & Herberstein in prep.) and a spider (Huber & PerezGonzalez 2001) also argues against the importance of specific fits. And an intra-specific analysis of a water strider showed no effects of the relationship between male and female morphology on male mating success (Arnqvist et al. 1997). There are exceptions (Callahan & Chapin 1960), but the lack of female “locks” is clearly widespread.

In addition, there is often no sign of the character displacement in males that is predicted in zones of sympatry of closely related species (McAlpine 1988, Ware & Opell 1979; Shapiro & Porter 1989). In addition, there is clear evidence of genitalic species-specificity in species that have evolved in complete or nearly complete physical isolation from any close relatives and that thus need no locks and keys, such as those endemic to oceanic islands and caves, and parasites isolated from all close relatives in their different hosts (Eberhard 1985, 1996; Shapiro & Porter 1989; Hedin 1997).

The lock and key hypothesis is still sometimes cited, and a few recent studies present data in favor.

In some noctuid moths male and female genital structures coevolve, as predicted (Mikkola 1992, 2008), but this support is weak because several other hypotheses are also compatible with such coevolution; Mikkola’s reason for dismissing cryptic female choice as an alternative explanation is unconvincing, nor is the evidence convincing that female genitalia are designed appropriately to exclude heterospecific males (Eberhard 1996). Lock and key arguments were also given to explain why in cross-specific pairings in *Carabus* beetles, the species-specific male copulatory piece does not fit easily in the a soft sac where it is lodged in the female’s reproductive tract, and sometimes causes fatal damage (Sota & Kubota 1998; Usami et al. 2006). These observations show the importance of a mechanical fit between male and female, but do not support the lock and key hypothesis. The hypothesis supposes that females evolve species-specific “locks” in order to gain (from avoiding cross-specific fertilization of their eggs), while in these carabids the female morphology causes them to lose (because of internal damage) when they mate with cross-specific males. Data for another proposed case in millipedes are limited to the genital consequences of differences in the size rather than shape, and are asymmetric (males of the larger species cannot fit into the smaller), and do not explain the diversity of genital forms in this genus (Tanabe & Sato 2008). There are also a few cases of geographic patterns of apparent character displacement in male genitalia that is predicted by lock and key, as in aedeagus length in populations of two closely related species of *Odontolabis* stag beetles that are sympatric at two sites (Kawano 2003). Such patterns are uncommon, however, and species which lack displacement have also been observed (Ware & Opell 1979; Tanabe et al. 2001; Taylor & Knouft 2006). Occasional displacement-like patterns might occur by chance, especially when ranges are not known in great detail or have changed historically (Shapiro & Porter 1989).

Further recent evidence also argues against lock and key. In several different groups female remating frequency is positively correlated with the amount of genital divergence (Eberhard 1985; Dixson 1987, 1998; Roig-Alsina 1993; Arnqvist 1998; Parag et al. 2006). This correlation is predicted by sexual selection hypotheses (below), but not by lock and key. One recent energetic defense of lock and key in Lepidoptera (especially Noctuidae) involves a major retreat, admitting that the substantial divergence

of the male genital structures that remain on the external surface of the female (“external” male genitalia) do not involve lock and key selection, because there are no female “lock” structures; only intromittent male structures are claimed to function as keys in internal female locks. In addition, the frequent divergence between the “internal” male genitalia of allopatric sister species (seen in 34 of 39 pairs of Holarctic noctuid species) is said to be due to drift rather than selection for species isolation. No explanation was given for why genitalia should drift more than all other traits (in only 17 of the 39 pairs did non-genital “habitus” traits differ). Such ad hoc retreats in the face of contradictory evidence are always possible in science, but reduce the credibility of the hypothesis.

A related idea, which is mentioned less often but is less strongly contradicted, is a stimulation version of lock and key: the female uses stimuli from the male’s genitalia to determine his species identity, and thus avoids cross-specific insemination (Patterson & Thaler 1982; Eberhard 1985), or the male uses stimuli from the female to avoid cross-specific sperm transfer (Tanabe & Sota 2008). This hypothesis can explain both species-specificity in males and the frequent lack of coevolution in female morphology in groups with rapid male divergence. It is contradicted, however, by the lack of character displacement in male genital morphology in zones of overlap (Eberhard 1985; Shapiro & Porter 1989), and the clear male divergence in many groups in which cross-specific pairing is impossible because of geographic or ecological isolation (Eberhard 1985, 1996). A stronger test, however, would be to search for female character displacement, as occurs in some other signals such as frog calls (Höbel and Gerhardt 2003). Sensory lock and key also does not necessarily predict the correlation between female polyandry and male genital divergence (Eberhard 1985; Roig-Alsina 1993; Dixon 1987, 1998; Arnqvist 1998; Parag et al. 2006).

Another hypothesis, motivated by the discovery that male genitalia can remove the sperm of other males from female storage organs in damselflies (Waage 1979) and the inspired speculations of Lloyd (1979), is that direct male–male sexual selection on the male’s ability to remove sperm from the female might be responsible for the diversity of male genitalia. Sperm competition (strictly speaking, “the competition within a single female between the sperm from two or more males for the fertilization of the ova” (Parker 1970); more commonly

extended to cover direct male effects on the sperm of other males within a female) has subsequently been documented in a variety of species (Simmons 2001). But without selection of some sort that causes rapid evolutionary changes in females that make different male designs better at removing sperm in different, closely related species (as expected under both cryptic female choice and sexually antagonistic coevolution—see below), sperm competition involving male genitalia seems unlikely to result in rapid divergent evolution of males by itself. In addition, in contrast with damselflies, the male genitalia in many (most) of the groups with diverse male genitalia do not reach sperm storage sites inside the female, and thus cannot physically remove sperm there (Eberhard 1985). Nevertheless, in some groups not all sperm from previous males are stored in the spermathecae, and they occur at sites such as the bursa or the vagina that are more accessible for the male. In addition, it can also be imagined (though it has never been convincingly demonstrated) that a male whose genitalia do not reach the sperm stored in the female can nevertheless flush them out with a douche-like spray (Eberhard 1985; Simmons et al. 1996; see Hosken et al. 1999 and Whitney et al. 2004 for refutations of this mechanism in a fly and a shark); physical sperm displacement with the male’s spermatophore does occur in one beetle (Förster et al. 1998). In some katydids (von Helversen & von Helversen 1991) and damselflies (Cordoba-Aguilar 1999) the male can induce the female to move sperm from her inaccessible spermathecae to other sites in her reproductive tract that he can reach with his genitalia.

In general, sperm competition could be linked with rapid divergence in two ways. If the species-specific aspects of a male’s genitalia allow him to overcome female-imposed barriers to gain access to otherwise inaccessible sperm and displace them, and if the female gains from having barriers by avoiding a cost of the male’s actions that reduces her production of offspring, then the male adaptations to overcome female barriers (and the female barriers) could represent adaptations favored by sexually antagonistic coevolution (below). If, on the other hand, these male genital traits serve to increase the male’s ability to induce female responses (such as those documented in the katydids and damselflies) that allow him to overcome female barriers and remove other males’ sperm, and if the female gains from having the barriers because they

enable her to bias paternity so as to obtain sons better able to overcome female barriers in following generations, this could represent a type of cryptic female choice (below).

There is, however, strong evidence against the generality of the sperm removal hypothesis that comes from the many species in which species-specific male structures clearly contact only sites in or on the female where sperm are never present, such as the many non-genital contact structures (Robson & Richards 1936; Eberhard 1985, 2004b). There are also numerous examples of species-specific genital structures that surely never come close to sperm in the female, including male surstyli in sepsid and tephritid flies (Eberhard & Pereira 1995, 1996; Eberhard 2001b), clasping gonocoxae and gonostyli in many dipteran families (reviewed in Eberhard 2004a), the stipes, volsella and squama in male bumble bees (figure 4.1) (Richards 1927), elongated genital setae in *Aelurus* wasps (Eberhard 2004c), and male cerci and associated setae in *Glossina* tsetse flies (Briceño et al. 2007), all of which remain outside the female's body during copulation.

Several newer, more neglected hypotheses have also been proposed. Møller (1998) proposed that the female uses the male's genitalia to judge his ability to resist infection by parasites. Perhaps due to the absence of any obvious reason to suppose that the form of a male's genitalia should be consistently responsive to such infections, this hypothesis has not to my knowledge received further attention. Simmons (2001) proposed a different sexual selection hypothesis involving direct male–male battles: complex male genital morphology diverged under selection to function as holdfast devices that defend copulating males against takeovers by other males. A possible reason for rapid divergent evolution of such holdfast devices, though none were given, would be to counteract the effects of rapid divergence in the behavior that other males use to displace copulating males. To my knowledge, however, no such divergent behavior has ever been documented. This hypothesis also has other serious problems. Many species-specific male genital structures surely do not function as holdfast devices. For example, in a list of functions attributed to 105 male genital structures in 43 species in 22 families of Diptera (Eberhard 2004), nearly half (46.7%) of the attributed functions were for penetrating the female and sperm transfer rather than for clasping. In many other groups with divergent intramittent

male genitalia, such as for instance nematodes, primates, rodents, and bats, the male clasps the female with structures other than his genitalia. In still other groups the male has a very powerful, species-specific clasping device which makes it essentially impossible to displace him from the female, but also has additional species-specific genital structures that enter the female that are not appropriately designed as hold-fast devices (see, e.g., Whitman & Loher 1984 on a grasshopper, Wood 1991 on several groups of flies, Briceño et al 2007 on tsetse flies). Still another problem is that in many groups with divergent male genital structures, displacement battles involving copulating males have never been observed; for instance, in some (probably many) spiders, male fights occur only when they are both out of contact with the female (Rovner 1968; Robinson & Robinson 1980; Eberhard & Briceño 1983; Mendez 2002).

Still another recent proposal is the “mate check” hypothesis of Jocqué (1998). As with Mayr's hypothesis, it supposes that pleiotropic effects on genital morphology are important. Key adaptations to environmental variables are thought to have pleiotropic effects on male genital morphology, and females are thought to use such genital traits as “guarantors” of male fitness. By responding preferentially to males with such morphological traits, the female would be able to increase the chances that her offspring would benefit from these adaptations. This idea suffers from the same serious problems mentioned above in connection with the Mayr's pleiotropism hypothesis, in particular, the unanswered question of why there should be a consistent association between fitness traits and the form of genitalia rather than other body parts (Eberhard 1985). It also fails to explain why “cheater” males lacking the key adaptations but possessing the preferred genital traits would not become common.

