

Unresolved questions in genitalia coevolution: bridging taxonomy, speciation, and developmental genetics

Amir Yassin¹

Received: 18 January 2016 / Accepted: 16 May 2016 / Published online: 24 May 2016
© Gesellschaft für Biologische Systematik 2016

Abstract Systematists and geneticists study biological diversity, but they use different approaches that rarely intersect. A very common pattern that is of interest for both researchers is the rapid evolution of genitalia, a trait of significant taxonomic utility in several sexually reproducing animal clades. The idea that both male and female genitalia are species-specific and play a role in reproductive isolation has long been controversial but has recently gained a renewed interest by speciation and developmental geneticists. Here, I highlight six unresolved questions in genitalia coevolution and I argue that systematists, with their well training in comparative morphology, usage of large and geographically diverse collections, and ability to apply molecular genetics techniques, can make important contributions. Such an extension of systematics into the speciation and developmental genetics realms is a promising opportunity to expand “integrative taxonomy” comparisons between DNA and morphology into more explanatory relationships between the two sources of taxonomic data.

Keywords Integrative taxonomy · Comparative morphology · Mechanical isolation · Introgressive hybridization · Character displacement · Pleiotropy · Evo-devo

Introduction

Delimiting species is a central endeavor in systematics. For a long time, this task has almost exclusively relied on

morphology, leading to the accumulation of a wealth of comparative data. Systematists have then rapidly recognized that male genitalia are often among the most informative diagnostic traits, being highly variable between species and relatively stable within them (Dufour 1844; Eberhard 1985). Even today, when more sophisticated genetic methods of species delimitation successfully discover cryptic species, subtle differences in male genitalia often constitute the sole diagnostics of a type specimen that are required for the proper naming of a new species.

The relationship between systematics, i.e., the study of *patterns* of diversity, and genetics, i.e., the study of *processes* generating such diversity, has not always been straightforward (Minelli 2015). Testing the congruence between genetic and morphological data in “integrative taxonomy” (Dayrat 2005; Schlick-Steiner et al. 2010; Will et al. 2005) is certainly fruitful, but both sources of data are interdependent. Morphological variation is the result of changes in underlying genes, whereas the directionality and fate of these changes depend on selection acting on morphology. The rapid evolution of male genitalia represents one of few cases where a highly useful trait for systematists is also of wide interest for geneticists, hence offering an opportunity for bridging the gap between the two disciplines.

Geneticists ask two major questions about genital evolution. Population geneticists investigate the driving forces (e.g., neutrality vs. selection) and possible effects on speciation (e.g., mechanical isolation), whereas developmental geneticists study the underlying genetic architecture which sets genitalia apart from the evolution of other traits. Central to the first question is the understanding of the function of the evolving parts of the genital apparatus, whereas determining pleiotropy is the focus of the second endeavor. Again, the two questions are not completely independent, since pleiotropy and the structure of the genetic architecture strongly influence the

✉ Amir Yassin
yassin2@wisc.edu

¹ Laboratory of Genetics, University of Wisconsin-Madison, 425G Henry Mall, Madison, WI 53706, USA

response to the selective pressure. My argument here is that the integration of both comparative and experimental approaches is essential if a complete picture of genital evolution is to be drawn.

Comparative morphology

1. How often do females morphologically coevolve with male genitalia?

Among the earliest explanations of the rapid evolution of genitalia was the “lock-and-key” hypothesis that was formulated by the French entomologist Léon Dufour (1844). This hypothesis postulates that both male and female genitalia are species-specific and that such specificity was the guarantee of species preservation against the vagaries of hybridization. However, this interpretation raised many critiques during the twentieth century (reviewed in Eberhard 1985; Shapiro and Porter 1989), mostly because of the apparent lack of female genital “locks” among closely related species compared to male “keys.” For example, Eberhard (2004, 2010a) reviewed the taxonomic literature for 43 insect and spider groups, in which the functional morphology of genitalia has been determined, and found that only 65 % of female genital morphologies that are contacted by species-specific male structures were also species-specific. Consequently, it has been suggested that female coevolution with rapidly evolving male genitalia is mostly sensorial, since sensorial coevolution can also be found in cases where morphological coevolution is also present.

However, this conclusion may be an underestimation of female morphological coevolution for two reasons. First, taxonomists are usually interested in describing diagnostic characters that can easily be checked on hard tissues of preserved specimens, while vaginal structures are rather difficult to describe due to their invagination or to preserve due to their softness. This would present a limitation to the usefulness of taxonomic literature in evaluating the diversity of female structures relative to those of males. Second, Eberhard’s (2004, 2010a) approach assumes that during copulation, the same male species-specific structure contact the same female genital part in all species in a clade. Male elaborate secondary sexual traits and female preferences for them show a rapid evolutionary turnover (Wiens 2001), and primary sexual traits, i.e., genitalia, may not be an exception.

Recent studies in *Drosophila* illustrate my two points. The apparent lack of interspecific differences in female genitalia relative to the taxonomic importance of male genital structures in this genus was used in arguments refuting the lock and key since the 1930s (Robson and Richards 1936) and continued until recent studies of copulation anatomy using SEM (Eberhard and Ramirez 2004; Jagadeeshan and Singh 2006). However, a detailed investigation using both experimental and

comparative (Kamimura and Mitsumoto 2011, 2012; Yassin and Orgogozo 2013) approaches in the nine species of the *melanogaster* (Meigen 1830) subgroup revealed the presence of several species-specific female genitalia that coevolved with contacting male species-specific structures. More importantly, similar male or female structures contact different structures in different species. For example, female depressions (e.g., pouches and furrows) harbor the large male grasping epandrial lobes in species of the *melanogaster* complex (Yassin and Orgogozo 2013) and the basal phallic spines in the *yakuba* (Burla 1954) complex (Kamimura and Mitsumoto 2012; Yassin and Orgogozo 2013), while phallic spines contact different internal vaginal and uterine sclerites in the *erecta* (Tsacas and Lachaise 1974) complex (Yassin and Orgogozo 2013). Given that *Drosophila* was included in recent reviews (Eberhard 2004, 2010a; Masly 2012) as an example of lack of male-female genital coevolution, these findings suggest that morphological genital coevolution might be more abundant than previously thought.

