



SYMPOSIUM

Causes and Consequences of Genital Evolution

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Synopsis The study of genital diversity has experienced rapidly burgeoning attention over the past few decades. This research has shown that male genitalia in internally fertilizing animals exhibit remarkably rapid and complex evolution. In recent years, a consensus has emerged that sexual selection is responsible for much of the observed genital diversity, with natural selection largely playing a subsidiary role. Despite enhanced understanding of the key proximate forms of selection responsible for genital evolution, we still have a poor grasp of the broader, ultimate causes and consequences of the striking diversity of genitalia. Here, we highlight three topics that have so far received comparatively little attention and yet could prove critically important. First, we encourage investigation of ecology’s direct and indirect roles in genital diversification, as ecological variation can influence selection on genitalia in several ways, perhaps especially by influencing the context of sexual selection. Second, we need more research into the effects of genital divergence on speciation, as genital differences could enhance reproductive isolation through either a lock-and-key process (where selection directly favors reproductive isolation) or as an incidental by-product of divergence. Third, we echo recent calls for increased research on female genitalia, as non-trivial female genital diversity exists, and multiple mechanisms can lead to rapid diversification of female genitalia. For all three topics, we review theory and empirical data, and describe specific research approaches for tackling these questions. We hope this work provides a roadmap toward increased understanding of the causes and consequences of the conspicuous diversity of primary sexual traits, and thus toward new insights into the evolution of complex traits and the phenotypic causes of speciation.

Introduction

Male genitalia in animals with internal fertilization are often highly complex in form and function and typically exhibit marked variation even among closely related species (Eberhard 1985, 1996; Edwards 1993; Birkhead 2000; Hosken and Stockley 2004). This widespread pattern of rapid genital divergence represents a longstanding puzzle in evolutionary biology, demanding an explanation for why genitalia should evolve more quickly than virtually any other trait. The past few decades have witnessed considerable effort toward elucidating the forms of selection responsible for this phenomenon (Fig. 1). Recently, a consensus has emerged that sexual selection represents a primary driver of genital evolution (Eberhard 1985, 1996, 2010, 2011; Arnqvist 1998;

Hosken and Stockley 2004; Simmons 2014). In particular, postmating sexual selection appears largely responsible for genital divergence (Eberhard 1985, 1996, 1998, 2001; Arnqvist 1998; Otronen 1998; Arnqvist and Danielsson 1999; Tadler 1999); although premating sexual selection, as well as natural selection, have some empirical support as well (Preziosi and Fairbairn 1996; Arnqvist and Rowe 2002; Bertin and Fairbairn 2005; Langerhans et al. 2005; Neufeld and Palmer 2008; Kahn et al. 2010; Reinhardt 2010; Langerhans 2011; Mautz et al. 2013). After first pointing to sexual selection as a major driver of genital diversity, this work shifted to testing the relative importance of alternative forms of sexual selection (e.g., mate choice, cryptic female choice, sperm competition, sexual conflict).

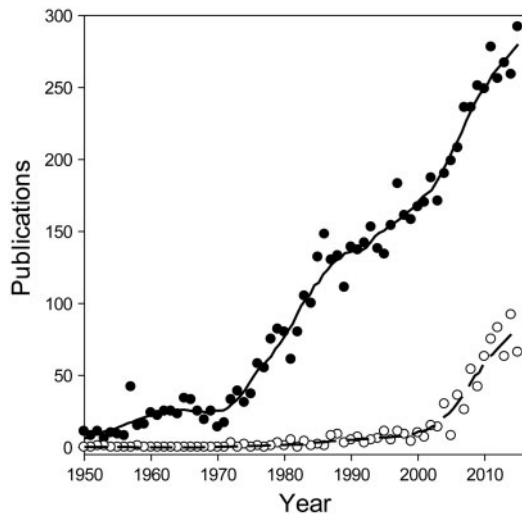


Fig. 1 Numbers of papers published on the topic of genital evolution during the past 65 years (solid symbols, solid line), with accompanying papers published on table tennis as a reference regarding overall increase in publications over time (open symbols, dashed line). Data from Web of Science searches of “genital* evolution” and “table tennis” conducted in July 2016.

This narrow focus on proximate mechanisms of sexual selection has proven useful, for instance, highlighting the importance of previously underappreciated evolutionary mechanisms such as cryptic female choice; yet this may come at the cost of careful consideration of the broader, ultimate causes and consequences of genital evolution (Reinhardt 2010; Langerhans 2011; Masly 2012).

