1 | INTRODUCTION

Genitalia are some of the fastest evolving characters in the animal kingdom. Such rapid evolution has led to a morphologically and functionally diverse suite of genital traits that are often the only means by which individuals of some species can be distinguished (Tuxen, 1970). Traditionally, male genitalia have been the overwhelming focus of evolutionary studies, and there are several potential reasons as to why this has been the case. From a practical point of view, the rigid, external genitalia of males are much easier to access and manipulate than the typically soft, internal genitalia of females (Eberhard, 1985; Simmons, 2014). But perhaps most of the bias towards studying only male genitalia has come from influential work which has suggested that across species, female genitalia are often uniform in shape and uninformative when compared to the genitalia of males (Eberhard, 1985).

In recent years, however, there has been a slow realization that female genitalia can in fact be highly diverse and covary with the genitalia of males. Here, we examine evidence for three mechanisms of genital evolution in females: species isolating ‘lock-and-key’ evolution, cryptic female choice and sexual conflict. Lock-and-key genital evolution has been thought to be relatively unimportant; however, we present cases that show how species isolation may well play a role in the evolution of female genitalia. Much support for female genital evolution via sexual conflict comes from studies of both invertebrate and vertebrate species; however, the effects of sexual conflict can be difficult to distinguish from models of cryptic female choice that focus on putative benefits of choice for females. We offer potential solutions to alleviate this issue. Finally, we offer directions for future studies in order to expand and refine our knowledge surrounding female genital evolution.

KEYWORDS
coevolution, female choice, genital evolution, lock-and-key, micro-computed tomography, pleiotropy, reproductive character displacement, sexual conflict, sexual selection
Not only are studies beginning to demonstrate that female genitalia have undergone divergent evolution, but there is also evidence that they coevolve with male genitalia. This seems intuitive, given the close interaction that occurs between both sets of organs for successful reproduction. One example of female genital diversification and female–male coevolution comes from stalk-eyed flies in the genera *Teleopsis*, *Cyrtaodiopsis* and *Megalabops* (Kotrba, Huber, & Feijen, 2014). In these taxa, the lengths of the female common and separate spermathecal ducts are highly divergent among 17 species, and the length of the common spermathecal duct has coevolved with the genital process of males (Kotrba et al., 2014). Several other studies have found similar patterns of female genital diversity and coevolutionary variation between female and male genitalia (Breed, Leigh, & Speight, 2013; Genevius, Caetano, & Schwertner, 2017; Hedrick, Natanson, Brennan, Antalek-Schrag, & Conith, 2019; Horton & Lewis, 2011; llango & Lane, 2000; Ishikawa, 1987; Simmons & García-González, 2011; Yoshizawa, Ferreira, Kamimura, & Lienhard, 2014).

In this review, we explore the mechanisms responsible for patterns in female genital evolution and coevolution. However, to put our review in context we must first consider the mechanisms of selection thought to act on male genitalia. Since the late 1980s, the majority of genital evolution studies have focused on sexual selection as being the driver of genital diversity (Eberhard, 1985, 2010; Hosken & Stockley, 2004; Simmons, 2014). Contemporary research on the effect of sexual selection on male genital evolution has now shifted towards understanding the exact mechanism(s) of sexual selection involved (Ah-King, Barron, & Herberstein, 2014; Simmons, 2014). There are three main sexual selection mechanisms that could be driving evolutionary change in male genitalia: cryptic female choice, sperm competition and sexual conflict.

Cryptic female choice occurs when females bias paternity towards preferred males during and after copulation (Thornhill, 1983). In the context of genital evolution, cryptic female choice could be achieved by female genitalia affecting a male's ability to engage his genitalia correctly for insemination and/or selectively storing and using a male's sperm for fertilization based on stimulatory cues delivered via male genitalia during copulation (Eberhard, 1985, 1996). The lock-and-key mechanism was the first proposed mechanism of genital evolution (Dufour, 1844), and represents a form of cryptic female choice in which females choose conspecific males over heterospecific males generating species isolation (Simmons, 2014). The most obvious reason why females would prefer conspecific over heterospecific males is the avoidance of genetic incompatibilities associated with hybridization (Andersson, 1994). However, more recently it has been suggested that within species, cryptic female choice of more ‘attractive’ genital variants is likely to be a more widespread form of sexual selection driving genital evolution than species isolating mechanisms such as the lock-and-key hypothesis (Eberhard, 1985, 2010). Differentiating between these hypotheses is difficult, but can be achieved in part by an examination of the tempo and mode of selection acting on genitalia: continuous directional selection and rapid evolution are expected when females select for the most “attractive” males, whereas stabilizing selection and slow divergence are expected where female genitalia select for males with species typical genital configurations (Simmons, 2014).

In some cases, the evolution of genitalia may be influenced by the elaborate genital morphologies that are favoured in males for the removal of sperm stored by females from previous matings (Gage, 1992; Kamimura, 2000; Robinson & Novak, 1997; Sekizawa, Goto, & Nakashima, 2019; Waage, 1979). In removing rival sperm, males can avoid sperm competition, which occurs when the sperm from more than one male overlap temporally in the reproductive tract of a female and compete to fertilize her ova (Parker, 1998; Simmons, 2001). However, sperm removal may not always be in the best interests of females, generating sexual conflict. Sexual conflict over who fertilizes a female’s eggs can favour the evolution of female genital traits that prevent males from manipulating their sperm stores, and the establishment of an antagonistic arms race as each sex evolves to maintain control over mating and fertilization events. Male genital traits that increase competitive fertilization success, such as genital spines or hooks, can also be harmful to females (Crudgington & Siva-Jothy, 2000), again favouring the evolution of defensive genital structures in females that reduce male harm. In this way, sexual conflict can drive the rapid evolutionary divergence of female and male genital morphology.

Few studies have been able to disentangle the relative importance of proposed mechanisms of sexual selection for the evolution of male genitalia, and indeed, these processes need not be mutually exclusive (Simmons, House, Hunt, & Garcia-González, 2009). Given the lack of attention that female genital evolution has received relative to that of males, the problem of understanding the effects of sexual selection on female genitalia is even more pronounced. Our aim in this review was to gather and synthesize current evidence both for and against sexual selection acting on female genitalia. We do this by examining individually the three different mechanisms of sexual selection that are candidate drivers of female genital diversity (Table 1). As with many other contributions to the field, we define genitalia to include secondary genital structures that make contact between males and females during copulation, but need not necessarily be intromittent organs (Barnard, Fincke, McPeek, & Masly, 2017; Brennan et al., 2007; Eberhard, 1985; Simmons, 2014). In reviewing this literature, we not only gain a better understanding of the relative importance of different mechanisms of sexual selection for the evolution of female genitalia, but also reveal areas of focus for future research.