2. How does morphological coevolution affect fitness?

The pattern of morphological coevolution between male and female genitalia may indicate the mode of selection driving this coevolution. Two sexual selection hypotheses have been proposed. On one hand, the cryptic female choice (CFC) hypothesis postulates that male genitalia stimulate females to favor the male’s own sperm at fertilization (Eberhard 1985). In this context, “CFC ... predicts that female external 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” (Eberhard 2010a, p. 12). On the other hand, the sexual antagonistic coevolution (SAC) hypothesis postulates that conflict over mating should result into an escalating arms race; males use their genitalia to force females into mating, with the costs that they may induce to the female probably reducing her chances to remate (Arnqvist and Rowe 2005). Consequently, female genitalia should evolve resistant morphologies, making the “correlated evolution between the sexes in morphology... is a rule rather than an exception” (Arnqvist and Rowe 2002, p. 941).

According to male species-specific genital morphologies with which they come into contact, female species-specific structures can be classified under two broad, non-exclusive categories: either invaginations or thickenings of the external and internal surfaces. Thickenings (e.g., sclerotized shields, cushions, pads) are common at places that contact males wounding (or apparently so) structures (e.g., spurs, spines, hooks, spines) whether on the internal (e.g., *Drosophila*: Yassin and Orgogozo 2013; isopods: Veuille 1978; some scorpions: Peretti 2003; beetles: Rönn et al. 2007) or external surfaces (e.g., sharks: Pratt 1979; gastropods: Valdés 2004)

and also on non-genital parts such as the paragenitalia of traumatically inseminating bugs (Siva-Jothy 2006; Tatarnic and Cassis 2010). Extreme forms of hardening, such as strongly sclerotized spines and claws, are less frequent in female genitalia than in males, yet they do occur in some cases (e.g., beetles: Cayetano et al. 2011; lepidopterans Sánchez et al. 2011). Such defensive structures may also evolve in the female genitalia of some hermaphrodite species when mating as males is preferred, such as the wounding “love darts” produced on the vaginal duct in many hermaphrodite snails and slugs (Koene and Schulenburg 2005).

External invaginations of the female genitalia (e.g., furrows, grooves, pits, pockets, etc.) are easier to detect and have been reported in a relatively higher number of cases (Eberhard 2004) and also on non-genital parts that are used in copulation (e.g., dragonflies head: Dunkle 1991; Johnson 1972; damselflies’ thorax: McPeck et al. 2009; waterstriders’ head: Khila et al. 2012). They usually coevolve with male elongated grasping organs (e.g., *Drosophila*: Kamimura and Mitsumoto 2012; Yassin and Orgogozo 2013). In many groups, the length of the vagina correlates with the length of the male intromittent organ (e.g., guppies: Evans et al. 2011; bugs: Horton and Lewis 2011). They may also result into the formation of dead end pockets that harbor male wounding phallic structures (e.g., deer mice: Patterson and Thaeler 1982; beetles: Sota and Kubota 1998; ants: Baer and Boomsma 2006; *Drosophila*: Kamimura 2007) or clockwise coiling coevolving with spiral, long male intromittent organs (e.g., waterfowls: Brennan et al. 2007; *Linyphia* spiders: Helsdingen 1969). The coevolution between the female seminal receptacle and sperm length in *Drosophila* may also be considered a particular form of internal invaginations (Pitnick et al. 1999).

Comparative studies alone in the lack of experimental measurement of the costs usually fail to determine which evolutionary process generated this pattern. For example, female genital thickenings could be defensive structures driven by SAC or CFC screens for traumatic males providing higher amount of nutrients or spermatozoa-rich ejaculates (Eberhard 2010b). Invaginations could be “selectively cooperative” to screen copulatory fittest males (Eberhard and Huber 2010) or anti-grasping organs in species with violent coercive mating wherein the female shows strong resistance to dislodge the male (Khila et al. 2012).

Speciation

3. How does morphological coevolution affect the directionality of cytoplasmic introgression?

Mechanical mismatch due to genital coevolution could play a role in the evolution, or at least the maintenance, of

reproductive isolation (Coyne and Orr 2004). However, male genitalia can also play a role in speciation even in the absence of morphological coevolution, as a sensorial signal for species recognition (Robertson and Paterson 1982). Masly (2012) has recently reviewed 13 experimental cases suggesting a role of genitalia in reproductive isolation and divided the mechanical isolation into two types: structural or sensorial. The major distinguishing criterion was the presence or absence of species-specific female genital morphologies coevolving with the divergent male genitalia. Accordingly, seven structural and three sensorial cases were identified. The three remaining cases (all including damselflies) were classified as both structural and sensorial, due to the presence of morphological coevolution between male grasping organs and female thoracic grooves and its absence between the male intromission organ and the female vagina. However, absence of evidence per se is a weak classificatory criterion. For example, one of the three sensorial cases included species of the *Drosophila simulans* (Sturtevant 1919) complex in which later more detailed investigation revealed the presence of morphological coevolution (Yassin and Orgogozo 2013).