When placed in a larger context, the phenomenon of rapid and divergent evolution of male genitalia presents a greater diversity of questions than simply what proximate forms of selection are responsible. First, considering the ample evidence for sexual selection’s role in genital evolution, combined with at least occasional importance of natural selection, we should more carefully question why these forms of selection should so frequently drive divergence across populations or species, rather than simply resulting in a single optimal morphology. This could arise from arbitrary preferences or mutation-order processes, but it could also arise from environmental heterogeneity, as ecological differences across space and time—e.g., variation in predation risk, parasite community, structural habitat, resource availability, climate—can not only alter natural selection, but also commonly alter the context of sexual selection in diverse taxa (Emlen and Oring 1977; Rowe et al. 1994; Zuk and Kolluru 1998; Grether et al. 1999; Candolin et al. 2007; Schwartz and Hendry 2007; Cornwallis and Uller 2010; Botero and Rubenstein

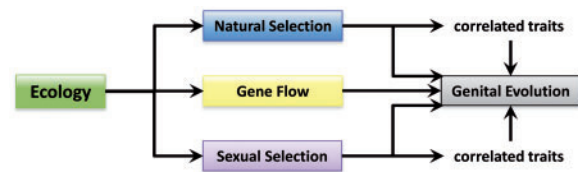


Fig. 2 Illustration of the four major ways that ecological variation can drive genital evolution: (1) directly altering natural selection on genitalia, (2) altering the context of sexual selection on genitalia, (3) altering selection on traits correlated with genitalia, and (4) driving divergent adaptation that results in reduced gene flow, indirectly facilitating genital divergence.

2012; Scordato et al. 2012). Recent studies have demonstrated that ecological variation can indeed play an important role in driving genital divergence (Cayetano et al. 2011; Evans et al. 2011; Heinen-Kay and Langerhans 2013; Heinen-Kay et al. 2014; Anderson and Langerhans 2015). Second, evolutionary changes in genital morphology can affect reproductive compatibility among populations, leading to reproductive isolation and speciation. Yet, so far we have a very limited understanding of how genital evolution might influence speciation. Finally, given the observed variation in male genital morphology, it is surprising how little attention female genital diversity has received. Recent work has revealed substantial variation in female genital morphology, and pointed to a number of reasons that female genitalia might experience rapid evolution and how understanding genital evolution may often require understanding variation in both sexes (Arnqvist and Rowe 2005; Rönn et al. 2007; Ah-King et al. 2014; Simmons 2014; Anderson and Langerhans 2015; Filippov et al. 2015). Here we discuss how further research into these three topics could uncover important insights into the causes and consequences of genital evolution.

Ecology and genital evolution

Ecology—broadly defined as the interactions between organisms and their environment—unequivocally plays a major role in promoting phenotypic diversification and speciation. Although receiving minimal research to date, ecology may influence genital evolution through four major routes: (1) eliciting direct natural selection on genital morphology, (2) influencing sexual selection on genitalia, (3) causing selection on traits genetically correlated with genitalia, and (4) exerting effects on gene flow among populations (Fig. 2). For instance, ecological agents can induce natural selection on genitalia through parasites or disease (Reinhardt 2010), or by the effects of

Table 1 Examples of ecological variation altering the context of sexual selection, leading to divergence in sexually selected traits

Taxon	Environmental factor	Altered context of sexual selection	Divergent traits	References
Field cricket, <i>Teleogryllus oceanicus</i>	Parasitoid	Mating behavior	Calling song	Zuk et al. (1993)
Firefly, <i>Photinus collustrans</i>	Predation	Mating behavior	Copulation duration	Wing (1988)
Blackeye goby, <i>Coryphopterus nicholsi</i>	Resource competition	Male-male competition	Body size, aggression	Breitburg (1987)
Guppy, <i>Poecilia reticulata</i>	Light intensity	Mate choice	Male coloration	Endler (1987)
Guppy, <i>P. reticulata</i>	Predation	Mate choice, mating behavior	Male coloration, courtship frequency, size and shape of male genitalia	Endler (1980), Stoner and Breden (1988), Kelly et al. (2000), Evans et al. (2011)
Bahamas mosquitofish, <i>Gambusia hubbsi</i>	Predation	Mate choice, mating behavior	Male coloration, copulation frequency, size and shape of male and female genitalia	Langerhans et al. (2005), Heinen et al. (2013), Heinen-Kay and Langerhans (2013), Martin et al. (2014), Anderson and Langerhans (2015)
Eastern newt, <i>Notophthalmus viridescens</i>	Anthropogenic pollution	Mate choice, mating behavior	Pheromone communication	Park et al. (2001), Park and Propper (2002)
Bark anole, <i>Anolis distichus</i>	Precipitation/temperature	Mate preference	Dewlap morphology	Ng et al. (2013)
Crested anole, <i>Anolis cristatellus</i>	Precipitation/temperature	Mate preference	Dewlap morphology	Leal and Fleishman (2004)
Hume's warbler, <i>Phylloscopus humei</i>	Climate	Mate choice	Timing of copulation, expression of sexually selected traits (e.g. wing bar size)	Scordato et al. (2012)

