

Shape matters: the evolution of insect genitalia

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This paper offers an overview of current knowledge of the evolution of the genitalia of insects, chelicerates and Myriapoda. The overcomplexity and extreme interspecific divergence of (especially the male) genitalia, can not be explained by either a function as 'lock-and-key' to prevent hybridisation, or a pleiotropic 'byproduct' of other evolutionary changes during speciation. Instead, current theory and empirical evidence strongly suggests that three aspects of sexual selection play a crucial role: Fisherian sexual selection by female choice, sperm competition, and male-female antagonistic coevolution. Probably the evolution of genitalia is a mosaic of the effects of all three. The data at hand do not offer a clear impression of how rapidly genitalia normally evolve, but it appears that their divergent evolution is relevant in speciation.

Keywords: genitalia, penis, sexual selection, sperm competition, female choice, lock-and-key hypothesis, sexually antagonistic selection, speciation, taxonomy, identification

Let us begin with a random example from systematic entomology: the beetle genus *Ptomaphagus*. Currently, 70 species are known in this Southeast-Asian taxon, and many still await description (Schilthuis & Perreau, 2003). In spite of this large number of recognised species, their external appearance is remarkably uniform. Most are between two and three millimeters long, brown, ovoid in habitus, and no special markings can usually be seen on their bodies. There are slight differences in the proportions of their antennal segments, and in the outline of pronotum and elytra, that is all. The bulk of their morphological diversity is, however, internal: the penis, especially its apex, is shaped in wildly different ways among the different species. Compared with the external morphology, genital diversity is so large and characteristic, that some authors do not even illustrate other parts of the body.

Ptomaphagus is in no way exceptional. Great divergence in genitalia morphology among species is the rule in insects, and in most groups, taxonomists use genitalia (especially male genitalia) for species identification and classification. A perusal of four recent issues of the *Tijdschrift voor Entomologie* (Netherlands Journal of Entomology) reveals that out of 26 taxonomic papers, genitalia played a prominent role in 23. A well-known examples of the value of genitalia in recognising species, are the honeybees, where males carry bizarre and widely disparate 'prongs' and spiny, evertible 'sacs' on and in their genitals (Simpson, 1970), but any taxonomic revision would serve to illustrate the phenomenon of extreme genital diversity at the species level.

Not only are genital systems diverse within taxa, they are also anatomically complex. Not rarely are they composed of a large number of interconnected and articulating pegs, plates, springs, and levers. The males of various species of flea (Siphonaptera), for example, carry large and complicated structures inside their abdomens that seem inordinately complex for the relatively simple task of fertilizing a female. The terminology in use by specialists is also indicative of a large morphological complexity. At least six terms (median lobe, paramere, apical orifice, internal sac, median stylet, ligula) are needed to describe distinguishable parts of the male genitalia of the above-mentioned genus *Ptomaphagus*, whereas orthopterologists need the same number (phallus, epiproct, paraproct, cercus, gonotreme, and titillator; Gwynne, 1998) in discussions of the male genitalia of crickets and grasshoppers. Eberhard (1985) has pointed out the resemblance of such elaborate structures to 'Rube Goldberg-machines'. The cartoonist Rube Goldberg is famous for inventing absurdly intricate contraptions for carrying out very simple tasks (like a pencil sharpener made up of no less than 19 components). If the penis is only a sperm-injecting organ, then both its complexity and its diversity would be as redundant as a Rube Goldberg machine.

Several explanations have been offered for this conundrum. In this paper I will give an overview of the more recent developments in this field, largely based on the seminal works by Eberhard (1985; 1996), which dealt with animal genitalia in general, updated with more recent

insights into the evolution of insect genitalia. In addition, I will speculate on the role genital evolution may have on insect speciation, and give suggestions for further research.

DISCREDITED HYPOTHESES

Pleiotropy

Mayr (1963) saw speciation as a largely random process of genetic reorganisation in small, isolated populations. Even though it may be initiated by selection on a small number of genes, many genes would follow suit due to pleiotropic interactions. He thought that the divergence in genitalia would be an example of such pleiotropic, ‘accidental’ response, especially since ‘as internal structures, they are less subject to the corrective influences of natural selection [...], provided the basic function of gamete transfer is not impaired’ (Mayr, 1963: 104).

Even ignoring the fact that there are many theoretical problems with Mayr’s model of speciation by ‘genetic revolution’ (Schilthuizen, 2000), pleiotropy as an explanation for genital divergence has several weaknesses (Eberhard, 1985). The most compelling of these is that, among internal organs, the genitalia are the only ones that vary this much. If Mayr’s argument had been correct, other internal organs would have shown the same pattern of variation. In addition, the empirical facts seem in contradiction as well. In spiders, for example, the external pedipalps have taken the role of genitalia, and these are as divergent between species as internal genitalia are in insects.