Classical evolutionary interpretations of the lock-and-key hypothesis viewed its role in reproductive isolation in terms of indirect costs of hybridization, i.e., the reduced fitness of the hybrids (Eberhard 2010b). Mechanical matching can help the female screening conspecific males during copulation. On the other hand, mechanical mismatching can have direct costs upon hybridization. One of the most convincing models of such direct costs in recent years was shown in *Carabus* beetles wherein mismatches lead to fatal vaginal injuries and sterilizing phallic mutilations (Sota and Kubota 1998). Elevated heterospecific female mortalities and genital wounds have been documented in other groups (e.g., moths: Standfuss 1896; flies: Vanderplank 1948; Matute and Coyne 2010; Yassin and David 2016; bugs: Ryckman and Ueshima 1964). However, in all these examples, death may not be caused by the wounds themselves, since many of the male seminal fluid proteins could be toxic to conspecific females, and their toxicity may be also higher to heterospecific females (e.g., mosquitoes: Bargielowski et al. 2013). Future genetic manipulations of male toxins in model species, e.g., *Drosophila*, should be able to elucidate the exact role of genitalia in heterospecific killing.

An important point for the molecular systematist is that mechanical matching needs not to be symmetrical between reciprocal crosses. For example, it is more difficult for a long intromittent organ to penetrate a heterospecific small vagina than for a small organ in a large vagina. Therefore, initial stages of mechanical isolation could involve a tendency toward asymmetric crosses. Recent advances in the phylogeographical analysis of DNA markers with uniparental inheritance have revealed a predominance of asymmetric introgression between species with incomplete reproductive

isolation (Toews and Brelsford 2012), and such an asymmetry may shed light on the relationship between genital evolution and mechanical isolation.

Asymmetric introgressions associated with genital size differences were observed in a number of cases (e.g., beetles: Nagata et al. 2007; Sota 2002; Takami et al. 2007; chipmunks: Good et al. 2003; millipedes: Tanabe and Sota 2008; Karner blue butterfly: Gompert et al. 2006; Lucas et al. 2008; *Drosophila*: Nunes et al. 2010; Yassin and Orgogozo 2013). In all these cases, maternally inherited mitochondrial DNA (mtDNA) was introgressed from the species with the larger genitalia to the species with the smaller genitalia, a pattern that Nagata et al. (2007) called the “hand-in-glove” analogy. In addition to size, closely related species may differ in the degree of male genital harmfulness and female genital resistance (Rönn et al. 2007). Jordal et al. (2006) found that on the Canary Island of La Palma, mtDNA has been introgressed from the bark beetle species *Aphanarthrum subglabrum* (Israelson 1972) whose male intromittent organ bears apical spines into the subspecies *Aphanarthrum glabrum nudum* (Israelson 1972) whose male intromittent organ is spineless.

Absence of detection of the opposite trend may be due to either the rejection of females to copulate with heterospecific males with larger or more harmful genitalia or to the extinction of the females with smaller or less resistant genitalia in sympatric regions due to high hybridization costs. For example, the invasive Asian tiger mosquito, *Aedes albopictus* (Skuse 1894), displaced resident populations of the yellow fever mosquito, *Aedes aegypti* (Linnaeus in Hasselquist 1762), in the southeastern USA and Bermuda through the sterilizing effects of their males’ accessory gland proteins (Bargielowski et al. 2013). However, no study to my knowledge has ever documented local extinction due to interspecific genital-induced costs.

4. Is there evidence for genital character displacement in sympatry?

Examples of genital displacement due to reinforcement in sympatric zones were usually lacking (Eberhard 1985; Shapiro and Porter 1989), but recent studies revealed some interesting cases (e.g., beetles: Kawano 2002, 2003; land snails: Kameda et al. 2009; grasshoppers: Kawakami and Tatsuta 2010; marine snails: Hollander et al. 2013; but see Sauer and Hausdorf 2009). A previous study on two North American araneid spiders of the genus *Larinoidea* compared 16 male genital and two body size measurements between adjacent populations and did not find evidence for character displacement (Ware and Opell 1989). However, when the data are re-analyzed in a more convenient way, i.e., comparing measurements between sympatric and allopatric populations in a single widespread species, two structures (the embolus and the conductor) both related to intromission in other

araneids (Eberhard and Huber 2010; Uhl et al. 2007) show evidence for displacement.

Most studies have focused on displacement in male genitalia. Kameda et al. (2009) found on simultaneous hermaphroditic land snails of the genus *Satsuma* evidence for genital displacement that was more pronounced for penis length than for vaginal length. Indeed, sympatric differentiation of vaginal length was only significant in the species with the smaller vagina, in which the vagina (and consequently the penis of conspecific males) becomes smaller in sympatry. Since a large penis may be harmful in a small vagina, this would suggest female resistance to drive the displacement of male genitalia. Genital displacement can also take place in harmful male structures such as in the case of the bark beetle *Aphanarthrum glabrum* (Wollaston 1860), wherein male genitalia of the subspecies *A. g. nudum* have lost the apical spines of their intromittent organs that are present in heterospecific sympatric *A. subglabrum* and conspecific allopatric populations of *A. g. glabrum* (Jordal et al. 2006).

Developmental genetics

5. What are the genes underlying the between-species divergence of genital traits?

At the end of their review of the lock-and-key hypothesis, Shapiro and Porter (1989) complained at the time poor knowledge of the genetic basis of genital development impeding a total understanding of genital evolution. Mayr (1963) postulated a major role of pleiotropy, i.e., shared genes and developmental networks between phenotypes, in the evolution of genitalia. Pleiotropy, whether developmental due to the effect of the same genetic locus, or selectional due to distinct fitness effects of a shared locus on multiple traits (Paaby and Rockman 2013), can affect genital coevolution through the correlation between either the genitalia of one sex with other non-genital traits or between the genitalia of the two sexes. At equal strengths, selection is expected to be more efficient on traits expressed in both sexes than on those expressed in a single sex, since the underlying genes of the latter can escape selection when unexpressed in the opposite sex (Parsch and Ellegren 2013), and theoretical models predict that the pace of coevolution between a male trait and a female preference depends on the degree of linkage of their underlying genes (Gavrilets 2004). The identification of the genetic basis of male and female genital divergence between closely related species may shed light on the relative role of pleiotropy in driving lock-and-key patterns and mechanical isolation.