genitalia on whole-organism performance attributes such as locomotion (Langerhans et al. 2005). Ecological variation can alter the context of sexual selection, for example, through the impacts of predation risk on mating systems (Heinen-Kay and Langerhans 2013). With the ubiquity of ecologically-driven selection on morphological traits (Kingsolver et al. 2001; Kingsolver and Pfennig 2007), this could prove important for genital evolution if genitalia exhibit strong genetic correlations with other traits that experience divergent selection. Additionally, any time ecologically-based divergent selection increases isolation between populations (e.g., ecological speciation), this reduced gene flow can enhance the rate of divergent evolution of genitalia. These four mechanisms are not mutually exclusive, and indeed multiple mechanisms might often operate simultaneously. Moreover, while divergent selection between dissimilar environments can obviously lead to divergent evolution, even similar selection in similar environments can lead to divergent genital evolution (Langerhans and Riesch 2013). That is, different populations might find different evolutionary solutions to the same selective problem. While still in early days, research so far suggests that ecology's effects on natural and sexual selection are more important than the latter two mechanisms. Owing to the widespread evidence for the role of sexual selection in genital evolution, it seems likely that ecology's indirect influence on genital divergence, by altering the context of sexual selection, could prove especially important. Thus, we concentrate on this particular route here.

Ecological variation commonly changes the context of sexual selection across environments, often resulting in divergence in sexually selected traits (Table 1). One way this can occur is by altering the cost-benefit balance for traits subject to tradeoffs between natural selection and premating sexual selection. This has been widely demonstrated for conspicuous sexual ornaments such as coloration, exaggerated morphological traits, acoustic signals, and courtship displays across diverse taxa in response to numerous ecological agents (e.g., Table 1). For instance, variation in predation risk may alter the strength and form of both natural selection against elaborate sexual traits and premating sexual selection favoring elaboration of the traits. For genitalia subject to such forms of selection, such as non-retractable genitalia that serve as sexual signals, a tradeoff between natural selection and premating sexual selection may generate rapid genital divergence. For example, male poeciliid fishes possess a large, non-retractable sperm-transfer organ which serves as a

pre-mating sexual signal in multiple species (Brooks and Caithness 1995; Langerhans et al. 2005; Kahn et al. 2010), experiences strong natural selection via its effects on locomotor performance (Langerhans et al. 2005; Langerhans 2011), and has repeatedly diverged in size between predatory environments (Kelly et al. 2000; Jennions and Kelly 2002; Langerhans et al. 2005). While small, retractable genitalia might not typically evolve via this mechanism, large, non-retractable genitalia might. With the added constraints on some genitalia, such as functional constraints for effective insemination and developmental constraints arising from developmental programs (e.g., gonopodia in poeciliid fish are modified anal fins), many organisms might be incapable of evolving solutions that “break free” from such tradeoffs. Thus, ecology’s role in genital divergence through the generation of tradeoffs between natural selection and pre-mating sexual selection deserves more attention in taxa with appropriate genitalia.

