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Abstract: Many hypotheses have been proposed to explain why male intromittent genitalia consistently tend to diverge more rapidly than other body traits of the same individuals in a wide range of animal taxa. Currently the two most popular involve sexual selection: sexually antagonistic coevolution (SAC) and cryptic female choice (CFC). A review of the most extensive attempts to discriminate between these two hypotheses indicates that SAC is not likely to have played a major role in explaining this pattern of genital evolution. Promising lines for future, more direct tests of CFC include experimental modification of male genital form and female sensory abilities, analysis of possible male-female dialogues during copulation, and direct observations of genital behavior.

Evolution of genitalia – theories, evidence, and new directions

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Abstract. Many hypotheses have been proposed to explain why male intromittent genitalia consistently tend to diverge more rapidly than other body traits of the same individuals in a wide range of animal taxa.

Currently the two most popular involve sexual selection: sexually antagonistic coevolution (SAC) and cryptic female choice (CFC). A review of the most extensive attempts to discriminate between these two hypotheses indicates that SAC is not likely to have played a major role in explaining this pattern of genital evolution. Promising lines for future, more direct tests of CFC include experimental modification of male genital form and female sensory abilities, analysis of possible male-female dialogues during copulation, and direct observations of genital behavior.

Hypotheses concerning the evolutionary forces responsible for the evolution of animal genitalia have a long history of controversy. Genital evolution requires special explanations because genitalia are often species-specific, and their forms are often more divergent among closely related species than are the forms of other traits such as legs, antennae, and eyes. This relatively rapid divergence of genitalia is extremely widespread taxonomically (Eberhard 1985), and has made them especially useful in distinguishing closely related species. Taxonomists have compiled a huge accumulation of data on genital evolution that is unparalleled in scope; in many groups, male genital morphology is better known than any other trait (reviewed in Eberhard 1985). What could be responsible for this evolutionary pattern? The major objective of this paper (which is condensed from a more extensive treatment in Eberhard in press, and was presented as part of a symposium on genital evolution in the 20th International Congress of Zoology in Paris) is to briefly review data and ideas that can help answer this question for a general audience. A few possible new directions to test theory more directly are also discussed critically.

Non-genital contact devices

Before beginning, it is necessary to explain that I will discuss both primary and secondary genitalia, and also non-genital species-specific male structures that are specialized to make contact with females prior to or during copulation (“non-genital contact devices”). I lump these structures with genitalia because they all show the same evolutionary pattern of consistent, relatively rapid divergent evolution, and thus probably require a similar explanation (Eberhard 1985; Robson and Richards 1936). The “secondary” male genitalia (which are distant from the primary genitalia, such as the pedipalps of spiders, but which house sperm received from the male’s gonopore and then introduce them into the female), show the same pattern of relatively rapid divergent evolution as primary genitalia, and are also generally included in discussions of genital evolution. Other structures that are not physically close to the genitalia, and that are specialized to contact non-genital parts of the female in sexual contexts also show the same pattern (see list of 63 groups in Eberhard 2004b; also Darwin 1871; Robson and Richards 1936, Eberhard 1985). In fact, some entomologists have included as “genitalia” some non-genital structures (*sensu strictu*) that are not associated with the segment on which the genital opening occurs; these include cerci and sternites near the “true” genitalia in groups in which these structures contact females directly during copulation, and they also show a pattern of rapid divergent evolution along with elaborate forms that is typical of more strictly genital structures (e.g. Tuxen 1970; Wood 1991). 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 in crustaceans, and the head, mandibles, antennae, pronotum, cerci, and wings of insects. 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, the same function that is performed by a large fraction of the male genital structures that are species-specific in form (summaries in Eberhard 1985, 2004a; Scudder 1971). The line between “true” genital claspers and non-genital claspers is, in the end, arbitrary (Chapman 1969; Darwin 1871; Eberhard 1985; see also Ghislen *in press*; Leonard and Cordoba-Aguilar *in press*).

Inclusion of non-genital organs specialized to contact females is especially useful because they have two advantages over “true” genitalia: the details of their physical interactions with the female are often better understood; and the female structures that they contact are often more easily manipulated and

studied, because contact is made with the female's outer surface rather than her internal genitalia. I will use the phrase "non-genital contact structures" for species-specific male structures that are not near his genitalia and that are specialized to contact females in sexual contexts.

Hypotheses to explain genital evolution

Lock and key

The classic explanation of rapid divergent genital evolution is species isolation by lock and key: females evolve genital structures into which only the male genitalia of their own species can fit, and thus avoid losing their large investment in eggs due to fertilization by heterospecific sperm; males also benefit, though to a lesser extent due to their less costly gametes. Although this theory can explain why genitalia are often species-specific in form and may be correct in particular cases, it has gradually lost favor as a general explanation for several reasons, and is probably in the process of being discarded definitively (Eberhard 1985; Shapiro and Porter 1989). Arguably the most important evidence against lock and key is that in many groups the supposed female locks simply do not exist (Eberhard 1985; Scudder, 1971; Shapiro and Porter 1989). There also other serious problems, including an almost complete lack of the predicted pattern of character displacement in males in zones of sympatry of closely related species (McAlpine 1988; Ware and Opell 1989), and clear evidence of genital species-specificity in species (such as those of species endemic to caves and oceanic islands, and of parasites isolated from all close relatives in their different hosts) that have probably evolved in complete or nearly complete physical isolation from all close relatives, and should thus not have evolved species-specific genitalia (Eberhard 1985, 1996; Hedin 1997; Shapiro and Porter 1989). While lock and key is very probably not a general explanation for genital evolution, and the evidence that has been adduced in its favor in particular studies (Mikkola 2008) has other possible interpretations (Eberhard in press), the possibility that it applies in any particular cases cannot be discarded without careful study.