2 | FEMALE GENITALIA AND THE SPECIES ISOLATING LOCK-AND-KEY HYPOTHESIS

The lock-and-key hypothesis proposes that species specificity in genital morphology is a mechanism against hybridization, where only males with genitalia of a specific morphology (the “key”) can fit the corresponding genitalia of the female (the “lock”) (Dufour, 1844). Unlike other evolutionary mechanisms which predict that
genitalia are under continuous directional selection, the lock-and-key hypothesis predicts that following rapid differentiation due to directional selection during the speciation process, genitalia should experience long periods of stabilizing selection where one particular genital morphology is favoured for the maintenance of species mate recognition (Hosken & Stockley, 2004; McPeek, Shen, Torrey, & Farid, 2008).

Traditionally, the lock-and-key hypothesis has been treated separately to mechanisms of sexual selection. However, female preferences can focus on traits that are cues to male quality within species, and on traits that ensure they mate with conspecifics (Andersson, 1994). Selection imposed by females during copulation, via genital interactions, is a form of sexual selection through cryptic female choice, whether this choice results in the directional selection for high-quality males better able to stimulate them or whether it results in females avoiding the reproductive costs of mating with heterospecifics (Simmons, 2014).

Despite its intuitive appeal, the lock-and-key hypothesis has been largely dismissed, most notably by Eberhard in the 1980s with his championing of a Fisherian model of directional female choice (Eberhard, 1985). Rejection of the lock-and-key hypothesis was due mostly to the apparent lack of evidence of diverse species-specific female "locks" across many taxa (Ah-King et al., 2014; Eberhard, 1985). However, the recent uncovering of significant morphological diversity in female genital structures, both within and among species, provides justification to reassess the importance of lock-and-key genital evolution (see Table 2).

There are a number of examples, particularly in invertebrates, where evidence points to the evolution of lock-and-key genitalia (Table 2). In Oohomopterus ground beetles, female (and male) genitalia have coevolved to be species specific in size and shape (Ishikawa, 1987). In two of these species, Carabus (Oohomopterus) maiyasanus and C. (O.) iwawakianus, genital lock-and-key appears to be important for the maintenance of a barrier against hybridization (Sota & Kubota, 1998). Firstly, Carabus maiyasanus and C. iwawakianus share a narrow hybrid zone, and when both species come into contact, males are unable to discriminate between females of the same or different species (Sota & Kubota, 1998). The absence of any apparent premating recognition system is expected to promote selection for species specificity of genital morphology to avoid costly hybridization. Indeed, it was shown that heterospecific matings were harmful for both sexes of these species, in that females suffered a high rate of mortality due to rupture of their genital membranes, and male C. maiyasanus often suffered genital breakage that would likely impede future copulatory attempts (Sota & Kubota, 1998). Moreover, heterospecific crosses produced much lower fertilization rates compared to homospecific crosses (Sota & Kubota, 1998). Clearly, genital mismatching between these species occurs, and so a lock-and-key mechanism plays a role in the maintenance of species integrity.

A more recent study on the sibling Drosophila species D. yakuba and D. santomea demonstrated marked differences in both female and male genital morphology, and that these species differences are maintained by lock-and-key evolution (Kamimura & Mitsumoto, 2014).
| Taxon        | Species                          | Sex examined (female/male/both) | Hypotheses of genital evolution | Reference(s)                                                                 |
|-------------|----------------------------------|---------------------------------|---------------------------------|-----------------------------------------------------------------------------|
| Diplopods   | Parafontaria spp.                | Both                            | Lock-and-key                    | Tanabe and Sota (2008, 2014)                                                |
|             | Levizonus spp.                   |                                 | Cryptic female choice           |                                                                             |
| Antichiropus | variableis                      | Male/Both                       | Sexual conflict                 | Wojcieszek et al. (2012); Wojcieszek and Simmons (2011, 2012, 2013)         |
| Insects     | Carabus (Ohomopterus) maiyasanus | Both                            |                                 | Sota and Kubota (1998)                                                     |
|             | C. (O.) wawakanus                |                                 |                                 |                                                                             |
|             | Carabus insulinicia              | Both                            | —                               | Takami et al. (2018)                                                       |
|             | Drosophila yakuba                | Both                            | Yes                             | Kamimura and Mitsumoto (2012)                                              |
|             | D. santomea                      |                                 |                                 |                                                                             |
|             | Enallagma spp.                   | Both                            | Yes                             | McPeak et al. (2008, 2009)                                                 |
|             | Chalcodoma Caucasus F. C. atalas | Both                            | —                               | Kawano (2002)                                                              |
|             | L.                                    |                                 | Maybe                           |                                                                             |
|             | Teleopsis spp.                   | Both                            | Maybe                           | Kotba et al. (2014)                                                        |
|             | Cyrtodopsis spp. Megalabop spp.  |                                 | Yes                             |                                                                             |
|             | Nicrophorus vespilloides         | Both                            |                                 | Hopwood et al. (2016)                                                      |
|             | Onthophagus taurus               | Both                            | —                               | Simmons and Fitzpatrick (2011); Simmons and García-González (2011)        |
|             | Metrioptera roeselii             | Both                            | —                               | Wulff et al. (2015, 2017, 2018); Wulff and Lehmann (2016)                 |
|             | Glossina spp.                    | Both                            | Yes                             | Brieseo and Eberhard (2009)                                                |
|             | Callosobruchus spp.              | Both                            | —                               | Caytano et al. (2011); Crudgington and Siva-Jothy (2000); Dougherty et al. (2017); Edvardsson and Tregenza (2005); Rönn et al. (2007) |
|             | Gerris spp.                      | Both                            | —                               | Arnqvist (1989); Han and Jablonski (2009); Perry and Rowe (2012); Rowe (1994) |
|             | Callopsyrphus xami               | Female                          | —                               | Ambriz et al. (2011)                                                       |
|             | Plodia interpunctella            | Both                            | —                               | McNamara et al. (2019)                                                     |
|             | Lepidopteran spp.                | Both                            | —                               | Sánchez and Cordero (2014); Sánchez et al. (2011)                          |
|             | Amorbia cuneana                  | Male                            | —                               | Camacho-García, Baixeras, and Cordero (2018)                                |
|             | Cordichusia                      | Both                            | Maybe                           | Tatamie and Cassis (2010)                                                  |
|             | Calopteryx haemorrhoidalis       | Both                            | Maybe                           | Córdoba-Aguilar (1999, 2005)                                               |
| Molluscs     | Satsuma eucosmia                 | Male/Both                       | —                               | Kameda et al. (2009); Hollander et al. (2013)                               |
|             | S. largillierti                  |                                 | —                               |                                                                             |
|             | Littorininae spp.                |                                 | —                               |                                                                             |
| Fish         | Poecilia reticulata              | Both                            | —                               | Evans et al. (2011, 2013)                                                  |
|             | Gambusia hubbi                   | Both                            | Yes                             | Anderson and Langerhans (2015)                                              |
| Birds        | Waterfowl spp.                   | Both                            | —                               | Brennan and Prum (2012); Brennan et al. (2007)                             |
| Mammals      | Cetacean spp.                    | Female/Both                     | —                               | Orbach et al. (2018); Orbach et al. (2017a)                                |
|             | Phoco vitulina                   |                                 | Yes                             |                                                                             |
|             | 163 different species            | Both                            | —                               | Lough-Stevens et al. (2018)                                                |
|             |                                  |                                 |                                 |                                                                             |
Estimates of neutral genetic and genital divergence among eight related species have provided evidence that male and female genitalia have co-evolved (McPeek et al., 2008). Together, these studies provide evidence that the lock-and-key species isolation hypothesis makes an important and unique prediction among potential mechanisms of genital evolution, which is that populations that have undergone lock-and-key evolution should show evidence of reproductive character displacement (RCD; Langerhans, 2011). RCD is where more geographically close populations (sympatric populations) should exhibit greater divergence in reproductive characters than more geographically isolated populations (allopatric populations; Langerhans, 2011; Langerhans, Anderson, & Heinen-Kay, 2016). This is because where populations are close to one another, individuals from those populations will encounter each other more frequently, favoring more divergent genital morphologies to prevent hybridization and other costs of interpopulation mating (Langerhans et al., 2016).