Finally, Jagadeeshan and Singh (2006) proposed a “male sex-drive” hypothesis in conjunction with their finding that in four closely related species of the *melanogaster* clade of *Drosophila*, in which male genital morphology is species-specific, a larger size of an evolutionarily derived male genital structure (the posterior process) may facilitate grasping the female oviscap during the first 5–10 min of copulation. A mechanical advantage of this sort may well sometimes be important in the early stages of the evolution of new genital traits; but it is not obvious why it would generally lead to great

genital diversity. More specifically, why would males of different species of *Drosophila* find that such different posterior process designs are best able to hold the essentially invariant portion of the female's anatomy (Eberhard & Ramirez 2004) that they grasp? More generally, many genital structures have no obvious mechanical grasping function.

WHY RELATIVELY RAPID DIVERGENCE? THE TWO MOST POPULAR HYPOTHESES

The two most popular hypotheses at the moment both invoke sexual selection: cryptic female choice, and sexually antagonistic coevolution. The basic arguments are the following.

Cryptic Female Choice (CFC)

Male genitalia are thought to be courtship devices. Sexual selection by female choice occurs after copulation has begun, with females favoring some male genital designs over others, via biases in post-copulatory processes such as sperm transport, oviposition, remating, etc. (Eberhard 1985, 1996). Male designs can be favored because they result in more effective stimulation of the female, or because they fit better with her genital morphology. The expected sequence of evolution can be outlined as follows.

- Females are inevitably stimulated by male genitalia during copulation in species with internal insemination (and also by non-genital male structures that contact them during sexual interactions). Natural selection on females favors female use of such stimuli to trigger certain reproductive processes, such as sperm transport, ovulation, oviposition, resistance to further copulation, secretion of products to help maintain sperm alive in storage sites, etc., that are otherwise kept inactivated until mating occurs. Triggering these same female processes is, incidentally, favorable to the reproduction of the current male.
- If, as is probably usually the case, females do not give 100% complete responses in all of these post-copulatory processes to every copulation (e.g., they do not ovulate or oviposit all available eggs, do not always dump all of the sperm of previous males, etc.), and if they are not strictly monogamous, then sexual

selection on males will favor the ability to increase the effectiveness of their stimulation of the female during copulation (including stimuli from their genitalia or non-genital contact devices) in eliciting more complete female responses.

- Selection on females will favor discrimination that allows them to bias paternity in favor of the males best able to deliver these stimuli, in order to obtain the benefit of sons whose genitalia and non-genital contact structures that are especially effective stimulators. This can result in a runaway process, which will tend to produce sustained, rapid divergent evolution of the corresponding male structures. Females could conceivably benefit from superior sons with respect to both good survivorship genes or good signaling genes, but theoretical expectations suggest a stronger correlation with signaling genes (Eberhard 1985, 1996). Direct empirical tests for a correlation between indicators of male "condition" with measures of genital size have been negative (Schulte-Hostedde & Alarie 2006; House & Simmons 2007). Because there are so many different ways a female may be stimulated, and because many types of stimuli are likely to have effects on triggering a variety of reproductive processes through the highly inter-connected nervous system of the female, divergence in male designs in different populations is likely.

Sexually Antagonistic Coevolution (SAC)

Male genitalia are thought to be devices to manipulate the female in ways that favor the male's reproduction but reduce the female's reproduction; females coevolve to counteract these negative male effects, resulting in an arms race between the sexes (Alexander et al. 1997; Holland & Rice 1998; Chapman et al. 2003; Arnqvist & Rowe 2005; Gilligan & Wenzel 2008). In this view, the sexual selection on males that results from female rejections is a side effect of natural selection on females (Rowe 1994; Arnqvist & Rowe 2005). The expected sequence of evolution can be outlined as follows.

- The male does something to the female with his genitalia or non-genital contact structures that increases his chances of paternity, but at the same time reduces the number of offspring produced by the female. For instance, the male might use spines or a rough surface on his

genitalia to scrape a hole in the lining of the female's reproductive tract, thus increasing the ability of his seminal products that induce the female to oviposit by giving them increased access to her body cavity and to her nervous system (figure 4.3). Selection on males could favor this mechanism of inducing rapid oviposition before the female mates with another male, even if it results in a decrease in overall female reproduction because of the physical damage to her reproductive tract, or because such rapid oviposition reduces the survival of her eggs because she was less selective in choosing oviposition sites.

- The female evolves defenses against the damaging effects of male genitalic manipulation. For instance, she might evolve a thicker lining

of her reproductive tract in the area that is abraded by the male, reducing the strength of his negative effects on her reproduction.

- The male evolves a way to overcome the new female defense. For instance, he might scrape at a different, unprotected site, or evolve longer or sharper scraping structures or stronger scraping movements at the old site. Sexual selection on the male will favor the development of such male traits, as long as the number of offspring he loses due to damage he inflicts on the female is less than the number of offspring he gains by manipulating her reproductive processes (such as oviposition). This coevolutionary arms race can result in relatively sustained rapid divergent evolution of male genitalia as long as neither sex evolves

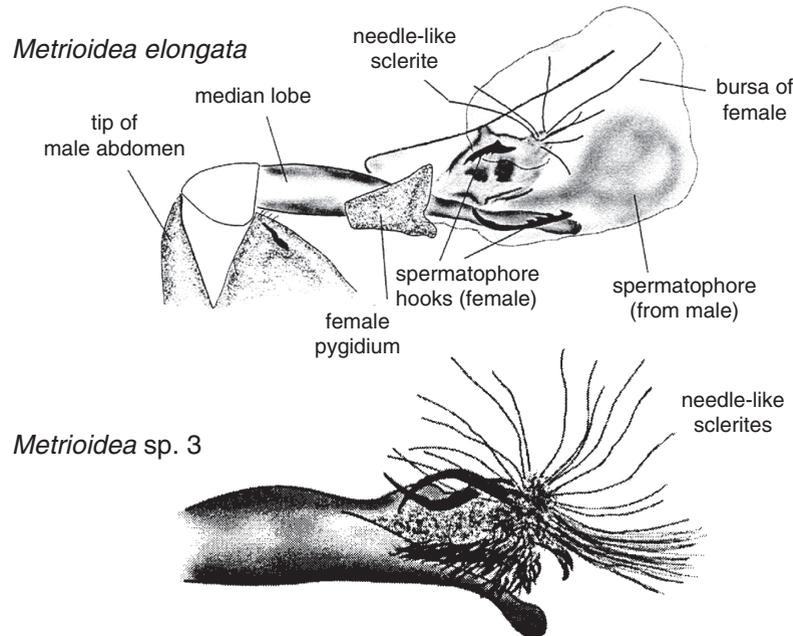


FIGURE 4.3 The needle-like sclerites on the male genitalia of *Metrioidea elongata* beetles flash-frozen in copula perforate the walls of the female bursa (above) (female abdomen dissected away). This damage to the female, perhaps a result of selection on the male to introduce seminal products into the female's body cavity where they will have more effect on her reproductive behavior or physiology, or perhaps to anchor himself more firmly or stimulate her more effectively, typifies the type of damaging male manipulation of the female that could give rise to SAC. The even longer sclerites in *M. sp. 3* (below) are also thought to perforate the female, because the bursae of field collected females had apparent scars resembling the scars in *M. elongata* (from Flowers & Eberhard 2006).

an unbeatable control mechanism. An outright “win” by one sex, however, would break the coevolutionary spiral, and remove selection on the other sex favoring antagonistic traits. Unassailable female defenses, such as a reproductive tract with a lining too strong for the male spines to perforate (figure 4.3), do not seem difficult to imagine. Thus coevolution might not be consistent and sustained over long periods of time.

SAC could involve different types of genital trait. A “physical coercion” version of SAC involves physical struggles between males and females (Alexander et al. 1997; Arnqvist & Rowe 2002a, b). For instance, males could evolve to seize females with genital claspers, females could then evolve defensive structures that impede such seizures, and males could respond by evolving modified claspers that overcome the female defenses. A second, “stimulation” version of SAC involves sensory traps: the male uses stimuli to which the female has already evolved under natural selection in other contexts both sensitivity and responsiveness (responses which, incidentally, favor the male) (Arnqvist 2006). Such traps are thought to be common in genital evolution, with males exploiting stimuli and the female responses to them that females evolved to control reproductive processes they need to trigger after copulation begins or has occurred (e.g., sperm transport, ovulation, oviposition, etc.) (Eberhard 1996). Sensory traps could be especially important during early stages of male–female evolutionary interactions. Under the stimulation version of SAC, male ability to induce a female response would reduce the female’s reproductive output, and thus select for changes in female sensitivity or responses to these stimuli. Female “escape” from these traps, by evolving changed sensitivities or responses, would be constrained by the original advantage of sensing and responding to these stimuli (Arnqvist 2006). The physical coercion version of SAC predicts common coevolution of male and female morphology; in contrast, the stimulation version of SAC does not predict that such easily observed coevolution should be common, because female coevolutionary adjustments could involve her sense organs and properties of her nervous system.

It should be noted that applying the stimulation version of the SAC hypothesis to genital evolution has complications that have not been previously noted.

The constraints on female responses to male manipulations that are posited by Arnqvist’s model (2006) are likely to be relaxed in genital evolution. This is because the female response that the male is attempting to alter (e.g., ovulation, oviposition, inhibition of remating, etc.) is the same response under which her sensitivity originally evolved. Or, from the female’s perspective, the message the female is under natural selection to obtain from the male’s stimuli remains the same: “copulation has occurred.” Thus only a small, presumably easy to evolve adjustment in the female’s sensitivity would be needed to counteract the disadvantageous effect (“overly emphatic” responses to the male’s signal) of a new male stimulus. Female adjustments to new male stimulatory adaptations could presumably be as simple as adding or subtracting a few synapses in her CNS, giving her the ability to retain the original function and also counteract the male-induced damage. This does not eliminate possible male–female SAC, but it implies that the durations of the periods when females are suffering costs from male sensory trap manipulations will tend to be brief. A similar consideration applies to at least some male manipulations by use of hormonal substances (signals) in his semen, unless they are also used in other contexts or have other side-effects in her body (Cordero & Eberhard 2005).

