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Comparison of phylogenetic signal between male genitalia and non-genital characters in insect systematics

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Abstract

It is generally accepted that male genitalia evolve more rapidly and divergently relative to non-genital traits due to sexual selection, but there is little quantitative comparison of the pattern of evolution between these character sets. Moreover, despite the fact that genitalia are still among the most widely used characters in insect systematics, there is an idea that the rate of evolution is too rapid for genital characters to be useful in forming clades. Based on standard measures of fit used in cladistic analyses, we compare levels of homoplasy and synapomorphy between genital and non-genital characters of published data sets and demonstrate that phylogenetic signal between these two character sets is statistically similar. This pattern is found consistently across different insect orders at different taxonomic hierarchical levels. We argue that the fact that male genitalia are under sexual selection and thus diverge rapidly does not necessarily equate with the lack of phylogenetic signal, because characters that evolve by descent with modification make appropriate characters for a phylogenetic analysis, regardless of the rate of evolution. We conclude that male genitalia are a composite character consisting of different components diverging separately, which make them ideal characters for phylogenetic analyses, providing information for resolving varying levels of hierarchy.

Among animals with internal fertilization, many species have species-specific male genitalia with morphological divergence among closely related species that is often dramatic and complex (Eberhard, 1985). This pattern is especially evident in insects, and male genitalia are considered one of the most important and useful species-diagnostic characters in insect systematics (Tuxen, 1970). At the same time, patterns observed throughout insect systematics show that male genitalia are also useful in resolving relationships in phylogenetic analyses. A number of higher classification schemes for different insect groups are based entirely on male genital structures (Sharp and Muir, 1912; Kennedy, 1919; Eyer, 1924; Peck, 1937; Dirsh, 1956). This versatile utility of male genitalia in insect systematics stems from the composite nature of the structure. Male genitalia are complex organs that consist of many component structures that are functionally different from each other (Huber, 2004; Huber et al., 2005; Song and Wenzel, 2008) and that are derived from tissues that differ in embryonic origin (Snodgrass, 1931, 1957; Scudder, 1971). For example, many insects have male genital structures that serve as grasping, stroking, rubbing, or pressing organs (Eberhard, 1985, 2004b), as well as sensory structures such as hairs, spines, barbs, and sensillae (Tuxen, 1970). They also have intromittent organs, the primary function of which is to transfer sperm to the opposite sex. Underneath these structures are muscle tissues and apodemes that support the movement of different genital components during copulation (Kumashiro and Sakai, 2001). Functionally different components of male genitalia can evolve separately from each other (Huber et al., 2005; Song and Wenzel, 2008) and thus are capable of providing useful characters across different taxonomic levels, but

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each of these structures serves a crucial and integral part of the male genitalia, working together to accomplish successful copulation.

A general consensus in the study of genital evolution is that male genitalia are under sexual selection (Eberhard, 1985, 2001, 2004b; Huber and Eberhard, 1997; Arnqvist, 1998; Arnqvist and Danielsson, 1999; Córdoba-Aguilar, 2005; House and Simmons, 2005). Specifically, cryptic female choice via Fisherian runaway selection (Eberhard, 1985, 1993, 1994) and sexually antagonistic coevolutionary arms race (Parker, 1979; Alexander et al., 1997; Arnqvist and Rowe, 2002a; Rowe and Arnqvist, 2002) have been proposed as possible mechanisms for driving the evolution of male genitalia. Because sexually selected characters tend to evolve rapidly (Lande, 1981; Kirkpatrick, 1982; West-Eberhard, 1983; Gavrilets, 2000), researchers generally agree that male genitalia evolve both rapidly and divergently (Eberhard, 1985; Arnqvist, 1997; Hosken and Stockley, 2004; Mendez and Cordoba-Aguilar, 2004). It is also a logical conclusion from observation that male genitalia are consistently useful as a taxonomic character at the species level, which suggests that they must acquire a new form in each new species (Eberhard, 1985). Moreover, there is an idea that the rate of genital evolution is extremely rapid, to the point that there may not be observable phylogenetic inertia left in the structures. For example, in studying the male genitalia of water striders, Arnqvist and Rowe (2002b, p. 944) asserted that they "found little correspondence between phenotypic and phylogenetic similarity across species [which] supports the prediction that sexually antagonistic coevolution leads to rapid character evolution". Eberhard (2004a, p. 143) examined the male genitalia of insects and spiders and suggested that, "the relatively rapid rate of change in male genitalia in many groups indicates that [phylogenetic] inertia has not been important". Both authors cited Losos (1999), who argued that no relationship may exist between degree of phylogenetic relationship and phenotypic similarity if rates of character evolution are high relative to speciation rate.

The idea that male genitalia as a whole do not have phylogenetic inertia is simply incorrect, as evidenced by the broad usage of genital characters in systematics. A brief survey of 89 phylogenetic analyses published in *Systematic Entomology* and *Annals of the Entomological Society of America* between 2000 and 2004 suggests that an overwhelming proportion of the studies (74 studies [80.9%], which included diverse arthropod lineages [two arachnid, two chilopod, and 14 hexapod orders]), found genital characters to be phylogenetically informative in forming clades. If the idea of rapid genital evolution is limited to only species-specific characters, however, the argument becomes valid. Because species-specific characters are confined to the members of one species, whether they are characteristics of shape, size, or both (Song, 2009), they must be considered autapomorphic characters, which certainly do not contain any phylogenetic signal. Nevertheless, the features of male genitalia are more than species-specific, and even non-species-specific components should be under direct or indirect influence of sexual selection because of the overall function of male genitalia. If male genitalia as a whole are under sexual selection, theories predict that they should evolve rapidly compared with non-genital characters that are not under such selective pressures. Surprisingly, there is little quantitative comparison of the pattern of character evolution between genital and non-genital traits. Arnqvist (1998) provided one such example that genital evolution was more than twice as divergent in polyandrous insects as in monandrous insects, while other external traits (wings, legs, and other body parts) were not divergent between two groups, but this type of explicit comparison is rare.