There is some empirical evidence of RCD occurring, but few studies take into account genitalic traits (Langerhans et al., 2016). Those that do include a study of two land snail species (Satsuma eucosmia and S. largillierti) where there was significantly greater divergence in penis length between sympatric populations of both species than between allopatric populations (Kameda, Kawakita, & Kato, 2009). Importantly, vaginal length was significantly more divergent between sympatric populations of S. eucosmia (Kameda et al., 2009). Preliminary studies on this snail genus have suggested that there is genital mismatch that causes early termination of copulation and failure of spermatophore exchange (Kameda et al., 2009), providing evidence for lock-and-key evolution in Satsuma and thus supporting the prediction that this type of evolution promotes RCD. A larger scale comparative study of 40 sister-pair species of littorinid snails also found evidence for RCD in that sympatric sister-pair species were found to have more divergent penis morphologies than those that were allopatric (Hollander, Smadja, Butlin, & Reid, 2013). This finding suggests a widespread pattern of lock-and-key genital evolution across the Littorininae. Interestingly, a recent study (Hollander et al., 2018) on one of the sister-pair species (Littoraria cingulata and L. filosa) included in the same comparative analysis found contradictory results in that there was no evidence for RCD in penial form. Unfortunately, these data are limited insofar as the corresponding female genital trait, vaginal length, was not examined.

Studies on Antichirus variabilis millipedes are also among the few that have assessed the mode of selection acting on genitalia. The first of these found that shape variation among last males to mate influenced paternity success (Wojcieszek & Simmons, 2011). Importantly, selection gradient analysis revealed stabilizing selection to be acting on male genitalia, consistent with lock-and-key selection in the current maintenance of species isolation (Wojcieszek & Simmons, 2011). Estimates of neutral genetic and genital divergence among eight different populations of A. variabilis and a subsequent \( Q_{ST} - F_{ST} \) analysis also found evidence of stabilizing selection acting on male genitalia among these populations (Wojcieszek & Simmons, 2013). Importantly, pairing males with females from different populations resulted in mechanical barriers to copulation indicating that genital divergence among these populations had established reproductive isolation between them (Wojcieszek & Simmons, 2013). Whereas the focus of these studies was primarily on male, rather than female, genitalia, given the evidence presented, particularly the genital mismatch between females and males from different populations (Wojcieszek & Simmons, 2013), it is not unlikely that female genitalia in this species of millipede have diversified between populations so as to correspond specifically with male morphology and vice versa. Indeed, using micro-computed tomography scanning, various male and female genital structures were found to complement each other in a tight “lock-and-key” fit (see Figure 1; Wojcieszek, Austin, Harvey, & Simmons, 2012).

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A study of two closely related rhinoceros beetle species (*Chalcosoma caucasus* F. and *C. atlas* L.) found that penis length, as well as other nongenitalic characters, was much more divergent between sympatric locations of *C. caucasus* and *C. atlas* compared to where these species occurred in allopatry (Kawano, 2002). In fact, penis length showed the greatest and most extreme divergence out of all characters between sympatric locations (Kawano, 2002). Whereas these results are highly suggestive of RCD and lock-and-key genital evolution occurring, as with studies of *Satsuma* and littorine snails, data are needed on female genital traits before firm conclusions can be drawn. Better evidence of RCD and lock-and-key genital evolution comes from studies of Bahamas mosquitofish (*Gambusia hubbsi*) (Anderson & Langerhans, 2015), where it was found that populations of fish with higher genetic relatedness (assumed to indicate higher levels of migration and mating opportunities) had greater divergence in female and male genital morphologies (see also Langerhans, 2011; Peden, 1972, 1975).

Given the evidence consistent with lock-and-key processes for both invertebrate and vertebrate species (Table 2), the dismissal of this hypothesis as an explanation for genital evolution seems premature. Further work is required to fully appreciate the extent to which selection for species isolation influences female genital divergence, and how it combines with other sexual selection mechanisms to drive divergence in animal genitalia more generally. However, as with all proposed mechanisms of genital evolution, the traditional focus on male genitalia seriously undermines any conclusions that can be drawn from studies of lock-and-key processes because too few studies have examined the species-specific female “lock.”