Lock-and-key

A much stronger possible explanation is the ‘lock-and-key’ hypothesis, an idea that goes back a long way. As early as 1844, Dufour advanced this hypothesis, saying that ‘*l’armure copulatrice [...] est la garantie de la conservation des types, la sauvegarde de la légitimité de l’espèce*’. More explicitly, the lock-and-key hypothesis states that, to avoid the production of maladapted hybrids, females have evolved specialised ‘locks’ to which only the ‘keys’ of the males of the same species will fit (Shapiro & Porter, 1989). Gosse (1883: 280) echoed Dufour more poetically when he wrote: ‘if I see a number of keys, of very minute and elaborate workmanship, all different, I cannot doubt that every one is intended to fit some special lock’.

A variant of this ‘mechanical lock-and-key’ hypothesis has been put forward by authors which have been struck by the fact that male genitalia appear designed for stimulation, rather than for a mechanical match with the female genitalia. For example Jeannel (1955) and de Wilde (1964), therefore, have proposed a ‘sensory lock-and-key’ hypothesis, where the male genitalia provide the female with species-specific stimuli during copulation, which allow her to assess whether she is about to be inseminated by a male of her own species or not.

It is not surprising that this hypothesis has been very popular, because intuitively it makes good sense. However, on close inspection, serious empirical and theoretical problems appear. First of all, copulations between different species and even different genera have been documented in abundance. However, it is usually not clear if such couplings produce result in fertilization, and if they do, whether fertilization rates are similar to intraspecific copulations. More compelling empirical evidence against the hypothesis is that genitalic divergence is just as strong in allopatric as in sympatric species. Insect species that are endemic to islands, such as *Oliarus* bugs on the Galápagos islands (Fennah, 1967), to separate cave systems (bathysciine beetles; Dupré, 1992) or specific to certain hosts, like parasitic lice (Emerson & Price, 1981) have quite divergent genitalia, even though they never come in contact with each other, so allospecific copulation will not occur. In addition, a stronger theoretical reason to be suspicious of the lock-and-key hypothesis is that, as remarked by Alexander (1964), one would expect natural selection to favour mechanisms that allow a female to ascertain the species identity of her mate at an earlier courtship stage than copulation.

A rigorous test of the lock-and-key hypothesis was carried out by Arnqvist (1998), who compared monandrous insect groups with polyandrous sister groups, and found that in the polyandrous groups the male genitalia were more divergent among species. This is strong evidence against the lock-and-key hypothesis, since one would expect that mistakes are selected against

more strongly in groups where females mate only once. In fact, Arnqvist's test supports a group of hypotheses that have become fashionable in recent years, and that rely on sexual selection (see below).

CURRENTLY POPULAR HYPOTHESES

Sexual Selection by Female Choice

Eberhard (1985) has advanced the idea that male genitalia are 'internal courtship devices' that stimulate the female by internal tactile display in an extension of the external courtship rituals (which include visual, auditory, olfactory and sensory displays). He suggested that exaggerated structures on the male genitalia are sexually selected by females in a Fisherian fashion, thus producing both structural complexity and interspecific divergence.

Eberhard presented a large amount of evidence in favour of this hypothesis, showing that genitalia indeed do have many stimulatory functions on top of their function as sperm injector and may thus be the target of selection by female choice. Examples from this body of evidence are: (1) male insects often have structures on their genitalia with which they tap females near the genital orifice prior to intromission; this happens in for instance beetles and wasps; (2) in certain groups, *e.g.*, spiders, millipedes, and beetles, males are known to perform a 'dry' copulation before sperm transfer takes place; (3) in Coleoptera and Lepidoptera, genital structures such as the parameres and male valves, respectively, remain outside the female during copulation and have been seen to rhythmically stimulate the outside of the female's abdomen; (4) rubbing and thrusting of the male intromittent organ are common in copulation, and the spines and knobs on some penises may thus cause strong tactile stimulation to the female; West-Eberhard (1984) observed autonomous movements of different parts of the male genitalia of the wasp *Parachartergus apicalis* and described these as 'the most fluid and subtly modulated movements I have ever observed in wasps'; (5) moths of the family Apatelodidae have stridulatory organs on the male genitalia that 'sing'.

In addition to this indirect evidence, a small number of direct experimental tests have been carried out. Rodriguez *et al.* (submitted, as cited in Eberhard, 1996) showed that in the tortoise beetle *Chelymormpha alternans*, males with a longer flagellum on the penis sired a larger number of offspring, and post-coital sperm dumping by the female appears to be the mechanism by which paternity is enhanced. Experimental shortening of the flagellum also reduced the manipulated males' rates of fertilisation success.