Sexually antagonistic coevolution and cryptic female choice

Recent developments in several fields facilitated forging new links between genital evolution and sexual selection by female choice (Birkhead 1996), and the more recent emphases on male-female conflicts

during copulation (Arnqvist and Rowe 2005; Parker 1979, 2005) have sparked new hypotheses. I will focus below on the two currently most popular sexual selection hypotheses to explain consistent rapid genital divergence: sexually antagonistic coevolution (“SAC”), and sexual selection by cryptic female choice (“CFC”). SAC proposes that male and female interests with respect to events associated with copulation, insemination, and fertilization are not identical, and that males and females are engaged in coevolutionary races for control over these processes (Alexander et al. 1997; Arnqvist and Rowe 2005; Chapman et al. 2003; Holland and Rice 1998). Male adaptations that increase male control are thought to entail damage to females, such as reduced longevity, reduced ability to produce optimum numbers of offspring at optimum times, etc. that are disadvantageous under natural selection on females. They are expected to lead to female adaptations to reduce such damage, and these adaptations in turn are expected to result in further selection on males for additional adaptations to control reproduction.

CFC also proposes that male and female interests are not identical, but supposes that there are important benefits to females from allowing some but not other males to fertilize their eggs, derived from the production of superior offspring. Male genitalia are thought to be courtship devices, often stimulating the female inside her reproductive tract. 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 1996). Male genital designs can be favored because they result in more effective stimulation of the female, or adjust mechanically more appropriately to her genital morphology. Sexual selection often leads to relatively rapid divergence in many other traits (e.g., structures and behavior involved in visual displays, songs) (Darwin 1871, West-Eberhard 1984) in evolutionary independent lines, because of the intense selective pressures, the multiple possible mechanisms by which males can improve their competitive abilities, and also because of the shifting settings in which competition is played out (e.g. female preferences, the abilities of other males).

The controversy between CFC and SAC explanations over genital evolution is part of a more general controversy swirling in the recent literature on sexual selection regarding phenomena formerly attributed to female choice (e.g. Arnqvist 2004; Arnqvist and Rowe 2005; Chapman et al. 2003; Cordero and Eberhard 2003, 2005; Kokko et al. 2003; Pizarri and Snook 2003, 2004). While there have been some misunderstandings (often due to the mistaken idea that conflict between the reproductive interests of males

and females implies that SAC rather than CFC is operating – Cordero & Eberhard 2003), there is a major contrast between the two hypotheses: under SAC the payoffs to a female from resisting the sexual attentions of some of the males that she encounters are to avoid reduction in the number of offspring she produces (direct reproduction); under CFC, her payoff is to obtain superior offspring (Chapman et al. 2003, Arnqvist and Rowe 2005 p.36). SAC presumes that resistance to males results in direct gains to the female in the number of her offspring; CFC supposes that the gains are from improved quality of her offspring. If genitalia are typical of other sexually selected traits, then resolution of the controversy for genitalia will point the way toward a more general resolution.

Discriminating between CFC and SAC explanations for genital evolution is difficult. The two hypotheses are not mutually exclusive. Theoretically they can act simultaneously and can either reinforce or counteract each other (Arnqvist and Rowe 2005; Cordero and Eberhard 2003, 2005; Eberhard 2004b; Hosken and Stockley 2004); they could also act one after the other on the same trait (Eberhard 2004b, below). Some predictions are the same for both, and direct measurements of some crucial variables involved in balancing potential costs and benefits is technically difficult (if not impossible in some cases) (Cordero and Eberhard 2003; see below).

Support for CFC and SAC

One clear prediction made by CFC is that the frequency of female remating in different groups should tend to correlate positively with the rate of genital divergence in that group (Arnqvist 1998; Eberhard 1985). In particular, if females consistently make genital contact with only a single male, then CFC among males on the basis of genital traits is not possible. SAC is less categorical, but also predicts a negative correlation with monandry (Arnqvist 1998). Conflict between male and female may 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). Selection favoring many possible male manipulations that might be disadvantageous to the female under natural selection (induce earlier oviposition, more massive ovulation, more resistance to subsequent mating, etc.) is eliminated in monandrous females. If females can benefit from polyandry but males “impose” monandry via use of their genitalia, then conflict could arise even in a species in which nearly all females are strictly monandrous.

Even if monandry is not imposed by the male, conflict could occur if the male provides the female with some resource that is in short supply (e.g. a large nutritious ejaculate), and if the male is polygamous and sometimes provides the monandrous female with less than she wants. Whether this sort of conflict could ever play out in genital morphology (e.g. the female attempting to induce greater male contributions) is not clear.

Thus CFC clearly predicts that groups with strictly monandrous females (or at least females that make genital contact with only a single male) should have male genitalia that are not species-specific in form, while the SAC expectation is for a mix of unspecified proportions, but probably mostly a 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 (Arnqvist 1998; Eberhard 1985), bees (Roig-Alsina 1993) primates (some males polygynous) (Dixson 1987, 1998; Verrell 1992), *Ischnura* dragonflies (Robinson and Novak 1997; Simmons 2001), mole rats (Parag et al. 2006), and in 16 other groups of insects (Arnqvist 1998). The prediction of reduced genital divergence was fulfilled in all cases, despite the fact that the prediction involves *rates* of genital divergence, while the data in most cases involved *amounts* of divergence, and in some groups the behavioral data were incomplete (e.g. Arnqvist 1998). There were generally no controls for the amount of time since divergence, although Arnqvist's finding (1998) that genitalia but not other structures correlated with the frequency of female remating suggests this was not a problem in his study. In general, this evidence favors both CFC and SAC.