### 3 DIRECTIONAL CRYPTIC FEMALE CHOICE

Eberhard (1985) argued that a Fisherian model of female choice provided a more general explanation for the evolution of male genital diversity, whereby females choose “attractive” males with genitalia better equipped to stimulate their own genitalia during copulation, and thereby produce “attractive” sons as well as daughters with the same preference for “attractive” males. Eberhard (2004) presented a broad review of the literature in support of his hypothesis. A second model of cryptic female choice does exist, the “good-genes” hypothesis, which, in addition to the benefits provided by the Fisherian model, predicts that females choose the most “attractive” males because they confer increased offspring viability. The “good-genes” hypothesis also predicts that genitalia should be condition-dependent, in that the degree of expression of a sexual trait should vary depending on the amount of resources an individual has to allocate between sexual and other traits related to fitness (Pomiankowski, 1987; Rowe, 1997).
& Houle, 1996). Much work on invertebrates has indicated that genitalia generally show weak levels of condition dependence (Cayetano & Bonduriansky, 2015; House & Simmons, 2007; House et al., 2016; though see Arnqvist & Thornhill, 1998). Although sexual selection through female choice is now widely accepted as playing a significant role in the evolution of male genitalia (Arnqvist, 1998; Orbach, Hedrick, Würsig, Mesnick, & Brennan, 2018; Simmons, 2014), few studies have provided direct evidence that female genitalia is a preference trait imposing sexual selection on male genitalia.

Increasing appreciation of the role of female genitalia as a preference trait is beginning to come from growth in the availablity and use of modern microscopy and histological techniques. The composition and structure of female genitalia can now be viewed and analysed at a level of detail previously unseen. The types of morphological and/or cellular detail expected to be seen in female genitalia that have evolved to facilitate and assess male genital structures might be morphologies that are more cooperative than defensive, or those that are composed of sensory cells. Three study systems that have utilized microscopy, histological and electrophysiological techniques to visualize male and female genital structures in such a way as to elucidate function are tsetse flies (Briceno & Eberhard, 2009), bushcrickets (Wulff & Lehmann, 2016; Wulff, Lehmann, Hipslsey, & Lehmann, 2015; Wulff, Schoeneich, & Lehmann, 2018; Wulff, van de Kamp, dos Santos Rolo, Baumbach, & Lehmann, 2017) and damselflies (Cordoba-Aguilar, 1999, 2002, 2005, 2006).

In tsetse flies, when the male genital structures that rhythmically squeeze the female abdomen during copulation were modified so that their function was inhibited, there was a decrease in ovulation and sperm storage by females, as well as an increase in the likelihood of female remating (Briceno & Eberhard, 2009). Subsequent experiments that included "blinded" females (females with blocked or damaged receptors) found results comparable to those from experiments using males with modified genitalia (Briceno & Eberhard, 2009). These results are consistent with a role for female sensory function, rather than male behaviour, in controlling female reproducution, and provide evidence of cryptic female choice against less stimulatory males in these tsetse flies (Briceno & Eberhard, 2009).

In the bushcricket Metrioptera roeseli, males possess a pair of sclerotized structures called titillators that are rhythmically inserted inside the female during copulation and tap against her subgenital plate (Wulff et al., 2015, 2017). The female subgenital plate is covered by a soft membrane and is composed of numerous sensory cells (Figure 2; Wulff & Lehmann, 2016). The female subgenital plate does not appear to present as a defensive structure and is not harmed by males during copulation (Wulff et al., 2015). These characteristics might be expected of female genitalia that were involved in Fisherian cryptic female choice. Furthermore, ablation of one or both male titillators incurs aggressive resistance behaviour from females, suggesting that the titillators induce cooperative female behaviour (Wulff & Lehmann, 2016). A recent study consolidated these observations of bushcricket genitalia, using neuroanatomical and electrophysiological methods. Electrophysiology revealed that female bushcrickets are able to perceive the tapping stimulation of male titillators on their subgenital plates; however, responses to such stimulation were greatly reduced following the application of a sensory blocking insecticide (pymetrozine; Wulff et al., 2018). Female bushcrickets also showed resistance behaviours towards males whose titillators were asymmetrically shortened (as demonstrated previously, see Wulff and Lehmann (2015)), but mechanoreceptor blocking experiments (similar to those in tsetse flies, Briceno & Eberhard, 2009) using both pymetrozine and UV-hardened glue produced females with a large decrease in resistance towards males (Wulff et al., 2018). The results from this study, along with previous work, clearly show that male titillators are used as stimulatory devices and that Fisherian cryptic female choice imposed on males via female genitalia has played an important role in the evolution of male genitalia in this group.

Finally, in damselflies, evidence suggests that females execute cryptic female choice via sperm ejection prior to oviposition (Cordoba-Aguilar, 2006). In the damselfly Calopteryx haemorrhoidalis, a series of experiments have shown that female genitalia control sperm ejection (Cordoba-Aguilar, 1999, 2002, 2005, 2006). During copulation, mechanoreceptor sensitive sensilla located on the vaginal plates of the female receive stimulation from the male's aedeagus, and it is this sensory perception that results in females ejecting rival sperm from their sperm stores (Cordoba-Aguilar, 1999). Specifically, the width of the male aedeagus is positively correlated with the amount of sperm ejected and the rate of sperm ejection increases with the number of sensilla on female genitalia (Cordoba-Aguilar, 1999, 2005). Interestingly, female C. haemorrhoidalis appear to have a reduced number of sensilla compared to females from other damselfly species, and this has been suggested to be a coevolved response to the stimulatory ability of males (Cordoba-Aguilar, 2005).