From a developmental point of view, the relationship between male and female genitalia can be classified under two categories: sexually dimorphic and serially homologous. Sexually dimorphic genitalia are those arising from the same

embryological tissue, such as the female clitoris and the male penis in mammals both differentiating from the embryonic genital tubercle (Cohn 2011). In spotted hyenas where penis-like clitoris have evolved in females, treatment with prenatal androgens results into a size reduction of the clitoris and the penis suggesting a correlated developmental basis in these species (Drea et al. 2002). Interestingly, such a pleiotropy may be selectionally antagonistic since a small clitoris increases the female fitness by reducing the number of her still-birth progeny whereas a small penis decreases the fitness of the males by reducing the chances of insemination (Drea et al. 2002). In primates, McLean et al. (2011) showed that a deletion of a ~61-kb-long non-coding DNA segment containing a highly conserved *cis*-regulatory element of the androgen receptor (*AR*) gene in the human lineage was associated with the loss of the vibrissae (presumably in both sexes) and to the loss of keratinized penile spines in men. Although the correlation with the loss of the vibrissae may support a role of pleiotropy with a non-genital trait, the chimpanzee's clitoris is a smooth body with no spines (Hill 1951). McLean et al. (2011) did not report effects of the loss of the *AR* element on the female clitoris, and this loss thus may not have played a role on genital coevolution between humans and chimpanzees.

Male and female genitalia are serially homologous in arthropods since they are appendages arising from different body segments. This gives an important role in the early patterning homeotic genes in driving or constraining the coevolution between the sexes. In spiders, for example, the male pedipalp is carried on the prosoma or cephalothorax part of the body the identity of which is determined by the expression of the anterior Hox gene *lab* (Damen et al. 1998). The female epigynum originates from the second segment of the opisthosoma or abdomen whose identity is determined by the posterior Hox gene *Abd-B* (Damen and Tautz 1999). In insects on the other hand, both male and female genitalia arise from adjacent abdominal segments. At early developmental stages, genital discs of both sexes share several appendage-patterning cascades (e.g., *Abd-A*, *Abd-B*, *Dll*, *hth*, *dac*) (Foronda et al. 2006; Gorfinkiel et al. 1999; Keisman and Baker 2001; Macagno and Moczek 2015; Sánchez and Guerrero 2001; but see Aspiras et al. (2011) for non-appendicular origin of male genitalia in *Tribolium* beetles). However, the number of genes that are differentially expressed increases at later stages (Chatterjee et al. 2011). This differential sharing of genes involved in genitalia development may partly explain the correlated response to experimental selection between male and female structures in dung beetles (Simmons and Garcia-Gonzalez 2011) and the partial overlap of quantitative trait loci (QTL) underlying male and female genital divergence between two *Carabus* beetles with a lock-and-key mode of isolation (Sasabe et al. 2010). In *Drosophila*, QTL analyses of genital divergence between closely related species have focused on male structures (Liu

et al. 1996; Macdonald and Goldstein 1999; McNeil et al. 2011; Peluffo et al. 2015; Schäfer et al. 2011; Takahara and Takahashi 2015; Tanaka et al. 2015; True et al. 1997; Zeng et al. 2000), but the genetic basis of the corresponding female structures remain unclear.

6. What are the genes underlying the within-species robustness of genital traits?

The idea that pleiotropy can disturb the developmental stability of genital traits leading to the appearance of new phenotypes has recently been illustrated in the model nematode *Caenorhabditis elegans* (Maupas 1900). The vulvae of the females or hermaphrodites develop from a row of three larval cells. A non-synonymous allele of the acetyltransferase gene *nath10*, that has probably recently evolved in laboratory strains, perturbs the robustness of vulval development by altering the number of larval precursor cells when the genetic system is disturbed by some mutations but not in the wild type (Duveau and Félix 2012). The allele was shown to confer higher fertility to laboratory strains over natural strains under laboratory conditions. Cell fate, pleiotropic relationships, and function of the different structures of the male copulatory apparatus of *C. elegans* have been determined using advanced methods such as laser ablation and mutagenesis as early as the 1980s (Hodgkin 1983), and evidence for copulation asymmetry between closely related species was demonstrated (Garcia et al. 2007). This may make *Caenorhabditis* one of the most-suitable model candidates to provide a complete picture of the developmental and evolutionary relationships between male and female genitalia.

Conclusion: the extension of integrative taxonomy

The objective of this article was to define a number of evolutionary biology and genetics questions to which systematists can make significant contribution. The relationship between DNA and morphology in integrative taxonomy should not be limited to strict comparisons testing the congruence (or lack of thereof) between the two sources of data in delimiting taxonomic groups. Causal explanations can be achieved if the two disciplines of systematics and evolutionary genetics are integrated to answer questions of morphological evolution, with genitalia being one of the most likely traits to find a common ground between the two disciplines. Systematists are usually well-trained in morphology and are more familiar with the use of large museum collections allowing better appreciation of the geographical variation in genitalia. Recent years have also seen a new generation of systematists who are also at ease with the use of molecular markers, population genetics principles, and whole-genome sequences in addressing taxonomy question. An extension of these skills to the realm of

experimental and evolutionary developmental genetics studies would certainly be a very promising approach, taking integrative taxonomy a step further toward explaining biological diversity.

