Coevolution between Male and Female Genitalia in the
Drosophila melanogaster Species Subgroup

Amir Yassin*, Virginie Orgogozo
Centre National de la Recherche Scientifique - Unité Mixte de Recherche 7592, Institut Jacques Monod, Université Paris Diderot, Paris, France

Abstract

In contrast to male genitalia that typically exhibit patterns of rapid and divergent evolution among internally fertilizing animals, female genitalia have been less well studied and are generally thought to evolve slowly among closely-related species. As a result, few cases of male-female genital coevolution have been documented. In Drosophila, female copulatory structures have been claimed to be mostly invariant compared to male structures. Here, we re-examined male and female genitalia in the nine species of the D. melanogaster subgroup. We describe several new species-specific female genital structures that appear to coevolve with male genital structures, and provide evidence that the coevolving structures contact each other during copulation. Several female structures might be defensive shields against apparently harmful male structures, such as cercal teeth, phallic hooks and spines. Evidence for male-female morphological coevolution in Drosophila has previously been shown at the post-copulatory level (e.g., sperm length and sperm storage organ size), and our results provide support for male-female coevolution at the copulatory level.

Introduction

In most animal species with internal fertilization, male external genitalia are the most rapidly evolving organs and they usually are the first organs to diverge morphologically following speciation [1]. Because of their rapid evolution and species-specificity, their illustration is a common feature of taxonomic literature to discriminate closely-related species. Among the various hypotheses proposed to explain such a rapid male genitalia evolution, two appear as the most plausible [2]. First, the cryptic female choice (CFC) hypothesis postulates that male genitalia evolution is driven by the ‘aesthetic’ sense of females [1]. This hypothesis considers the great diversity of male external genitalia comparable to the rapid evolution of exaggerated sexual ornaments (e.g., feather colors) that are used to charm or lure females. Second, the sexually antagonistic coevolution (SAC) hypothesis postulates that the reproductive optimum of one sex is in opposition to that of the other, setting up an escalating arms race of antagonistic traits in males and females. Morphological traits under SAC include male genitalia that cause damage to the female, in order to directly or indirectly maximize the use of the male’s own sperm, in particular by preventing females from remating [3–5].

The coevolution between male and female genitalia expected under CFC differs from the one expected under SAC [2]. On one hand, CFC predicts that female changes will probably involve female modifications should be unraveled by future neurobiological research, such as examinations of female reproductive tract neurons. CFC is also compatible with a certain degree of morphological coevolution between male and female genitalia, which would be on a “cooperative basis”, such as grooves and furrows helping males to grasp the female, or helping females to sense the male. Such a pattern of cooperative coevolution has been widely documented in Pholcidae spiders between male cheliceral apophyses and female epigynal pockets [6–8]. On the other hand, SAC predicts that female genitalia might evolve in response to male aggressive genital structures on a “defensive basis” in order to resist the harm induced by males. Few instances of resistant female structures coevolving with male harmful genitalia have been documented and even fewer appear to be defensive [2,9]. These examples include the genital pads in Malabar ricefish [10,11], the thickness of vaginal connective tissues in seed beetles [12], the genital spines in water striders [13], the paragenital systems in bedbugs [14], the vaginal coils in waterfowl [15] and morphometrical covariations in female guppies [16] and dung beetles [17]. Most of these cases involve species with coercive mating and reduced courtship, suggesting that the lack of female ‘aesthetic’ senses in these species may have led to the evolution of such cases [18–20].

Two comparative studies of genitalia in various fruit flies of the genus Drosophila concluded that in contrast to rapidly evolving male genitalia, female genital morphology is “practically invariable” among closely-related species that have diverged 3 million years ago (Ma) [21], and that their general form remained identical between distantly-related species that have diverged 40–60 Ma [22]. Because courtship is elaborate in Drosophila species and involves different aspects that appear to influence female choice [23], CFC has been thought to be the primary factor explaining the rapid evolution of male genitalia in these flies [21]. However, Drosophila copulation anatomy has recently been investigated in detail, and a general pattern seems to emerge,
with male genitalia causing copulatory wounds to the female tract, mainly via phallic auxiliary organs known as posteri parames or inner paraphyses [24,25] or via phallic spikes [26]. Whether these wounds reduce survival of mated females is unknown, although they were shown to trigger a localized immune response [27]. In D. melanogaster, a few harmful seminal proteins such as the sex peptide are known to enter the female hemolymph through the intima of the anterior margin of the vagina [28,29] where the mating wounds form [25]. Comparative investigations of copulation anatomy between species also revealed two female genital structures coevolving with male parts. First, in the four species of the melanogaster complex, a membranous pleural pouch before the anterior margin of the female oviscapt (sternite 8) and below tergite 8 harbors the male epandrial posterior lobes at the late stages of copulation [30,31]. The size of this female pouch covaries with male lobe size between the four species. Second, in two species of the yakuba complex, a furrow at the antero-dorsal margin of the oviscapt harbors the male phallic basal spikes during intromission [26,27]. The sizes of these female furrows and male spikes also covary between species of the yakuba complex.