Do male genitalia exhibit a different pattern of evolution from non-genital characters because of sexual selection? How much phylogenetic signal do male genitalia have compared with non-genital traits? In this study, based on 41 cladistic analyses of insects, we attempt to answer these questions by quantifying and comparing the level of phylogenetic signal between male genital traits which are presumably under sexual selection, and non-genital traits that are not likely to under sexual selection, in order to test if these two sets of traits display different patterns of character evolution. Our goal is to provide a pattern-oriented perspective to the study of genital evolution, which is already well equipped with process-oriented theories, thereby presenting a broader and more comprehensive view on this interesting morphological trait.

Materials and methods

Assumptions

First, we assumed that all genital characters included in each of 41 cladistic analyses would be under sexual selection. This idea would be difficult to assess without explicit experiments, but the descriptions of genital characters given by the original authors allowed us to assume that the structures may reasonably be under such selective pressure. We also assumed that genital characters coded by the original authors would be under sexual selection across different taxonomic levels.

Second, we took a position that inclusion of characters of interest in a phylogenetic analysis would not be circular. There has been much discussion on the practice of including characters of interest in phylogenetic analyses, and whether or not this practice biases the resulting phylogenies (Coddington, 1988; de Queiroz,

1996; Luckow and Bruneau, 1997; Grandcolas et al., 2001). In this study, we were interested in comparing phylogenetic signal between genital and non-genital traits, both of which must be tested through simultaneous phylogenetic analyses. Because both these traits could provide discrete homology statements regardless of any selective pressures they might be influenced by (Luckow and Bruneau, 1997), the inclusion of both traits would lead to a robust solution, which would become the basis of comparing phylogenetic signal. Therefore we included genital traits in all our analyses.

Third, we assumed that a phylogeny based on morphological characters could provide an appropriate benchmark of comparing phylogenetic signal between different morphological character sets. While some would argue that one should use neutrally evolving molecular markers to study the pattern of morphological character evolution effectively (Bond et al., 2003; Huber, 2003), we believed that morphological phylogenies could provide sufficient information about how included characters might have evolved through character optimization or by comparing measures of fit.

Finally, we assumed that two measures of fit (consistency index [CI, Kluge and Farris, 1969] and retention index [RI, Farris, 1989]) used in our study would serve as adequate statistics for calculating phylogenetic signal in the characters of interest. Although other measures, such as the partitioned Bremer support values (PBS) (Baker and DeSalle, 1997) or the number of phylogenetically informative characters (PIC) (Meier and Lim, 2009), have been used to describe phylogenetic signal, we considered CI and RI to be the simplest and most straightforward character statistics that could adequately describe the levels of homoplasy and synapomorphy, respectively. Similar justification has been made by Sanderson and Donoghue (1989, 1996) and de Queiroz and Wimberger (1993).

Selection of studies

We surveyed cladistic analyses published in *Systematic Entomology* between the years 2000 and 2006, identifying a total of 75 cladistic studies that used genital traits in their analyses. From these studies, we selected a total of 41 studies for our analysis, based on the following criteria. First, we used studies that relied on equal weights as the basis of reciprocal illumination of character sets so that we could readily replicate and obtain comparable results. Hence studies with final results based on successive weighting and implied weighting were excluded (this is not to be taken as a comment on those methods, but is only an operational concern for evaluating measures of fit). Second, we did not include studies that were based entirely on genital characters because our goal was to compare the phylogenetic signal of genital traits with non-genital traits. Finally, we included only studies with explicit character descriptions and data matrices. This was a particularly important criterion because in many cases the authors used the terms ''male terminalia'' and ''male genitalia'' interchangeably, despite the fact that these do not necessarily mean the same thing. By examining each character description, we were able to filter out these discrepancies. The 41 studies included in our study encompassed 11 insect orders (Table 1). Thirty-four studies focused on the five major orders (Coleoptera, Diptera, Hemiptera, Lepidoptera, and Hymenoptera), and there were two studies on Orthoptera and one study for each of the smaller orders, including Megaloptera, Neuroptera, Odonata, Phthiraptera, and Trichoptera (Fig. 1A). These studies also focused on different taxonomic levels from superfamily to genus, with lower-level analyses being more prevalent than higher-level analyses (Fig. 1B). The number of taxa included in the studies ranged between 8 and 115, and the total number of characters ranged between 15 and 155 (Table 1). On average, the genital traits constituted about 30.2% of the total characters included in any given analysis. Among the genital traits, the overwhelming proportion was male genitalia (92.9%) while female genitalia were rarely used (7.1%). In fact, 24 studies did not include any female genital structures at all (Table 2).

Phylogenetic analysis and calculation of fit statistics

From each of the 41 studies, we recoded the data matrix using WinClada (Nixon, 2002). Because we were interested only in how genital traits performed against non-genital traits in a phylogenetic framework, we coded all characters unordered with equal weight. This character recoding affected the original coding of only two of 41 studies (references 7 and 8 in Table 1) and did not affect the resulting overall pattern.