Differences in male genital morphology have been found to correlate with paternity when a female mates with more than a single male in five insect species: two in the water strider genus *Gerris* (Arnqvist and Danielsson 1999; Danielsson and Askenmo 1999); two distantly related scarab beetles, *Onthophagus taurus* and *Anomala orientalis* (House and Simmons 2003; Wenninger and Averill 2006); and the chrysomelid beetle *Chelymorpha alternans* (Rodriguez et al. 2004). In addition, experimental modifications in the sepsid fly *Archiseopsis diversiformis* of both the morphology of a non-genital clamping structure 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). These cases support CFC, but some are also compatible with SAC.

SAC is unlikely in probably the best understood species, *A. diversiformis*. The male's clamp fits very precisely with the female's wing (Eberhard 2001a), but experimental modification of the male's clamp did not impair his ability to hold onto the female with his front legs (on the basis of the durations of riding times) (Eberhard 2002a), arguing against a SAC interpretation. Female receptors that sense stress in the cuticle occur in the area 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 - Ingram et al. in press), 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 (a possible prediction of SAC). Parenthetically, male damage to female wings by clasping them was claimed in another sepsid with similar male grasping devices (Mühlhäuser and Blankenhorn 2002), but the wing damage that was observed was in other parts of the wing, and likely occurred when 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 (in the genera *Archisepsis*, *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, unpub.; Ingram et al. in press).

None of the other species allow such clear discrimination between CFC and SAC. 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 deposited in this duct, but it is also deposited outside the duct in the female's bursa, and the significance of bursal sperm remains unclear. No male-inflicted damage to female reproduction (as predicted under SAC) is known, and the highly sclerotized spermathecal 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 pers. comm.). In the oriental beetle, *A. orientalis*, the male sclerite whose size correlates with paternity hooks the female just inside her vagina, where it probably stimulates her and may also provide purchase for deeper thrusting by other, inflatable portions of his genitalia (Wenninger and Averill 2006). Possible

damage to the female was not checked. Very little is known about how the male genital structures that correlate with paternity are used in *Gerris*. A failure to even consider stimulation of the female as a possible function in the study of genital function in *O. taurus* (Werner & Simmons 2008) makes functional interpretations difficult to evaluate in this species.

The data from four of these species have another possibly important limitation. Except for the chrysomelid and sepsid studies, only correlations, rather than cause and effect relationships were demonstrated. In addition, no control was devised in the chrysomelid study for the effects of the operation itself (it was not feasible to cut the male's genitalia and then glue them back together). It should be noted, however, that the tests in all six species were conservative, in that they did not take into account the possible effects of male genitalia on many other female reproductive processes, such as decreased remating or increased oviposition.

One type of evidence that supports CFC and some but not other 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 in which stimulation is least controversial is stridulation, which has been observed directly in the tipulid fly *Bellardina* sp. (Eberhard and Gelhaus in press), and inferred from male morphology in moths (Forbes 1941; Gwynne and Edwards 1986) and wasps (Richards 1978). Copulation in various mammals also involves behavior apparently designed to stimulate the female with the genitalia (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 and Preston 2004). In addition, the male genitalia of several insects and spiders perform long, highly rhythmic series of taps, 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), several sepsid flies in different genera (Eberhard and Pereira 1996; Eberhard 2001b, 2003, 2005), a pholcid spider (Huber and Eberhard 1997; Peretti et al. 2006), some scathophagid flies (Hosken et al. 2005), several species of tsetse flies (Briceño et al. 2007; Briceño and Eberhard in prep.), and the hesperiid butterfly *Urbanus dorantes* and the katydid *Idiathron* sp. (W. Eberhard unpub.). In those groups in which the genital behavior of more than one congeneric species is known (the spider, tsetse flies, and the sepsid flies *Microsepsis* and *Archisepsis*), the

temporal patterning of squeezes differs among species (Briceño and Eberhard in prep.; Eberhard 2001b; Eberhard and Pereira 1996; A. Peretti, pers. comm.), as would be expected to often be the case if this behavior is under sexual selection by CFC. Alternative SAC interpretations based on physical coercion can be ruled out in some of these cases. Direct male effects on internal female genital structures with squeezing behavior are not possible on morphological grounds in the sepsids and the crane fly. Possible external physical damage to the female resulting from male movements may occur in some but not all of the tsetse flies and the pholcid spider, but not in the sepsids or the katydid (data are not sufficient to judge in the others groups).

SAC cannot be ruled out, however, because it is possible that male stimulation of the female sometimes leads to losses of offspring for the female (Arnqvist 2006) when males use sensory traps. Such traps may have played a large role in the early stages of cryptic female choice on genitalia (Eberhard 1996). Female countermeasures could be in her sense organs and nervous system, and thus invisible externally. If such a coevolutionary struggle between males and females did not “spill over” into struggles involving physical coercion, it would not be visible in studies of external morphology such as those reviewed in this paper (Eberhard 2004a).

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 and Rowe 2002a, b; Rowe and Arnqvist 2002). Such clamping helps the male hold onto the female during her energetic struggles when 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 morphological traits (both genital and non-genital) probably coevolved. Another type of possible female counter-adaptation would be species-specific resistance behavior that is appropriate to free her from species-specific male grasping structures; but such specialized behavior has never been to my knowledge observed for any species.