We conducted here a detailed comparative analysis of male and female genitalia in the nine species of the melanogaster subgroup. We found several new female characters whose evolution between species correlates with changes in contacting male structures.

Materials and Methods

Fly Culture and Morphological Analyses

Males and females were obtained from laboratory cultures of the nine species of the melanogaster subgroup (Table 1) and reared on standard Drosophila medium at 21°C. Cultures were kindly provided by Jean R. David (CNRS, Gil-sur-Yvette) and we confirmed the identification of each species based on species-specific male genitalia traits [32–38]. Genitalia of at least 10 individuals per sex and per species were dissected, mounted on microscopic slides in DMHF mounting medium (Entomopraxis A9001) and photographed under a Keyence VHX-2000 light microscope. Outlines of male epandrial posterior lobes and female oviscapt pouches were drawn manually on the light microscope images and their areas were estimated with the ImageJ software package [39]. Measurements were taken on well-dissected and correctly oriented preparations for a single pouch per female (D. melanogaster, N = 17; D. simulans, N = 20; and D. sechellia, N = 19) and from a single epandrial posterior lobe per male (D. melanogaster, N = 8; D. simulans, N = 9; and D. sechellia, N = 5). In addition, 10 D. simulans virgin females were examined for the presence of an oviscapt pouch. These virgin females were selected at the pupal stage based on sex comb absence and adults were grown on standard food for 8 days before dissection. Scanning electron microscopy (SEM) was performed using standard protocol.

For the two species of the melanogaster subgroup whose copulation anatomy has never been described, D. orena and D. erecta, pairs were dissected in copula to investigate the position of male and female genital structures during mating. For each species, 20 virgin females were kept in a vial for five days, and then mated en masse to 4–5 days old males. Ten tubes (N = 200 females) were used for each species. At 3–5 minutes from the start of matings, flies were killed by ether and conserved in absolute ethanol. Thirty mating pairs were dissected, mounted in DMHF and observed under a Leica DMZ light microscope for each species. Ether has also been used efficiently to kill copulating pairs in several other species of the melanogaster subgroup (Jean David, personal communication) and D. orena flies were killed as rapidly as D. erecta in presence of ether. We therefore think that the superficial penetration in D. orena is not an artifact due to rapid withdrawal of their genitalia before death.

Phylogenetic Analysis of Male-female Genital Coevolution

Coevolution between male and female structures was inferred using Pagel’s [40] phylogenetic correlation (λ) test as implemented in the MESQUITE software package [41]. Male and female characters were binary coded (0 = absent, 1 = present) and mapped on the phylogenetic tree of the nine species inferred from Obbard et al. [42] (File S1). For each characters pair, likelihood ratios are compared between two models, one with independent rates of character evolution and the other with the rate of one character depending on that of the second character. Significance was estimated from simulation data after 100 or 1000 iterations using MESQUITE, and False Discovery Rate (FDR) control [43] was applied to correct for multiple comparisons, as implemented in the LBE 1.22 software package in R [44].

Results

Species-specific Female Genitalia

In contrast to previous reports [21,22], our detailed examination of the nine species of the D. melanogaster subgroup uncovered several novel female genitalia structures that are species-specific. These female structures can be classified under two categories: external pouches and internal vaginal shields. We discovered sclerotized depressions of distinctive sizes and shapes at the postero-dorsal margin of the oviscapt in five species. They differ from the membranous pleural pouches described previously by Robertson [30] and Kamimura and Mitsumoto [31] that are located anteriorly at the junction between the oviscapt and the eighth tergite. These newly described sclerotized structures were recently found independently by Kamimura and Mitsumoto [26] in two species, D. yakuba and D. teissieri. Furthermore, we detected sclerifications on internal walls of the vagina, that we named vaginal shields, in three species. Those of D. orena were previously described by Tsacas and David [35]. We provide below a detailed account of these female structures.