For each study, we examined the character description of each character in detail with the aid of the ''Taxonomist's glossary of genitalia in insects'' (Tuxen, 1970) to identify whether or not a given character was a genital feature. Because many authors used the term genitalia loosely, we restricted the definition of genitalia to mean reproductive structures that would be explicitly involved in copulation and internal courtship, thus under sexual selection (Eberhard, 1985). For instance, abdominal sternites and tergites that were initially considered to be genitalia by the original authors were not recognized as genitalia in our study because it was evident that the original designation was based purely on a physical location of the structures, not their function. Similarly, we recognized only female internal genital structures as genital, such as spermathecae and bursa copulatrix, and did not recognize ovipositors or female cerci. However, we did recognize secondary sexual characters of Odonata (reference 37 in Table 1)

Table 1
Cladistic analyses used in this study for comparison of character consistency index (CI) and retention index (RI) within data sets

					G			NG		
References	Order	Taxon	Rank	Taxa	CHAR	CI	RI	CHAR	CI	RI
1	Coleoptera	Sericine beetles	3	49	15	0.29	0.78	90	0.35	0.74
2	Coleoptera	Aleocharine beetles	3	40	17	0.44	0.78	131	0.39	0.65
3	Coleoptera	*Nordus* rove beetles	4	43	11	0.36	0.62	74	0.38	0.67
4	Coleoptera	Cerophytid beetles	2	26	9	1.00	1.00	9	0.90	0.98
5	Coleoptera	Derelomine weevils	3	115	20	0.95	0.99	135	0.61	0.94
6	Coleoptera	Hoplandriine rove beetles	3	26	19	0.25	0.41	109	0.29	0.50
7	Coleoptera	*Neoclypeodytes* beetles	4	27	1	1.00	1.00	21	0.46	0.76
8	Coleoptera	*Leptochromus* beetles	3	8	3	0.60	0.33	14	0.73	0.70
9	Coleoptera	Arrowinine rove beetles	3	19	4	0.53	0.71	85	0.47	0.59
10	Diptera	Coenosiine flies	3	49	6	0.30	0.72	61	0.31	0.63
11	Diptera	*Empis setitarsus*-group flies	4	23	6	0.78	0.92	9	0.80	0.91
12	Diptera	*Lordiphosa* flies	4	41	28	0.40	0.72	40	0.49	0.81
13	Diptera	*Thricops* flies	4	46	7	0.67	0.92	37	0.45	0.83
14	Diptera	Eudoryline flies	3	60	69	0.40	0.76	60	0.29	0.73
15	Diptera	*Colocasiomyia* flies	4	35	11	0.31	0.66	51	0.40	0.73
16	Diptera	*Bonjeania* flies	4	16	21	0.63	0.79	31	0.48	0.66
17	Diptera	Agapophytine flies	3	26	12	0.64	0.77	39	0.44	0.71
18	Diptera	Tabanomorpha flies	1	15	5	0.55	0.78	34	0.69	0.81
19	Hemiptera	*Aquarius* water striders	4	53	15	0.51	0.93	45	0.41	0.85
20	Hemiptera	Evacanthine leafhoppers	3	40	24	0.19	0.46	66	0.25	0.55
21	Hemiptera	Cosmopsaltriine cicadas	3	97	31	0.68	0.95	17	0.58	0.90
22	Hemiptera	Nepomorpha bugs	1	43	4	0.50	0.90	61	0.87	0.97
23	Hemiptera	*Iolania* plant hoppers	4	10	12	1.00	1.00	7	0.70	0.63
24	Hemiptera	Acleridid scales	2	22	9	0.43	0.70	67	0.42	0.65
25	Hemiptera	*Solonaima* plant hoppers	4	18	11	0.38	0.29	20	0.50	0.47
26	Hymenoptera	*Augochlorella* bees	4	20	24	0.57	0.71	24	0.53	0.52
27	Hymenoptera	*Agapostemon* bees	4	45	32	0.26	0.54	109	0.25	0.51
28	Hymenoptera	Pseudomyrmecine ants	3	76	42	0.38	0.71	100	0.26	0.70
29	Hymenoptera	Microgastrine wasps	3	55	2	0.45	0.87	50	0.20	0.51
30	Lepidoptera	Hepialid moths	2	17	26	0.70	0.82	29	0.74	0.80
31	Lepidoptera	*Charis* butterflies	4	23	30	0.79	0.90	6	0.47	0.79
32	Lepidoptera	Scopuline moths	3	59	34	0.38	0.72	52	0.39	0.69
33	Lepidoptera	*Hypanartia* butterflies	4	14	16	0.76	0.91	19	0.62	0.80
34	Lepidoptera	*Dircenna* butterflies	4	15	12	0.88	0.89	37	0.65	0.81
35	Megaloptera	Chauliodine fishflies	3	24	31	0.65	0.88	24	0.67	0.87
36	Neuroptera	Neuroptera	1	23	8	0.58	0.62	28	0.51	0.70
37	Odonata	*Enallagma* damselflies	4	66	15	0.27	0.30	22	0.28	0.30
38	Orthoptera	*Agnotecous* crickets	4	16	27	0.65	0.80	29	0.51	0.56
39	Orthoptera	*Panacanthus* katydids	4	10	1	1.00	1.00	29	0.81	0.83
40	Phthiraptera	Goniodid lice	2	31	5	0.40	0.68	57	0.33	0.63
41	Trichoptera	*Otarrha* caddis flies	4	36	49	0.60	0.78	7	0.79	0.86

Rank is the taxonomic rank of the terminal taxa in each study. The following notation is used: 1, superfamily/order; 2, family; 3, subfamily/tribe; 4, genus. G CHAR and NG CHAR are the total number of genital (both male and female) and non-genital characters that are phylogenetically informative. G CI and NG CI, and G RI and NG RI are the ensemble CIs and RIs for the total genital and non-genital characters, respectively.