Even in *Gerris* it is difficult to rule out CFC, however. Possible additional, stimulatory effects on females from male genitalia have never been checked (e.g., by covering or inactivating sense organs at the tip of her abdomen). In addition, the expectation that such a clear case of SAC might lead to morphological diversity in males and females is not clearly fulfilled. The morphological designs of both sexes of *Gerris* do 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 paludrum*, but spines also occur in males (where they are proportionally longer); female fertility in captivity is increased rather than decreased (as predicted by SAC) by additional matings in this species (Ronkainen et al. 2005).

Several other species provide possible 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 and Pollock 1975; Merrett 1989). Species-specificity in female defensive morphology, and the question of whether female reproduction is actually reduced by copulatory damage both remain to be checked, however. The possibility also remains that stimulation (which seems likely to occur) induces female responses favoring the male, so CFC can not be ruled out.

Summarizing, very few species give direct evidence that compellingly discriminates between the CFC and SAC hypotheses. I think the clearest data favoring CFC over SAC come from the front leg grasping organs of sepsid flies, and from some species with male genitalia that are designed to stimulate the female. The strongest support for SAC comes from *Gerris* water striders, but here no attempts have been made to rule out CFC.

Discriminating between SAC and CFC

SAC and CFC predictions vary in some contexts, and the massive data bank on genital evolution that is available in the taxonomic literature permits the use of huge sample sizes to test them. I will discuss what I consider the two most powerful sets of evidence available regarding the likely generality of SAC and CFC explanations for genital evolution (for a more complete discussion, see Eberhard in press). Both tests strongly favor CFC over SAC.

1. Groups in which males can and cannot coerce females to mate

This test is based on a SAC prediction made by Alexander et al. (1997), who 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 that are engaged in other activities, and without any preliminaries attempt to grasp the female's genitalia with their own. Females often struggle forcefully to dislodge males and to prevent genital coupling. The example of non-coercive mating was in the cricket genus *Gryllus*, in which males produce a calling song; 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 (i.e., she is protected from unwanted male attentions). Alexander et al. reasoned that SAC in male and female genitalia would be more likely to occur in grasshoppers because male and female interests were more clearly in conflict. The two groups they discussed fit their prediction: male genitalia are often species-specific in grasshoppers, but are simple and not divergent in species of *Gryllus* (Alexander et al. 1997).

Subsequently this sample was greatly enlarged by using publications on behavioral ecology and taxonomy to discriminate between SAC and CFC (Eberhard 2004a). Discrimination is possible, because CFC predicts 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 is the opposite – there should be greater genital divergence in non-coercive mating systems).

A list of groups (in most cases genera) in which females are more or less likely to be coerced into mating by males was compiled from publications on the behavioral ecology of insects and spiders. 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. For each genus in which behavioral evidence suggests that females are consistently either protected or unprotected, taxonomic studies were consulted to determine whether male genitalia are or are not useful in distinguishing closely related species.

The data clearly failed to conform to the SAC prediction (Fig. 1a). A total of 75.4% of 236 genera with protected females have species-specific male genitalia, while 68.8% of 125 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 the trend was in the opposite direction from that predicted by SAC. Re-analyses designed to correct for possible biases in the data (over-use of genitalia by taxonomists due to tradition, under-use of genitalia due to the difficulty of studying them, inadvertent biases with respect to which groups were included in the study, and phylogenetic inertia) all failed to result in the predicted trend. Analyses of more taxonomically restricted groups, such as the large fly family Chironomidae, in which additional behavioral details increase confidence in the lack of male-female conflict, also failed to fit the SAC prediction. These numbers, in fact, strongly underestimate the strength of the evidence against SAC, because data from the large order Lepidoptera (approximately 250,000 species) also contradict SAC predictions but were omitted from the totals. 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). Contrary to SAC predictions, the genitalia of most lepidopterans are nevertheless elaborate and species-specific in form, as shown in taxonomic compendia that review thousands of species in the North American and Palearctic fauna (Amsel et al. 1965-2000; Dominick et al. 1971-1998; Forster and Wohlfahrt 1952-1981; Huemer et al. 1996).

The SAC prediction of Alexander et al. (1997) of a negative correlation between species-specificity in genitalia and protection of females from male harassment needs to be tempered, however, because even though a female is receptive to copulation and thus to genital coupling, she might not be receptive to genital manipulations performed after copulation has begun. To estimate how frequently different species-specific male genital structures function in different 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 prediction of Alexander et al. is reasonable, though not absolute. Of 105 cases in which a function

was attributed to a species-specific male genital structure (in 43 species in 22 families), 85.7% were functions in which male-female conflict should be reduced or absent in species with protected females (39.0% of the attributed functions involved clasping the female, and 46.7% facilitating penetration and sperm transfer) (Eberhard 2004a). These precise percentages are not especially meaningful, because of probable biases in the ease of documentation of these particular functions compared with others (e.g., stimulation), and a bias in the possible functions that were considered by the authors. But the percentages show clearly that the SAC predictions should have been met in an appreciable number of genital traits. Unless Diptera are somehow unrepresentative of other insects in this respect (there is no obvious reason to suspect this; species-specific claspers are common, for instance, in Lepidoptera), these percentages indicate that the proposed survey was indeed a valid test of SAC predictions. The large sample in this study, made possible of course by the huge taxonomic literature on genitalia, is rare in comparative evolutionary studies. A sample of this size should have been sufficient to reveal even a weak trend in the predicted direction. Thus the lack of this trend constitutes strong evidence against SAC as a general explanation.