TESTING THE CFC AND SAC HYPOTHESES

The controversy between CFC and SAC explanations of genital evolution is part of a more general controversy currently swirling in discussions of sexual selection that concern many phenomena that were previously attributed to female choice (e.g., Pizarri & Snook 2003; Chapman et al. 2003; Kokko et al. 2003; Cordero & Eberhard 2003, 2005; Arnqvist 2004; Arnqvist & Rowe 2005). The major contrast between the two hypotheses revolves around the payoffs that a female obtains from resisting the sexual attentions of some of the males (Arnqvist & Rowe 2005). CFC presumes that she benefits from obtaining increased offspring quality. Such gains are thought to outweigh possible losses in direct reproduction (numbers of offspring) from male effects and the process of rejection itself; it can result in female behavior and morphology that is designed to give selective *cooperation* with males. SAC, in contrast, presumes that the female gains in

the number rather than quality of her offspring from resisting males, and that these gains outweigh potential losses from the process of rejection, and from her inability to screen males and thus increase the quality of her offspring (Arnqvist & Rowe 2005; Cordero & Eberhard 2005); SAC should result in female behavior and morphology that is appropriate for non-selective resistance to males, rather than selective cooperation. On the male side, trait exaggeration under CFC is impelled by female response criteria that evolve to increase offspring quality, while under SAC male trait exaggeration is impelled by the evolutionary responses of the female to these costs (Arnqvist & Rowe 2005). Resolution of the controversy for genitalia may point the way toward more general conclusions regarding sexual selection.

Discriminating between CFC and SAC explanations of genital evolution is difficult. The two hypotheses are not mutually exclusive (Cordero & Eberhard 2003, 2005; Hosken and Stockley 2004; Eberhard 2004b; Arnqvist & Rowe 2005). In addition, some predictions are the same for both, and direct measurements of some crucial variables involved in balancing potential costs and benefits is technically very difficult, if not impossible (Cordero & Eberhard 2003) (see final section of this chapter). The two types of selection can reinforce each other, or act against each other with respect to a given female trait, and they could act at the same time or in sequence on a particular trait (Cordero & Eberhard 2005; Eberhard 2004b; Arnqvist & Rowe 2005). For example, in the SAC example above, the original female payoff from evolving a defense against male genital scraping such as a thicker lining in her reproductive tract could be that it enabled her to avoid damage to her reproductive interests inflicted by his genitalia (a SAC type payoff); but she could also benefit, via superior sons, if the thicker lining also resulted in a bias that favored the males that were more potent manipulators (CFC-type payoffs) (Cordero & Eberhard 2005). Either type of payoff (or both) could be involved, for example, in the tendency for relatively high penile spinosity in male primates to be associated with relatively short durations of female receptivity within the ovarian cycle (Stockley 2002). Even this complex example of male damage to the female reproductive tract may be oversimplified compared with the real world; the females of a bruchid beetle that are damaged in this way also benefit, at least in terms of fecundity, from longer

copulations (which may result in more male damage) (Edvardsson & Canal 2006; Eady et al. 2007).

SUPPORT FOR CFC AND SAC

One prediction made by CFC and SAC is that the frequency of female remating in different groups should tend to correlate positively with the rate of genital divergence in that group (Eberhard 1985; Arnqvist 1998). If females consistently mate with only a single male (strict monandry), then CFC among males is not possible. Conflict between male and female that could lead to SAC may also be reduced or eliminated by female monandry, especially if the male is also monogamous (in which case conflict should not occur, and male and female genitalia should not coevolve). The SAC prediction is somewhat less sweeping, however. If females can benefit from polyandry but the polygamous males “impose” monandry via use of their genitalia, then conflict could occur even in a species in which nearly all females are strictly monandrous. In addition, conflict is possible even if monandry is not imposed by the male. For instance, if the male provides the female with some resource that is in short supply (e.g., a large nutritious ejaculate), a polygamous male might provide the monandrous female with less than she wants. Whether this sort of conflict could ever play out in genital morphology (e.g., the female attempts to induce greater male contribution) is not clear, but it has been inferred in non-genital female copulatory courtship in a fly (Ortiz 2002).

Thus CFC clearly predicts that groups with strictly monandrous females should have genitalia that are not species-specific in form, while the SAC expectation is also for a bias toward lack of species-specificity. Possible correlation between female monogamy and genital divergence has been tested in 22 different groups, including termites (males also monogamous) and *Heliconius* butterflies (Eberhard 1985; Arnqvist 1998), bees (Roig-Alsina 1993) primates (males polygynous in some, monogynous in others) (Dixson 1987, 1998; Verrell 1992), *Ischnura* dragonflies (Robinson & Novak 1997; Simmons 2001), mole rats (Paraq et al. 2006), and in 16 other groups of insects (Arnqvist 1998). The predictions of reduced genital divergence were fulfilled in all cases, despite several complications. The predictions concern *rates* of genital divergence,

while the data in most cases involved *amounts* of divergence, and in some groups the behavioral data were not complete (e.g., Arnqvist 1998). There were generally no controls for the amount of time since divergence, although Arnqvist's (1998) finding that genitalia but not other structures correlated with the frequency of female remating suggests this was not a problem in his study. Another possible problem is that the particular morphological aspects of genitalia that were studied were chosen at least in part because they were easier to study; there was no guarantee that they are the aspects that most strongly influence female responses (CFC) or do the most damage to her (SAC). These weaknesses make the consistent confirmations even more impressive.

It should also be noted, however, that data from one group, the bumblebee genus *Bombus*, contradict the predictions. The male genitalia are quite complex and strongly divergent among 18 species of *Bombus* (Richards 1927) (figure 4.1), but contrary to the prediction of CFC, females are thought to be strictly monandrous in at least seven of eight species of *Bombus* on the basis of both molecular and behavioral evidence (Schmid-Hempel & Schmid-Hempel 2000). Expectations of the physical coercion version of SAC are also contradicted in *Bombus* because some of the species-specific portions of the male's genitalia contact a relatively featureless portion on the external surface of the female's abdomen (figure 4.1) (Richards 1927; comment by O.W. Richards in Alexander 1964). There is thus no sign of the expected female defensive coevolution that could have selected for the divergence in the males.

Partial confirmation of CFC comes from the correlation between differences in male genital morphology and paternity when a female mates with more than a single male in six species: two in the water strider genus *Gerris* (Arnqvist & Danielsson 1999; Danielsson & Askenmo 1999); two distantly related scarab beetles, *Onthophagus taurus* and *Anomala orientalis* (House & Simmons 2003; Wenninger & Averill 2006) (figure 4.4); the damselfly *Calopteryx haemorrhoidalis* (Cordoba-Aguilar 1999, 2002, 2005); and the chrysomelid beetle *Chelymorpha alternans* (Rodriguez et al. 2004). In addition, experimental modifications in the sepsid fly *Archiseptis diversiformis* of both the morphology of a non-genital contact courtship organ on the male's front leg, and of the female's ability to sense this organ reduced the likelihood of

female acceptance of copulation (Eberhard 2002a), and experimental modifications of male genital structures and female receptors that they contact during copulation in the tsetse fly *Glossina pallidipes* affected female cryptic choice mechanisms such as ovulation, sperm transfer, and tendency to remate as predicted by CFC theory (Briceño & Eberhard 2009).

These cases support CFC, but possible SAC cannot be ruled out in five of the seven. Very little is known about how the male genital structures that correlate with paternity are used in *Gerris*, and a study of genital function in *O. taurus* failed to even consider the possible role of female stimulation by male structures that pinch her at several sites and are thrust up her rectum (Werner & Simmons 2008). In the oriental beetle, *A. orientalis*, the male sclerite that affected paternity hooks the female just inside her vagina, where it is likely to stimulate her and may also provide purchase for deeper thrusting by other, inflatable portions of his genitalia (Wenninger & Averill 2006). Possible damage to the female was not checked.

In *C. alternans*, the increased paternity associated with greater length of one male genital structure, the effects of experimental shortening this structure, morphological studies of how male genitalia engage the female during copulation, and the dramatic variation in the ducts of females of different species (Rodriguez 1994; Rodriguez et al. 2004), suggest that mechanical fit in the female's rigid, tortuous spermathecal duct, rather than stimulation, may be an important determinant of sperm precedence. Sperm is also deposited, however, outside the duct in the female's bursa, and its significance remains unclear. No male-inflicted damage to female reproduction (as predicted under SAC) is known, and the highly sclerotized spermatheca duct seems unlikely to be damaged by the male; nevertheless damage has never been searched for, and might occur deeper in the female (e.g., the spermathecal valve) (D. Windsor personal communication).

SAC is very unlikely, however, in the two other cases. In the fly *A. diversiformis* the male's clamp fits very precisely with the female's wing (Eberhard 2001a), but experimental modification of the form of the male's clamp did not impair his ability to hold on to the female with his front legs for extended periods, despite shaking behavior by the female (Eberhard 2002a), arguing against a SAC interpretation. Female stress receptors occur in the area

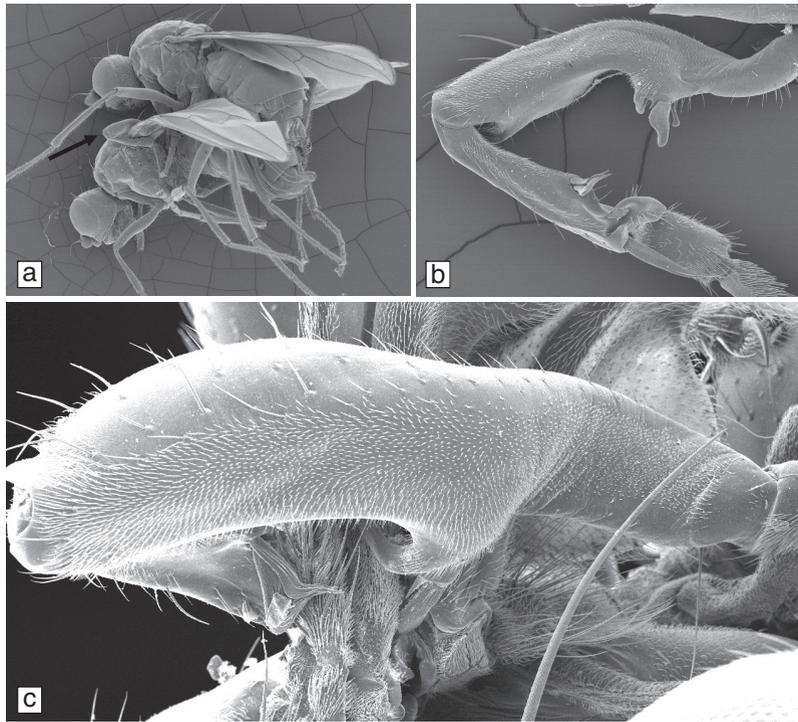


FIGURE 4.4 A detailed understanding of how the spectacularly elaborate, species-specific non-genital male foreleg clasper function permits confident rejection of a SAC explanation for male foreleg morphology in the appropriately named sepsid fly *Themira superba*. The males of this and other sepsid flies clamp the base of the female wing prior to copulation with their modified front legs (arrow in (a)). The form of the male foreleg is elaborate and species-specific (b), and the tibial and femoral modifications fit against the stem and costa veins in the base of the female's wing (c). Despite the striking diversity of male forms, female wing designs are quite uniform in this genus, and indeed throughout the entire family, and they show few signs of resistance structures that might explain the male diversity. Experimental alteration in one species of male foreleg morphology (or of female wing touch receptors) did not reduce the male's ability to hold on; instead, females rejected male copulation attempts (Eberhard 2002). (from Ingram et al., 2008; courtesy of R. Meier).