References: 1 Ahrens, 2006; 2 Ashe, 2005; 3 Chatzimanolis, 2005; 4 Costa et al., 2003; 5 Franz, 2006; 6 Hanley, 2002; 7 Miller, 2001; 8 O'Keefe, 2002; 9 Solodovnikov and Newton, 2005; 10 Couri and Pont, 2000; 11 Daugeron and Grootaert, 2003; 12 Hu and Toda, 2001; 13 Savage et al., 2004; 14 Skevington and Yeates, 2001; 15 Sultana et al., 2006; 16 Winterton et al., 2000; 17 Winterton et al., 2001; 18 Zloty et al., 2005; 19 Damgaard and Cognato, 2006; 20 Dietrich, 2004; 21 Duffels and Turner, 2002; 22 Hebsgaard et al., 2004; 23 Hoch, 2006; 24 Hodgson and Millar, 2002; 25 Soulier-Perkins, 2005; 26 Coelho, 2004; 27 Janjic and Packer, 2003; 28 Ward and Downie, 2005; 29 Whitfield et al., 2002; 30 Brown et al., 2000; 31 Harvey and Hall, 2002; 32 Sihvonen and Kaila, 2004; 33 Willmott and Lamas, 2006; 34 Willmott et al., 2001; 35 Liu and Yang, 2006; 36 Aspöck et al., 2001; 37 May, 2002; 38 Desutter-Grandcolas and Robillard, 2006; 39 Montealegre-Z and Morris, 2004; 40 Smith, 2000; 41 Blahnik, 2002;.

as genitalia because a number of studies have shown that these structures effectively serve as courtship devices and are under sexual selection (Córdoba-Aguilar, 2005). Therefore a precise definition of male genitalia *sensu* Eberhard (1985) was adopted. We recognized a set of genital traits (G), which consisted of male genitalia (G-MG) and female genitalia (G-FG) from each study. The remaining characters were automatically considered non-genital traits (NG). However, NG includes many different types of character set that may be under different selective pressures, therefore a direct comparison between G and NG would not be fair or adequate

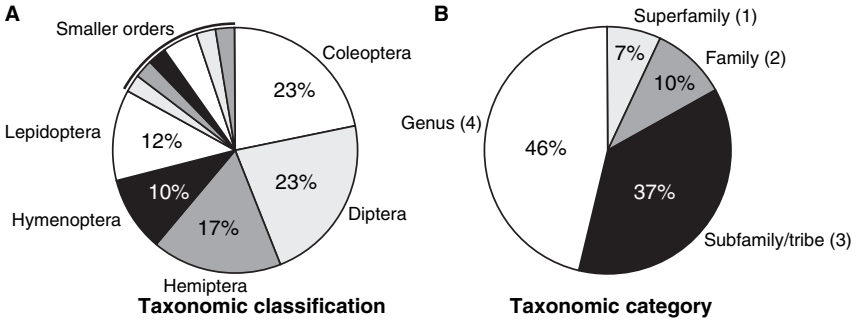

Fig. 1. Summary of 41 studies analysed in the present study. (A) Studies sorted by taxonomic classification. Smaller orders include Orthoptera, Megaloptera, Neuroptera, Odonata, Phthiraptera and Trichoptera. (B) Studies sorted by taxonomic category.

in some cases. Therefore we selected a set of non-genital characters that did not appear to be under sexual selection, such as leg and thorax characters, to perform a fair comparison with G-MG. We designated these traits as non-sexually selected non-genital characters (NG-NS). We realize that to determine whether NG-NS are truly not under sexual selection, they must be subjected to empirical studies, but for the purpose of this study we made a conservative assumption when selecting NG-NS (Table 2).

We performed a parsimony analysis in NONA (Goloboff, 1995) on each of the recreated data matrices using the following commands: `rs 0; hold 1000; mult* 50; best`. Due to the relatively small sizes of each data set, the TBR and SBR search methods were sufficient to find all most parsimonious trees (MPTs). After the search, we calculated the number of minimum and maximum steps for each character from individual data matrices and corresponding observed steps from the strict consensus phylogeny of the MPTs using WinClada, which were used to calculate fit statistics. We calculated the ensemble consistency index (CI) and the ensemble retention index (RI) for G, G-MG, G-FG, NG and NG-NS for each study to investigate the level of homoplasy and synapomorphy for these particular suites of characters (Farris, 1989). Because phylogenetically uninformative characters (characters that are not useful in tree construction because they are invariant or autapomorphic) are known to influence the calculation of these measures (Brooks et al., 1986; Sanderson and Donoghue, 1989), we did not include such characters in our calculation, despite the fact that many such characters (both genital and non-genital) are published. Although calculation of CI and RI based on the strict consensus of all MPTs is not a standard practice because the consensus tree is necessarily a solution less optimal than any one of the MPTs, nevertheless we took this approach from a practical standpoint. Among the 41 studies we examined, 10 studies resulted in a single MPT, three studies resulted in more than 100 MPTs, while the majority fell between two and 36 MPTs. Moreover, most of the studies presented the strict consensus as a preferred tree in discussing phylogenetic implication of their results. Despite the fact that observed number of steps should be calculated either on all MPTs or on a randomly selected MPT, we felt that such calculation would be impractical and would not shed any new light compared with when the observed number of steps was calculated on the strict consensus. Because we treated G and NG alike, our calculation was not affected by our practice, and presented a more conservative estimate on the phylogenetic signal of both character sets.

Statistical analyses

The ensemble CI and RI from each study served as raw data for various statistical analyses. Before comparing between different variables, we tested them for normality. While some variables were normally distributed, the majority of the variables were not. However, we found that the paired differences of CI and RI between genital and non-genital traits followed a normal distribution, and therefore we used a paired *t*-test which would be appropriate for testing the mean difference between paired observations (de Queiroz and Wimberger, 1993). To test a null hypothesis that the level of homoplasy in genital traits is not different from that in non-genital traits, we compared the CI between G and NG, and between G-MG and NG-NS. To test the null hypothesis that the level of synapomorphy is not different between genital and non-genital traits, we compared the RI between G and NG, and between G-MG and NG-NS. A limited set of studies used female genitalia in phylogenetic reconstruction, and we compared the CI and RI between G-MG and G-FG in these 17 studies.

