



The evolution of animal genitalia: distinguishing between hypotheses by single species studies

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Rapid evolution of genitalia is one of the most general patterns of morphological diversification in animals. Despite its generality, the causes of this evolutionary trend remain obscure. Several alternative hypotheses have been suggested to account for the evolution of genitalia (notably the lock-and-key, pleiotropism, and sexual selection hypotheses). Here, I argue that thorough intraspecific studies are the key to gaining insight into the patterns and processes of genitalic evolution. Critical assumptions and predictions that may be used to distinguish between the different hypotheses are identified and discussed. However, current knowledge of selection on genitalia, or even of the degree of phenotypic and genotypic variability of genitalic morphology, is highly limited, allowing only a very tentative assessment of the various hypotheses. In-depth single species studies of current patterns and processes of selection on genitalia are badly needed, and a single species research program is briefly outlined.

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INTRODUCTION

One of the most striking and general patterns in the evolutionary radiation of animal taxa is morphological diversification of male genitalia. In animals with internal fertilization, male genitalic morphology typically differs considerably and

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consistently between species, even if general morphology differs very little. In his seminal contribution, Eberhard (1985) reviewed the pattern of genitalic elaboration and diversification across a wide range of animal taxa, and assessed the various hypotheses that have been put forth to account for this evolutionary phenomenon. However, as has been stressed by several authors (Scudder, 1971; Eberhard, 1985, 1990; Shapiro & Porter, 1989; Andersson, 1994; Arnqvist & Rowe, 1995; Alexander, Marshall & Cooley, 1996), there is a serious shortage of empirical studies dealing with genitalic evolution, and the basis for distinguishing between different hypotheses is currently very weak. In particular, relevant in depth single species studies are virtually lacking. Considering the generality and form of genitalic evolution relative to several other domains of morphological adaptation, our current ignorance of the processes responsible for genitalic evolution is truly perplexing.

The reasons for our lack of understanding of genitalic evolution are partly historic. Darwin (1871) distinguished between two types of sexually dimorphic traits; primary and secondary sexual traits. Traits being directly connected with the act of reproduction, largely gonads, ducts and copulatory organs, were considered primary sexual traits favoured by natural selection. Being copulatory organs, genitalia have been considered primary sexual traits and have, as such, been essentially disqualified from studies of sexual selection, which have almost exclusively been concerned with secondary sexual traits (Bradbury & Andersson, 1987; Andersson, 1994; Johnstone, 1995). However, there are very good reasons to question the validity of this distinction, and there are at least four reasons to abandon the dichotomy between primary and secondary sexual traits (see also Eberhard, 1985; Andersson, 1994). First, Darwin (1871) had already realized that it is often impossible to separate between primary and secondary sexual traits on the basis of function alone. For example, many non-genitalic male traits, which hardly qualify as primary sexual traits, are used as 'copulatory organs' (e.g. spider pedipalps and various male claspers). Second, many primary sexual traits function, at least in part, in competition over mates or fertilizations. Male genitalic traits may be involved in competition for mates or fertilizations (Waage, 1979, 1984; Siva-Jothy & Tsubaki, 1989; Birkhead & Hunter, 1990), and even glands, such as testes, may commonly be subjected to sexual selection by sperm competition among males (Warner & Robertson, 1978; Harcourt *et al.*, 1981; Eberhard & Cordero, 1995). Third, new theories on the evolution of animal genitalia (Eberhard, 1985, 1990, 1993a; Arnqvist & Rowe, 1995; Alexander *et al.*, 1996) suggest that primary sexual traits may become elaborated as a result of sexual selection, in much the same way as secondary sexual traits (Andersson, 1994). Fourth, many primary sexual traits evolve rapidly and are species specific, indicating important functional significance. In closely related *Drosophila* species, for example, not only male and female genital morphology, but also traits such as sperm morphology, ovary size, testis size, testis colour, seminal products and gonadal proteins, evolve rapidly and differ considerably between species (Mahowald & Kambyzellis, 1980; Coyne, 1985; Jamieson, 1987; Joly *et al.*, 1991; Pitnick & Markow, 1994; Eberhard & Cordero, 1995; Civetta & Singh, 1996). Thus, the distinction between primary and secondary sexual traits is not only gratuitous and ambiguous on the basis of function, but it may also be essentially flawed on the basis of evolutionary theory. Similar evolutionary mechanisms potentially affect both types of sexual traits: sexual selection may favour elaboration and natural selection may counteract this trend. Abandoning the dichotomy between primary and secondary sexual traits, and thus addressing the evolution of both types of traits in much the