To identify the male parts that contact these female structures during copulation, we examined the anatomy of copulating pairs. Based on previous reports for seven D. melanogaster subgroup species [21,22,25,26,30,31] and our observations for two species for which no data were available, we identified male organs that contact each female structure during copulation. Phylogenetic correlation analysis revealed significant correlated evolution of these interacting male and female genitalia structures in the D. melanogaster subgroup.

Female Oviscapt Pouches

In a monograph on European drosophilids, Bächli et al. [38] noted the presence of a large depression at the postero-dorsal margin of the oviscapt of D. simulans that they suggested to “hold the large male epandrial posterior lobe during copulation.” We examined the oviscapt of D. simulans and observed a large depression as indicated by Bächli et al. [38], named hereafter oviscapt pouch (Fig. 1D–J’). This pouch was present in both virgin (N = 10) and mated females (N = 10). We also examined the remaining three species of the melanogaster complex and found smaller oviscapt pouches in two species, D. melanogaster (Fig. 1A–A’) and D. sechellia (Fig. 1G–G’) and no pouch in D. mauritiana (Fig. 1J–J’; N = 10).

Mating descriptions in species of the melanogaster complex [21,22,25,30,31] indicate that at the beginning of copulation the
postero-dorsal margin of the oviscapt contacts male grasping organs known as epandrial posterior lobes. Epandrial posterior lobes provide the strongest discriminatory characters between species of the melanogaster complex (Fig. 1B–C, E–F, H–I, K–L) and have been subject to extensive investigations aiming at identifying the genetic basis of morphological divergence [45–50]. We found
that average female pouch area correlates with average male lobe area in the melanogaster complex species (Spearman's rank correlation: \( r = 1.00, P < 0.157 \); Fig. 2). In D. mauritiana, the epandrial posterior lobe is reduced to a small rod (Fig. 1K). Although a slight depression at the postero-dorsal margin of D. mauritiana oviscapt might be perceptible on SEM photos (arrow in Fig. 1J), we did not detect any oviscapt pouch in dissected D. mauritiana oviscapts under a conventional light microscope.

**Female Oviscapt Furrows**

In the yakuba complex, we also detected a depression at the postero-dorsal margin of the oviscapt in D. teissieri (white arrowheads in Fig. 3A) and in D. yakuba (Fig. 3D) but not in D. santomea (Fig. 3G; \( N = 10 \)). Similar observations were made independently by Kamimura and Mitsumoto [26] in these three species. This depression forms a slit in D. teissieri (Fig. 3A) and an oval pocket in D. yakuba (Fig. 3D, see also Fig. 1e–c’ in Kamimura and Mitsumuto [26]) and is called hereafter oviscapt furrow, as it lacks the oval shape typical of the oviscapt pouches of the melanogaster complex.

Small protrusions were also detected in D. teissieri, D. yakuba and D. santomea males in the part of the epandrium that harbors epandrial posterior lobes in species of the melanogaster complex (Fig. 3B, E, H). These structures can thus be considered as small epandrial lobes. Lobes of D. teissieri (Fig. 3B; [32]) are larger than those of D. yakuba (Fig. 3E; [51]), while those of D. santomea (Fig. 3H, not reported previously) are of equal size to those of D. yakuba. Kamimura and Mitsumoto [26] did not describe the role of these lobes during copulation, but according to their microscopic preparations of mating couples, these lobes do not contact female oviscapt furrows during copulation. The female oviscapt furrows of D. yakuba were shown to hold two basal phallic processes during copulation that Kamimura and Mitsumoto [26] called phallic
spikes. Phallic spikes are longer in *D. teissieri* than in *D. yakuba* and are absent in *D. santomea* (Fig. 3C, F, I, [26]). The elongated slit-like shape of the *D. teissieri* frutless suggests that, like in *D. yakuba*, they hold phallic spikes during copulation. In the four species of the *melanogaster* complex, no phallic spikes are found and the female pouches contact male epandrial posterior lobes during copulation [21,25,31].

In *D. orena* and *D. erecta*, no female oviscapt depressions were found (*D. orena*, *N*= 10, Fig. 4D–D'), [35]; *D. erecta*, *N*= 10, Fig. 4G–G', [33]), nor male epandrial posterior lobes (data not shown). The phalli of these species are the largest among the *melanogaster* subgroup species [32]. Phalli of the *erecta* complex strongly discriminate the two species, and their basal protrusions are different from each others and from the phallic spurs of the *yakuba* complex (Fig. 4F, I). We called these protrusions phallic hooks in *D. orena* (Fig. 4F) and phallic spines in *D. erecta* (Fig. 4I).