We also examined CI and RI in the context of taxonomic category and classification. We categorized the studies into four taxonomic levels: superfamily (1), family (2), subfamily/tribe (3), and genus (4). To test whether different levels of analysis consistently yielded a similar CI and RI, we performed an analysis of variance

Table 2
Comparison of character consistency index (CI) and retention index (RI) within data sets

References	G-MG	CI	RI	G-FG	CI	RI	NG-NS	CI	RI	Type
1	14	0.35	0.74	1	1.00	1.00	20	0.36	0.72	Leg
2	16	0.42	0.77	1	1.00	1.00	11	0.34	0.66	Leg
3	11	0.36	0.62	0	n/a	n/a	14	0.33	0.70	Thorax
4	9	1.00	1.00	0	n/a	n/a	3	1.00	1.00	Leg
5	17	1.00	1.00	3	0.75	0.94	9	0.36	0.78	Leg
6	14	0.25	0.41	5	0.27	0.40	10	0.42	0.59	Thorax
7	1	1.00	1.00	0	n/a	n/a	6	0.56	0.85	Leg
8	3	0.60	0.33	0	n/a	n/a	3	1.00	1.00	Leg
9	4	0.53	0.71	0	n/a	n/a	6	0.40	0.54	Leg
10	5	0.30	0.73	1	0.33	0.67	10	0.21	0.57	Leg
11	6	0.78	0.92	0	n/a	n/a	1	1.00	1.00	Thorax
12	27	0.41	0.73	1	0.17	0.44	5	0.33	0.70	Thorax
13	7	0.56	0.90	0	n/a	n/a	13	0.53	0.83	Leg
14	69	0.40	0.76	0	n/a	n/a	10	0.18	0.66	Leg
15	11	0.31	0.66	0	n/a	n/a	5	0.56	0.84	Leg
16	18	0.62	0.75	3	0.75	0.93	16	0.43	0.59	Thorax
17	10	0.62	0.74	2	0.83	0.92	7	0.35	0.69	Leg
18	5	0.55	0.78	0	n/a	n/a	4	0.63	0.82	Thorax
19	12	0.47	0.90	3	0.80	0.99	9	0.35	0.73	Thorax
20	24	0.19	0.46	0	n/a	n/a	21	0.29	0.62	Head
21	31	0.68	0.95	0	n/a	n/a	3	0.80	0.98	Head
22	4	0.50	0.90	0	n/a	n/a	10	1.00	1.00	Mouthpart
23	12	1.00	1.00	0	n/a	n/a	4	0.57	0.40	Head
24	9	0.43	0.70	0	n/a	n/a	19	0.42	0.63	Head
25	11	0.38	0.29	0	n/a	n/a	12	0.53	0.52	Head
26	24	0.57	0.71	0	n/a	n/a	6	0.60	0.43	Leg
27	32	0.26	0.54	0	n/a	n/a	32	0.25	0.50	Thorax
28	42	0.38	0.71	0	n/a	n/a	20	0.29	0.73	Mouthpart
29	2	0.45	0.87	0	n/a	n/a	3	0.24	0.38	Head
30	13	0.70	0.81	13	0.69	0.82	8	0.63	0.70	Mouthpart
31	28	0.80	0.90	2	0.75	0.83	4	0.63	0.88	Wing
32	25	0.41	0.73	9	0.32	0.68	6	0.34	0.60	Head
33	14	0.75	0.91	2	0.80	0.86	11	0.63	0.84	Wing
34	12	0.88	0.89	0	n/a	n/a	2	0.50	0.67	Wing
35	29	0.63	0.87	2	1.00	1.00	10	0.77	0.93	Wing
36	8	0.58	0.62	0	n/a	n/a	13	0.57	0.81	Head
37	10	0.27	0.33	5	0.28	0.22	5	0.42	0.13	Larvae
38	25	0.65	0.80	2	0.67	0.67	2	1.00	1.00	Fastigium
39	1	1.00	1.00	0	n/a	n/a	4	1.00	1.00	Pronotum
40	5	0.40	0.68	0	n/a	n/a	6	0.47	0.85	Thorax
41	36	0.61	0.78	13	0.54	0.79	5	0.90	0.94	Wing

G-MG, G-FG and NG-NS are the total number of male genital, female genital and non-sexually selected non-genital characters that are phylogenetically informative. CI and RI are the ensemble CIs and RIs for particular character types. NG-NS type is the type of a character used to represent non-genital characters that are assumed to be not sexually selected. For References, see Table 1.

(ANOVA) with the taxonomic levels as independent variables. Similarly, we identified the five most frequently studied insect orders and tested whether the characters were used differently across taxonomic classification based on ANOVA. All statistical analyses were performed in Minitab 13.20 (Minitab Inc., State College, PA)

Results

In comparing between genital and non-genital traits, we found a statistically significant difference between both the ensemble CI of NG and G and the ensemble RI of NG and G (Table 3). In both CI and RI, G actually had higher values, indicating that the level of homoplasy was lower and the level of synapomorphy was higher in genital traits than in non-genital traits. When male genital traits and non-genital, non-sexually selected traits were compared, we found no difference between both CI and RI. For a limited data set (17 studies), we compared the phylogenetic signal between male and female genitalia and found no difference in both CI and RI (Table 3). A similar pattern was found across different taxonomic levels (Fig. 2; Table 4), suggesting that the phylogenetic signal was similar between genital and non-genital traits regardless of the level of divergence. However, in all cases

Table 3
Comparison of level of phylogenetic signals between genital and non-genital traits

Measure of fit	Comparisons	*N*	*T*	*P*
Consistency index	G *vs* NG	41	−2.35	0.024*
	G-MG *vs* NG-NS	41	0.56	0.576
	G-MG *vs* G-FG	17	−1.63	0.122
Retention index	G *vs* NG	41	−2.08	0.044*
	G-MG *vs* NG-NS	41	0.86	0.398
	G-MG *vs* G-FG	17	−0.42	0.680

G, NG, G-MG, G-FG and NG-NS are genital, non-genital, male genital, female genital and non-sexually selected non-genital traits. *N* is the number of studies; *T* and *P* are values resulting from a paired *t*-test.