same way, will greatly improve the prospects of gaining a thorough understanding of the evolution of animal genitalia.

Here, I argue that single species studies, largely using methods that have been successfully applied in other areas of evolutionary biology/ecology where the focus is on causes and effects of intraspecific morphological variation, offer novel possibilities to gain insights into the evolutionary processes responsible for genitalic evolution. Further, I identify the critical predictions that can be used to distinguish between different hypotheses for genitalic evolution.

The discussion below primarily concerns male intromittent genitalia. The reason for this is fivefold (cf. Eberhard, 1985). First, male genitalia seem to be more variable and more elaborated than female genitalia, implying a more rapid evolutionary divergence of male genitalia compared to female genitalia. Second, it is typically easier to measure/quantify morphological variation in male genitalia, since it is composed by identifiable morphological structures to a larger extent (e.g. sclerotized parts in invertebrates). Third, female genitalia are, for these reasons, less often described in the literature. Fourth, we have a comparatively good understanding of the evolution of male non-intromittent genital traits, such as various types of genital claspers (e.g. Thornhill, 1984; Arnqvist, 1989; Arnqvist & Rowe, 1995; Sakaluk *et al.*, 1995). Fifth, some of the suggested evolutionary mechanisms apply, for theoretical reasons, to selection on males only. However, there is undoubtedly much to learn from including, or even focusing on, female genitalia in particular cases (Arnqvist & Rowe, 1995; Rice, 1996; see also below).

THE HYPOTHESES

Three main hypotheses can be considered as offering general, and more or less plausible, scenarios for the evolutionary processes responsible for genitalic evolution (see Eberhard, 1985; for a review of several different hypotheses). These are (1) the lock-and-key hypothesis, (2) the pleiotropy hypothesis and (3) the sexual selection hypothesis.

The lock-and-key hypothesis is deeply rooted among many biologists: it was originally proposed in the pre-Darwinian era (Dufour, 1844), and has long been considered a valid explanation for genitalic evolution. In short, this hypothesis holds that species specific genitalia evolve via selection for pre-insemination reproductive isolation (i.e. avoidance of hybridization), so that male genitalia evolve to be species specific, invariant and unique (the key) in order to fit appropriately in female genitalia (the lock). Despite its intuitive appeal and the widespread and long-standing consent to this hypothesis, recent comparative studies of genitalic diversification have failed to conform with the predictions of the lock-and-key hypothesis, and it has been considered a less-plausible hypothesis on these grounds (see Scudder, 1971; Eberhard, 1985; Shapiro & Porter, 1989; for reviews). Also, the underlying assumption of general hybrid inferiority is currently being questioned (see Arnold & Hodges, 1995; for a review).

Mayr (1963) concluded that differences in genitalic morphology appear to play a very minor role, at best, in premating mechanical isolation. Instead, he suggested that variation in genitalic morphology is largely selectively neutral. Under the pleiotropy hypothesis, genitalic evolution is an indirect result of evolution of genetically correlated characters, via accumulated pleiotropic effects of genes that code

for both genitalic and general morphology. Pleiotropic effects on genitalic morphology (or analogous traits, e.g. pedipalps in spiders) are not selected against since genitalic variation is assumed to be neutral, in contrast to most other morphological traits, thus allowing rapid, but arbitrary, genitalic evolution.