**Female Vaginal Shields**

Our microscopic investigation of the internal morphology of female genitalia revealed strong sclerites (hereafter vaginal shields) that are found only in *D. teissieri, D. erecta* and *D. orena*. In *D. teissieri*, these sclerites are located at the ventral margin of the vagina (Fig. 4A–A', B; hereafter ventral vaginal shields) and absent from the vagina of its two closely-related species *D. yakuba* and *D. santomea*. During copulation, this part of the vagina contacts male cerci in the four species of the *melanogaster* complex (Fig. 6 in Eberhard and Ramirez [22]; [18,22,26]). Interestingly, *D. teissieri* male cerci harbor a set of teeth that are stronger and stouter than in the other species of the *melanogaster* subgroup (Fig. 4C; [32]), and whose number and disposition differ among geographically isolated populations [53,54]. Vaginal shields in this species may thus have evolved as a protection against those strong cercal teeth.

In *D. orena*, we found a sclerification above the female vulva (Fig. 4D–D', E; hereafter vulval shield; [35]). In *D. erecta*, we found a large sclerite at the dorsal margin of the vaginal duct leading to the uterus (Fig. 4G–G', H; hereafter uterine shield).

**Copulation Anatomy of *D. orena* and *D. erecta***

To determine which male parts come into contact with the vaginal shields in *D. orena* and *D. erecta*, we mounted copulating pairs at 3–5 minutes after copulation started and examined their anatomy. General patterns of the copulation anatomy of *D. orena* and *D. erecta* resembles those of the remaining species of the subgroup (Fig. 5). As in the other species of the subgroup [21,22,25,26,30,31], the male abdomen bends at 180° to penetrate the female and the epandrial lobes, which lack epandrial posterior lobes, grasp female oviscapers at the dorso-distal margins while the surstyli grasp them on the ventro-distal margins. The male cerci grasp the female oviscap at their ventro-medial margin. The male phallus and the two pairs of paraphyses (the inner and outer pairs) penetrate the female vagina. Like in other species [25,26,31], the paraphyses spread into the female vagina laterally, with the outer pairs pressing on the female dorso-lateral walls and the inner pairs pressing on her ventro-lateral walls. Phallic penetration was deep in *D. erecta* (Fig. 5C) and superficial in *D. orena* (Fig. 5A).

Accordingly, most copulating pairs of *D. orena* fixed in alcohol separated from each other during dissection (17 out of 30 pairs), in contrast to *D. erecta* pairs which were strongly fixed and never detached from each other (N=30 pairs). Our observations show that species-specific vaginal shields in *D. orena* and *D. erecta* contact species-specific phallic hooks and spines, respectively, during copulation (arrowheads in Fig. 5B, D).

**Phylogenetic Analysis of Coevolution**

Male and female genital traits (presence/absence) were mapped on the phylogeny of the nine species in order to test their coevolution (File S1; Fig. 6). Table 2 shows the distribution of Pagel’s phylogenetic correlations (λ) between the different male and female genital traits described here, and their corresponding probability values after FDR correction for multiple comparisons. With the exception of the negative correlation between male epandrial posterior lobes in the *melanogaster* complex and the small lobes of the *yakuba* complex (λ = 3.34; q = 0.01), the highest correlation values were found between male and female structures and they all correspond to positive correlations: epandrial posterior lobes with oviscapt pouches (λ = 6.09; q = 0.019; Fig. 6B), phallic spikes with oviscapt furrows (λ = 4.76; q = 0.017; Fig. 6C), phallic hook with vulval shield (λ = 3.10; q = 0.019; Fig. 6D), phallic spines with uterine shield (λ = 3.09; q = 0.019; Fig. 6D) and cerical teeth with ventral vaginal shields (λ = 3.11; q = 0.017; Fig. 6D). Interestingly, each of these coevolving structure pairs comes in contact with each other during copulation (see above). The male epandrial posterior lobes of the *melanogaster* and *yakuba* complexes did not show significant coevolution with the female oviscapt depressions which include both pouches and furrows, in these two complexes (λ = 2.01; q = 0.052), in concordance with the observation that the female pouches and furrows contact distinct male organs during copulation.

**Discussion**

**Species-specific Evolution of Female Genitalia**

In contrast to previous reports [21,22], our detailed investigation of female external genitalia in the *Drosophila melanogaster* species subgroup shows them to be both species-specific and coevolving with the male structures that they contact during copulation. We not only uncovered a correlation between male lobes and female pouches size (Fig. 2G), but also several qualitative associations between male and female genitalia: ventral vaginal shields and cerical teeth in *D. teissieri*, vulval shields and phallic hooks in *D. orena*, and uterine shields and large serrated phallus in *D. erecta* (Fig. 6D).