In sum, data from literally hundreds of thousands of species failed to show the negative relation between species-specificity in genital morphology and female protection from male harassment predicted by SAC, even when analyses were modified to attempt to take into account possible biases against SAC. If anything, the trend was in the opposite direction.

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 studies are available on the functional morphology of species-specific male structures. Species were included in which studies have determined 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 physical coercion version of the SAC hypothesis makes several clear predictions for these structures: the female morphology should generally 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, and in particular against the action of his

species-specific structures. Female structures that can hold the male away or impede his access should be common. Finally, because under SAC females need to mate at least once even though they resist other males, an especially likely design would be species-specific female structures that can facultatively defend against males. Moveable structures such as erectable spines, inflatable sacs, or sliding barriers that could be moved out of the way to facilitate the copulation or copulations needed to obtain sperm, 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 and perhaps even more likely with respect to how their CNS processes the information from these sense organs. Females can also screen males on the basis of their morphological fit with the female, however, so some cases of male-female morphological coevolution are expected. The designs of females are expected to often be “selectively cooperative” (below), rather than defensive, as expected with the physical coercion version of SAC.

I assembled a collection that included 43 species-specific male genital structures in 34 taxonomic groups and 63 species-specific male non-genital contact structures in 53 taxonomic groups, mostly insects and spiders. The assembled groups were then checked for female traits. Once again, the SAC predictions clearly failed. Of 106 structures in 84 taxonomic groups (Fig. 1b), in more than half (53.8%) female morphology was inter-specifically uniform even though the male morphology was species-specific (the respective percentages for genital and non-genital structures were 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, 55.1% were not even feasible as defensive devices (57.1% of 28 genital structures and 52.4% of 21 non-genital structures). The female designs seemed 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) rather than defensive. In total, females failed to confirm to these SAC predictions in 79.2% of 106 structures.

In addition, the female design that constituted arguably the strongest prediction by the physical coercion of the SAC hypothesis, facultatively defensive structures, was completely absent (0% of 106). An

extended 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 facultatively deployable defensive device among thousands of species included in general faunal studies and recent reviews.

It would be possible to rescue the physical coercion version of the SAC hypothesis from these apparently contradictory data if it turned out that in the many species in which females that lack species-specific defensive morphology, females use species-specific defensive behavior instead of morphology, and that this behavior 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 it must be admitted that perhaps female behavior is seldom studied with sufficient detail). In addition, the details of male-female interactions in 21 genera of the 84 taxonomic groups allow confident rejection of the female defensive behavior possibility; species-specific female resistance that could select for the variant designs seen in different species 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 the female has no need for special "resistance" behavior that could have favored the male's species-specific morphology (Eberhard 2004b). In sum, female morphology in an entirely independent, large sample of taxa also fits CFC predictions much better than predictions of the physical coercion version of the SAC hypothesis. The stimulation version of SAC does not suppose male-female morphological coevolution, so is not affected by these observations.

Conclusions regarding CFC and SAC

The current balance of evidence is tilted against SAC as a general explanation (for a more complete discussion, see Eberhard, in press). This is not to argue that SAC on genitalia and non-genital contact structures never occurs. Even in cases in which SAC seems especially unlikely to have shaped current morphology, it may nevertheless have occurred at certain moments in evolution. Take, for instance, the sepsid flies, a relatively well-studied 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. The area near the

base of the female wing that is contacted by the elaborate, species-specific clamping devices on the males' front legs is relatively uniform in different species and genera, and seldom has potentially defensive traits, so female morphological coevolution with males has generally not occurred (Eberhard 2001a; Ingram et al. in press). In addition, experimental data support the female choice rather than the SAC hypothesis (Eberhard 2002a). Nevertheless, it is quite possible that SAC played a role at early stages in the evolution of the clamping structures of male sepsids. Energetic female shaking behavior that could dislodge males is widespread in sepsids (Eberhard 2005; Ingram et al. in press; Parker 1972; Ward 1983), and also in other related flies in which the male's front legs are not modified (Crean and Gilburn 1998; Eberhard 2000). Shaking may have originally evolved in sepsids due to male-inflicted losses to females when males began to ride them for long periods at oviposition sites; a female with a riding male is probably less able to avoid predators. 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; similar modifications have also occurred in the males of some other related flies (Dodson 2000). Subsequently, however, it is likely that further modifications of the sepsid male legs that resulted in the great diversity of forms in modern species evolved under sexual selection by female choice (Eberhard 2004b).

New frontiers

Speculating on where scientific research will go in the future is difficult. There are, however, some types of missing data that would clearly help solve present problems in understanding.

1. Paradoxical species

Further study of species that seem anomalous under the two presently popular hypotheses is likely to be especially rewarding. The bumblebee genus *Bombus* appears to falsify predictions of both CFC and SAC hypotheses. The complete lack of modification of the portion of the female's abdomen that is contacted by the diverse, species-specific stipes, volsella and squama of the male genitalia (Richards 1927) clearly contradicts the prediction of the physical coercion version of SAC that female morphology will coevolve with that of males. Female *Bombus* are also thought to be strictly monandrous but male genitalia

are nevertheless complex and strongly species-specific in form (see Eberhard in press for details), contradicting CFC predictions and failing to follow the less strict trend predicted by SAC.