Since genitalia are so obviously involved in reproductive events, sexual selection may be responsible for genitalic evolution and diversification. There are three suggestions of how genitalia may evolve via sexual selection. (1) Eberhard (1985) suggested that sexual selection by cryptic female choice (Thornhill, 1983; Eberhard, 1994, 1996) is responsible for genitalic evolution. According to the cryptic female choice hypothesis, male genitalia function as internal, tactile courtship devices that stimulate/titillate multiply-mated females to selectively use sperm from males with superior stimulatory capabilities (i.e. genitalic morphology), over that of others, to fertilize their eggs. While non-random fertilization success among males has been documented in some species (Watson, 1991a, b; LaMunyon & Eisner, 1993; Dickinson, 1996), this has never been unambiguously related either to female sperm use or to male genitalic morphology. (2) It is known that conflicts between the sexes over control of reproductive decisions may drive coevolution of male and female sexual traits. There is empirical evidence in insects showing that conflicts over mating decisions can lead to the evolution of non-intromittent genital claspers in males and female counteradaptations to these (e.g. Thornhill, 1984; Arnqvist, 1989; Arnqvist & Rowe, 1995; Sakaluk *et al.*, 1995). The sexual conflict hypothesis, however, can be expanded and generalized to include male intromittent non-clasping genitalia (Lloyd, 1979; Alexander *et al.*, 1996). According to this scenario, genitalic evolution is a result of sexual conflict over control of fertilization decisions (as opposed to mating decisions). Intromittent male genitalia may function to manipulate female internal sperm use, to induce the female to use more of a particular male's sperm to fertilize her eggs even if this is counter to her own interests. (3) Male genitalia may also become elaborated as a result of sexual selection by sperm competition, so that male genitalia evolve to be efficient at displacing or dislocating sperm from previous males within the female reproductive tract or to induce non-receptivity in females (Smith, 1984). There is evidence for a sperm removal function of male intromittent organs in some insects (Waage, 1979, 1984; Smith, 1984; Siva-Jothy & Tsubaki, 1989; Birkhead & Hunter, 1990).

A SINGLE SPECIES RESEARCH PROGRAM

Previous studies of genitalic evolution have primarily been based on various types of comparative data (Scudder, 1971; Eberhard, 1985, 1990, 1993a; Shapiro & Porter, 1989), and the relatively few single species studies available are largely restricted to functional morphology (e.g. Heming-Van Battum & Heming, 1989; Bao & Robinson, 1990; Eberhard, 1992, 1993b, c; Huber, 1993, 1994). This is partly due to the fact that most data have been gathered in a taxonomic or systematic framework. As a matter of fact, very few explicit tests of hypotheses have been carried out at all, and these have been based on various comparisons between closely related taxa (e.g. Porter & Shapiro, 1990; Tadler, 1993). Also, the tests so far suggested for discriminating between different hypotheses are based either on functional morphology of genitalia or on comparative data (Eberhard, 1985; Shapiro

TABLE 1. The main hypotheses for the evolution of male genitalia, and their testable predictions and assumptions

Prediction	Lock-and-key	Pleiotropy	Sexual selection
1. Male fitness component affected	Mating success ^b	None ^a	Fertilization success
2. Type/form of selection	Stabilizing	None ^a	Directional
3. Degree of genotypic variability of genitalia	Low	High	High
4. Condition dependent expression	Weak	Strong	— ^b
5. Degree of phenotypic variability of genitalia	Very low	High	High
6. Genetic correlation between genitalia and 'functional' traits	No	Yes	— ^b
7. Assortative mating by genitalic traits	Yes	No ^a	No

^a True only for studies where multiple traits are analysed simultaneously. Indirect selection on genitalic morphology may occur via selection on correlated characters.

^b See text.