contacted by the male's front leg in this species (Eberhard 2001a) (as well as in other sepsid species with species-specific male front legs—Ingraham et al. 2008) (figure 4.4), and could thus enable her to sense his grip, supporting a CFC interpretation. The female's wing base is quite sturdy, and there were no signs of damage in *A. diversiformis* (a possible prediction of SAC) (damage inflicted by male claspers to female wings was claimed by Mühlhäuser and Blankenhorn (2002) in another sepsid with similar male grasping devices; but the wing damage that they observed was in other parts of the wing, and likely occurred when female flies beat their

wings against the walls of their small glass containers—see Baena and Eberhard 2007). In addition, in only one of the >10 sepsid species that have been checked (in *Archiseptis*, *Microsepsis*, *Palaeosepsis*, *Sepsis*, and *Themira*) is there any even potentially defensive modification of the female's wing in the area where the species-specific modifications of the male's front legs grasp her (Eberhard 2001a, 2005, unpublished; Ingram et al. 2008).

In the damselfly, the male manipulation of the female (he replaces the sperm of a previous male with his own in the female's reproductive tract) is not likely to inflict the types of naturally selected

costs (reduction in numbers of offspring) to the female that are specified by SAC theory (Chapman et al. 2003; Arnqvist & Rowe 2005). Some aspects of this case are still puzzling under both SAC and CFC, however. The male genital trait (penis width) shows no sign of the extravagant elaboration that is often associated with genital evolution. In addition, penis width varies both geographically and seasonally in this and in another species in which it also affects the male's ability to remove sperm (Cordoba-Aguilar 2009).

These studies have some possibly important limitations. Except for the chrysomelid, sepsid and damselfly studies, only correlations were demonstrated, rather than cause and effect relationships. The possibility thus remains that paternity was actually affected directly by other, correlated variables rather than by genital form itself. In the chrysomelid study no control was devised for the effects of the operation itself (it was not feasible to cut the male's genitalia and then glue them back together). On the other hand, the tests in all species were conservative in that they did not take into account

possible effects of male genitalia on many additional female reproductive processes, such as decreased remating, increased oviposition, etc.

One type of evidence that clearly supports CFC but is incompatible with physical coercion versions of SAC comes from a growing number of observations of genitalia used in ways that are appropriate to stimulate the female, but not to physically coerce her. Probably the genital behavior that is least controversial is stridulation, which has been observed directly in the tipulid fly *Bellardina* sp. (Eberhard & Gelhaus 2009) (figure 4.5), and inferred from male morphology in moths (Forbes 1941; Gwynne & Edwards 1986) and wasps (Richards 1978). Copulation in various mammals also involves genital behavior that is apparently designed to stimulate the female (summary Eberhard 1996; see also Dixson 1998), and some aspects of copulation behavior in rodents correlate with indicators of increased probability of competition with sperm from other males (Stockley & Preston 2004). In addition, the male genitalia of several insects and spiders perform long, highly rhythmic series of taps,

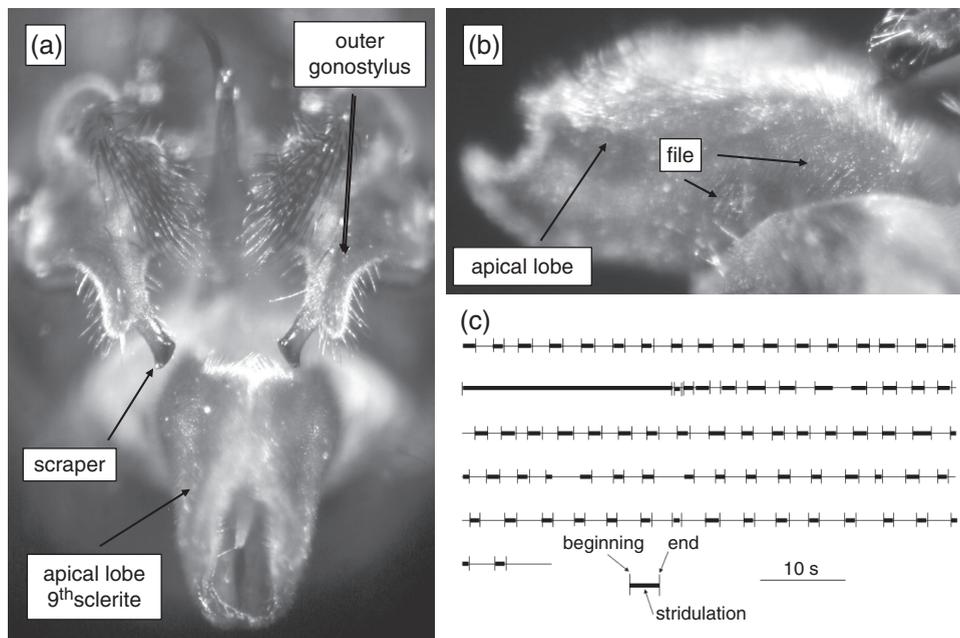


FIGURE 4.5 Male genital structures whose function to stimulate the female seems incontrovertible—the scraper (a) and file (b) of the male genitalia of the tipulid fly *Tipula* (*Bellardina*) sp. Direct behavioral observations show that the scraper is rubbed against the file to produce a highly rhythmic “song” (c) during copulation (from Eberhard & Gelhaus 2010).

or squeezes on membranous portions of the female, that also suggest that stimulation of the female is important; these include a dryomyzid fly (Otronen 1990), a buprestid beetle (Eberhard 1990), a sciarid fly (Eberhard 2001c), sepsid flies in several genera (Eberhard & Pereira 1996; Eberhard 2001b, 2003, 2005), a pholcid spider (Huber & Eberhard 1997; Peretti et al. 2006), some scathophagid flies (Hosken et al. 2005), several species of tsetse flies (Briceño et al. 2007; Briceño & Eberhard 2009), and the hesperiid butterfly *Urbanus dorantes* and the katydid *Idiathron* sp. (W. Eberhard unpublished). In those groups in which details of the genital behavior of more than one species are known (the spider, tsetse flies, and the sepsid flies *Microsepsis* and *Archisepsis*), the temporal patterning of squeezes differs among congeneric species, as is likely if this behavior is under sexual selection by CFC (A. Peretti, personal communication; Briceño & Eberhard in 2009; Eberhard 2001b; Eberhard & Pereira 1996). Alternative SAC interpretations based on physical coercion can be discarded in some of these cases. Morphological considerations rule out direct male effects on female internal genital structures with squeezing behavior in the sepsids and the crane fly. Possible external physical damage to the female resulting from male movements may occur in some tsetse flies and the pholcid spider, but not in the sepsids, tsetse flies, or the katydid (data are not sufficient to judge in the others).

The stimulation version of the SAC hypothesis cannot be ruled out, however, because it is possible that male stimulation of the female sometimes leads to reproductive losses to the female, especially when males are using sensory traps (Arnqvist 2006). Female counter-measures to male stimuli could occur in her sense organs or her nervous system, and thus be invisible externally. If such a coevolutionary struggle between males did not “spill over” into battles involving physical coercion, it could not be observable in studies of external morphology.

The strongest support for SAC in genitalia comes from water striders in the genus *Gerris*. Dorsally projecting spines near the female’s genitalia are elongated to different degrees in different species, and have independently become especially elongate in *Gerris incognitus* and *G. odontogaster*. Longer female spines impede male attempts to clamp the tip of the female’s abdomen with his genitalia (Arnqvist & Rowe 2002a,b; Rowe & Arnqvist 2002) (clamping the female’s abdomen helps the

male hold on during her energetic struggles to escape after he mounts, and is a necessary prelude to intromission. There is a cross-specific correlation between the relative development of several different male structures, including elongate grasping male genitalia, and the relative development of female defensive structures. An independent contrasts analysis based on a robust phylogeny showed that changes in male and female traits (both genitalic and non-genitalic) probably coevolved. Even in *Gerris* CFC cannot be ruled out, however. The possibility that male genitalia have additional, stimulatory effects on females has never been checked (e.g., by inactivating sense organs at the tip of her abdomen). In addition, the expectation that such a clear case of SAC would lead to morphological diversity in males and females is less clearly fulfilled. The morphological designs of both sexes of *Gerris* differ somewhat among species, but both male and female structures are relatively simple and practical. A morphologically similar abdominal spine that can fend off males also occurs in female *Aquarius paludum*, but its functional interpretation is not clear, because spines also occur in males (where they are proportionally longer), and female fertility is increased rather than decreased in captivity by additional matings (Ronkainen et al. 2005). Finally, it may be that male–female interactions in water striders are not typical of those in other groups, because their essentially 2-dimensional world may make male harassment of females unusually feasible (Eberhard 2006).

There are several other possible cases of less complete support for SAC. In *Lucilia* blowflies, complex, species-specific male genital asperities (Aubertin 1933) rub holes in apparently defensive thickenings in the lining of the female’s reproductive tract (Lewis & Pollock 1975; Merrett 1989). Species-specificity in female morphology and the question of whether female reproduction is actually reduced by copulatory damage both remain to be checked, however. In addition, the possibilities remain that stimulation (which seems likely) induces female responses favoring the male, and that females gain by producing superior sons as a result of the thickened lining, so CFC cannot be ruled out.