Male-Male Conflict

Although Eberhard (1985) included direct sperm competition in his sexual selection hypothesis, the two are conceptually somewhat different. In sexual selection by female choice, it is the female that has control over the sperm of which male she will use for fertilization; in direct male-male competition (*i.e.*, sperm competition; Parker 1970), the battle is fought directly between males, with the female reproductive tract as their arena.

A famous example of a male genital structure involved with direct male-male competition is the ligula of damselflies, which Waage (1979) showed to remove sperm from previous males. Other insects also remove sperm of previous males from females' genitalia, using 'scraper'-like parts on their own genitalia. Ono *et al.* (1989) showed that the majority of a female *Truljalia hibinonis* tree cricket's offspring are fathered by the last male she mates with. The ejaculation of this last male pushes out most of the ejaculate of previous males. This 'old sperm' then sticks to the male's penis, and is withdrawn after copulation is finished. In the katydid *Metaplastes ornatus*, during 'phase I coupling' (a kind of pre-copulation), the male's subgenital plate mimics the movement of an egg down the female's reproductive tract, which induces the release of (a previous male's) sperm from her spermatheca. This sperm is then pulled out by the current male and subsequently eaten by the female herself. After this the actual copulation takes place (von Helversen & von Helversen, 1991).

In spite of such studies, Eberhard (1985) has argued that male-male conflict via sperm removal can not be seen as a general explanation for divergence in male genital morphology, since most

male genitalia during copulation do not reach the site where previous males' sperm is kept. However, later, he conceded that perhaps this conclusion had been premature, as sperm is now known to be present in other parts of the female reproductive tract, and may sometimes be accessible to the male's genitalia after all.

Male-Female Conflict

Recently, the evolution of male and female reproductive organs and their secretions have been interpreted in terms of male-female conflict, or, antagonistic coevolution ('chase-away sexual selection' sensu Holland & Rice, 1998). In other words, selection pressures on females may be different from those on males, and traits in males may evolve because they benefit males even if they give a disadvantage to females, and vice versa. This has been clearly demonstrated in the cocktails of chemicals that accompany sperm and act as antiaphrodisiacs on females (which, to the females' detriment, reduces remating and increases a male's fertilization success). Not only do these chemicals prevent females from selecting further males, they also reduce females' longevity.

Chapman *et al.* (1995) discovered just how damaging sex can be to *Drosophila melanogaster* female health. They divided female banana flies into three groups. The ones in the first group were mated with genetically engineered males, which could produce only sperm and none of the additional fluids. Those in the second group were mated with another batch of transgenic males, but in these males the situation was reversed: they produced empty ejaculates, with no sperm, but only the accessory fluids. The third group were control females, who had no males around. The study found that the females who were exposed only to the males' sperm, and not to the ejaculates, lived just as long as unmated females, up to thirty-five days. Those that were exposed to the males' ejaculates, however, lived no longer than twenty-five days.

One may thus expect that evolutionary steps taken by males will be followed by evolutionary countermeasures of the female part of the population. Such an arms race could lead to rapid divergence in reproductive properties.

Here we are concerned primarily with the morphology of genitalia. Machada de Barros (1959) mated male tsetse flies from one part of Africa with females from another part and found that copulation led to such mechanical damage that it was lethal to the females. This may be taken to mean that females that have not coevolved with males do not have the proper 'defences' against male manipulative structures. Antiaphrodisiacs are not just inserted with the male's sperm. Semen of the housefly, *Musca domestica*, leaches a hole in the female's vagina, which allows the antiaphrodisiacs to pass directly into her hemolymph, and in the blowfly *Lucilia sericata*, the spikes on the male's penis inject manipulative substances into the female (Eberhard, 1996).

A recent study by Crudgington & Siva-Jothy (2000) showed that the spines on the penis of the bean weevil *Callosobruchus maculatus* are not involved in sperm competition, but, like in *Lucilia*, puncture the wall of the female genital tract, possibly to inject antiaphrodisiacs. Females try to avoid copulations by kicking the male, and those who mate frequently die younger. This is clearly an example of male-female conflict.

Another example involves the famous 'traumatic insemination' of bedbugs (*Cimex lectularius*). In these insects, males use their sabre-like penis to pierce the body-wall of the female and inject their sperm directly into the body cavity of the female. The sperm then migrate through the female's body to reach the ova. Insertion of the penis, however, does not happen at a random locality, but at a set of 'secondary genitalia' on the left-hand side of the female's abdomen. This so-called 'spermalege' is thought to have evolved as an evolutionary response of the female to regain control over fertilization (Eberhard, 1985). Nevertheless, the repeated wounding of the female cuticle during copulation brings a roughly 25% cost in female longevity and female reproductive output (Stutt & Sive-Jothy, 2001).