& Porter, 1989). In contrast, I suggest that a research program similar to that which has so successfully been adapted to the study of other domains of evolutionary biology (e.g. evolution of life histories, behavioral ecology, sexual selection) be applied to the study of genital evolution. The different hypotheses of genital evolution all make numerous predictions about occurrence of selection, patterns of morphological variation and inheritance of morphological traits in single species. Thus, a research program consisting of in-depth studies of intraspecific variation in genital morphology, using a multitude of methods, has the potential of illuminating the processes of genitalic evolution. However, since this program focuses on current evolutionary processes rather than the results of past evolutionary events, it should be viewed as a necessary complement, rather as an alternative, to other approaches. In other words, the program is primarily concerned with the maintenance of genitalic elaboration, which does not necessarily account for the origin of these traits (Johnstone, 1995). Thus, I believe that a more diversified view, where the research program outlined here is linked with comparative and historical studies as well as with those of functional morphology, will prove to be the most fruitful path to a thorough and complete understanding of genitalic evolution.

In single-species studies, five major methodological pathways may be exploited. First, measurements of phenotypic selection on genitalic traits should be carried out in natural populations. This is important, since the different hypotheses make different predictions about the occurrence and type of selection occurring on genitalic traits (Table 1). Multivariate methods of measuring selection (e.g. Lande & Arnold, 1983; Arnold & Wade, 1984a, b; Manly, 1985; Endler, 1986; Crespi & Bookstein, 1989) should be used, since it is critical to distinguish between direct and indirect selection on genitalic traits.

Second, the different hypotheses make different predictions about which fitness components should be affected by genitalic morphology. Specifically, provided that genital morphology influences male reproductive success, it is critical to distinguish between effects due to mating success versus fertilization success in species where females mates multiply. Thus, selection on males should be partitioned into components or episodes (e.g. survival, mating success or number of achieved inseminations, fertilization success and offspring viability/performance). Again, it is important to include multiple traits in the analysis, to enable a distinction between direct and indirect selection on genitalic traits (e.g. Arnold & Wade, 1984a, b).

Third, since different degrees of phenotypic variability in genitalic versus general morphological traits are expected under various hypotheses, thorough biometric studies are needed. Based on our current state of knowledge, it is not even possible to assess whether genitalic traits are more or less variable than other traits (Eberhard, 1993a). Recent methodological developments in biometrics, both in terms of increasing measurement accuracy and in terms of statistical methods of processing and summarizing complex morphometric variation, have opened up new possibilities along these lines (Bookstein, 1989, 1991; Rohlf & Marcus, 1993; Liu *et al.*, 1996). The methodological problems, which have previously partly obstructed detailed morphometric studies of genitalia, are hence greatly reduced. Still, the importance of assessing measurement error in morphometric studies cannot be underestimated, by calculating the repeatability of one's measures (Lessels & Boag, 1987).

Fourth, quantitative genetic studies are needed to determine the patterns of inheritance of genitalic traits. While a few studies of closely related species show that genitalic traits are polygenic (Coyne, 1983, 1985; Coyne & Kreitman, 1986; Porter & Shapiro, 1990; Liu *et al.*, 1996), I am unaware of any studies addressing the level of genetic variation in genitalic morphology in natural populations, or whether genes coding for genitalia have pleiotropic effects. The contending hypotheses make relatively hard predictions about the pattern of inheritance of genitalia, so quantitative genetic data would be very illuminating (Falconer, 1989). In particular, the degree of additive genetic variance of genitalic traits should be assessed, and the degree of genetic correlation between genitalic and general morphology estimated.

Fifth, various hypothesis make different predictions about the degree of canalization/plasticity of the phenotypic expression of genitalic traits. Thus, it is important to determine to which degree genitalic morphology is condition dependent relative to other traits (Andersson, 1982, 1994; Nur & Hasson, 1984; Grafen, 1990a, b; Price, Schluter & Heckman, 1993; Johnstone, 1995). This could be assessed in controlled laboratory rearing experiments, allowing estimations of the relative contributions of genetic factors versus various conditional factors (e.g. food availability, parasite load) to phenotypic variance in morphology. An unbiased evaluation of the degree of condition dependence in phenotypic expression of sexual traits actually requires experimental manipulation of environmental conditions (biotic and/or abiotic) (see Johnstone, 1995; for a review).