Our observations show that one cannot infer faster morphological evolution of genitalia in males than in females based on genitalia drawings in taxonomic literature, as descriptions of male structures are usually overrepresented in current literature [1,2,9]. Female genitalia of all species of the *melanogaster* subgroup except *D. yakuba* and *D. santomea* were previously drawn in taxonomic papers [32–36,38], but only the oviscapt pouch of *D. simulans* [38] and the vulval shield of *D. orena* [35] were outlined. The *D. melanogaster* pouch can be seen on the SEM micrographs of Eberhard and Ramirez [22] and on the light micrographs of Kamimura [25] but the authors did not comment on it. The female genitalia traits that we uncovered here are either external depressions or internal sclerifications. These structures are not as conspicuous as the protrusions (epandrial posterior lobes, phallic spines, etc.) identified previously on male external and internal genitalia in the *D. melanogaster* subgroup species. Although *D. mauritiana* and *D. santomea* female genitalia did not display any species-specific sclerotized structures, their oviscapers exhibited other species-specific morphological traits, e.g. *D. mauritiana* oviscapt are larger, elongated and with stouter peg-like bristles (Fig. 1J, 3G, 6B, C).

Our observations also suggest that male- or female-specific structures located at similar anatomical positions might contact distinct female- or male-specific structures, respectively, in
different species. For example, female pouches and furrows located at similar positions contact male lobes in the melanogaster species complex and phallic basal spikes in the yakuba species complex, respectively. Furthermore, the male phallic basal hooks contact a vaginal shield in D. orena whereas their corresponding structure in the yakuba complex, the basal spikes, contacts female furrows.

In our presently limited state of knowledge regarding the genetic and developmental basis of most of the genital traits described here, it is difficult to formulate homology hypotheses and to precisely determine whether similar traits have been lost or represent independent evolutionary innovations. For example, the various vaginal shields located at different positions in the female lower reproductive tract in diverse species may have diverged from a single ancestral shield or may be true independent innovations. We chose here to code each species-specific vaginal shield as an independent character, and the most parsimonious scenario

Figure 4. Micrographs of female vaginal shields (A–B, D–E, G–H) and male cerci (C) and phalli (F, I) in D. teissieri (A–C), D. orena (D–F) and D. erecta (G–I). White arrowheads indicate apparently harmful male phallic structures. Each oviscapt picture is duplicated (A’, D’, G’), with the contours of the vaginal shields and oviscapt pouches outlined with continuous and dotted lines, respectively; ov: oviscapt; vu: vulva. Scale bar is 50 μm.
doi:10.1371/journal.pone.0057158.g004
Evolutionary Causes and Consequences of Male-female Genital Coevolution in *Drosophila*

At the post-copulatory level, intra- and interspecific size coevolution between male sperm and female sperm storage organs have been documented in *Drosophila* [55–57]. Given that several male seminal proteins are toxic to females [58], most notably the sex peptide which also controls sperm release from sperm storage organs [59], SAC has been proposed to be a major factor driving the rapid evolution of post-copulatory reproductive traits in *Drosophila*.
Our study reveals that female genital structures appear to coevolve with male structures in the *melanogaster* species subgroup. Such a pattern is consistent with the SAC hypothesis (antagonist coevolution), with the CFC hypothesis (cooperative evolution) and with another evolutionary hypothesis known as the lock-and-key [60], which posits that male and female genitalia coevolve rapidly to prevent or reduce copulation between closely-related species [61]. Divergence in genitalia morphologies is clearly not sufficient to prevent interspecific mating in the *melanogaster* species subgroup. Hybrids between *D. santomea* and *D. yakuba* have been found in natural populations on the island of São Tomé [62] and interspecific crosses can be performed in the laboratory between multiple species pairs in the *D. melanogaster* species subgroup [63].