These conclusions are not completely convincing, however. The molecular evidence that a single male sires all of a female's offspring does not necessarily demonstrate the strict female monandry; the female might have genitalic contact with other males, some copulations might occur without insemination, or some copulations may involve complete dumping of sperm from previous males. Perhaps further observations will reveal attempted couplings in which the male stipes, volsella and squama contact other portions of the female that fail after contact and thus give evidence of female rejections on this basis (contact seems to usually lead, however, to copulation – P. Schmid-Hempel, pers. comm.). The displacement of sperm from previous males (despite apparent mating plugs – Sauter et al. 2001) might obliterate traces of polyandry, and save the CFC hypothesis from these apparently contradicting data. Alternatively, further understanding of *Bombus* may lead to a new 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 its subsequent sclerotization and narrowing) have not occurred in step with the evolution of associated traits of the male genitalia (various modifications of the tip of the median lobe) that fit into the pouch; instead, changes in females have preceded those in males (Liebherr 1992). Perhaps the female pouch has other functions; evolution of female structures adapted only to male structures that have not yet evolved is paradoxical under any of the hypotheses. This group merits further study.

2. Genital dialogues between males and females

The emphasis in studies of copulation behavior has generally been on the behavior of males. For instance, my own study of copulatory courtship in insects and spiders (Eberhard 1994) concentrated on the possibility that males perform courtship during copulation, and neglected the possibility that females also perform communicatory behavior during copulation that can influence paternity. Nevertheless, female behavior during copulation that could be communicatory in function is apparently common. A conservative count indicates that it occurred in at least 12.2% of 131 species of insects and spiders in that study (see

Peretti et al. 2006). It seems likely that many of the female signals during copulation are functionally related to the behavior of male's genitalia, and that they may represent a window on understanding what the male genitalia are doing. A recent study of stridulation by a female spider during copulation showed that understanding both the male's genital behavior and his paternity success was improved by taking into account this female communicative behavior (Peretti et al. 2006).

3. Direct observations of the behavior of genitalia

Direct observations of the behavior of male genitalia are often difficult, because they are generally hidden within the female during copulation. Nevertheless, the dream of directly observing their behavior is now in reach. The removal of the male's head or anesthesia (sometimes combined with mild stimulation of the male genitalia) releases genital behavior in some species (Roeder 1967 on mantids; West-Eberhard 1984 on a wasp; Eberhard and Pereira 1995 and Briceño, Chinea-Cano, Wegrzynek, and Eberhard in prep. on flies). In addition, it is possible to observe male genital behavior within the female in some species such, as the tsetse fly *G. pallidipes* (Briceño, Chinea-Cano, Wegrzynek, and Eberhard in prep.), using real time phase contrast synchrotron X ray videos; some of the same coordinated and surprisingly energetic movements seen in headless males occur inside the female during copulation with intact males.

4. Female sense organs

The CFC hypothesis predicts that in all groups, other than those lacking rigid species-specific female structures that might be filtering males on the basis of mechanical fit (e.g. most spiders - see Eberhard and Huber in press), females should have sense organs in the area contacted by species-specific portions of male genital structures. Female sense organs are also possible, though not necessarily predicted, on rigid structures that are contacted by species-specific male structures (e.g. the wing bases of sepsid flies). This prediction thus constitutes a strong test of CFC for some types of female structures, but female sense organs have almost never been studied (see, however Battin 1993 and Robertson and Paterson 1982 on the thorax of damselflies; Córdoba-Aguilar 2005 on the oviduct of a damselfly; Eberhard 2001a, 2005 and Ingram et al. in press on the wings of sepsid flies; and Djernaes et al. in prep. on genital sclerites in four species of cockroaches). It is also not clear whether females utilize generalized receptors with other

functions that were already present in the area that is contacted by the male, or whether they tend to evolve special sensors whose placements or other characteristics coevolve with the form of the male. Both distributions are compatible with CFC, because even without specially-positioned receptors in each species, female preferences could result from differences in processing deeper in the CNS. Receptors that have other functions in addition to sensing males would seem less likely to show special distributions that reflect the shapes of the male organs that stimulate them. The presence or absence of sensors is not a test for the stimulation version of the SAC hypothesis, which is compatible with both (Arnqvist 2006).

5. Experimental manipulations of male and female structures

A general weakness of most supposed demonstrations of CFC-type effects of male genitalia on female reproductive processes is that they have only documented correlations, rather than direct cause and effect relations. Experimentation, in particular by alteration of the species-specific aspects of the male and the corresponding traits of the female, is needed to establish cause and effect (e.g., Rodriguez et al. 2004). Such experiments are feasible in groups with male genitalia that are large and tough enough to resist experimental modifications (e.g. removal of at least portions of particular, non-inflatable structures) without disabling the male; the use of lasers to modify small structures is particularly promising (M. Polak, pers. comm. on *Drosophila*). A good example of the possible payoffs from this type of experiment comes from a recent study of the tsetse fly *Glossina pallidipes*. Males have sexually modified, species specific cerci and other genital structures that clasp the female's abdomen during copulation (Briceño et al. 2007). Alteration of the species-specific form of the male cerci and of his abdominal sternites elicited changes in post-copulatory female reproductive responses, suggesting that the male structures are under sexual selection by cryptic female choice: ovulation and the amount of sperm arriving in the spermathecae were reduced, and female tendency to remate was increased (Briceño and Eberhard in prep.). In addition, inactivation or modification of possible sense organs at the sites on the portions of the female's external surface where these male grasping structures contact her produced similar female responses. Together with data from physiological studies showing that physical stimulation of the female during copulation rather than male seminal products or products released from spermathecae that contain sperm elicit these female responses (Gillott and Langley 1981; Saunders and Dodd 1972), these experiments give strong evidence

that CFC explains the evolution of these species-specific male structures. The sites on the female show no sign of coevolution with the male; they lack defensive species-specific adaptations that could impede male attempts to grasp her, and species-specific resistance behavior to these structures does not occur, arguing against SAC.