*Statistical significance at $\alpha = 0.05$. In both cases where statistical significance is found, G has higher measures of fit than NG.

the variance was much larger in the lower-level analyses than in the higher-level analyses (Table 5). When the same comparison was made among five major insect orders, there was no difference in both CI and RI between genital and non-genital traits (Fig. 3; Table 6). Although Hymenoptera had slightly lower overall CI than other orders, no statistical difference was found among the orders in terms of CI and RI, and the variance was similar across the classification (Table 5).

Discussion

Phylogenetic signal in male genitalia in insect systematics

Because theories predict that characters under sexual selection tend to evolve rapidly (Lande, 1981; Kirkpatrick, 1982; West-Eberhard, 1983; Gavrilets, 2000), one might consider that such characters should have less phylogenetic signal than characters that are not under such selective pressures (Losos, 1999; Arnqvist and Rowe, 2002b). Although levels of homoplasy (CI) or synapomorphy (RI) cannot serve as direct indicators of the rate of character evolution, they can reveal information about the relative amount of phylogenetic signal in the characters of interest compared with other

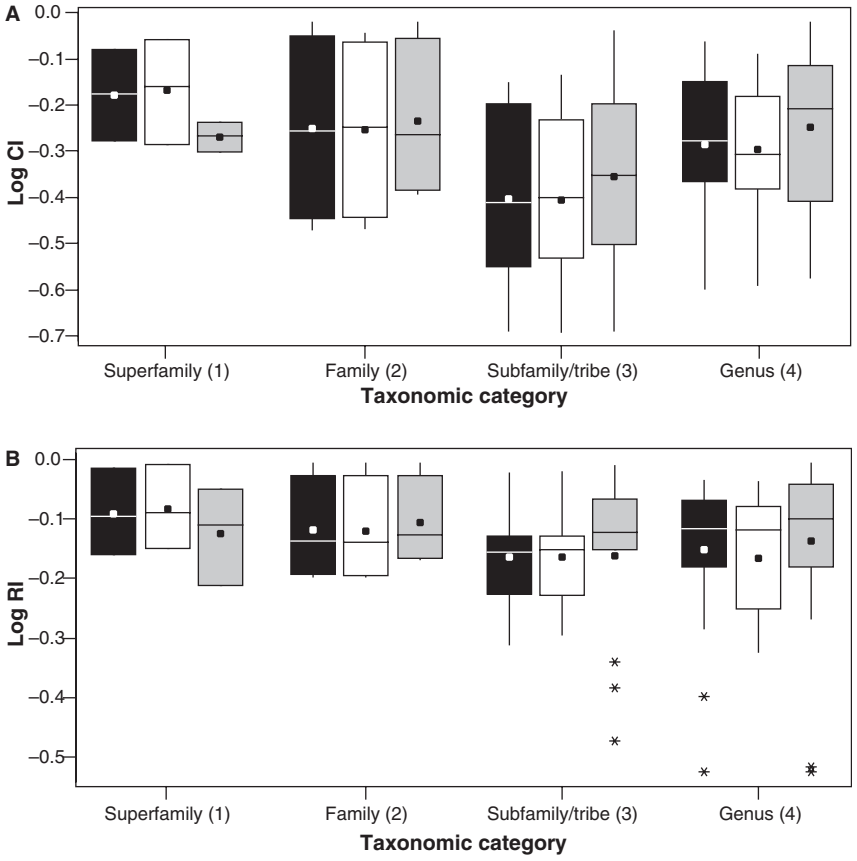

Fig. 2. Box plots showing the results of ANOVA of log-transformed measures of fit between different taxonomic categories. Black box, total informative characters; white box, non-genital traits; grey box, genital traits. The horizontal line in the box is the median; the dot in the box is the mean. Vertical lines extending to either side indicate the general extent of the data. Asterisks indicate an outlier. (A) ANOVA of log CI between taxonomic categories; (B) ANOVA of log RI between taxonomic categories.

Table 4
Comparison of level of phylogenetic signals between genital and non-genital traits in the context of taxonomic hierarchy

Measure of fit	Taxonomic category	Comparisons	*N*	*T*	*P*
Consistency index	1	G *vs* NG	3	1.15	0.369
		G-MG *vs* NG-NS	3	−1.20	0.353
	2	G *vs* NG	4	−1.04	0.376
		G-MG *vs* NG-NS	4	0.12	0.913
	3	G *vs* NG	15	−1.85	0.085
		G-MG *vs* NG-NS	15	0.90	0.384
	4	G *vs* NG	19	−2.37	0.029*
		G-MG *vs* NG-NS	19	0.54	0.597
Retention index	1	G *vs* NG	3	3.26	0.083
		G-MG *vs* NG-NS	3	−2.50	0.130
	2	G *vs* NG	4	−3.88	0.030*
		G-MG *vs* NG-NS	4	0.06	0.955
	3	G *vs* NG	15	−0.74	0.471
		G-MG *vs* NG-NS	15	0.36	0.725
	4	G *vs* NG	19	−2.33	0.032*
		G-MG *vs* NG-NS	19	1.27	0.219

1, Superfamily; 2, family; 3, subfamily/tribe; 4, genus. G, NG, G-MG and NG-NS are genital, non-genital, male genital and non-sexually selected non-genital traits. *N* is the number of studies within each taxonomic category. *T* and *P* are values resulting from a paired *t*-test.

*Statistical significance at $\alpha = 0.05$.