In the lack of experimental data testing the costs induced to the female by the species-specific male structures identified here, it is difficult to conclude whether CFC or SAC is the prevalent force driving genital coevolution in the *melanogaster* subgroup. According to their anatomy and the male organs that they contact during copulation, the various vaginal shields discovered in this study might protect from apparently harmful phallic ornaments (in *D. erecta* and *D. arena*) or from cercal teeth (in *D. teissieri*) during copulation. These shields are devoid of grooves and furrows, suggesting that they might not facilitate genital coupling during copulation. Similarly, *D. yakuba* and *D. teissieri* oviscapt furrows might protect from harmful phallic spikes. Accordingly, contamination risk via matings wounds caused by these spikes in *D. yakuba* are higher in interspecific crosses with *D. santomea* females lacking oviscapt furrows than in intraspecific crosses [27]. The main force driving coevolution of lobes and pouches in the *melanogaster* complex is less clear. The oviscapt pouches may have evolved to screen males for the ones having the most compatible lobes or to help them grasp, in agreement with CFC. Alternatively, the oviscapt pouches and furrows may act as anti-grasping organs that help to dislodge the mating male. At present, it is difficult to interpret from comparative data alone the main driving force of lobe-pouch coevolution.

Recent experimental techniques such as laser surgery provide promising tools to understand the function and fitness consequences of microscopic genital structures. Experimental and genetic approaches have recently helped to understand the adaptive role of a few male grasping structures in *Drosophila* such as the mechanosensilla of the surstylus in *D. melanogaster* [64], the spine-like dorsal portion of the surstylus (known as secondary claspers) in *D. bipectinata* [65] and in *D. ananassae* [66], and the asymmetric epandrial lobes of *D. pachea* [67]. Alteration of these structures decreased male mating success, but the effect on female fitness was not determined. Future examination of the fitness consequences of experimental modifications of the male and female structures identified in this study would probably provide useful data to test which sexual selection hypothesis drives genital coevolution in the *melanogaster* species subgroup.

Theoretical models suggest that sexual selection on reproductive traits drives male and female coevolution along a line of equilibrium within populations, hence ultimately leading to populations differentiation and speciation [68]. However, empirical tests are lacking, probably due to the scarcity of cases where clearly coevolving male-female genital structures are known to vary in natural populations or between incompletely-isolated, nascent species. Geographical variation in male epandrial posterior lobes in the *melanogaster* complex [47] and in number of male cercal teeth in *D. teissieri* [43,44] has been reported. Future analysis of the geographical variation of the corresponding coevolving female structures identified here might reveal interesting patterns.

With high-throughput sequencing methods and powerful genetic tools, the genes responsible for genitalia morphological differences between species of the *Drosophila melanogaster* subgroup are now within reach and should soon be identified. Having these data in hand will then allow us to explore important yet
unanswered evolutionary questions, such as whether coevolving male and female traits share similar developmental basis and which selective forces drive male-female genitalia coevolution.

Supporting Information

File S1  A nexus file describing male and female genital characters distribution in the nine species of the Drosophila melanogaster species subgroup. (NEX)

Figure 6. Mapping of male-female genital coevolution on the phylogeny of the nine species of the melanogaster subgroup (A) drawn after Obbard et al. [42]: male epandrial posterior lobes and female oviscapt pouches in the melanogaster species complex (B), male phallic spikes and female oviscapt furrows in the yakuba species complex (C), and male phallic spines and hooks and cercal teeth and female uterine, vulval and vaginal shields in D. erecta, D. orena and D. teissieri, respectively (D).
doi:10.1371/journal.pone.0057158.g006

Acknowledgments

We thank Jean David for providing strains and for mounting mating pairs, Leondas Tsacas (National Museum of Natural History, Paris) for sharing SEM images, David Montero and the Scanning Electron Microscopy platform of the ITDYS laboratory in Université Paris 7 Diderot for their help in SEM preparation and observation. We also thank two anonymous referees for their constructive criticisms on an earlier version of this manuscript.

Author Contributions

Conceived and designed the experiments: AY VO. Performed the experiments: AY. Analyzed the data: AY VO. Contributed reagents/materials/analysis tools: AY VO. Wrote the paper: AY VO.

References

1. Eberhard WG (1985) Sexual Selection and Animal Genitalia. Harvard University Press. 256 p.
2. Eberhard WG (2010) Evolution of genitalia: theories, evidence, and new directions. Genetica 130: 5–18. doi:10.1007/s10709-009-9356-y.
3. Stutt AD, Siva-Jothy MT (2001) Traumatic insemination and sexual conflict in the bed bug Cimex lectularius. Proc Natl Acad Sci USA 98: 5683–5687. doi:10.1073/pnas.101440698.
4. Arnqvist G, Rowe L (2005) Sexual Conflict: Princeton University Press. 360 p.
5. Hosken DJ, Stockley P, Tregonza T, Wedell N (2009) Monogamy and the battle of the sexes. Annu Rev Entomol 54: 361–378. doi:10.1146/annurev-ento.54.110807.090608.
6. Huber BA (1999) Sexual selection in pholcid spiders (Araneae, Pholcidae): artful chelicerae and forceful genitalia. J Arachnol 27: 135–141.
7. Huber BA (2003) Southern African pholcid spiders: revision and cladistic analysis of Quantiana gen. nov. and Spernaphora Hentz (Araneae: Pholcidae), with notes on male-female covariation. Zoo J Linn Soc 139: 477–527.
Drosophila mauritiana