4 | CRYPTIC FEMALE CHOICE AND SEXUAL CONFLICT

Sexual conflict is expected as a direct consequence of cryptic female choice because the reproductive interests of choosy females will be opposed to those of disfavoured males. Moreover, traits that evolve in males to achieve reproductive success can be harmful to females and such harm can take a variety of forms. Males might impose direct harm on females via damage to their genitalia or alterations to their physiology, or indirect harm through reductions in offspring number and/or quality that result from circumvention of cryptic female choice. Reductions in female fitness from male harm are expected to generate a coevolutionary arms race between males and females, with genital traits in one sex driving the evolution of counteradaptive genital traits in the other (Arnqvist & Rowe, 2002a; Birkhead & Pizzari, 2002). Sexual conflict can also arise when genital traits evolve in males in the context of sperm competition over fertilizations with rivals, since these traits can be harmful to females, generating adaptations in female genitalia to reduce the collateral damage imposed through sperm competition (Chapman, Arnqvist, Bangham, & Rowe, 2003; Stockley, 1997).
Many studies of genital evolution have found it difficult to separate the effects of cryptic female choice and sexual conflict, and therefore, the relative importance of one compared to the other is difficult to gauge. For example, an analysis of vaginal morphology of 20 species of cetaceans revealed extensive diversification in vaginal fold size, number, positioning and shape (Orbach et al., 2017b), although the functional role of these vaginal folds in sexual selection is not immediately clear (Figure 3). Further studies have found evidence for both congruent (genitalia are complementary) and antagonistic (genitalia are combative) coevolution between male and female genitalia in marine mammals, hinting at roles for either Fisherian cryptic female choice or sexual conflict mechanisms (Orbach et al., 2017a).

In species showing congruent coevolution, female genitalia offer no physical barriers to males and the shape of male and female genitalia appears to match, whereas in species showing antagonistic coevolution, the female genital tract presents barriers to male intromission and the penis tip of males is elongated, possibly in order to navigate such barriers (Orbach et al., 2017a).

One method to clearly distinguish Fisherian cryptic female choice from other evolutionary mechanisms, particularly sexual conflict, is to measure and compare fitness costs and benefits incurred by females (Fricke, Perry, Chapman, & Rowe, 2009). Where females obtain net benefits from genital interactions, cryptic female choice is likely to be the main driver of female genital evolution. Studies of the dung beetle Onthophagus taurus suggest that cryptic female choice may be a prominent mechanism of genital coevolution (Simmons & García-González, 2011). In this species, the aedeagus of the male must make contact with genital pits within the reproductive tract of the female before copulation and ultimately ejaculation can occur (Werner & Simmons, 2008). Experimental evolution was used to examine how female genital traits evolved in response to the presence or removal of sexual selection. Under a regime of sexual selection, female genital pits moved closer together away from the outer edge of the pygidium, and in a correlated response, male aedeagus length increased (Simmons & García-González, 2011). Furthermore, significant genetic correlations between the sexes were found for these genital traits (Simmons & García-González, 2011). The authors conclude that the evolutionary changes in female genitalia and the coevolution with males were most likely due to cryptic female choice, because previous studies of this species had found offspring viability
is increased both by female choice and by multiple mating, without any apparent costs to females (García-González & Simmons, 2011; Simmons & Holley, 2011; Simmons & Ridsdill-Smith, 2011). Most recently, in a comparative study of 33 species in the genus Onthophagus, Simmons and Fitzpatrick (2019) found that female genital traits are diverging nearly three times faster than the male genital traits with which they interact, suggesting that female genitalia are driving the evolutionary divergence of male genitalia via cryptic female choice.

Perhaps one of the clearest examples of the influence that sexual conflict can have on female genital evolution occurs in seed beetles (Callosobruchus spp.). Male beetles possess spines on their genitalia that puncture the female reproductive tract (Figure 4), thereby facilitating the movement of seminal fluid proteins into the female’s bloodstream where they affect her subsequent use of the copulating male’s sperm (Yamane, Goenaga, Rönn, & Arnqvist, 2015). Genital damage due to male spines is well known to have costly fitness consequences for females (Crudgington & Siva-Jothy, 2000; Dougherty & Simmons, 2017; Edvardsson & Tregenza, 2005; Rönn, Katvala, & Arnqvist, 2007), and selection for increased length of genital spines appears to have driven the coevolution of thicker reproductive tracts in females in response to male harm, both among (Rönn et al., 2007) and within species (Dougherty et al., 2017).

Cayetano, Maklakov, Brooks, and Bonduriansky (2011) attempted to halt sexual selection by imposing monogamy on populations of C. maculatus and found that after 18–21 generations, male spine length reduced in size in large males, supporting results from previous studies (Crudgington & Siva-Jothy, 2000; Edvardsson & Tregenza, 2005; Rönn et al., 2007) by showing that male spines are directly harmful to females. Interestingly, female genitalia in terms of reproductive tract thickness did not respond to monogamy in this time frame, which was interpreted to be characteristic of defensive traits in that their evolution often lags behind that of offensive traits (Cayetano et al., 2011).

Sexual conflict as an evolutionary driver of female genital evolution is also well documented in Gerridae water striders (Arnqvist & Rowe, 2002a; Perry & Rowe, 2012). Two studies in particular have demonstrated clear evidence of an effect of sexual conflict in this system. Females from most species experience male harassment and forced copulations, and typically try to resist matings because excessive mating carries fitness costs (Arnqvist, 1989, 1992; Rowe, 1994; Watson, Stallmann, & Arnqvist, 1998). Several traits in females and males are predicted to be influenced by such premating conflict, such as abdominal shape and appendage size/shape. A comparative analysis of 15 Gerris species found that there was coevolution between the sexes in abdominal structures: females in species where males
have evolved grasping adaptations were found to have coevolved morphologies to increase the difficulty for male intromission, such as elongated abdominal spines and shape changes to the genital tip (Arnqvist & Rowe, 2002b). In addition, their analysis offered support for the theoretical prediction that coevolution of female armaments should occur rapidly and in a bidirectional manner (Arnqvist & Rowe, 2002b). A second study that focused on behavioural traits again found evidence of sexual conflict in this system, providing support for the theoretical prediction that sexual conflict can be difficult to detect in a system where females and males are equally armed; it is only when one sex increases armament level relative to the other that fitness consequences can be detected (Arnqvist & Rowe, 2002a).

Interestingly, a later study found that in one species, *Gerris gracilicornis*, sexual conflict appears to have driven the evolution of a novel female genital morphology where the female genitalia are no longer exposed, but rather are concealed behind body segment 7 (Figure 5; Han & Jablonski, 2009). Such hidden genitalia prevent forced intromissions and so give the female the upper hand in the coevolutionary arms race. However, it appears that males have evolved a behavioural counteradaptation to the female genital shield. Male *G. gracilicornis* use a tapping behaviour on the water surface to seemingly attract predators to intimidate females into exposing their genitalia (Han & Jablonski, 2010). Nevertheless, this particular species of water strider demonstrates well the cycles of sexually antagonistic coevolution by which females can evolve novel genital morphologies.