Ecology can also alter the context of post-mating sexual selection, a known major driver of genital divergence. For instance, ecological agents, such as predation risk or resource availability, can affect the opportunity for post-mating sexual selection by influencing the frequency of multiple mating of females, frequency or duration of courtship behaviors, frequency of coercive mating, or strength of sexual conflict (Wing 1988; Lima and Dill 1990; Endler 1991; Magnhagen 1991; Magurran and Seghers 1994; Sih 1994; Candolin 1997; Jennions and Petrie 1997). How might these ecology-driven changes in post-mating sexual selection drive genital evolution? This question has so far received very little attention (though see Evans et al. 2011; Heinen-Kay and Langerhans 2013; Anderson and Langerhans 2015), but considering that genital morphology often exhibits associations with copulation duration, sperm-transfer rate, and insemination and fertilization success, it seems logical that ecology-mediated shifts in post-mating sexual selection might often produce genital divergence. Although this notion has so far received little research, and was not even discussed in several recent reviews (Hosken and Stockley 2004; Eberhard 2010, 2011; Reinhardt 2010), we suggest this mechanism could prove widespread.

Variation in predation risk and ecological factors that affect population demographics seem particularly promising areas for future research in this area. Elevated levels of predation risk might often elicit different forms of selection on genital morphology, perhaps for mediating efficient sperm transfer and effective fertilization under scenarios of varying levels of risk. Further, changes in population

demographics, such as density or sex ratio, can commonly alter sexual selection (Clutton-Brock and Parker 1992; Kvarnemo and Ahnesjö 1996; Prohl 2002; Magurran 2005; Kokko and Rankin 2006; Knell 2009), and may thus often alter post-mating sexual selection on genitalia. In support of this notion, social structure appears to influence genital morphology, with eusociality leading to highly divergent genitalia in naked mole rats (Seney et al. 2009). More studies are needed to better understand how ecological conditions may affect the nature of post-copulatory sexual selection, and whether ecological variation may play an important role in promoting genital diversity.

Despite the scant research to date on the topic, clear evidence for a role of ecology in genital evolution exists for several taxa (e.g., Langerhans et al. 2005; Neufeld and Palmer 2008; Evans et al. 2011; Langerhans 2011; Oneal and Knowles 2013). For example, it appears that changes in sexual selection caused by variation in predation risk has led to divergent evolution of genitalia in Bahamian mosquitofish (Heinen-Kay and Langerhans 2013; Heinen-Kay et al. 2014; Anderson and Langerhans 2015); wave action influences genital morphology in barnacles (Neufeld and Palmer 2008); and Caribbean cricket genitalia have diverged between dry and wet forests (Oneal and Knowles 2013). Importantly, researchers must build on existing knowledge and conduct new tests to determine whether ecological variation has resulted in altered natural or sexual selection on genitalia. Renewed focus on ecological causes of genital diversity appear warranted, and could especially benefit from a combination of comparative approaches (comparing genitalia between environments) and functional or experimental approaches (testing how ecological factors alter selection and how genital morphology mediates fitness).

Genital evolution and speciation

Male genitalia often exhibit a high degree of species specificity, and have long represented a key trait used to distinguish between closely related, and otherwise phenotypically similar, species. Although this well-known pattern provides circumstantial evidence for a role of genital morphology in speciation, whether genital diversification plays a direct role in promoting the evolution of reproductive isolation per se remains unclear. Indeed, genital evolution comprised the first proposed mechanism of speciation (Dufour 1844), as implications of genital divergence for reproduction seemed obvious. Because genitalia are directly involved in the mating process, rapid

divergence of genital size or shape among populations could quickly enhance mechanical isolation, where males may be physically incapable of properly copulating, inseminating, or fertilizing a heterospecific female, regardless of behavioral traits. However, empirical studies to date have yielded mixed results.

Genital evolution can facilitate the evolution of reproductive isolation through two distinct pathways:

- (1) Lock-and-key mechanism: selection directly favors reproductive isolation, leading to genital divergence as a means of reducing costly inter-population mating.
- (2) Byproduct mechanism: enhanced reproductive isolation occurs as an incidental consequence of divergence in genitalia caused by mechanisms other than selection against inter-population mating.