Acknowledgments I am much grateful to an anonymous reviewer, Virginie Orgogozo, and Jean R. David for their constructive criticisms on the manuscript.

Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

References

- Amqvist, G., & Rowe, L. (2002). Correlated evolution of male and female morphologies in water striders. *Evolution*, *56*(5), 936–947.
- Amqvist, G., & Rowe, L. (2005). *Sexual conflict*. Princeton University Press.
- Aspiras, A. C., Smith, F. W., & Angelini, D. R. (2011). Sex-specific gene interactions in the patterning of insect genitalia. *Developmental Biology*, *360*(2), 369–380.
- Baer, B., & Boomsma, J. J. (2006). Mating biology of the leaf-cutting ants *Atta colombica* and *A. cephalotes*. *Journal of Morphology*, *267*(10), 1165–1171.
- Bargielowski, I. E., Lounibos, L. P., & Carrasquilla, M. C. (2013). Evolution of resistance to satyriization through reproductive character displacement in populations of invasive dengue vectors. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(8), 2888–2892.
- Brennan, P. L. R., Prum, R. O., McCracken, K. G., Sorenson, M. D., Wilson, R. E., & Birkhead, T. R. (2007). Coevolution of male and female genital morphology in waterfowl. *PLoS ONE*, *2*(5), e418.
- Burla, H. (1954). Zur Kenntnis der Drosophiliden der Elfenbeinküste Französisch West-Afrika. *Revue Suisse de Zoologie*, *61*(suppl), 1–218.
- Cayetano, L., Maklavov, A. A., Brooks, R. C., & Bonduriansky, R. (2011). Evolution of male and female genitalia following release from sexual selection. *Evolution*, *65*(8), 2171–2183.
- Chatterjee, S. S., Uppendahl, L. D., Chowdhury, M. A., Ip, P.-L., & Siegal, M. L. (2011). The female-specific *doublesex* isoform regulates pleiotropic transcription factors to pattern genital development in *Drosophila*. *Development*, *138*(6), 1099–1109.
- Cohn, M. J. (2011). Development of the external genitalia: conserved and divergent mechanisms of appendage patterning. *Developmental Dynamics*, *240*(5), 1108–1115.
- Coyne, J. A., & Orr, H. A. (2004). *Speciation*. Sinauer Associates, Incorporated Publishers.
- Damen, W. G., & Tautz, D. (1999). *Abdominal-B* expression in a spider suggests a general role for *Abdominal-B* in specifying the genital structure. *The Journal of Experimental Zoology*, *285*(1), 85–91.
- Damen, W. G. M., Hausdorf, M., Seyfarth, E.-A., & Tautz, D. (1998). A conserved mode of head segmentation in arthropods revealed by the expression pattern of Hox genes in a spider. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(18), 10665–10670.
- Dayrat, B. (2005). Towards integrative taxonomy. *Biological Journal of the Linnean Society*, *85*(3), 407–415.
- Drea, C. M., Place, N. J., Weldele, M. L., Coscia, E. M., Licht, P., & Glickman, S. E. (2002). Exposure to naturally circulating androgens during foetal life incurs direct reproductive costs in female spotted hyenas, but is prerequisite for male mating. *Proceedings of the Royal Society B: Biological Sciences*, *269*(1504), 1981–1987.
- Dufour, L. (1844). Anatomie générale des Diptères. *Annales des Sciences Naturelles*, *1*, 224–264.
- Dunkle, S. W. (1991). Head damage from mating attempts in dragonflies (Odonata: Anisoptera). *Entomological News*, *102*(1), 37–41.
- Duveau, F., & Félix, M.-A. (2012). Role of pleiotropy in the evolution of a cryptic developmental variation in *Caenorhabditis elegans*. *PLoS Biology*, *10*(1), e1001230.
- Eberhard, W. G. (1985). *Sexual selection and Animal Genitalia*. Harvard University Press.
- Eberhard, W. G. (2004). Rapid divergent evolution of sexual morphology: comparative tests of antagonistic coevolution and traditional female choice. *Evolution*, *58*(9), 1947–1970.
- Eberhard, W. G. (2010a). Evolution of genitalia: theories, evidence, and new directions. *Genetica*, *138*(1), 5–18.
- Eberhard, W. G. (2010b). Rapid divergent evolution of genitalia: theory and data updated. In J. Leonard & A. Cordoba-Aguilar (Eds.), *The evolution of primary sexual characters in animals* (pp. 40–78). Oxford: Oxford University Press.
- Eberhard, W. G., & Huber, B. A. (2010). Spider genitalia: precise maneuvers with a numb structure in a complex lock. In J. Leonard & A. Cordoba-Aguilar (Eds.), *The evolution of primary sexual characters in animals* (pp. 249–284). Oxford: Oxford University Press.
- Eberhard, W., & Ramirez, N. (2004). Functional morphology of the male genitalia of four species of *Drosophila*: failure to confirm both lock and key and male–female conflict. *Annals of the Entomological Society of America*, *97*(5), 1007–1017.
- Evans, J. P., Gasparini, C., Holwell, G. I., Ramnarine, I. W., Pitcher, T. E., & Pilastro, A. (2011). Intraspecific evidence from guppies for correlated patterns of male and female genital trait diversification. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1718), 2611–2620.
- Foronda, D., Estrada, B., de Navas, L., & Sánchez-Herrero, E. (2006). Requirement of *Abdominal-A* and *Abdominal-B* in the developing genitalia of *Drosophila* breaks the posterior downregulation rule. *Development*, *133*(1), 117–127.
- García, L. R., LeBoeuf, B., & Koo, P. (2007). Diversity in mating behavior of hermaphroditic and male–female *Caenorhabditis* nematodes. *Genetics*, *175*(4), 1761–1771.
- Gavrilets, S. (2004). *Fitness landscapes and the origin of species*. Princeton, N.J.: Princeton University Press.
- Gompert, Z., Nice, C. C., Fordyce, J. A., Forister, M. L., & Shapiro, A. M. (2006). Identifying units for conservation using molecular systematics: the cautionary tale of the Kameron blue butterfly. *Molecular Ecology*, *15*(7), 1759–1768.
- Good, J. M., Demboski, J. R., Nagorsen, D. W., & Sullivan, J. (2003). Phylogeography and introgressive hybridization: chipmunks (genus *Tamias*) in the northern Rocky Mountains. *Evolution*, *57*(8), 1900–1916.
- Gorfinkiel, N., Sánchez, L., & Guerrero, I. (1999). *Drosophila* terminalia as an appendage-like structure. *Mechanisms of Development*, *86*(1–2), 113–123.
- Hasselquist, F. (1762). *Reise nach Palästina in den Jahren 1749 bis 1752*: Rostock.
- Heldsingen, P. J. V. (1969). *A reclassification of the species of Linyphia Latreille based on the functioning of the genitalia (Araneida, Linyphiidae)*. Brill Archive.
- Hill, W. C. O. (1951). The external genitalia of the female chimpanzee; with observations on the mammary apparatus. *Proceedings of the Zoological Society of London*, *121*(1), 133–145.
- Hodgkin, J. (1983). Male phenotypes and mating efficiency in *Caenorhabditis elegans*. *Genetics*, *103*(1), 43–64.