Ro¨nn J, Katvala M, Arnqvist G (2007) Coevolution between harmful male genitalia and female resistance in seed beetles. Proc Natl Acad Sci USA 104: 10921–10925. doi:10.1073/pnas.0701170104.

Arnqvist G, Rowe L (2002) Antagonistic coevolution between the sexes in a group of insects. Nature 415: 787–789. doi:10.1038/415757a.

Carayon J (1966) Traumatic insemination and paragenital system. In: Usinger R, editor. Monograph of Cimicidae (Hemiptera, Heteroptera). Entomol Soc. America. 88–166.

Brennan PLR, Prum RO, McCracken KG, Sorenson MD, Wilson RE, et al. (2010) Intraspecific evidence from guppies for correlated patterns of male and female genital trait diversification. Proc Biol Sci 277: 2611–2620. doi:10.1098/rspb.2010.2453.

Simmons LW, Garcia-Gonzalez F (2011) Experimental coevolution of male and female genital morphology. Nat Commun 2: 374. doi:10.1038/ncomms1379.

Valher V (n.d.) Coercive copulation in the alpine bushcricket tettigoniidae alpinae. Zoology 110: 1065–1075.

Peretti AV, Willems A (2006) Sexual coercion does not exclude luring behavior in the climbing camel-shepherd Otusca chauvin (Arachnida, Solifugae, Atacadochidae). J Ethol 24: 29–39. doi:10.1007/s10164-006-0201-y.

Dickson BJ (2008) Wired for sex: the neurobiology of Drosophila. J Zool 276: 197–213. doi:10.1111/j.1420-9101.2006.01099.x.

Joly D, Schiffer M (2010) Coevolution of male and female reproductive behavior and genital coupling in Drosophila melanogaster. Proc Natl Acad Sci USA 107: 2611–2615. doi:10.1073/pnas.0911070107.

Eberhard WG (2004) Rapid divergent evolution of sexual morphology: genitalia reduce the possible infection risk caused by male copulatory wounding. Proc Biol Sci 271: 1107–1114. doi:10.1098/rspb.2007.0192.

Brennan PLR, Prum RO, McCracken KG, Sorenson MD, Wilson RE, et al. (2007) Evolutionary novelties in islands: metaphyly in Southeast Asian pholcid spiders: the case of Drosophila ananassae species from Sao Tome. Proc Biol Sci 274: 1478–1495. doi:10.1098/rspb.2007.1169.

Bachli G, Velea CR, Ecsedy SA, Saura A, Bachli G, et al. (2004) The Drosophilidae (Diptera) of Fennoscandia and Denmark. Fauna Entomol Scand 39: 1–362.

Abramoff MD, Magalhães PJ, Ram SJ (2004) Image processing with ImageJ Biophotonics Internat 11: 36–42.

Pagel M (1994) Detecting correlated evolution on phylogenies: A general method for the comparative analysis of discrete characters. Proc Biol Sci 255: 37–45.

Maddison WP, Maddison DR (2012) Mesquite: a modular system for evolutionary analysis. Version 2.75. Available-http://mesquiteproject.org.

Ohbahn DJ, Maclean J, Kim K-W, Rambaut A, O’Grady PM, et al. (2012) Estimating divergence dates and substitution rates in the Drosophila phylogeny. Mol Biol Evol 29: 3459–3473.

Benjamin Y, Hochberg Y (1995) Controlling the false discovery rate: a practical approach to multiple testing. J R Stat Soc B 57: 289–300.

Dalmauco C, Brot P, Moreau T (2005) A simple procedure for estimating the false discovery rate. Bioinformatics 21: 660–663.

Coyne JA (1985) Genetic basis of differences in genital morphology among three sibling species of Drosophila. Evolution 39: 1101–1118.

Coyne JA, Run, J, David JR (1991) Genetics of morphological differences and hybrid sterility between Drosophila simulans and its relatives. Genet Res 57: 113–122.