First, the lock-and-key mechanism describes the process whereby selection against inter-population mating favors genital incompatibilities between populations, resulting in evolution of complementary genitalia in males (key) and females (lock) within populations/species (Dufour 1844; Shapiro and Porter 1989). This essentially represents the postmating analog of reinforcement, the well-studied phenomenon describing the similar evolution of premating isolation among populations (both mechanisms describe prezygotic isolation). Selection against inter-population or inter-species mating can occur for a wide variety of reasons (anything causing reduced fitness of hybrids or individuals that mate with the “wrong” form). While originally controversial, reinforcement selection has now been demonstrated in numerous cases (Littlejohn and Watson 1985; Butlin 1987; Nosil et al. 2003; Pfennig 2003; Servedio and Noor 2003; Schwartz et al. 2010). While intuitive, the lock-and-key mechanism traditionally received little convincing support despite a long history of inquiry (Eberhard 1985, 2010; Ware and Opell 1989; Porter and Shapiro 1990; Arnqvist 1998; Eberhard and Ramirez 2004). However, recent work employing comparative and functional approaches has pinpointed lock-and-key as a plausible mechanism for speciation by genital evolution (Sota and Kubota 1998; Kawano 2004; McPeck et al. 2009; Langerhans 2011; Masly 2012; Wojcieszek and Simmons 2013; Simmons 2014; Anderson and Langerhans 2015). Might this mechanism represent a common way that genital evolution influences reproductive isolation, and how might we answer this question?

Because reinforcement selection can only occur when individuals from different populations

encounter one another (e.g., sympatry or parapatry), the lock-and-key mechanism makes a specific, unique biogeographic prediction: reproductive character displacement (RCD). That is, differences in genital morphology should be greater among populations that experience higher probabilities of inter-population mating opportunities; for instance, greater genital divergence is predicted for sympatric populations compared to allopatric populations. No other mechanism of genital divergence makes this prediction. Empirical studies demonstrating RCD are numerous (Murray and Clarke 1980; Johnson 1982; Cooley et al. 2001; Geyer and Palumbi 2003), though a minority establish the presence of RCD in traits associated with postmating, prezygotic isolation, e.g., genital morphology (though see Brown and Wilson 1956; Marshall and Cooley 2000; Kawano 2002; Smith and Rausher 2008; Langerhans 2011; Anderson and Langerhans 2015). Further, few studies of RCD have uncovered the source or magnitude of reduced fitness incurred by inter-population mating, or how genital divergence might actually influence reproductive isolation. That said, patterns demonstrated in some cases so far, such as elevated divergence in both male and female genital morphology for Bahamas mosquitofish populations experiencing greater levels of gene flow (Anderson and Langerhans 2015), strongly implicate the lock-and-key mechanism as an important driver of genital evolution, with obvious implications for speciation. In light of the evidence to date, it appears that dismissal of the lock-and-key mechanism in the literature was premature, and it may prove to represent at least a semi-frequent occurrence, driving genital diversification and speciation. While comparative evidence has been accumulating, future work should center on functional investigations, revealing how interactions between male and female genitalia during copulation affect insemination and fertilization. This knowledge is critical for understanding the possible operation of the lock-and-key mechanism.

The second way that genital evolution can facilitate speciation is through the by-product mechanism, where divergence in genitalia—caused by any mechanism other than selection against inter-population mating—incidentally results in enhanced reproductive isolation between divergent populations. We already know that genitalia often evolve very rapidly, resulting in highly divergent forms among closely related species. Regardless of whether this rapid genital divergence resulted from cryptic female choice, sexual conflict, sperm competition, female mate choice, or natural selection, if genital differences affect insemination or fertilization success