Resolution

It appears clear that we have to turn to sexual selection to understand the cause for the complexity and diversity in insect genitalia that have always been such a blessing for insect taxonomists. However, at least three different schools of thought are popular at the moment: 'Fisherian' sexual selection, sperm competition, and sexually antagonistic selection.

It should be pointed out, however, that these three hypotheses are not mutually exclusive, and the differences between them may even be less strict than they appear. First of all, the net effect of Fisherian sexual selection and sperm competition is not different: both result in a female preferentially using mainly a particular male's sperm, and whether this results through rejecting of other males' sperm by the female or removal of such sperm by the male, is not relevant for the outcome. The differences between these two and sexually antagonistic selection, however, are greater. In the latter situation, an additional natural selection comes into play, in the form of increased mortality or morbidity of the female due to manipulation by the male. However, as pointed out by Cordero & Eberhard (2002), this does not necessarily mean that a female's fitness will be reduced if she chooses to suffer these consequences. If we take into account the fact that her sons will inherit similarly manipulative abilities, females may ultimately benefit rather than suffer from mating with such males.

Also, different portions of the genitalia may be under different kinds of selection. Spines on the penis' internal sac, for example, may inject manipulative substances, whereas its parameres are purely for tactile stimulation, and a third structure on the same penis may remove sperm from a predecessor. Such mozaic evolution should be borne in mind when trying to understand the evolution of genital morphology in a particular group.

THE IMPACT OF GENITAL EVOLUTION ON SPECIATION

The above suggests that genitalia shape can evolve rapidly. It would be interesting to know exactly how rapidly. Some authors have argued that genital evolution is not rapid at all, since, given that they are so useful for identification, there obviously is little variation in populations that selection can act on: genital shape would actually be very conserved within a species (see Eberhard, 1985). However, this is a misconception, as studies of intra- and interspecific variability in genital morphology show that genitalia are in fact quite variable intraspecifically, while retaining more or less relative interspecific uniformity (*e.g.*, Shelley, 1981; Tanabe *et al.*, 2001).

Another indication that genitalia diverge rapidly should come from studies where some dating is possible, *i.e.*, paleontological or molecular clock studies. So far, few data are available, but it appears that both rapid and slow evolution can take place. For example, the pair of butterfly sister species *Lycaeides idas* and *L. melissa* show clear differences in their male genitalia, but are genetically indistinguishable, which suggests that they have diverged extremely recently (Nice & Shapiro, 1999). Conversely, Coope (1979) remarked that, by their preserved genitalia, fossil beetles as old as the late Tertiary and early Pleistocene can still be assigned to recent species. Hence, it appears that genital evolution can be either rapid or slow, and it is as yet not clear what the evolutionary rates depend on.

Whether rapidly evolving or not, genitalic evolution may play an important role in speciation. However, the assessment depends on one's view of species and speciation. If species are seen as cohesive, distinct 'gene clusters', then genitalia evolution certainly is a key factor, as many insect species differ (morphologically, at least) mainly in their genitalia. However, this is a somewhat circular argument. The question should be: does divergence in genitalia aid in bringing about divergences in other traits as well? Here, it appears that their chief role would be in disrupting gene flow between incipient species. The tsetse fly example above, shows that reproduction is no longer possible between males and females that have evolved in isolation, possibly due to the lack of coadaptation between male and female offence and defence. Theory predicts that the evolutionary steps in Fisherian sexual selection, sperm competition and male-female reproductive conflict will follow a tortuous and unpredictable route. Populations that are allopatric will cease to run in synchrony where such developments are concerned (Iwasa & Pomiankowski, 1995). Consequently, reproductive isolation will evolve. This may thus be an important factor in allopatric speciation; its role in sympatric speciation is not clear yet, however (Schilthuisen, 2000).

Future studies could use molecular tools to evaluate the pivotal role of genital evolution in speciation. Similar studies could be done as the one by Ting *et al.* (2000), who used a molecular genealogy for the 'speciation gene' *Odysseus* to demonstrate its key role in *Drosophila* speciation. Very little is known on the genetic basis of genital morphology, but this undoubtedly will change,

and it should then be possible to see whether divergences in these genes predate divergences in other parts of the genome.

Other studies may focus on disentangling the relative effects on genital evolution of sexual selection by female choice, sperm competition, and male-female antagonistic coevolution. Again, molecular studies might help: if the latter aspect is important, then genes expressed in the development of the female genitalia should be as divergent as those expressed in the formation of the male genitalia. In any case, I hope this paper will have confirmed the assertion of Burns (1987; as cited in Shapiro & Porter, 1989), who said that 'genitalia deserve all the respect and attention they can get'.

Acknowledgements I wish to thank Jan Bruin, the Netherlands Entomological Society (N.E.V.), and the University of Amsterdam for making it possible for me to give this lecture and to attend the 'Entomologendag'.

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