- Hollander, J., Smadja, C. M., Butlin, R. K., & Reid, D. G. (2013). Genital divergence in sympatric sister snails. *Journal of Evolutionary Biology*, 26(1), 210–215.
- Horton, D. R., & Lewis, T. M. (2011). Variation in male and female genitalia among ten species of North American *Anthocoris* (Hemiptera: Heteroptera: Anthocoridae). *Annals of the Entomological Society of America*, 104(6), 1260.
- Israelson, G. (1972). Male copulatory organs of Macaronesian species of *Aphanarthrum* Wollaston. With designations of lectotypes and descriptions of new taxa (Col. Scolytidae). *Insect Systematics and Evolution*, 3(4), 17–257.
- Jagadeeshan, S., & Singh, R. S. (2006). A time-sequence functional analysis of mating behaviour and genital coupling in *Drosophila*: role of cryptic female choice and male sex-drive in the evolution of male genitalia. *Journal of Evolutionary Biology*, 19(4), 1058–1070.
- Johnson, C. (1972). Tandem linkage, sperm translocation, and copulation in the dragonfly, *Hagenius brevistylus* (Odonata: Gomphidae). *American Midland Naturalist*, 88(1), 131–149.
- Jordal, B. H., Emerson, B. C., & Hewitt, G. M. (2006). Apparent “sympatric” speciation in ecologically similar herbivorous beetles facilitated by multiple colonizations of an island. *Molecular Ecology*, 15(10), 2935–2947.
- Kameda, Y., Kawakita, A., & Kato, M. (2009). Reproductive character displacement in genital morphology in *Satsuma* land snails. *The American Naturalist*, 173(5), 689–697.
- Kamimura, Y. (2007). Twin intromittent organs of *Drosophila* for traumatic insemination. *Biology Letters*, 3(4), 401–404.
- Kamimura, Y., & Mitumoto, H. (2011). Comparative copulation anatomy of the *Drosophila melanogaster* species complex (Diptera: Drosophilidae). *Entomological Science*, 14(4), 399–410.
- Kamimura, Y., & Mitumoto, H. (2012). Lock-and-key structural isolation between sibling *Drosophila* species. *Entomological Science*, 15(2), 197–201.
- Kawakami, Y., & Tatsuta, H. (2010). Variation in the shape of genital appendages along a transect through sympatric and allopatric areas of two brachypterous grasshoppers, *Parapodisma setouchiensis* and *Parapodisma subastris* (Orthoptera: Podisminae). *Annals of the Entomological Society of America*, 103(3), 327–331.
- Kawano, K. (2002). Character displacement in giant rhinoceros beetles. *The American Naturalist*, 159(3), 255–271.
- Kawano, K. (2003). Character displacement in stag beetles (Coleoptera: Lucanidae). *Annals of the Entomological Society of America*, 96(4), 503–511.
- Keisman, E. L., & Baker, B. S. (2001). The *Drosophila* sex determination hierarchy modulates *wingless* and *decapentaplegic* signaling to deploy dachshund sex-specifically in the genital imaginal disc. *Development*, 128(9), 1643–1656.
- Khila, A., Abouheif, E., & Rowe, L. (2012). Function, developmental genetics, and fitness consequences of a sexually antagonistic trait. *Science*, 336(6081), 585–589.
- Koene, J. M., & Schulenburg, H. (2005). Shooting darts: co-evolution and counter-adaptation in hermaphroditic snails. *BMC Evolutionary Biology*, 5(1), 25.
- Liu, J., Mercer, J. M., Stam, L. F., Gibson, G. C., Zeng, Z. B., & Laurie, C. C. (1996). Genetic analysis of a morphological shape difference in the male genitalia of *Drosophila simulans* and *D. mauritiana*. *Genetics*, 142(4), 1129–1145.
- Lucas, L. K., Fordyce, J. A., & Nice, C. C. (2008). Patterns of genitalic morphology around suture zones in North American *Lycaeides* (Lepidoptera: Lycaenidae): implications for taxonomy and historical biogeography. *Annals of the Entomological Society of America*, 101(1), 172–180.
- Macagno, A. L. M., & Moczek, A. P. (2015). Appendage-patterning genes regulate male and female copulatory structures in horned beetles. *Evolution & Development*, 17(4), 248–253.