Box 1 Five nonmutually exclusive mechanisms of female genital diversification

- (1) **Pleiotropy**—Female genitalia could diversify owing to shared genetic/developmental bases with male genitalia, which rapidly evolve for separate reasons. In contrast to the pleiotropy hypothesis for male genital evolution, which relies on genetic correlations with other non-genital traits, this hypothesis narrowly targets genetic correlations between male and female genitalia. This mechanism may be most important when genitalia share homologous structures between the sexes, and when male genitalia are known to evolve rapidly for reasons unrelated to female genital morphology. This mechanism seems least likely when the sexes do not share homologous structures in relevant genitalic traits. So far, this mechanism has not received much attention, but might provide a null hypothesis in cases where mechanisms of male genital divergence are known but mechanisms of female divergence are unknown. This hypothesis can be tested directly by quantifying genetic correlations.
- (2) **Ecology**—Ecological interactions can influence genital evolution in several ways (see “Ecology and genital evolution” section; Fig. 2). We suggest the mostly likely routes for ecology to drive female genital evolution are through changes in natural and sexual selection on female genital morphology (see text). Divergent selection between different environments seems most likely to drive divergence, and could result in parallel evolution across environmental gradients. This can also lead to coevolution of male and female genitalia if the sexes both independently respond to shared environmental variation. For instance, divergent natural selection on female genitalia via differences in disease or parasites across environments can lead to female genital divergence, while divergent sexual selection owing to shifts in the mating environment can also spur divergence. On the other hand, similar ecologically-derived selection across populations can also lead to divergence if fitness surfaces are complex, with multiple female genital morphologies having similarly high fitness within certain environments. For evaluating the importance of this mechanism, studies can test for ecologically-associated genital divergence and examine how ecological variation alters selection on female genitalia. Little work to date has investigated this mechanism, but recent work has found a strong role of ecology in driving female genital divergence in Bahamian mosquitofish (Anderson and Langerhans 2015).
- (3) **Sexual conflict**—Sexually antagonistic selection can result in a coevolutionary arms race among male and female genitalia. That is, males and females often have conflicting interests regarding mating and fertilization, which can lead to the evolution of male genitalia that bypass female choice (at a cost to the female), then causing female genitalia to evolve defenses against the male armaments and regain control over mating or fertilization (at a cost to the male), and so on. This reciprocal selection should lead to correlated evolution of the sexes across populations/species regardless of the ecological environment. Tests of this hypothesis include testing for male–female coevolution of genitalia and functional studies of male–female genital interactions; tests of coevolution are strengthened when a role of shared ancestry is excluded by statistically or experimentally controlling for genetic relatedness. This mechanism has been viewed by many as a primary explanation for female genital diversity, with empirical evidence accumulating (e.g., Arnqvist and Rowe 1995, 2005; Rönn et al. 2007; Kuntner et al. 2009; Brennan et al. 2010; Perry and Rowe 2014).
- (4) **Female choice**—Female genitalia can provide a means through which females exert preference for male genital morphologies, such as via mechanical or sensory components of genitalia. Thus, female genital morphology can partially represent a manifestation of cryptic female choice. Similar to sexual conflict, this hypothesis also posits that females bias insemination or fertilization success of males based on male genital morphology, but in this case females obtain net fitness gains by selecting high-quality males instead of reducing mating costs through restriction of copulation. This can also lead to coevolution of male and female genitalia, and thus distinguishing this mechanism from sexual conflict requires more than comparative data (Fricke et al. 2009). Cryptic female choice appears to play an important role in rapid male genital evolution (Eberhard 1996, 2010; Hosken and Stockley 2004; Andersson and Simmons 2006; Simmons 2014), but because few studies have focused on its role in female genital evolution we do not yet understand its putative importance for females.

(5) **Lock-and-key**—This mechanism describes selection against inter-population mating (either mating *per se*, or hybridization, or both) that favors genital incompatibilities between populations (see “Genital evolution and speciation” section). This results in rapid evolution of complementary genitalia in the male (key) and female (lock) within populations/species, reducing the probability of inter-population mating. This mechanism can also lead to correlated evolution among male and female genitalia, potentially across dissimilar or similar ecological environments, depending on fitness consequences of inter-population mating. Thus, patterns of male–female coevolution may not distinguish this mechanism from others. However, the lock-and-key hypothesis makes a unique prediction of genital divergence: RCD, where populations/species differ more strongly in genital morphology when experiencing higher probabilities of inter-population mating opportunities (see text). This mechanism does not occur for completely allopatric populations, but can drive the evolution of mating incompatibilities for populations experiencing low-moderate frequencies of inter-population encounters (Servedio and Kirkpatrick 1997; Servedio and Noor 2003; Servedio 2011). This mechanism has had a variable history in the literature, and some studies have found no evidence for its occurrence; however, recent work has provided supportive evidence in a variety of taxa (see text). The importance of this mechanism in explaining genital diversification remains an open question, but its likelihood as a major player has been increasing recently with gathering empirical evidence.

during inter-population mating, then genital evolution can result in mechanical isolation as a byproduct of divergence. So far, we know very little in most cases about how genital variation actually influences insemination or fertilization (but see e.g., Polak and Rashed 2010; Hotzy et al. 2012; Kwan et al. 2013; Bookmythe et al. 2016). In the cases where this knowledge exists, seldom have researchers investigated this variation in the context of ongoing or recent speciation. This is the knowledge that will shed crucial insight into how, and how commonly, genital divergence might incidentally enhance reproductive isolation and cause speciation. The scant knowledge we have today on the role of genital evolution in speciation is surprising considering the obvious implications of divergence in genitalia on reproductive compatibility among populations, combined with the considerable attention both topics independently receive—i.e., many people study genital evolution and many people study the causes of speciation, but few actually study how genital evolution might drive speciation. We hope this soon changes.