Summarizing, few species give evidence that compellingly discriminates between the CFC and SAC hypotheses for genital evolution. I think the clearest data favoring CFC over SAC come from the front leg grasping organs of sepsid flies, the

cercal claspers of tsetse flies, and from some species with male genitalia that are obviously designed to stimulate the female. The strongest support for SAC comes from *Gerris* water striders, but CFC has not been ruled out in these animals.

DISCRIMINATING BETWEEN SAC AND CFC

There are several other contexts in which SAC and CFC predictions differ. The massive data bank on genital evolution available in the taxonomic literature permits one to utilize huge sample sizes, and tests using these data are to my mind the most powerful evidence available regarding the likely generality of SAC and CFC explanations for genital evolution. Four different tests (all involving >100 species) have been made.

1. Comparing Groups in Which Males Can and Cannot Coerce Females to Mate

The most extensive test of SAC predictions regarding genitalia, in terms of the numbers of species included (up to several hundred thousand, depending on how one adds them up), is based on a prediction formulated by Alexander et al. (1997). They distinguished between coercive and non-coercive circumstances in which males attempt to obtain copulations. Grasshopper males were cited as mating coercively, because they often jump onto females which are engaged in other activities without any preliminaries, and attempt to grasp the female's genitalia with their own. Females often struggle forcefully to dislodge males and to prevent genital coupling. The cricket genus *Gryllus* was cited as not mating coercively, because males produce a calling song and the receptive female, with no overt coercion by the male, approaches the male and positions herself to allow him to couple with her. The female cannot be physically coerced, because she only encounters the male if she seeks him out. She is thus protected from unwanted male attentions. Alexander et al. reasoned that SAC in male and female genitalia would be more likely to occur in a group like grasshoppers in which male and female interests are more clearly in conflict — those in which females are less protected and in which male coercion occurs. Grasshoppers and *Gryllus* fit their prediction: male genitalia are often

species-specific in grasshoppers, while they are not divergent and not useful to distinguish species of *Gryllus* (Alexander et al. 1997).

A sample of two, of course, is not very convincing, and I undertook a larger survey (Eberhard 2004a), using information from the behavioral ecology and taxonomic literatures. Discriminating between SAC and CFC is possible, because CFC suggests that no trend should occur: female use of male genitalia to bias paternity could occur equally well in species with protected or unprotected females (unless unprotected females are more likely to be monandrous due to male manipulations, in which case the prediction would be the opposite—greater genital divergence in non-coercive mating systems).

First, publications on the behavior and ecology of insects and spiders were consulted to determine whether or not females of different groups were likely to be coerced into mating by males. Protection of females from coercion was assumed in species in which males attract females by chemical signals or singing, females attract males with attractant pheromones, females emit light signals at night in response to light signals from the males that allow the male to find them, males form leks or swarms that are not associated with resources needed by females such as oviposition or feeding sites, and in spiders in which males are dwarfs in comparison with females (and the female can thus easily kill a harassing male). In contrast, species in which females are not protected from harassment included those in which males station themselves near oviposition or feeding sites and attempt to mate with arriving females, and those in which males station themselves at sites where females are emerging from pupae and mate with them while they are still relatively defenseless. Second, for each genus in which behavioral evidence suggests that females are consistently either protected or unprotected, the taxonomic literature was then consulted to determine whether male genitalia are or are not useful in distinguishing closely related species.

The data clearly did not conform to the SAC prediction that the male genitalia in groups with unprotected females should diverge more rapidly, and thus that these groups would tend to more often have species-specific male genitalia (figure 4.6a). Analyzed in terms of genera, 75.4% of 223 genera with protected females have species-specific male genitalia, while 68.8% of 105 genera with unprotected females have species-specific male

genitalia (data from 113 families in 10 orders). The difference is not significant, $X^2 = 1.82$, d.f. = 1, $p = 0.17$, and is in any case in the opposite direction from that predicted by SAC. Several modified analyses that attempted to correct for possible biases in the data (over-use of genitalia by taxonomists due to custom, under-use of genitalia due to the difficulty of studying them, inadvertent bias in groups that were included in the study, and phylogenetic inertia) also failed to result in the predicted trend (figure 4.6a). More taxonomically restricted analyses of groups, such as the large fly family Chironomidae in which additional behavioral

details increase the confidence of the lack of probable male–female conflict, also failed to fit the SAC prediction.

The data in figure 4.6a strongly underestimate the strength of the evidence against SAC, because data from the large order Lepidoptera (which includes something like 250,000 species) were omitted because they are so uniform. Female lepidopterans are nearly all protected from pre-copulatory male coercion, because females throughout the order attract males with long distance attractant pheromones (Phelan 1997). And, contrary to SAC predictions, the genitalia are elaborate and species-

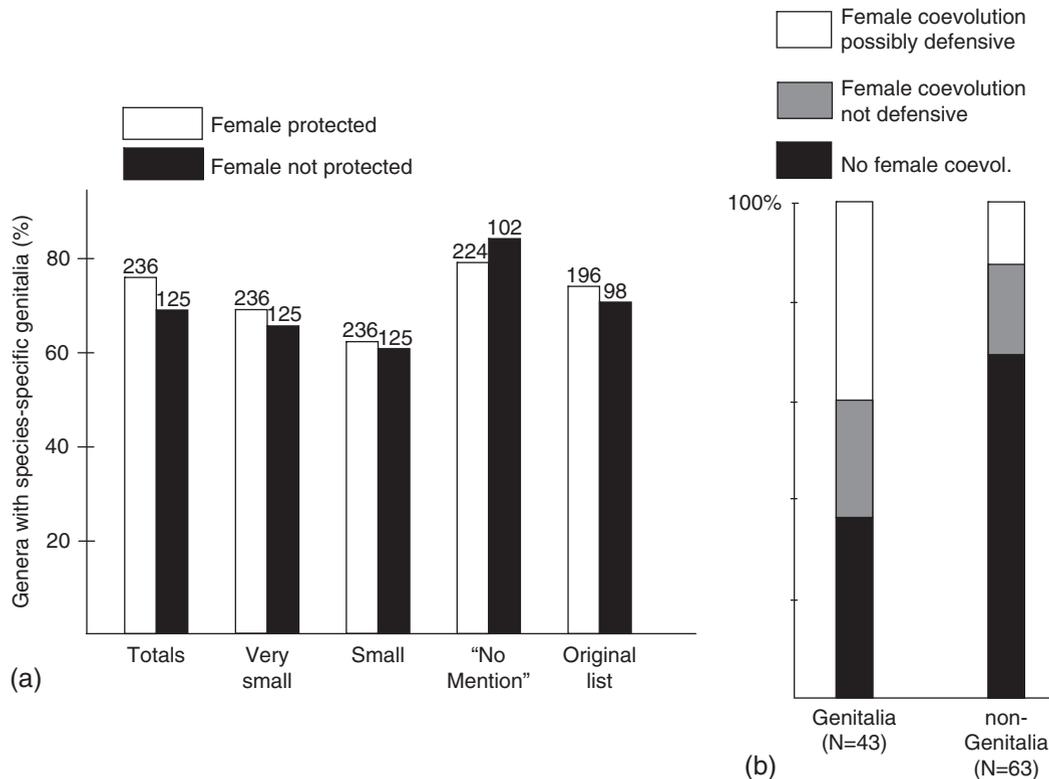


FIGURE 4.6 Summaries of two large survey studies that documented failures to confirm predictions of the SAC hypothesis. (a) Percentages of genera in which male genitalia are and are not species-specific in groups in which non-receptive females are and are not protected from sexual harassment by males. The totals (left pair of bars) include all groups examined; the other pairs of bars represent data that were modified in different ways to attempt to take into account different possible biases in the data against SAC predictions (see text) (numbers at tops of bars are area sample sizes). The SAC prediction that the dark bars would be higher was not confirmed. (b) Conservative estimates of fractions of the 84 taxonomic groups with species-specific male genitalia (left) and non-genital contact structures (right) that did (white) and did not (grey and black) conform to SAC predictions of species-specific defensive female coevolution (a) from Eberhard 2004a; (b) based on data from Eberhard 2004b).

specific in form throughout the order, as shown in taxonomic compendia that review thousands of species in the North American and Palaearctic fauna (Dominick et al., 1971–1998; Amsel et al. 1965–2000; Forster & Wohlfahrt 1952–1981; Huemer et al. 1996).

A possible problem with these results is that the SAC prediction of Alexander et al. (1997) may be overly simple. The reduction in male–female conflict in species with protected females may not be complete, even in species in which no male–female contact occurs unless the female is receptive. This is because once a pair has formed, the male could attempt to manipulate post-copulatory female behavior such as remating or oviposition, and thus reduce female reproduction. Even though a female was receptive to copulation, her reproductive interests might be damaged by such manipulations, and she might evolve to reduce this damage from the genitalia of manipulative males. To estimate how frequently different species-specific male genital structures function in these possibly conflictive ways, I made a separate literature survey of studies of the functional morphology of male genitalia in the order Diptera. The results indicated that the SAC prediction of Alexander et al. is likely to be a strong trend rather than absolute. Of 105 cases in which a function was attributed to species-specific male genital structure (in 43 species in 22 families), the majority (85.7%) were functions in which male–female conflict should be reduced or absent in species with protected females (39.0% apparently function to clasp the female, and 46.7% to facilitate penetration and sperm transfer) (Eberhard 2004a). The precise percentages are probably not especially meaningful, because of several probable biases (including the ease of documentation of these particular functions compared with others such as stimulation; and a bias in the set of possible functions considered by the authors). But the percentages clearly show that the SAC predictions should be met in an appreciable number of genital traits. Even if Diptera are somehow unrepresentative of other insects in this respect (there is no obvious reason to suspect this), the SAC prediction failed when Diptera were analyzed apart from others (Eberhard 2004a). The survey was thus a valid test of SAC predictions.

In sum, data from literally hundreds of thousands of species failed to show the trend predicted by SAC; if anything, the trend was in the opposite direction. The immense number of species in this

sample, made possible of course by the huge taxonomic literature on genitalia, is rare in evolutionary studies. A sample of this size should have been sufficient to reveal even a weak trend in the predicted direction, so the lack of this trend constitutes strong evidence against SAC as a general explanation.