Non-genital contact devices of males are particularly attractive objects for further experimental studies of this type to test CFC predictions: their forms are relatively easily modified; their mechanical functions are frequently easily understood (usually clasping the female); and the sites on the female that they contact are also easily modified.

6. Limited usefulness of experimental measurements of fitness

Experimental alteration of selective regimes on genitalia and female monogamy/polyandry in captive populations has illuminated aspects of genital evolution and male-female conflicts (e.g., Miller and Pitnick 2003, Arnqvist and Rowe 2005). Experimental manipulation of the intensity of male-female conflicts (e.g. by imposing strict monogamy on males and females) is also a powerful tool (Arnqvist and Rowe 2005). Nevertheless, I do not share the optimism of some of the leading workers on CFC and SAC (e.g. Hosken and Stockley 2004; Holman and Snook 2006; Moore et al. 2003; Orteiza et al. 2005; Pizzari and Snook 2003; Rice and Chippendale 2001) that another use of studies of captive populations, to study the overall reproductive costs and benefits to females in the laboratory, is likely to resolve the relative importance of SAC and CFC in the evolution of genitalia (or other traits). I say this despite the fact that the crucial difference between CFC and SAC models hinge on the balance between a female's overall gains from traits that result in rejecting some males in terms of direct fitness. Direct gains (fewer offspring lost when she avoids male manipulations) are expected to be larger if SAC is operating (Arnqvist and Rowe 2005; Chapman et al. 2003), while indirect gains (improved offspring quality) are expected to be larger if CFC is operating (Eberhard 1996). The most direct means of resolving the CFC-SAC controversy would seem to be to measure these costs and benefits. But any direct comparison requires accurate measurements of the magnitudes of both types of fitness to determine the sign of the difference between them. It is not enough (as is often true in many evolutionary studies) to simply determine the sign of the effect - whether it is positive or negative. 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. A large part of the difficulty stems from the trite but true idea that the costs and benefits to the female must be measured under "ecologically realistic" conditions (Cordero and Eberhard 2003). The costs and benefits must be similar in sign and magnitude to those under which the animals evolved, if one wishes to make arguments concerning why some traits and not others occur in present-day organisms. Unfortunately, precise measurements of both direct and indirect payoffs in the field are extremely difficult to obtain; they will be impossible in model species such as *Drosophila melanogaster* and *Tribolium castaneum*, in which the natural habitat is 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. Attempts to solve the "ecological realism" problem by using organisms that have spent many generations in captivity (Orteiza et al. 2005) are also problematic, because adaptations to the new captive environments are likely to be only partial (for a specific case in *Drosophila*, see Eberhard in press). Female reproductive processes cannot be assumed to be finely adjusted to conditions in captivity.

In sum, partial evolutionary responses in captivity will not be enough to justify confident conclusions regarding the ambitious undertaking of obtaining measurements of the balance between absolute values of direct and indirect effects on female fitness that can be used to evaluate the relative importance of the SAC and CFC evolutionary theories. Tests in captivity can be (and often are) very sophisticated technically; nevertheless they are only relatively crude in their theoretical implications for the SAC-CFC controversy.

Conclusion

Genitalia are surely the best known traits in the animal kingdom with respect to how species-level divergence evolves. With what other traits could one imagine the possibility of further, yet-to-be-done tests of SAC and CFC ideas using data that have already been collected, with a taxonomic sweep ranging from leeches, molluscs and mites to primates, snakes and bats to nematodes? The profound coverage down to the level of species for literally hundreds of thousands of species is unparalleled in any other trait. Genitalia

may be especially useful in understanding sexual selection action on “arbitrary” male signals to females (Eberhard 1993). Many related questions remain unanswered, and answers may have important implications for understanding genital evolution and sexual selection in general. Happily this potential is beginning to be realized. Much work is yet to be done to harvest the abundant payoffs promised by the pioneering discoveries of taxonomists.