What about Females?

Much of the research on genital evolution has so far focused on males. Indeed, nearly twice as many studies have examined male genitalia as compared to female genitalia (Ah-King et al. 2014). Yet the prevailing hypotheses for rapid evolution in male genitalia may also cause diversification of female genitalia (Arnqvist and Rowe 2005; Rönn et al. 2007; Ah-King et al. 2014; Simmons 2014; Anderson and

Langerhans 2015). Evidence to date indicates that rather than uniformity in female genital morphology, female genitalia can evolve rapidly and exhibit considerable diversity, with an increasing call for greater attention to females (Brennan et al. 2007; Kuntner et al. 2009; Evans et al. 2011; Langerhans 2011; Simmons and Garcia-Gonzalez 2011; Ah-King et al. 2014; Simmons 2014; Tanabe and Sota 2014; Anderson and Langerhans 2015). Recent work has pointed to five nonmutually exclusive mechanisms that could lead to rapid genital evolution in females (Box 1). Each mechanism is plausible and requires further study for better understanding the causes of female genital diversity. We are still in the infancy of this field of inquiry.

If female genitalia can evolve rapidly, and may have important consequences for understanding the evolution of primary sexual traits and how genital divergence can influence speciation, then why the lack of attention? Recent work suggests this male bias in the study of genital evolution derives mainly from assumptions about female genitalia exhibiting little variation and males generally experiencing stronger sexual selection for ornamented traits (Ah-King et al. 2014). Studies in the past decade have thoroughly demonstrated that females can exhibit marked genital variation and experience strong selection on genitalic traits. Further, female genitalia are typically much less obvious and accessible for study than males: e.g., often involve more soft tissue, internal features, and show relatively subtle external, superficial variation. Moreover, female genitalia not only interact with

intromittent organs during copulation, but are also involved in ejecting eggs or young, and this multifunctionality may confound simple interpretations and constrain diversification. These female-specific features surely explain some of the bias toward males in the investigation of genital evolution. Yet, as recent studies illustrate, to gain a better understanding of both the causes of genital evolution, and its consequences for speciation, we must address both sexes. This not only demands inclusion of measurements of female genitalia to quantify variation and perform comparative tests, but also functional studies of male–female genital interactions.

The study of male genital evolution has benefitted from a clear framework that began taking shape with the classic work of Eberhard (1985), but the study of female genital evolution has so far lacked a clear framework. Most work on female genitalia has focused on coevolution with male genitalia; the vast majority of studies (85%) that have examined female genitalia also examined male genitalia, while about half of the studies on male genitalia (53%) exclusively focused on males (Ah-King et al. 2014). Box 1 provides the outline of the research agenda that is currently forming for the investigation of female genital evolution, and thus providing a more thorough framework for understanding the evolution of primary sexual traits more generally.

Conclusions

Understanding the ultimate causes and consequences of genital evolution relies not only on understanding the proximate mechanisms underlying male genital evolution, but also on the comprehension of three understudied topics: ecology's role in driving genital evolution, genital evolution's role in the speciation process, and the causes of female genital evolution. Ecology may often provide an ultimate driver of evolutionary change in both male and female genitalia, especially by altering the context of sexual selection in different environments (e.g., population demographics, predation risk, climate, background environment). We clearly need more comparative studies testing hypotheses of phenotype–environment associations for genitalia, and more ecologically-relevant functional studies that elucidate how ecological factors influence genital morphologies. We have a surprisingly poor knowledge of how genital variation influences reproductive isolation, but functional and comparative studies should soon shed light on the roles of the lock-and-key and byproduct mechanisms on speciation. With greater understanding of how male and female genitalia function during copulation

and result in insemination and fertilization—within the context of ongoing speciation—we will gain crucial insights into the ways that genital divergence affects speciation. Females exhibit more genital variation than traditionally thought, with growing evidence for several explanations for the evolution of this diversity. Future work might particularly focus on the roles of ecology, sexual conflict, and lock-and-key in female genital evolution. We now have a set of clear hypotheses for explaining rapid evolution of both male and female genitalia, and their consequences for speciation. We need focused research employing this research agenda to gain important insights into the causes and consequences of the evolution of some of the most important, rapidly evolving, and complex animal traits: genitalia.

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