2. Female Defensive Coevolution with Males

A second broad survey (Eberhard 2004b) examined a different set of predictions in 61 families, mostly of insects and spiders, in which the functional morphology of species-specific male structures has been studied. Species were only included if morphological studies have determined both the site on the female that is contacted by the species-specific portions of the male structure and the mechanical details of the fit between them. The sample included 43 male genital structures in 34 taxonomic groups, and 63 male non-genital contact structures in 53 taxonomic groups. SAC on the basis of physical coercion (Alexander et al. 1997; Arnqvist & Rowe 2002a, b) makes several clear predictions for these structures: the female morphology should often coevolve with the species-specific aspects of the male; the species-specific female structures of related species should interact mechanically with the species-specific portion of the male; and the designs of the species-specific aspects of the female structures should often be appropriate to defend her against the male, especially against the action of his species-specific structures. Female structures that can hold the male away or impede his access are predicted to be common. Finally, because females under SAC need to mate at least once but resist other males, an especially likely design would be species-specific female structures that can be used facultatively against males. Moveable structures such as erectable spines, inflatable sacs, or sliding barriers that could be moved out of the way to facilitate one (necessary) copulation, but interposed to reject others are expected.

CFC, in contrast, predicts that external female morphology will often (but not always) not vary when females are screening males on the basis of the stimuli they produce. Rather, females are expected to coevolve with respect to their sense organs (sometimes visible externally, as in some damselflies—Robertson & Paterson 1982; Battin 1993—but often not), and with respect to how their CNS processes information from these sense

organs (completely invisible externally) (see below). Females can also screen males on the basis of their morphological fit with the female, and in these cases male–female morphological coevolution is expected. In addition, the designs of females are expected to be often “selectively cooperative” (figure 4.7, below), rather than defensive as is expected with SAC.

The assembled groups were then checked for female traits. Once again, the SAC predictions clearly failed. Of 106 structures in 84 taxonomic groups, in more than half (53.8%) (figure 4.6b) female morphology was inter-specifically uniform while male morphology was species-specific (the respective percentages for genitalic and non-genitalic structures 34.9% of 43, and 68.3% of 63).

In addition, the designs of over half of those female structures that did coevolve with species-specific structures of males did not have the predicted defensive designs: among 49 coevolving female structures in 39 taxonomic groups, they were not even feasible as defensive devices in 55.1% of the structures (57.1% of 28 genitalic structures and 52.4% of 21 non-genitalic structures). The female designs seem to be selectively cooperative in many species (grooves and furrows used by a male with the appropriate design as sites to support or strengthen their grip on the female) (figure 4.7) rather than defensive. In total, females failed to confirm to SAC predictions in 79.2% of 106 structures (figure 4.6b). This finding that female morphology frequently fails to coevolve with that

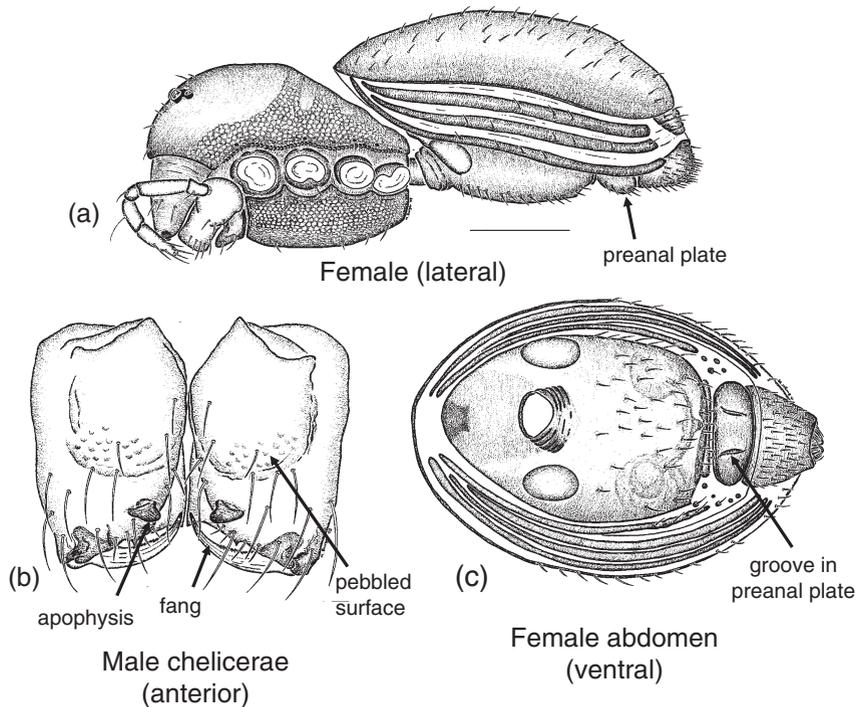


FIGURE 4.7 A recently discovered illustration in the tiny armored spider *Indicoblemma lannaianum* of the general trend for females to have selectively cooperative rather than defensive structures. Males of this genus are distinguished by bearing apophyses and other modifications on the anterior surfaces of their chelicerae (b) that are species-specific in form. Males use these projections to grasp the preanal plate of the female (a) during copulation. The female’s preanal plate has “selectively cooperative” grooves (c), which facilitate rather than impede the male’s grasp with his fangs. (scale line in (a) = 0.2 mm; drawings and behavioral observations after Burger 2005).

of males echoes the findings of previous surveys performed to test possible lock and key functions in the genitalia of other arthropod groups (Robson and Richards 1936; Kraus 1966; Eberhard 1985; Shapiro & Porter 1989; for further examples, see Djernaes et al. in preparation, and the discussion of lock-and-key above).

In addition, the female design that constituted arguably the strongest prediction by SAC, facultatively defensive structures, was completely absent (0% of 106). A search for defensive designs in an additional, large set of spider species (in which drawings of the female genitalia are routinely included in taxonomic descriptions) failed to reveal a single example of such a defensive device that could be facultatively deployed among the descriptions of thousands of species in general faunal studies and recent reviews (Eberhard 2004b) (see also Eberhard & Huber chapter in this book).

Data on these topics are more limited for other taxonomic groups. The recent discovery of coevolution between male and female genitalia in waterfowl (Brennan et al. 2007) fits SAC predictions better than most of the arthropod data. In some mammals female genital morphology has also coevolved with male penis morphology (Coe 1969; Patterson & Thaeler 1982). Nevertheless, lack of female coevolution with male morphology is common in some other groups with species-specific male genitalia. The bursae of male nematodes and the spermatophores of scorpions are often species-specific in form, but the areas of the female's body that they contact seem not to differ between species (Chitwood & Chitwood 1974; Peretti 2003). Antagonistic female coevolution of female genital morphology with male morphology is also apparently absent in primates, a group with numerous elaborate, species-specific male genitalia: "I have been unable to identify a single case among the primates where the mechanical conflict of interest hypothesis might be applicable" (Dixson 1998: p. 247). Clearly, the predicted defensive female coevolution with males is not a general rule.

It might be possible to rescue the physical coercion version of the SAC hypothesis from these apparently contradictory data in at least some species if it turned out that in the many species in which females that lack species-specific defensive morphology, the females instead use species-specific defensive behavior that selects for diversity in male contact structures (Eberhard 2004b). To my knowledge, however, not a single case of such female

behavior has ever been documented (though female behavior may seldom be studied with sufficient detail). Such a rescue is ruled out by the details of male–female interactions in several of the 84 taxonomic groups (Eberhard 2004b). In 21 genera, species-specific female resistance that could select for the species-specific designs of males is either mechanically impossible or female behavior has been observed with sufficient detail to rule it out (Eberhard 2004b). In nine other genera, it is the female that approaches the male and actively maintains contact with him, rather than vice versa; she is thus free to break away at any time, so female "resistance" behavior is simply not biologically realistic (Eberhard 2004b) (see figure 4.2). One further reason to doubt that as yet unstudied the female behavior will rescue SAC is that it is not clear why females should so often fail to use potential morphological counter-adaptations to males, and rely instead on behavior. Simple spines like those found in some *Gerris* females, for instance, would seem to offer relatively cheap, simple, and effective defenses to females. The stimulation version of the SAC hypothesis is less clearly contradicted, because it predicts only occasional rather than consistent coevolution of the female's morphology, and is thus compatible with the many cases in which such coevolution has not occurred (figure 4.6b).

3. Evolutionary Patterns When Males Inflict Damage on Females

I examined the CFC–SAC controversy over genital evolution from still another angle, that of groups in which current knowledge indicates that male genitalia are especially likely to inflict damage on females. I found 16 groups of insects in which male genital damage to females has evolved independently (Eberhard 2006, plus the recent discoveries of traumatic insemination in mirid bugs—Tatarnic et al. 2005, and *Drosophila* flies—Kamimura 2007). Damage included traumatic insemination (the male punctures the female's exoskeleton and introduces his sperm and seminal fluid into her body cavity), producing perforations of her exoskeleton or internal organs grasping her, or clasping with the genitalia or (in one case) other specialized male structures that increases her susceptibility to predation or decreases her ability to feed. I then consulted the taxonomic literature on these groups to determine whether the male traits that are used

to do the damage have undergone sustained divergent evolution, and whether females have evolved defensive morphology against these male traits, as expected under SAC (I made the usual assumption that males can impose at least some copulations on females; the predictions of SAC are weaker to the extent that such coercion is not possible). CFC, in contrast, predicts at least some selectively cooperative female designs in these groups.

The data gave one weak confirmation and two rejections of the SAC predictions. The prediction that male genitalia or grasping organs would evolve relatively rapidly and divergently in these groups was confirmed. Taxonomists of these groups have generally used the morphology of these damage-inflicting structures to distinguish congeneric species (there were two clear exceptions). If one counts (conservatively) any family which has at least a few genera in which genitalia are species-specific as being families that are typified by species-specificity, then 16 of 18 families show rapid divergent genital evolution. (Eberhard 2006). This fraction is higher than that of 71% of 328 genera in the general survey described above (Eberhard 2004a), although the difference is not statistically significant ($p = 0.12$ with X^2 Test).

Two other predictions, however, were not confirmed. With two clear exceptions (*Lucilia* and *Drosophila* flies), the male structures showed only modest complexity, and relatively small differences between congeneric species, compared with similar structures in groups in which male damage to females has not been documented. The trends to simplicity and small differences were especially clear in two relatively large groups with traumatic insemination, cimicoid bugs and Strepsiptera. The male genitalia of both these groups are secondarily reduced and highly simplified, and have entirely lost structures that were present ancestrally (Eberhard 2006). Male designs are typically utilitarian: for instance, cimicid bugs have simple, sword-like genitalia that are obviously well designed for penetrating the exoskeleton of females. Interestingly, this male evolutionary conservatism contrasts with the evolution of male structures known to function as weapons in male–male battles. Both species-specificity and diversity of design is typical of beetle horns, unguulate horns and antlers, and earwig cerci (Arrow 1951; Geist 1966; Otte & Stayman 1979; Enrodi 1985; Brindle 1976). This contrast is especially striking given the fact that both sets of male traits often function to solve

similar mechanical problems, such as grasping and stabbing another animal.