Table 5
Analysis of variance on the measures of fit between taxonomic category and taxonomic classification

Comparison	Measure of fit	d.f.	*F*	*P*
Taxonomic category	Total characters log CI	3	2.61	0.066
	NG log CI	3	2.92	0.047
	G log CI	3	1.24	0.311
	Total characters log RI	3	0.50	0.682
	NG log RI	3	0.73	0.538
	G log RI	3	0.21	0.888
Taxonomic classification	Total characters log CI	4	2.30	0.082
	NG log CI	4	2.21	0.092
	G log CI	4	0.92	0.468
	Total characters log RI	4	1.44	0.245
	NG log RI	4	2.32	0.081
	G log RI	4	0.51	0.727

NG and G are non-genital and genital traits. Detailed comparisons are found in Figs 2 and 3.

characters within a given data set (Sanderson and Donoghue, 1989; de Queiroz, 1996). Therefore, if genitalia have less phylogenetic signal relative to non-genital traits, it is possible to predict that they would be more homoplasious (lower CI) and less synapomorphic (lower RI) than non-genital characters in a simultaneous analysis of both traits. The pattern observed in our study is considerably different from this prediction. Genitalia are statistically less homoplasious and more synapomorphic than non-genital traits, and the level of homoplasy and synapomorphy is similar between male genitalia and non-sexually selected, non-genital characters (Table 3). This pattern is found consistently across taxonomic levels (Table 4) and different insect orders (Table 6), and none of the comparisons provides evidence for more homoplasious or less synapomorphic genitalia. When a statistically significant difference is seen, it is always the genital characters that have higher measures of fit than the non-genital characters. Therefore our study demonstrates conclusively that genitalia have similar phylogenetic signal compared with non-genital traits across divergent insect groups.

Why do male genitalia have similar phylogenetic signal compared with non-genital characters, despite the fact that male genitalia as a whole are under sexual selection? Rapid evolution does not necessarily equate with the lack of phylogenetic signal. Characters that evolve by a pattern of descent with modification make appropriate characters for a phylogenetic analysis, regardless of the rate of evolution. It is therefore possible that the characters can be directly or indirectly shaped by sexual selection and still be informative in forming clades. Soulier-Perkins (2001) demonstrated that male and female genitalia of a hemipteran family Lophopidae were under sexual selection via sexually antagonistic coevolution and that inclusion of these genital characters in phylogenetic reconstruction resulted in phylogenetic accuracy, and our study concurs with her findings. The composite nature of male genitalia may also be a key to understanding the observed pattern. It is possible that some genital components could be phylogenetically conserved, such as features that may be functionally constrained, while other characters could be phylogenetically much more labile, perhaps because they are involved in copulation. Huber et al. (2005) expressed a similar opinion in the study of litter-dwelling *Metagonia* spiders, and suggested that presence or absence of genital structures and their ''bauplan'' would be conserved while the shapes,

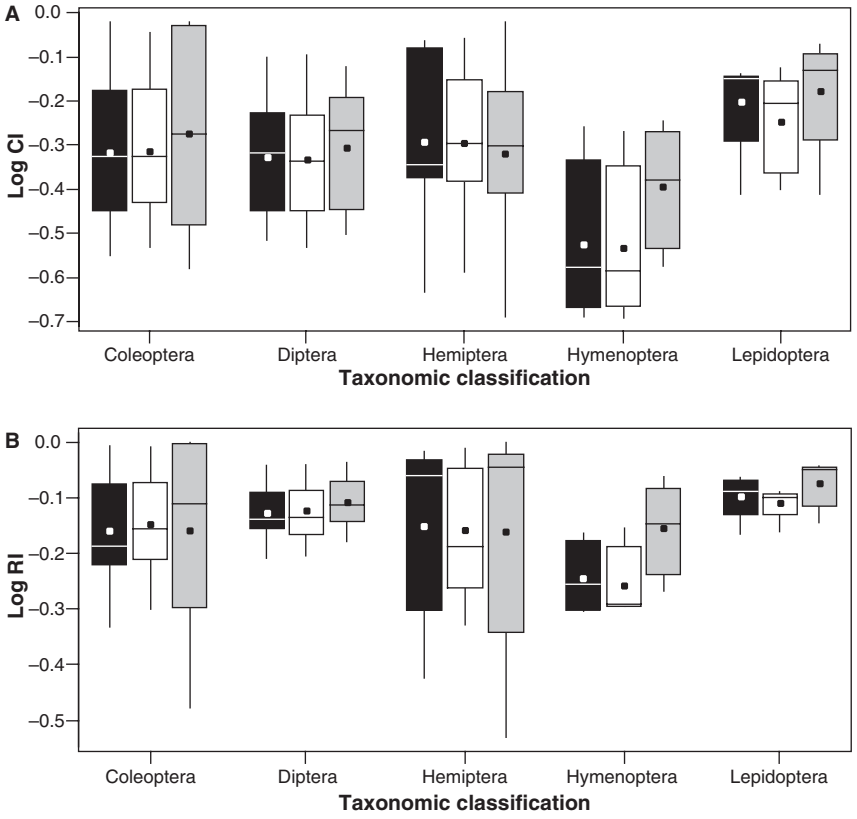

Fig. 3. Box plots showing the results of ANOVA of log-transformed measures of fit between five major insect orders. Black box, total informative characters; white box, non-genital traits; grey box, genital traits. The horizontal line in the box is the median; the dot in the box is the mean. Vertical lines extending to either side indicate the general extent of the data. (A) ANOVA of log CI between orders; (B) ANOVA of log RI between orders.

numbers, and sizes of individual genital structures would evolve rapidly. Such phylogenetically conserved characters are useful in forming clades in typical cladistic analyses, and coded in a fashion similar to non-genital characters in a given matrix. We find that the CI and RI of both genital and non-genital traits are nearly consistent across taxonomic levels (Fig. 2), which suggests that male genitalia have phylogenetically conserved components at a shallow level (between species) as well as at a deeper level (between families). Our findings collectively suggest that male genitalia used as characters in cladistic analyses are by no means special (no better or worse than non-genital characters), regardless of any selective pressures they might be under.