Finally, morphological traits to include in the analysis have to be chosen carefully. Genital traits which have evolved rapidly (species specific traits), and hence are presumably the targets of selection, should obviously be included. It is critical, for several reasons, not to restrict studies to include genitalic traits only. Results on genitalic traits will have to be related to corresponding results on general morphological traits. Traits which are known, or can be assumed, to be functionally important (e.g. body size, length of wings and appendages) should be included, as well as some general traits which are directly comparable with genitalia in absolute size, and thus presumably in their measurement error (for example, measurements of eyes or mouthparts in arthropods). Further, one particularly interesting aspect of morphology to include would be fluctuating asymmetry. Fluctuating asymmetries in bilaterally symmetrical traits result from the inability of individuals to undergo identical development on both sides of the body, and is believed to reflect the more general inability of individuals to cope with environmental stress. Thus, fluctuating asymmetry may be an especially revealing indicator of individual condition/quality and has recently received much attention in the study of sexual selection (Møller &

Pomiankowski, 1993; Watson & Thornhill, 1994; Swaddle, Witter & Cuthill, 1994). The degree of fluctuating asymmetry in genitalia may, for example, be informative of the relative degree of canalization versus condition dependence, or of relative costs of trait expression.

TESTABLE ASSUMPTIONS AND PREDICTIONS

Recent reviews have concluded that the interspecific pattern of genitalic divergence fits poorly with the classical theories of genitalic evolution, i.e. the lock-and-key and pleiotropism hypotheses (Eberhard, 1985, 1990; Shapiro & Porter 1989). Based on this lack of general agreement, Eberhard (1985) suggested that genitalia have diversified under sexual selection. Below, I show how results from studies such as those outlined above could be used to distinguish between the different hypotheses, by identifying critical assumptions and predictions of the various hypotheses. They are numbered according to the sequence shown in Table 1.

The lock-and-key hypothesis

(1) Under this hypothesis, male genitalia are selected to correspond closely with female genitalic morphology. If the fit is poor, genitalic coupling and/or insemination is rendered more difficult, less likely or even impossible. Thus, sexual selection on genitalic traits is predicted, which should be manifested as a relationship between male genitalic morphology and the number of achieved copulations/inseminations (i.e. male mating success).

(2) Optimal male genitalic morphology in a given population will, in general, be the one that most closely corresponds to the average female genitalic conformation. Since all potential deviations from this optimal male genitalic morphology are as likely to decrease the fit with female genitalia, sexual selection should generally be stabilizing rather than directional (non-linear rather than linear) (Alexander *et al.*, 1996). A possible exception could be relatively brief periods of directional selection in sympatric populations that undergo interspecific divergence.

(3) Due to consistent stabilizing/non-linear selection on genitalia, the degree of genetic variance for genitalic traits should be low (cf. Pomiankowski & Møller, 1995). Thus, genitalia are expected to exhibit insignificant or low degrees of additive genetic variance relative to many other traits.

(4) Due to the critical importance of having the 'right' key, development of genitalia should evolve to be highly canalized, so that fitting genitalia are produced in spite of perturbations and stress that might occur during ontogeny (Alexander *et al.*, 1996). Thus, in direct assessments, genitalic traits should not be condition dependent in their expression. As a result of this, any morphological variation in genitalic traits should also be relatively independent (e.g. low phenotypic correlations) of typical condition dependent traits, such as body size.

(5) As a result of (3) and (4) above, genitalic traits should exhibit low degrees of phenotypic variation within populations.

(6) Because of (3) and (4) above, genitalic morphology should not be genetically correlated with other morphological traits.

(7) Considering the close 'fit' between male and female genitalia assumed under the lock-and-key hypothesis, populations should exhibit positive assortative mating at least by certain genitalic traits (true assortment, *sensu* Arnqvist *et al.*, 1996), to the extent that there is measurable morphological variation in male and female genitalia.