Finally, the SAC prediction that females would possess species-specific defensive structures at sites contacted by males, was clearly not fulfilled. In most groups (with four and possibly five exceptions—*Gerris* water striders, dytiscine water beetles, *Coridromius* plant bugs, *Drosophila* flies, and perhaps *Lucilia* blowflies) female morphology in the area contacted by the male's piercing genitalia or grasping structure was not species-specific (Eberhard 2006; Tataric et al. 2005; Kamimura 2007). Female morphology was also generally not defensive in design, in the sense that it lacked design features that could potentially prevent the undoubted physical damage inflicted by traumatic insemination.

Females of the six *Drosophila* species known to have wound-producing male genitalia have small “pockets” into which the penetrating portions of the male genitalia fit, but the clear photos of Kamimura (2007) show no sign of any thickening or sclerotization that would make penetration more difficult, and that would thus select for changes in the male genitalia as predicted under SAC. In fact, the body wall is “especially thin” at the bottom of the pockets in the species complex in which four species of males perform traumatic insemination using divergent genital structures (Y. Kamimura, personal communication.). In some cimicoid bugs and orthoptera, females instead have structures such as grooves or pits that guide the male and give him greater purchase on the female, and thus appear to be “selectively cooperative” instead of defensive as expected under SAC. Female “mimicry” of certain male designs in one cimicid strongly suggest SAC, however (Reinhardt et al. 2007).

In some cimicoid bugs, and perhaps also in strepsipterans, females have diverse internal structures at sites where males penetrate, suggesting that instead, females have evolved internal mechanisms to control sperm (as expected under CFC) or seminal products or invasive pathogens, rather than to avoid the physical damage and infections that result from copulation itself. Lack of external defenses is not predicted by SAC. The damage to the female comes from the act of insemination (physical injury to the female's tissues, and the increased risk of infection) (Stutt & Siva-Jothy 2001; Morrow & Arnqvist 2003), and to defend against physical damage, females would be expected to evolve defenses against penetration per se. Females could

evolve internal defenses against infection at the site of insemination, but such a defense might not set off a coevolved race between males and females, because males would gain nothing (and probably lose) from improving their ability to infect their mates with venereally transmitted pathogens. Coevolution with such internal female defenses could occur, however, if they also killed the male's sperm. This "classic" example of male–female conflict may have a cooperative aspect. Selection on males to cooperate with internal female defenses against infection could explain an otherwise puzzling behavior of males (Siva-Jothy 2006), which insert their hypodermic genitalia just at the site where the female's internal paragenital structures can digest his sperm (Carayon 1966). The possibility that internal female traits like paragenitalia also exercise cryptic female choice by manipulating the sperm and/or seminal products within her body has not to my knowledge been tested.

Limitations of this study include the fact that the taxonomic data may be biased by a trend for taxonomists to over-utilize genitalia to distinguish species (see above); this bias would favor confirmation of the SAC hypothesis. The sample size was substantially smaller than those in the first surveys (only 114 genera with perhaps 500–1000 species in total), and the traits of many species are undoubtedly not entirely independent among closely related species. Nevertheless, the classic trend for genitalia to diverge relatively rapidly suggests that phylogenetic inertia is not especially strong in genital traits. Finally, the lack of SAC-predicted female defensive morphology could be explained using the same argument regarding yet-to-be-discovered species-specific female defensive behavior.

4. Genital Allometry

If male genitalia are under selection to overcome physical resistance from females, one likely way for males to overcome female resistance is physical force (Lloyd 1979). This expectation, that at least some fraction of male genital structures function as physical weapons in battles with females, yields a strong prediction regarding the allometry of these structures: those male genital structures that are used as weapons should tend to be relatively large in larger individuals when conspecific males of different sizes are compared (they should show relatively high allometric slopes and "positive static allometry"). This prediction is derived from

the well established empirical observation that male structures which are used as weapons in battles with other males usually show positive allometry; the larger males usually have disproportionately large weapons compared with smaller conspecific males in deer antlers, crab claws, spider chelicerae, beetle horns, earwig forceps, and the armed legs of bugs and beetles (summaries in Huxley 1932 [1972] and Kodric-Brown et al. 2006; for exceptions see Bonduriansky 2007). This prediction is especially clear for male structures such as claspers that remain outside the female and are not constrained to act within possibly restrictive female ducts. Thus the expectation of SAC for species in which genital force is used to overcome females is that larger males of a given species should generally have disproportionately large genitalia.

This expectation of positive static allometry in male genitalia is clearly not met. In fact, there is a very strong trend in just the opposite direction, toward lower allometric slopes in the genitalia of insects and spiders: larger individuals almost always have disproportionately smaller genitalia. In 195 of 208 genital structures in 101 species, the allometric slopes was lower than the median allometric slope for other, non-sexually selected, non-genital traits of the same individuals (Eberhard 2009). Counting by species, the median slope for genitalia was lower than the median slope for non-genital structures in 96 of 101 species. "One size fits all" hypotheses that emphasize the importance of physical fits between male and female structures may explain this negative allometry (which also includes female genitalia) (Eberhard et al. 1998; Eberhard 2009). Perhaps some of the genital structures that were measured in these studies do not function to exercise force on the female or are constrained because they must perform in restricted spaces within the female's reproductive tract, and thus may not be expected to follow this SAC weapon prediction. Nevertheless, some structures such as the claspers of 13 species of scathophagid flies (Hosken et al. 2005), two species of sepsid flies (Eberhard et al. 1998; Eberhard 2001b), five species of moth (Ohno et al. 2003; Mutanen & Kaitala 2006; Mutanen et al. 2006) and the parameres of *Onthophagus* and *Macroductylus* beetles (Palestrini et al. 2000; Eberhard et al. 1998; Eberhard 1993a), perform potentially physically coercive grasping functions; but in 21 of the 22 species they nevertheless showed the typical tendency to negative allometry.

There may also be other possible functions of male genitalia in sperm competition that reduce the numbers of expected offspring for females, as proposed by SAC and that would also show low slopes. Schmitz et al. (2000) mentioned that sperm removal structures might be expected to need to fit precisely with the female; but given the emphasis in SAC theory on male effects on female losses in quantity rather than quality of offspring, SAC seems unlikely to act on a male's sperm removal abilities (see above).

CONCLUSIONS REGARDING CFC AND SAC

In a recent summary, Hosken and Stockley (2004) concluded that current evidence strongly favors sexual selection as the primary force driving rapid divergent evolution of genitalia, but that it is not clear whether SAC or CFC sexual selection mechanisms are responsible. I believe the current balance is more strongly tilted against SAC than they thought. In the first place, further evidence not in accord with expectations of the physical coercion versions of the SAC hypothesis has appeared subsequent to their paper, showing a general lack of female defensive coevolution in groups with species-specific male genitalia and non-genital contact devices, and only weak genital diversification in groups with likely intense male–female conflicts (Eberhard 2004b, 2006). Additional extensive data on genital scaling show a strong trend that is opposite to that predicted by the physical coercion version of SAC (Eberhard 2009). Stimulation versions of SAC are also contradicted, though less thoroughly. Females protected from males should also be less subject to damaging male stimulation, yet the especially large sample sizes (Eberhard 2004a) failed to show a trace of the trend predicted by SAC. In addition, one likely female defense against male use of sensory traps with their genitalia (though not the only one—other possibilities include modifications of the female's CNS) would be defensive morphology associated with their genitalia; but arguably the most likely morphological design (facultatively deployable defensive structures) was completely absent. In sum, there is strong evidence against the physical coercion version of SAC, and less conclusive evidence against the stimulation version for both genitalia and non-genital contact structures.

In the second place, Hosken and Stockley argued that the conclusions from the large-scale study of genital evolution in species with females that are and are not protected from male harassment (Eberhard 2004a; see above) were inconclusive, because male–female conflict over fertilization (rather than mating per se) could influence genital evolution even in species with protected females. This possibility is surely reasonable (as noted above, also Eberhard 2004a). But the combination of the immense sample sizes (hundreds of thousands of species, when one includes Lepidoptera in the 2004a study), and the lack of even a trace of the trend in the direction predicted by SAC, means that the SAC effect due to conflict over fertilization, if it exists, must be tiny. The flip side, that there is only relatively modest genitalic diversity in species in which male–female conflict is especially clear, also argues against the importance of SAC. This constitutes evidence against both stimulation and physical coercion versions of SAC. If SAC has acted, it has apparently been brief, weak, or inconsistent; most of the modern diversity of genitalia is apparently due to some other factor.

This is not to argue that SAC, even of the less favored physical coercion type, never occurs on genitalia. Even in cases in which SAC seems especially unlikely to have shaped current morphology, it may nevertheless have played an important role at certain moments in evolution. Take, for instance, the sepsid flies (figure 4.4), a group in which SAC seems especially unlikely to explain the present-day morphology of the modified male front legs that clamp the female's wing (above). Nevertheless, SAC may have played a role in the early stages of the evolution of the clamping structures of male sepsids. Energetic female shaking behavior to dislodge males is widespread in other related flies in which the male's legs are not modified (Crean & Gilburn 1998; Eberhard 2000), as well as in sepsids (Parker 1972; Ward 1983; Eberhard 2005; Ingram et al. 2008). Shaking may thus have originally occurred in sepsids due to male-inflicted losses to females when males began to ride them for long periods at oviposition sites; there is a likely female cost, because a riding male appears to make her less able to avoid predators (personal observation). Early modifications of the male's femur that allowed him to couple his leg more tightly to the female's wing may have represented an antagonistic coevolutionary male response to female shaking behavior, as in some other flies (Dodson 2000). Subsequently,

however, the further modifications of the legs of male sepsids that resulted in the great diversity of forms in modern species more likely evolved under sexual selection by female choice.

FRONTIERS

Speculating on where scientific research will go in the future is difficult. I can, however, point to some types of missing data that would help solve presently perceived problems, and also explain why I believe that one currently popular type of research is unlikely to be helpful.