- Macdonald, S. J., & Goldstein, D. B. (1999). A quantitative genetic analysis of male sexual traits distinguishing the sibling species *Drosophila simulans* and *D. sechellia*. *Genetics*, 153(4), 1683–1699.
- Masly, J. P. (2012). 170 years of “lock-and-key”: genital morphology and reproductive isolation. *International Journal of Evolutionary Biology*, 2012, 1–10.
- Matute, D. R., & Coyne, J. A. (2010). Intrinsic reproductive isolation between two sister species of *Drosophila*. *Evolution*, 64(4), 903–920.
- Maupas, E. (1900). Modes et formes de reproduction des nématodes. *Archives de Zoologie Expérimentale et Générale*, 8, 463–624.
- Mayr, E. (1963). *Animal species and evolution*. Cambridge: Belknap Press of Harvard University Press.
- McLean, C. Y., Reno, P. L., Pollen, A. A., Bassan, A. I., Capellini, T. D., Guenther, C., et al. (2011). Human-specific loss of regulatory DNA and the evolution of human-specific traits. *Nature*, 471(7337), 216–219.
- McNeil, C. L., Bain, C. L., & Macdonald, S. J. (2011). Multiple quantitative trait loci influence the shape of a male-specific genital structure in *Drosophila melanogaster*. *G3: Genes, Genomes, Genetics*, 1(5), 343–351.
- McPeck, M. A., Shen, L., & Farid, H. (2009). The correlated evolution of three-dimensional reproductive structures between male and female damselflies. *Evolution*, 63(1), 73–83.
- Meigen, J. W. (1830). *Systematische Beschreibung der bekannten Europäischen zweiflügeligen Insekten*. Schulze.
- Minelli, A. (2015). Biological systematics in the Evo-Devo era. *European Journal of Taxonomy*, 125, 1–23.
- Nagata, N., Kubota, K., Yahiro, K., & Sota, T. (2007). Mechanical barriers to introgressive hybridization revealed by mitochondrial introgression patterns in *Ohomopterus* ground beetle assemblages. *Molecular Ecology*, 16(22), 4822–4836.
- Nunes, M. D. S., Orozco-Ter Wengel, P., Kreissl, M., & Schlotterer, C. (2010). Multiple hybridization events between *Drosophila simulans* and *Drosophila mauritiana* are supported by mtDNA introgression. *Molecular Ecology*, 19(21), 4695–4707.
- Paaby, A. B., & Rockman, M. V. (2013). The many faces of pleiotropy. *Trends in Genetics*, 29(2), 66–73.
- Parsch, J., & Ellegren, H. (2013). The evolutionary causes and consequences of sex-biased gene expression. *Nature Reviews Genetics*, 14(2), 83–87.
- Patterson, B. D., & Thaler, C. S. (1982). The mammalian baculum: hypotheses on the nature of bacular variability. *Journal of Mammalogy*, 63(1), 1–15.
- Peluffo, A. E., Nuez, I., Debat, V., Savisaar, R., Stern, D. L., & Orgogozo, V. (2015). A major locus controls a genital shape difference involved in reproductive isolation between *Drosophila yakuba* and *Drosophila santomea*. *G3: Genes|Genomes|Genetics*, g3.115.023481.
- Peretti, A. V. (2003). Functional morphology of spermatophores and female genitalia in bothriurid scorpions: genital courtship, coercion and other possible mechanisms. *Journal of Zoology*, 261, 135–153.
- Pitnick, S., Markow, T., & Spicer, G. S. (1999). Evolution of multiple kinds of female sperm-storage organs in *Drosophila*. *Evolution*, 53(6), 1804–1822.
- Pratt, H. L. (1979). Reproduction in the blue shark *Prionace glauca*. *Fishery Bulletin*, 77, 445–470.
- Robertson, H. M., & Paterson, H. E. H. (1982). Mate recognition and mechanical isolation in *Enallagma* damselflies (Odonata: Coenagrionidae). *Evolution*, 36(2), 243–250.
- Robson, G. C., & Richards, O. W. (1936). *The Variation of Animals in Nature*. London, New York [etc.] Longmans, Green and Co.
- Rönn, J., Katvala, M., & Arnqvist, G. (2007). Coevolution between harmful male genitalia and female resistance in seed beetles.