Liu J, Mercer JM, Stam LF, Gibson GC, Zeng ZB, et al. (1996) Genetic analysis of a morphological shape difference in the male genitalia of Drosophila simulans and D. melanogaster. Genetics 141: 1129–1145.

Macdonald SJ, Goldstein DB (1999) A quantitative genetic analysis of male sexual traits distinguishing the sibling species Drosophila simulans and D. sechellia. Genetics 153: 1683–1699.

Zeng Z-B, Liu J, Stam LF, Kao C-H, Mercer JM, et al. (2000) Genetic architecture of a morphological shape difference between two Drosophila species. Genetics 154: 299–310.

Mashy JP, Daloune JE, Srivastava S, Chen L, Archerman MN (2011) The genetic basis of rapidly evolving male genital morphology in Drosophila. Genetics 189: 357–374. doi:10.1534/genetics.111.138015.

Sánchez L, Santamaria P (1997) Reproductive isolation and morphogenetic evolution in Drosophila analyzed by breakage of ethological barriers. Genetics 147: 231–242.

Lachaise D, Camp F, Caruso M-L, Doly J, Lemeunier F, et al. (2004) Nine relatives from one African ancestor: population biology and evolution of the Drosophila melanogaster subgroup species. In: Singh RS, Uyenoyama MK, editors. The Evolution of Population Biology. Cambridge University Press. 351–353.

Lachaise D, Lemeunier F, Veuille M (1991) Clinical variations in male genitalia in Drosophila tiera. Tsacca. Am Nat 117: 600–608.

Doly J, Caruso M-L, Mihanga-Matungadura T, Lachaise D (2010) Male terminalia variation in the rainforest dwelling Drosophila tiera contrasts with the sperm pattern and species stability. Genetica 130: 139–152. doi:10.1007/s10709-009-9423-4.

Pinnick S, Markow T, Spicer GS (1999) Evolution of multiple kinds of female sperm-storage organs in Drosophila. Evolution 53: 1804–1822. doi:10.2307/264044.

Miller GT, Pinnick S (2002) Sperm-female coevolution in Drosophila. Science 298: 1290–1293. doi:10.1126/science.1076068.

D. mauritiana

Joly D, Schiffer M (2010) Coevolution of male and female reproductive structures in Drosophila. Genetica 138: 105–118. doi:10.1007/s10709-009-9392-5.

Mueller JL, Page JL, Wolfner MF (2007) An ectopic expression screen reveals the protective and toxic effects of Drosophila seminal fluid proteins. Genetics 175: 777–783. doi:10.1534/genetics.106.065316.

Avida RW, Racemit K, Blech Qin MC, Wolfner MF (2010) Sex peptide is required for the efficient release of stored sperm in mated Drosophila females. Genetics 186: 595–600. doi:10.1534/genetics.109.118735.

Dufour L (1814) Anatomie générale du Díperotes. Annls Sci Nat 1: 224–264.

Dauty JP (2012) 170 years of “Lock-and-Key”: genital morphology and reproductive isolation. Internat J Evol Biol 2012: 1–10. doi:10.1155/2012/247352.

López A, Lachaise D, Coyne JA (2005) An anomalous hybrid zone in Drosophila. Evolution 59: 2602–2607.

Caruso M-L, Silvain JF, Daubin V, Da Lage JL, Lachaise D (2001) Divergence between Drosophila simulans and allopatric or sympatric populations of D. yakuba using paralogous amylase genes and migration scenarios along the Cameroon volcanic line. Mol Ecol 10: 649–660.

Acres A, Codd M, Ferrer J-F (2003) Species-specific effects of single semilunar ablation on mating position in Drosophila. J Exp Biol 206: 3093–3100. doi:10.1242/jeb.005522.

Polak M, Rashid A (2010) Microscale laser surgery reveals adaptive function of male intromittent genitalia. Proc Biol Sci 277: 1371–1376. doi:10.1098/rspb.2009.1729.

Grieshop K, Polak M (2012) The proropulatory function of male genital spines in Drosophila ananassae [Dedeschall] (Diptera: Drosophilidae) revealed by laser surgery. Evolution 66: 2637–2645. doi:10.1111/j.1558-5646.2012.01631.x.
67. Lang M, Orgogozo V (2012) Distinct copulation positions in *Drosophila pachon* males with symmetric or asymmetric external genitalia. Contribs Zool 81: 87–94.

68. Ritchie MG (2007) Sexual selection and speciation. Ann Rev Ecol Evol Syst 38: 79–102. doi:10.1146/annurev.ecolsys.38.091206.095733.