Systematic bias

Although our study demonstrates clearly that the level of phylogenetic signal is comparable between genital and non-genital traits, there is a possibility that this pattern might have been affected by systematic bias of the original authors. We identify two possible sources of bias: exclusion of phylogenetically uninformative characters, and improper weighting of genital characters. Sanderson and Donoghue (1989) provided an excellent discussion on other sources of bias that can influence the calculation of measures of fit, such as character distribution, multistate characters, and missing data. We do not discuss these issues in this paper.

In a cladistic analysis, some researchers will exclude phylogenetically uninformative characters *a priori* during character coding or *a posteriori* during the process of secondary homology assessment or recursive discovery (Carpenter, 1988). Characters that are expected to have a high level of homoplasy due to rapid evolution, or that are invariable, are often excluded at the outset. This is a step accidentally or intentionally left unreported in the published methods section of a manuscript, but it is considered a common phylogenetic practice. About one-third of all the studies we examined (13/41) included only the phylogenetically informative characters, implying that the uninformative characters might have been already excluded by the authors. More than half of the studies we analysed (25/41) did not have any uninformative genital characters, and 12 studies had only one or two genital characters that are phylogenetically

Table 6
Comparison of level of phylogenetic signals between genital and non-genital traits in the context of taxonomic classification

Measure of fit	Taxonomic classification	Comparisons	*N*	*T*	*P*
Consistency index	Coleoptera	G *vs* NG	9	−1.32	0.222
		G-MG *vs* NG-NS	9	0.79	0.455
	Diptera	G *vs* NG	9	−0.82	0.434
		G-MG *vs* NG-NS	9	0.53	0.613
	Hemiptera	G *vs* NG	7	0.06	0.953
		G-MG *vs* NG-NS	7	−0.40	0.700
	Lepidoptera	G *vs* NG	5	−1.82	0.142
		G-MG *vs* NG-NS	5	2.72	0.053
	Hymenoptera	G *vs* NG	4	−1.91	0.151
		G-MG *vs* NG-NS	4	1.33	0.275
Retention index	Coleoptera	G *vs* NG	9	−0.17	0.867
		G-MG *vs* NG-NS	9	−0.32	0.758
	Diptera	G *vs* NG	9	−1.02	0.337
		G-MG *vs* NG-NS	9	0.78	0.460
	Hemiptera	G *vs* NG	7	−0.44	0.675
		G-MG *vs* NG-NS	7	0.46	0.663
	Lepidoptera	G *vs* NG	5	−3.56	0.024*
		G-MG *vs* NG-NS	5	3.32	0.029*
	Hymenoptera	G *vs* NG	4	−1.83	0.164
		G-MG *vs* NG-NS	4	1.74	0.179

Five most frequently studied insect orders were examined. G, NG, G-MG and NG-NS are genital, non-genital, male genital and non-sexually selected non-genital traits. *N* is the number of studies within each taxonomic category. *T* and *P* are values resulting from a paired *t*-test.

*Statistical significance at $\alpha = 0.05$.

uninformative due to autapomorphy or invariability. Some of these ''pre-excluded'' characters might have been species-specific (autapomorphic) genital characters that are most affected by sexual selection. One might argue that our study is incomplete because these rapidly evolving genital characters are already excluded by design and we are comparing only between slowly evolving genital characters and other non-genital traits. However, we believe that the exclusion of phylogenetically uninformative characters does not negatively affect our conclusion. Because we explicitly assume that all genital components, both species-specific and conserved, would be under sexual selection because of the overall function of the structures, we argue that the genital characters included in the analysis should not be understood simply as slowly evolving. The rate of evolution is relative by nature, and even rapidly evolving characters can still be phylogenetically informative.

Another source of bias may be the inflated sense of taxonomic importance in dealing with genital characters (Huber, 2003). Although some researchers would intentionally avoid the inclusion of genital characters in phylogenetic analyses, due to their potential to be too rapidly evolving to contain phylogenetic signal, many insect systematists would actually prefer to include genital characters in their analyses. This is because genitalia traditionally have been considered highly informative in insect taxonomy not only at the species level but also for higher-level relationships (Dirsh, 1956; Keffer, 2004; Yoshizawa and Johnson, 2006). Indeed, genitalia seem to occupy a special place among the characters used in insect systematics because of their versatile taxonomic utility (Cohn, 1994). Perhaps because of this, researchers tend to focus on genitalia more than on other characters (Eberhard, 1985) and possibly find more phylogenetic signal in them. Our finding that the overall CI and RI of genital characters are higher than those of non-genital traits (Table 5) may be explained by this bias. A close examination of the measures of fit indicates that four studies (references 4, 7, 23 and 39 in Table 1) found perfect CI and RI in genitalia, while none of the studies found non-genital characters to have the perfect measures of fit. Twelve studies found genital characters to have an RI over 0.9, while only three studies did so in non-genital characters.

Conclusion and future prospects for the study of genital evolution

Our study provides the first empirical demonstration that genital characters have similar or better phylogenetic signal relative to non-genital characters, contrary to the idea that genitalia do not contain enough phylogenetic inertia (Arnqvist and Rowe, 2002b). Certain features of genitalia useful in distinguishing closely related species may be evolving more rapidly than other genital components under sexual selection, and they have been the focus of recent studies in genital evolution. However, genitalia also contain

phylogenetically informative characters regardless of the rate of evolution. Certain levels of systematic bias may inflate the phylogenetic utility of genital traits, but the overall pattern is clearly in support of genitalia being useful in phylogenetics, perhaps even more so than certain other non-sexually selected traits. Because of the preconceived notion of rapid genital evolution, some researchers may avoid the inclusion of genital characters in phylogenetic analyses. This type of practice is unsubstantiated in light of the composite nature of male genitalia, and genital characters should not be excluded *a priori* simply due to the fact that they are potentially under sexual selection. Instead, we argue that the very composite nature of genitalia makes them ideal characters for phylogenetic analyses, providing information for resolving varying levels of hierarchy.

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