Sexual conflict has been suggested to drive widespread changes in female genital morphology across the Lepidoptera. In numerous species, females have evolved sclerotized spines (signa) within the corpus bursa (Figure 6). The corpus bursa is the same structure within which males deposit the spermatophore, and the signa are used to tear open the spermatophore envelope in order to access the resources inside (Sánchez, Hernandez-Banos, & Cordero, 2011). It is known that many female lepidopterans become unreceptive following mating and that the time it takes for females to regain receptivity is negatively correlated with the amount of spermatophore remaining in the corpus bursa (Sánchez et al., 2011). Therefore, in order to gain a greater share of paternity, it would be advantageous for any individual male to increase the refractory period of a female, and in species where females are polyandrous, males are predicted to evolve thicker spermatophore envelopes that would take longer for females to break down (Sánchez et al., 2011). Conversely, for females, mating with multiple males and gaining access to numerous spermatophores are advantageous and so the evolution of signa is therefore thought to have come about as an antagonistic response to thicker spermatophore envelopes (Sánchez et al., 2011).

Three separate studies provide varying levels of support for the evolution of the female signa via sexually antagonistic coevolution. The first is a phylogenetically controlled study looking at the relationship between mating system and the presence or absence of signa across 37 lepidopteran taxa (Sánchez et al., 2011). As predicted, it was found that the females of most polyandrous species had evolved signa whereas the females of most monandrous species had lost signa (Sánchez et al., 2011). In monandrous species, male–male competition is negligible to absent and so it is predicted that the selection pressure for thicker spermatophore envelopes is lost, and in a coevolved response, signa should be lost from females. Whereas such among-species correlations are supportive of the sexual conflict hypothesis driving changes in female genitalia, conclusive support requires experimental evidence.

A second, more recent study provided further substantiation for the role of sexual conflict in the evolution of signa in females. Using eleven species of Heliconiinae butterflies, Sánchez and Cordero (2014) firstly found that the spermatophore envelope from a polyandrous species with signa was thicker than that of two monandrous species.

**FIGURE 5** Schematic drawings of the ventral view of the abdomens of female (a) *Gerris lacustris* (typical *Gerris* female abdomen) and (b) *G. gracilicornis*. The dotted lines show the difference between the genitalia of a typical *Gerris* female and the genitalia of *G. gracilicornis* females whose genitalia are hidden behind segment 7. SEM image (c) corresponds to schematic (b). Scale bar is 0.1 mm. Adapted from Han and Jablonski (2009)
species that lack signa. Secondly, they looked at whether the spermatophore envelopes from three monandrous species that have signa were thicker than those of related polyandrous species with signa, which was also found to be true for two of the monandrous species (Sánchez & Cordero, 2014). This second finding is in line with predictions from the sexual conflict hypothesis because it is expected that in monandrous species with signa, males have enforced monogamy on females via the evolution of extra thick spermatophore envelopes (Sánchez & Cordero, 2014). The authors recognize, however, that male enforced monogamy as an explanation for monandrous females with signa requires experimental support (Sánchez & Cordero, 2014).

Ambriz, Mota, and Cordero (2011) used a different approach to try to detect an effect of sexual conflict on female genital evolution by looking for additive genetic variation in signa traits in the butterfly *Callophrys xami*. No significant additive genetic variation was found in components of signa shape, which is suggestive of an effect of directional selection driving evolution towards a particular shape (potentially via sexual conflict), thus removing genetic variation for this trait from the population (Ambriz et al., 2011). Interestingly, between two different populations, there were significant differences in a suite of traits, including signa width and three components of signa shape (Ambriz et al., 2011). A more recent study on the Indian meal moth (*Plodia interpunctella*) has found somewhat conflicting results. This study looked at signa shape and spermatophore envelope thickness in individuals reared from two experimentally evolving populations with either a female-biased or male-biased sex ratio (differing levels of sexual conflict intensity; McNamara, Dougherty, Wedell, & Simmons, 2019). Given the results from previous studies, it was expected that coevolution between signa and spermatophore morphology should occur, driven by the level of sexual conflict. Although females from populations with reduced sexual conflict were found to have evolved wider shaped signa, no coevolution between signa traits and spermatophore thickness was detected (McNamara et al., 2019). The contrast between the findings of experimental and correlational studies of coevolution highlights the importance of experimental approaches for confirming process from pattern. Clearly, more experimental work on this interesting female genital trait is required before its functional significance can be confirmed, but what is clear from the *Plodia* work is that female genital traits can and do respond to variation in sexual selection.

An interesting system where sexual conflict appears to have driven diversity in female genital morphology is in traumatically inseminating species. Traumatic insemination occurs when males pierce the female abdominal wall with their genitalia and ejaculate inside the body cavity (Tatarnic, Cassis, & Siva-Jothy, 2014). In the true bug infraorder Cimicomorpha, traumatic insemination has evolved independently at least three times and has likely driven the evolution of novel and diverse female genital morphologies via sexual conflict (Tatarnic et al., 2014). A study on the plant bug genus *Coridromius* (within Cimicomorpha) found that female and male genital complexity are highly correlated; as female genitalia became more complex, male genitalia changed shape in a corresponding manner (Tatarnic & Cassis, 2010). The study also found that elaborate female genitalia have arisen multiple times across the plant bug genus (Tatarnic & Cassis, 2010). Whereas other factors cannot be ruled out as driving genital evolution and coevolution in *Coridromius*, the authors conclude that given the strong evidence for a role of sexual conflict in a
related group of traumatically inseminating insects (bed bugs), there is a strong possibility that sexual conflict also plays a role in this system (Tatarnic & Cassis, 2010).

Lastly, in ground beetles (Carabus insulicola), the evolution of divergent female (elongation of the vaginal appendix) and male (elongation of the copulatory piece) genital morphologies could be driven by sexual conflict, because crosses between males and females derived from divergent populations exhibited a higher rate of egg fertilization than within-population crosses, a pattern expected when males and females evolving under sexual conflict are mated to opposite sex partners with whom they have not coevolved (Takami, Fukuharu, Yokoyama, & Kawata, 2018). Moreover, in this study the effective population size was found to decrease with increasing size of female and male genital traits, potentially exposing populations to increased risk of extinction (Palstra & Ruzzante, 2008). In theory, sexual conflict can increase the risk of population extinction (Rankin, Dieckmann, & Kokko, 2011). Interestingly, increased male expenditure on genitalia has been a driver of extinction among fossil ostracods (Martins, Puckett, Lockwood, Swaddle, & Hunt, 2018).