The pleiotropism hypothesis

(1) Under this hypothesis, male genitalic morphology should not correlate directly with any fitness components, i.e. there should be no direct phenotypic selection on genitalia. However, this is true only for studies where multiple traits are considered simultaneously. If traits that are genetically correlated with genitalia are also phenotypically correlated, the pleiotropism hypothesis actually predicts indirect selection on genitalia which is caused by fitness effects of functional traits that are phenotypically correlated with genitalic morphology. In other words, non-zero selection on genitalia can be accommodated or even predicted for univariate estimates of selection (selection differentials), but not for multivariate estimates including the true targets of selection (selection gradients).

(2) No direct selection is expected, and the predicted indirect selection could assume any form.

(3) Since genitalic variation is essentially neutral, genetic variation should be relatively high compared to other traits, or at the very least of similar magnitude.

(4) Due to the basic assumption of genotypic correlations between genitalia and other traits, and since the hypothesized neutrality of genitalic variation *per se* implies weak or no selection for canalization, the pleiotropism hypothesis predicts a fairly strong condition dependency in expression of genitalic traits. Thus, we also expect morphological variation in genitalic traits to be phenotypically correlated with many other traits.

(5) Due to the above, genitalic traits should exhibit a phenotypic variability comparable to, or even larger than, that of other similar sized traits.

(6) Under this hypothesis, genitalia evolve through indirect selection on genetically correlated characters. Thus, non-zero genetic correlations between genitalic morphology and functional general morphological traits is a key assumption of the hypothesis. This may be especially applicable for genitalic and general traits which have diverged relative to other closely related species (species specific traits).

(7) Variation in genitalic morphology is essentially non-functional, so no assortative mating between male and female genitalic conformation is expected.

The sexual selection hypothesis

(1) Under this hypothesis, variation in male intromittent genitalic traits is predicted to be related to male fertilization success. This could be due to female cryptic choice based on male genitalia, or to variation in either male ability to control/coerce female sperm use or to compete with sperm from other males (see Discussion). Irrespective of which mechanism is operating, non-random fertilization success among males is expected. Such non-random fertilization success could be brought about by a variety of factors, not only female internal sperm use and differential sperm mortality, but also factors such as differential female oviposition/remating

behaviour or differential female reproductive investment (Eberhard, 1996). In either case, sexual selection is predicted, which should be manifested as a relationship between male genitalic morphology and the number of fertilized eggs when mated to multiply mated females (male fertilization success rather than mating/insemination success).

(2) Sexual selection is typically directional in form (Andersson, 1994; Pomiankowski & Møller, 1995; Andersson & Iwasa, 1996). This should be true for any genital variation that is related to fertilization success as well: males with extreme, rather than intermediate, genital configuration (and thus stimulatory/coercive/competitive ability) should be most successful (Alexander *et al.*, 1996). Thus, sexual selection should be directional.

(3) For traits under directional selection, genetic variation should in theory be exhausted (cf. Andersson, 1994). However, a multitude of factors may alter this expectation by maintaining variation in face of selection, and recent reviews of the amount of genetic variation in sexually selected traits have revealed high or intermediate degrees of genetic variation for such traits (e.g. Pomiankowski & Møller, 1995). Thus, it is difficult to make conclusive predictions for data from single experiments, and the expectations also depend on the type of sexual selection, but an overall expectation would be that genitalic traits would exhibit significant and fairly high levels of additive genetic variance compared to other traits.

(4) Depending on which type of sexual selection is operating, phenotypic expression of genitalia may or may not evolve to be condition dependent (Andersson, 1994; Johnstone, 1995).

(5) Traits under sexual selection typically exhibit relatively high phenotypic variance (Andersson, 1994; Pomiankowski & Møller, 1995). There is no reason why this should not be true for genitalic traits as well.

(6) Whether genitalic traits should evolve to be genetically correlated with other morphological traits in males depends critically on the type of sexual selection involved, so no clear general predictions can be made (Andersson, 1994).