- Proceedings of the National Academy of Sciences of the United States of America*, 104(26), 10921–10925.
- Ryckman, R. E., & Ueshima, N. (1964). Biosystematics of the *Hesperocimex* complex (Hemiptera: Cimicidae) and avian hosts (Piciformes: Picidae; Passeriformes: Hirundinidae). *Annals of the Entomological Society of America*, 57, 624–638.
- Sánchez, L., & Guerrero, I. (2001). The development of the *Drosophila* genital disc. *BioEssays*, 23(8), 698–707.
- Sánchez, V., Hernández-Baños, B. E., & Cordero, C. (2011). The evolution of a female genital trait widely distributed in the Lepidoptera: comparative evidence for an effect of sexual coevolution. *PLoS ONE*, 6(8), e22642.
- Sasabe, M., Takami, Y., & Sota, T. (2010). QTL for the species-specific male and female genital morphologies in Ohomopterus ground beetles. *Molecular Ecology*, 19(23), 5231–5239.
- Sauer, J., & Hausdorf, B. (2009). Sexual selection is involved in speciation in a land snail radiation on crete. *Evolution*, 63(10), 2535–2546.
- Schäfer, M. A., Routtu, J., Vieira, J., Hoikkala, A., Ritchie, M. G., & Schlötterer, C. (2011). Multiple quantitative trait loci influence intra-specific variation in genital morphology between phylogenetically distinct lines of *Drosophila montana*. *Journal of Evolutionary Biology*, 24(9), 1879–1886.
- Schlick-Steiner, B. C., Steiner, F. M., Seifert, B., Stauffer, C., Christian, E., & Crozier, R. H. (2010). Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology*, 55(1), 421–438.
- Shapiro, A. M., & Porter, A. H. (1989). The lock-and-key hypothesis: evolutionary and biosystematic interpretation of insect genitalia. *Annual Review of Entomology*, 34(1), 231–245.
- Simmons, L. W., & Garcia-Gonzalez, F. (2011). Experimental coevolution of male and female genital morphology. *Nature Communications*, 2, 374.
- Siva-Jothy, M. T. (2006). Trauma, disease and collateral damage: conflict in cimicids. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, 361(1466), 269–275.
- Skuse, F. A. A. (1894). The banded mosquito of Bengal. *Indian Museum Notes*, 3(5), 20.
- Sota, T. (2002). Radiation and reticulation: extensive introgressive hybridization in the carabid beetles *Ohomopterus* inferred from mitochondrial gene genealogy. *Population Ecology*, 44(3), 145–156.
- Sota, T., & Kubota, K. (1998). Genital lock-and-key as a selective agent against hybridization. *Evolution*, 52(5), 1507–1513.
- Standfuss, M. R. (1896). *Handbuch der paläarktischen Gross-Schmetterlinge für Forscher und Sammler*. Jena: G. Fischer.
- Sturtevant, A. H. (1919). A new species closely resembling *Drosophila melanogaster*. *Psyche*, 26(6), 153–155.
- Takahara, B., & Takahashi, K. H. (2015). Genome-wide association study on male genital shape and size in *Drosophila melanogaster*. *PLoS ONE*, 10(7), e0132846.
- Takami, Y., Nagata, N., Sasabe, M., & Sota, T. (2007). Asymmetry in reproductive isolation and its effect on directional mitochondrial introgression in the parapatric ground beetles *Carabus yamato* and *C. albrecthi*. *Population Ecology*, 49(4), 337–346.
- Tanabe, T., & Sota, T. (2008). Complex copulatory behavior and the proximate effect of genital and body size differences on mechanical reproductive isolation in the millipede genus *Parafontaria*. *The American Naturalist*, 171(5), 692–699.
- Tanaka, K. M., Hopfen, C., Herbert, M. R., Schlötterer, C., Stern, D. L., Masly, J. P., et al. (2015). Genetic architecture and functional characterization of genes underlying the rapid diversification of male external genitalia between *Drosophila simulans* and *Drosophila mauritiana*. *Genetics*, 200(1), 357–369.
- Tatarnic, N. J., & Cassis, G. (2010). Sexual coevolution in the traumatically inseminating plant bug genus *Coridromius*. *Journal of Evolutionary Biology*, 23(6), 1321–1326.
- Toews, D. P. L., & Brelsford, A. (2012). The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, 21(16), 3907–3930.
- True, J. R., Liu, J., Stam, L. F., Zeng, Z.-B., & Laurie, C. C. (1997). Quantitative genetic analysis of divergence in male secondary sexual traits between *Drosophila simulans* and *Drosophila mauritiana*. *Evolution*, 51(3), 816–832.
- Tsacas, L., & Lachaise, D. (1974). Quatre nouvelles espèces de la Côte-d'Ivoire du genre *Drosophila*, groupe *melanogaster*, et discussion de l'origine du sous-groupe *melanogaster* (Diptera: Drosophilidae). *Annales de l'Université d'Abidjan*, 7, 193–211.
- Uhl, G., Nessler, S. H., & Schneider, J. (2007). Copulatory mechanism in a sexually cannibalistic spider with genital mutilation (Araneae: Araneidae: *Argiope bruennichi*). *Zoology*, 110(5), 398–408.
- Valdés, A. (2004). Morphology of the penial hooks and vaginal cuticular lining of some dorid nudibranchs (Mollusca, Opisthobranchia). *American Malacological Bulletin*, 18, 49–54.
- Vanderplank, F. L. (1948). Experiments in crossbreeding tsetse-flies, *Glossina* species. *Annals of Tropical Medicine and Parasitology*, 42(2), 131–152.
- Veuille, M. (1978). Biologie de la reproduction chez *Jaera* (Isopode Asellote) II. Evolution des organes reproducteurs femelles. *Cahiers de Biologie Marine*, 19, 385–395.
- Ware, A. D., & Opell, B. D. (1989). A test of the mechanical isolation hypothesis in two similar spider species. *Journal of Arachnology*, 17(2), 149–162.
- Wiens, J. J. (2001). Widespread loss of sexually selected traits: how the peacock lost its spots. *Trends in Ecology & Evolution*, 16(9), 517–523.
- Will, K. W., Mishler, B. D., & Wheeler, Q. D. (2005). The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology*, 54(5), 844–851.
- Wollaston, T. V. (1860). On additions to the Madeiran Coleoptera. *Annals and Magazine of Natural History*, 3(5), 358–365.
- Yassin, A., & David, J. R. (2016). Within-species reproductive costs affect the asymmetry of satyrization in *Drosophila*. *Journal of Evolutionary Biology*, 29(2), 455–460.
- Yassin, A., & Orgogozo, V. (2013). Coevolution between male and female genitalia in the *Drosophila melanogaster* species subgroup. *PLoS ONE*, 8(2), e57158.
- Zeng, Z.-B., Liu, J., Stam, L. F., Kao, C.-H., Mercer, J. M., & Laurie, C. C. (2000). Genetic architecture of a morphological shape difference between two *Drosophila* species. *Genetics*, 154(1), 299–310.