Much less is known of the role for sexual conflict in the evolution of vertebrate genitalia, although a well-known example comes from waterfowl. Across 16 different waterfowl species, Brennan et al. (2007) found a high level of variability in vaginal morphology, which ranged from simple, short reproductive tracts to more complex, elaborate structures. Increasing vaginal complexity was found to be correlated with increasing penis length, which in turn was positively correlated with the level of forced extra-pair copulations (Brennan et al., 2007). Increasing vaginal complexity saw the appearance of a number of blind-ended sacs as well as vaginal coiling, which occurred in the opposite direction to penis coiling (Brennan et al., 2007). Apart from forced copulation, most waterfowl species also have elaborate courtship displays where females are able to assess and choose preferred males with which to pair-bond (Brennan & Prum, 2012). The evolution of complex female genitalia in waterfowl may have been driven by selection on females to prevent males from gaining paternity over their chosen males via forced copulation (Brennan & Prum, 2012).

A second vertebrate system known to display sexually antagonistic behaviours is the guppy, Poecilia reticulata. The polyandrous mating system of this species and the high levels of sexual harassment that can be experienced by females point to the guppy as a target for genital coevolution via sexual conflict. Indeed, there is evidence to show that there is a divergence in male and female genital morphology among populations that experience different levels of sexual harassment, as well as coevolution between female oviduct width and male gonopodium tip length (Evans et al., 2011).

5 | REVERSED MATING ROLES

It is worth briefly mentioning how in exceptional cases, sexual selection on female genitalia may derive from male mate choice. Perhaps the best example of this occurs in species of cave insects from the genera Neotroglia and Afrotroglia (tribe Sensitibillini) (Yoshizawa, Ferreira, Lienhard, & Kamimura, 2019; Yoshizawa, Ferreira, Yao, Lienhard, & Kamimura, 2018; Yoshizawa et al., 2014). Sex role reversal has occurred where females from these species possess an intromittent organ (gynosome), whereas male genitalia are described as simple. Females insert the gynosome into a male to receive a potentially nutritious spermatophore, anchoring herself inside the male while doing so. Both the gynosome of females and the genitalia of males are species specific and appear to have coevolved. For example, in Neotroglia species where females possess a gynosome covered with spines, males have genitalia that contain corresponding pockets to receive these spines (Yoshizawa et al., 2014). It has been suggested that the evolution of the gynosome and the coevolution between female and male genitalia in Neotroglia and Afrotroglia are driven by direct sexual selection imposed on females by males who choose the most “attractive” females that are competing for access to nutritious ejaculates in their oligotrophic cave environments (Yoshizawa et al., 2014, 2018, 2019). Female–female mating competition is also likely intensified by the possession of two seminal gift slots on the female spermathecal plate which allows one female to collect nutritious ejaculates from multiple males (Yoshizawa et al., 2019). Similar conclusions have been drawn in an earlier study regarding a secondary copulatory organ in female ground weta (Hemiandrus pallitarsis) which varies in morphology between closely related species (Gwynne, 2004, 2005). Female intromittent organs are common in sea horses where females deliver their eggs into the male brood pouch (Stöltting & Wilson, 2007), yet to our knowledge there have been no studies of their adaptive evolution. Additionally, the intromittent organs of female sea horses differ from the female penis of Sensitibillini species because they are used as an ovipositor, whereas in cave insects, the female penis is used in seminal product receipt (Yoshizawa et al., 2019).

6 | PLEIOTROPY AND NATURAL SELECTION

Mayr (1963) argued that because genital structures are largely internal, they may be protected from selection, allowing them to diverge randomly via correlated responses to selection on traits that are visible to selection. Thus, a reasonable alternative hypothesis to adaptive evolution is that internal female genital structures may not be under direct selection at all (Hosken & Stockley, 2004; Mayr, 1963). Since the increased interest in the effect of sexual selection on genital evolution, this pleiotropy (neutral evolution) hypothesis has been largely neglected. However, there are studies which appear to be consistent with the pleiotropy hypothesis.

In one of these studies, individuals from a natural population of Gerris incognitus water striders were subject to food stress during development, and the effects of food stress on the phenotypic expression of a number of genital and morphological traits were measured (Arnqvist & Thornhill, 1998). An important finding from this study consistent with the pleiotropy hypothesis was that genetic correlations were found between genital size and shape and measures of...
body size and leg length (Arnvist & Thornhill, 1998). Given that linkage was assumed to be negligible, the authors concluded it was likely that the set of genes that influence genital morphology were the same set that influence the development of other morphological traits in G. incognitus (Arnvist & Thornhill, 1998).

A more recent study used a phylogenetic approach to examine the presence or absence of genital bones across 163 mammalian species (Lough-Stevens, Schultz, & Dean, 2018). The baculum is the bone found inside the penis of many mammalian species, and the baubellum is the equivalent bone found within the clitoris of females (Lough-Stevens et al., 2018). The baubellum was found to be much more labile than the baculum in terms of its evolution and development; the baubellum showed many more gains and losses than the baculum and exhibited greater developmental variation (Lough-Stevens et al., 2018). The baubellum was also shown to be significantly more variable in morphology than the baculum (Lough-Stevens et al., 2018). Together, these results suggest that the baubellum may not be a target of sexual selection but rather a vestigial and nonfunctional trait in females.

Whereas these two studies provide evidence consistent with the pleiotropy hypothesis and show that female genitalia may not always be under selection, given the considerable evidence of coevolution and sexual selection acting on female genitalia presented in Table 2, it is likely safe to assume that pleiotropy is not a major contributing factor to the diversity we see in female genital morphology across the majority of animal species.

Finally, female genital traits are undoubtedly subject to natural selection and changes in female genital morphology in other contexts are likely to impose selection on male genital morphology and drive their coevolution. For example, in the invasive pest Drosophila suzukii, the saw-like ovipositor of females has become adapted to pierce the hard skin of ripening fruits that are not used by related species of Drosophila. Muto, Kamimura, Tanaka, and Takahashi (2018) have found that ecologically driven changes in the ovipositor have driven correlated changes in male genital traits, leading them to abandon the use of the hook-like parameres in order to achieve genital coupling in the presence of the novel elongated ovipositor in the females of this species. We anticipate that with increasing study of female genital traits, natural selection acting on female traits is likely to prove a major source of selection driving male genital evolution.