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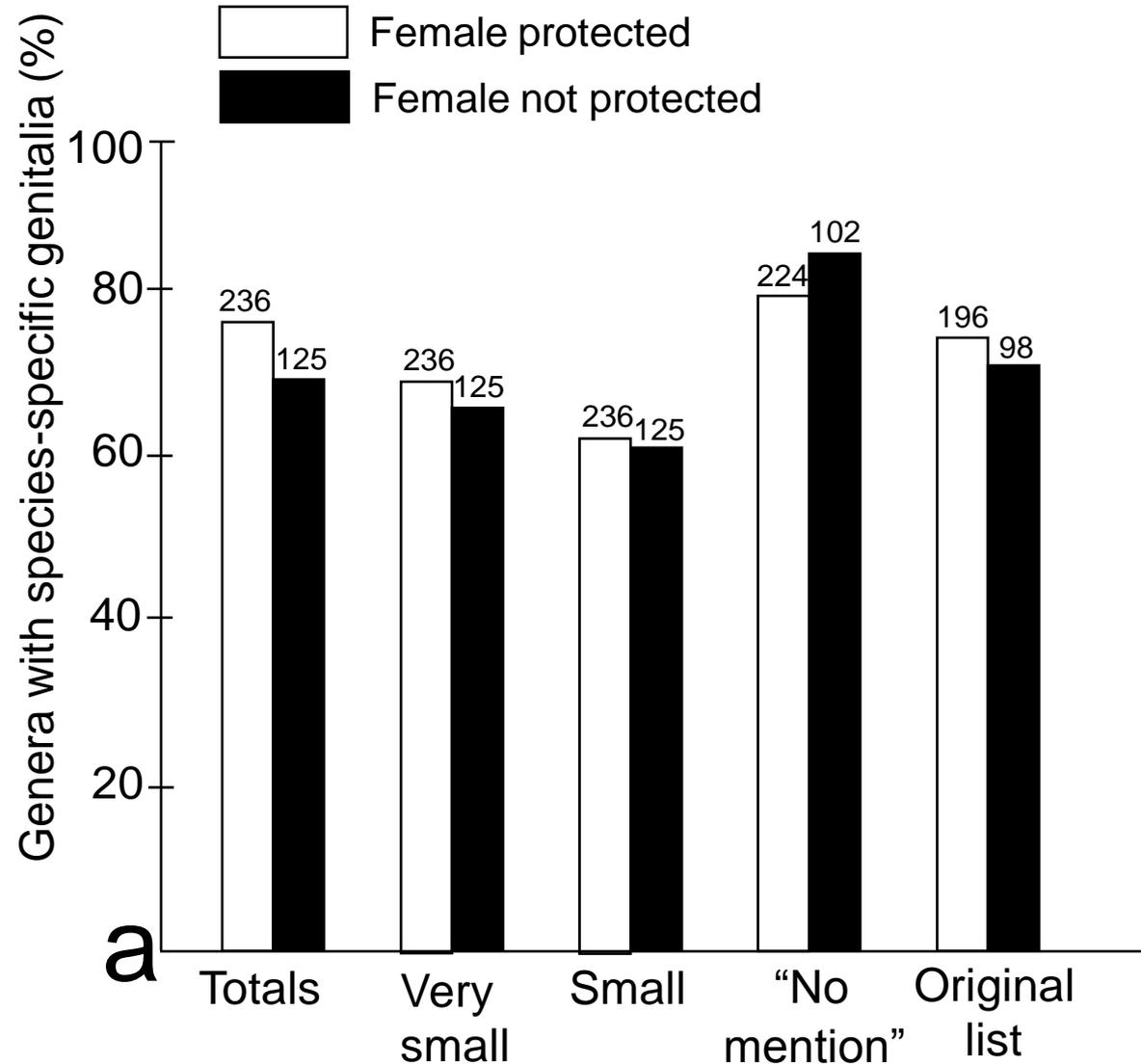
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Figure caption

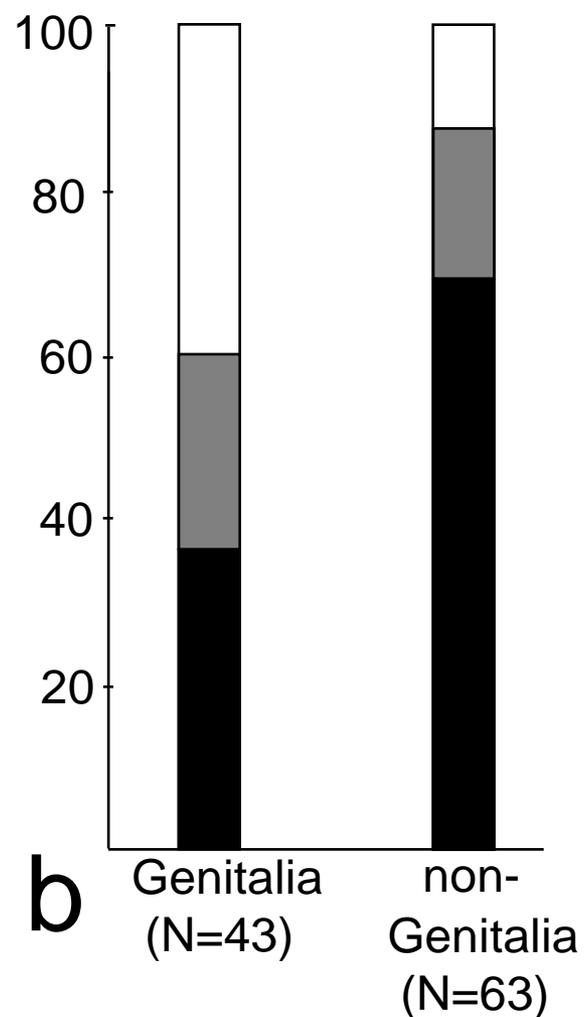
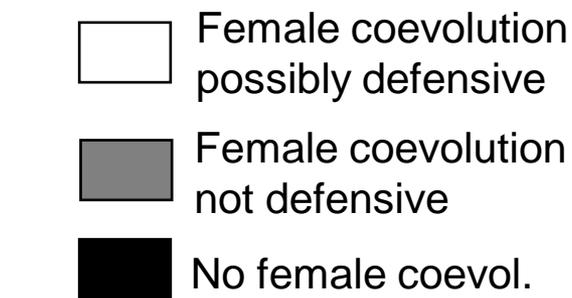
Fig. 1. Summary of data from two large survey studies documenting failures of confirm predictions of the SAC hypothesis. a) Percentages of genera in which male genitalia are and are not species-specific, contrasting groups in which non-receptive females are protected from sexual harassment by males with groups in which non-receptive females are not protected. 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 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 (black) and did not (grey and white) conform to predictions of species-specific defensive female coevolution from physical coercion versions of the SAC hypothesis (after Eberhard in press; data from Eberhard 2004a,b).

figure

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a



b