1. Paradoxical Species

Further study of species that seem anomalous under presently popular hypotheses is likely to be especially rewarding. As noted above, the apparently strict monogamy of female bumblebees appears to falsify predictions of the CFC hypothesis, because male genitalia are elaborate and species-specific (figure 4.1). The implications of these data are not, however, entirely conclusive. The evidence for female monogamy is molecular (a single male sires all of a female's offspring), but a female might sometimes make genital contact with other males that are rejected before sperm transfer, or sperm might sometimes be discarded. Claims of strict female monandry based on behavioral data have had a poor track record in other animals (summary in Eberhard 1996). Further observations of events involving genitalia (e.g., attempted couplings that fail) (contact seems to usually lead, however, to copulation—P. Schmid-Hempel, personal communication), and of possible sperm dumping (despite apparent mating plugs—Sauter et al. 2001) might save the CFC hypothesis from these apparently contradicting data. I do not see how to save the physical coercion version of the SAC hypothesis from the lack of female coevolution in the area contacted by the elaborate male genital structures that remain on the outer surface of her body (Richards 1927) (stippled portions in figure 4.1). Or perhaps further understanding of *Bombus* will lead to a new, alternative theory?

Another paradoxical group is the carabid beetle genus *Platynus*, in which changes in female genital traits (development of a dorsal pouch of the bursa, and its subsequent sclerotization and narrowing) apparently preceded rather than occurring in step

or following the evolution of associated traits of the male genitalia (various modifications of the tip of his median lobe) (Liebherr 1992). Development of female structures adapted to male structures that have not yet evolved is paradoxical under any of the hypotheses, and this group merits further study.

2. Female Sense Organs

The CFC hypothesis predicts that in all groups lacking rigid species-specific female structures that might be filtering males on the basis of mechanical fit (see for instance the chapter by Eberhard & Huber on spiders in this book), females should have sense organs in the area that is contacted by species-specific portions of male genital structures. Sense organs are also possible, though not necessarily predicted, on rigid female structures that are contacted by species-specific male structures (e.g., the wing bases of sepsid flies—figure 4.4). SAC, on the other hand, is compatible with both the presence and absence of such sense organs regardless of the possible importance of mechanical fit. The CFC sense organ prediction has almost never been tested. Two techniques are available: morphological or histological studies to reveal sense organs; and experimental behavioral studies in which potential female receptors are covered or otherwise inactivated (e.g., Eberhard 2002; Briceño & Eberhard 2009), and then possible changes in female responses to the male are checked. The behavioral technique is especially useful for more difficult to find possible female receptors in membranous areas. Females may utilize generalized receptors that were already present in the area that is contacted by the male, or evolve special sensors that coevolve with the form of the male. Both distributions of sensors are compatible with CFC, because even if there are no receptors located at species-specific sites, differences in female preferences could result from differences in processing of stimuli deeper in their nervous systems.

The only animals I know with data on possible changes in the locations of female sense organs are the damselflies *Enallagma* (Robertson & Paterson 1982), *Coenagrion* (Battin 1993), and *Calopteryx haemorrhoidalis* (Córdoba-Aguilar 2005), sepsid flies in the genera *Archiseptis*, *Themira*, and *Sepsis* (Eberhard 2001a, 2005; Ingram et al. 2008), and four species of cockroaches (Djernaes et al. in preparation). In all cases, female sense organs exist as

predicted by CFC, but their placement patterns vary. Female *Enallagma* & *Coenagrion* damselflies have arrays of sense organs whose distribution varies between species in ways that reflect the sites contacted by the species-specific clasping organs of males (Robertson & Paterson 1982; Battin 1993). Female sensory coevolution with male genitalia has also occurred in *C. haemorrhoidalis*, but in the opposite direction. The male trait (increased aedeagus diameter) induces the female to expel larger numbers of sperm stored from previous matings (Córdoba-Aguilar 2005), and the female adjustment to the male has been to *reduce* the number of sensilla that are stimulated by the male's aedeagus (Córdoba-Aguilar 2005). This change could result from either CFC or SAC, as a female mechanism to discriminate in favor of males with an especially thick aedeagus (a CFC explanation), or as a female defense against male manipulation (a SAC explanation).

The wings of the female sepsids and some genital sclerites of the female roaches, in contrast, have stress sensors (campaniform sensilla) that are near but not exactly at the sites where the male's front legs (sepsids) and genitalia (roach) make contact, but they do not reflect the species-specific differences in male form. There are only slight differences between male and female sepsids in the distribution of the sense organs on their wings. Nevertheless, as mentioned above, experimental modifications of either the male's legs or of the female sense organs in one species resulted in sharp increases in female rejections of males (Eberhard 2002), demonstrating that the females can indeed sense the form of the male's front legs (or at least the gross differences in form involved in this experiment) even without a species-specific array of female sensors. Females of this species also appeared to reject mounts by heterospecific males especially vigorously, even though their clamping structures differ only subtly from those of conspecific males (Eberhard 2002). These observations show that the lack of species-specific female sense organs in other groups, such as the roaches, does not rule out the possibility of CFC (Djernaes et al. in preparation).

3. Experimental Manipulations of Male and Female Structures

As noted above, one weakness of most "demonstrations" of the CFC-type effects of male genitalia on female reproductive processes is that they have

documented correlations, rather than cause and effect relations. Direct experimentation, such as alterations of species-specific aspects of the male or blockage or alteration of corresponding female sensory traits, is needed to establish cause and effect. Lasers offer a promising technique for altering very small structures (M. Polak personal communication on *Drosophila*). Blocking female sense organs is especially important to control for the possibility in male alteration experiments that changes in female responses are due to changes in the male's behavior that result from changes in his morphology. Experimental ablations of species-specific male genital structures have suggested several possible functions, involving possible natural selection, female choice, and sperm competition (Rodríguez 1993; Rodríguez et al. 2004; Moreno-García & Cordero 2008; H. Brailovsky, personal communication., Takami 2003; Méndez 2002; Méndez & Eberhard in preparation., Nessler et al. 2007) (in none of these species were female sensory structures modified as controls). As with any experimental study, the conclusions that can be drawn are limited by the possible response variables that are measured. This can constitute an especially serious weakness for studies of genital function, because so many different female reproductive responses could be important (more than 20 female mechanisms are known for CFC—Eberhard 1996).

4. Direct (and Indirect) Observations of Genital Behavior

Some simple but nevertheless infrequently used techniques can give insights into how male genitalia are used. Simply observing a copulating pair under a dissecting microscope sometimes leads to surprising discoveries, such as genital stridulation by a crane fly (figure 4.5). In some insects removal of a male's head sometimes results in spontaneous behavior of the male genitalia which can reveal unsuspected functions (e.g., the pushing action of an inflatable sac that gradually inches the male genitalia through the long narrow vagina of the female medfly—Eberhard & Pereira 1995), and rapid, energetic "swimming" motions of inflatable spiny sacs in a tsetse fly (Briceño et al. in preparation). Combining direct observations with studies of musculature can also reveal probable movements of some structures that are hidden from direct view (Briceño et al. 2007). Techniques involving more sophisticated technology include real time phase

contrast X-ray imaging of a fly (Briceño et al. in preparation.), and magnetic resonance imaging of human copulation (Schultz et al. 1999) (observing flies rather than humans has the advantage that the subjects are less inhibited by being observed!).

5. Limited Usefulness of Experimental Measurements of Fitness

I do not share the optimism of some of the most outstanding workers on CFC and SAC (e.g., Moore et al. 2003; Pizzari & Snook 2003; Hosken and Stockley 2004; Rice & Chippendale 2001; Orteiza et al. 2005) that laboratory studies of the overall reproductive costs and benefits to females are likely to resolve questions concerning the relative importance of SAC and CFC in the evolution of genitalia (or other traits such as seminal products). Even though the most direct means of resolving the CFC–SAC controversy would be to measure these costs and benefits to females of mating, any direct comparison requires precision in the technically difficult measurements of the magnitudes of both types of fitness; measurements must be accurate enough to correctly determine the sign of the difference between the two values. Pizzari and Snook (2003, 2004) make a related point: it is necessary to utilize male and female fitness, rather than arbitrary phenotypic traits, if experimental approaches to testing SAC are to be useful.

Measuring fitness accurately is not child's play, to say the least. It is trite but true that the costs and benefits to the female must be measured under “ecologically realistic” rather than artificial conditions, if one wishes to make arguments concerning why some traits and not others occur in present-day organisms (Cordero & Eberhard 2003). Unfortunately, precise measurements of both direct and indirect payoffs in the field are extremely difficult to obtain; they are impossible in model species such as *Drosophila melanogaster* and *Tribolium castaneum* in which the natural habitat(s) are not even known. There is no guarantee that the balance of gains and losses under captive conditions is a reliable indicator of the balance under natural conditions. For instance, even such an “obvious” cost to females as reduced life span (Miller & Pitnick 2003) is not necessarily selectively important, if females in nature do not survive long enough to reap all of the benefits of an increased potential life span. For *Drosophila* flies, for example, one may

need to quantify the effects that a bewildering array of factors in nature, such as variations in limitations in oviposition substrate, nutrients in different types of food for larvae and adults, survival rates of adults, rates of parasitism of larvae and pupae at different population densities, microorganisms and secondary compounds present in different types of food that could influence larval and pupal survival, and densities of males and females that affect male–male competition and also female mating frequency. It is likely that there are interactions between some factors of this sort (Eady et al. 2007), making accurate analysis even more difficult.

Attempts to solve this “ecological realism” problem by using strains that have spent many generations in captivity (Orteiza et al. 2005) are problematic, because adaptations to captive environments are likely to be only partial. This is illustrated by a strain of *D. melanogaster* that has been used for sexual selection studies for several hundred generations in captivity (Orteiza et al. 2005). The rearing protocol for this strain has been to use eggs laid after the adult female was over two weeks old to raise the offspring for the subsequent generation of flies (Orteiza et al. 2005). This constitutes intense selection against oviposition early in the female's life. Nevertheless the females of this strain continue to lay many eggs during the first two weeks of their adult lives. If the females were truly adapted to this new selective environment, they would not lay eggs until reaching two weeks of age.

The point is that these (and thus other) female reproductive processes cannot be assumed to be finely adjusted to conditions in captivity, even in captive strains. Therefore measurements of direct and indirect female gains and losses from responding to male manipulations cannot be assumed to indicate the balance between gains and losses that occurred when these responses evolved. In summary, tests involving experimental evolution can be (and often are) very sophisticated technically, but nevertheless only relatively crude in their theoretical implications for the SAC–CFC controversy.

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