7 | WHERE TO NEXT FOR THE STUDY OF FEMALE GENITALIA?

Whereas it is clear that studies on female genital evolution are lacking in comparison with those focusing on male genitalia, progress is beginning to be made in terms of detailed visualizations of female genitalia, elucidation of potential functions(s) and deciphering of their coevolutionary relationships with male genitalia. Coevolutionary studies are important because information about the relationship between male and female genital morphology can provide much more insight into the mechanisms and consequences of genital evolution than information about genital morphology from a single sex alone.

Most studies that have reported on female genital shape variation have utilized two-dimensional (2D) geometric morphometric techniques (Evans, van Lieshout, & Gasparini, 2013; Hopwood et al., 2016; Orbach et al., 2018; Polihronakis, 2006; Simmons & García-González, 2011). However, with the increase in the accessibility of three-dimensional (3D) scanning technologies and analysis software, more studies are beginning to utilize 3D techniques to capture a level of variation that is not possible with 2D methods (Semple, Peakall, & Tatarin, 2019; Tatsuta, Takahashi, & Sakamaki, 2018).

Much of the work on genital evolution that has utilized 3D technology has been discussed above. Some of the earliest examples come from the work of McPeek et al. (2008, 2009) on damselfly genital evolution. Other studies that utilize 3D technology to visualize and study female genitalia include those on millipedes, seed beetles, bushcrickets, mantophasmatodeans, barklouse and spiders (Cheng & Yoshizawa, 2019; Dougherty & Simmons, 2017; Küpper, Klass, Uhl, & Eberhard, 2019; Schendel, Junghanns, Bilde, & Uhl, 2018; Wojcieszek et al., 2012; Wulff et al., 2015). Some studies have pioneered the use of micro-computed tomography (micro-CT) to obtain 3D images. One clear advantage of technology such as this is the ability to visualize internal reproductive structures that in the past have been too difficult or delicate to dissect from females. Unlike males, female genital traits are often internal, and this is believed to be a key reason as to why they have been so poorly studied relative to the genitalia of males. Not only does 3D technology allow the visualization of the diverse form of female genitalia across species, but it allows us to better understand the way in which female genitalia interact with the genitalia of males and even has the potential to allow the elucidation between different mechanisms of genital evolution, particularly cryptic female choice and sexual conflict. A good example comes from bushcrickets, where scanning electron microscopy allowed the visualization of sensory cells and hairs on the female subgenital plate, indicative of this structure being used to evaluate male stimulatory quality and thus function more as a means of cryptic female choice than as a defensive structure that has resulted from sexual conflict (Figure 2; Wulff et al., 2015).

Other studies have taken advantage of the fact that 3D scanning technologies allow the visualization of female and male genitalia “in copula,” providing a means by which the “fit” between female and male genitalia can be visualized, and the female and male traits most likely to be subject to coevolutionary divergence identified (Figures 1 and 4). A clear example of this was shown in work on marine mammals where micro-CT scanning was performed on the genitalia of females and males in simulated copulation (Orbach et al., 2017a). These scans revealed strong patterns of coevolution between male and female genital morphologies. As 3D scanning technologies are relatively new, they are so far underutilized in the study of genital evolution.

Whereas 3D scanning technologies are a relatively new asset to genital evolution studies, refining some of the approaches that have already been used is also important in order to elucidate the
evolutionary mechanisms responsible for female genital evolution. Historically, experimental evolution studies have been key to understanding what evolutionary mechanisms are acting on female and male genitalia. Many have been described above (Table 2), but at least one study stands out as being able to distinguish between the different hypotheses of genital evolution. Using artificial selection lines on the burying beetle, *Nicrophorus vespilloides*, Hopwood et al. (2016) selected for high or low mating rate finding that after only 10 generations, the genital morphology of beetles in the lines selected for high and low mating rates had diverged relative to control lines. Furthermore, male and female genitalia were found to coevolve within selection lines. Importantly, the divergence in female and male genital morphology from the control lines could be attributed to sexually antagonistic selection and not to effects of cryptic female choice or male–male competition, because males and females were forced to mate monogamously. Future experimental evolution studies should adopt similar, well-thought-out designs that are able to distinguish between the different mechanisms of genital evolution if we are to make advancements in the field.

More broadly, an understanding of the extent of female genital diversity and its influence on the evolution of male genitalia is critical to increase our understanding of two of fundamental yet still debated topics: reproductive isolation and speciation. It is currently understood that genital evolution can contribute to reproductive isolation and eventually speciation either directly via selection against hybridization (genital lock-and-key) or indirectly as a result of mechanisms that were not directly selecting for reproductive isolation, such as Fisherian cryptic female choice and sexual conflict (Langerhans et al., 2016). Until recently, the study of both genital evolution and speciation has been largely decoupled, and a recent review (Langerhans et al., 2016) highlighted the need for further work in this area. We strongly endorse these sentiments. Recent progress in this area examined female and male genital variation in the fish *Poecilia mexicana*, both within and between populations living in sulphidic and nonsulphidic habitats (Greenway et al., 2019). It appears that this species is experiencing ongoing ecological speciation as the authors found that female and male genitalia have diverged rapidly and in a correlated fashion across pairs of populations that have been subject to divergent selection (Greenway et al., 2019). Populations occurring in the same habitat types, however, show evidence of convergent evolution of genital traits between the sexes. Together, the results from this study provide timely evidence to suggest that genital evolution can contribute to the early stages of divergence and speciation (Greenway et al., 2019). It is interesting to note that in other systems, however, it seems that female and male genital divergence has not been sufficient to isolate species allowing for the formation of hybrid swarms (Langton-Myers, Holwell, & Buckley, 2019).

We believe that more studies of reproductive character displacement in genital traits are required in order to assess the role of lock-and-key processes in species isolation, because this is a unique prediction of this form of genital evolution. Where other mechanisms underlying genital divergence between populations are supported, we need to better understand how female genitalia interact with those of males, and to employ comparative analyses and experimental evolution in order to determine the macro- and micro-evolutionary responses to selection in genitalia, and how these divergences affect reproductive isolation and the speciation process. One thing is for certain, a wealth of new information about female genital variation and evolution is soon to be revealed and the answers that it will provide for the field of evolutionary biology will be profound.

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