(7) Since genitalic morphology does not affect the probability of pairing under this hypothesis, no positive assortative mating by genitalic conformation is expected.

Taken together, the different hypotheses for the evolution of animal genitalia make a number of unique predictions with regards to the expected patterns of selection and trait variation. However, some predictions are clearly 'harder' than others. Predictions 1, 2, 6 and 7 above are comparatively straightforward and thus particularly useful. Predictions 3–5 are all based on relative amounts of trait variation found in genitalic traits compared to general traits. These predictions are all 'softer', in the sense that they alone provide less conclusive evidence for or against different hypotheses. They are, nevertheless, informative and important, and comparisons between genital and other types of traits should greatly facilitate interpretation of empirical results of this type.

DISCUSSION

Two within-species patterns of genital morphology have previously been held as being 'general' (1) size of genitalia does not correlate with body size and (2) genitalic traits are less phenotypically variable than 'other traits'. However, these beliefs are

deeply rooted in old confounding assumptions of the lock-and-key hypothesis, and factual assessments of these patterns are very rare. The highly limited amount of data that is available, actually show that neither of these patterns are generally present (Kunze, 1959; Coyne, 1983; Eberhard, 1985, 1993a; Porter & Shapiro, 1990; Goulson, 1993; Liu *et al.*, 1996).

Single species studies have typically been concerned with typological descriptions of the functional morphology of genitalia, and very few have given intraspecific variation in genitalia and its potential causes and effects any attention at all. Goulson (1993) found large phenotypic variation in male genitalia of the butterfly *Maniola jurtina*, but failed to find any effect of genitalic morphology on male mating success in a natural population. Moreover, genital size was tightly correlated with body size. Similarly, Kunze (1959) found large intraspecific variation in male genitalia in the cicadellid *Euscelis plebejus*, but failed to find any relationship between genital morphology and successful copulations. Sengün (1944) went one step further, when he experimentally altered male genital morphology in silkworms, *Bombyx mori*, and found that this did not prevent successful copulations.

These intraspecific studies are at least in tentative agreement with the conclusions of comparative studies: that the 'classical' lock-and-key hypothesis is a less plausible explanation for genitalic evolution (Eberhard, 1985; Shapiro & Porter, 1989). However, knowledge of current selection on genitalic traits, patterns and causes of morphometric variation of genitalia, and an understanding of the mechanisms involved are necessary to enable a future resolution of the evolutionary problem that animal genitalia constitute. It is clear that we need thorough single species studies to enable a future synthesis of the causes of the evolution of animal genitalia; the main hypotheses make numerous more or less exclusive predictions for several different types of data.

Sexual selection

As mentioned above, there are three different ways in which sexual selection could be responsible for genitalic evolution. All take their starting point in non-random fertilization success in males. They differ only by which mechanism that is hypothesized to cause this variation. Eberhard's (1985, 1990, 1993a, 1996) cryptic female choice hypothesis suggests that variation in fertilization success among males is caused by adaptive female manipulation of sperm inside their reproductive tract, and that variance in male stimulatory ability (caused by variance in genital morphology) is responsible for this. The sexual conflict scenario, on the other hand, suggests that phenotypic variation in intromittent male genitalia is related to their ability to control/coerce female internal sperm use, often in conflict with female interests (Lloyd, 1979; Alexander *et al.*, 1996). The sperm competition hypothesis, finally, suggests that variation in male genitalia is related to fertilization success because of different abilities to compete with sperm of other males. These different routes to non-random fertilization success may also certainly grade into one another (see below).

Recent findings of non-random male fertilization success in multiply mated females are in support of the most basic assumption of the sexual selection hypotheses: there appear to be intricate mechanisms by which sperm of some males are favoured over those of others (Eberhard, 1991, 1994, 1996; Otronen & Siva-Jothy, 1991; Watson,

1991a, b; LaMunyon & Eisner, 1993; Ward, 1993; Eberhard & Cordero, 1995; Dickinson, 1996). Unfortunately, non-random fertilization success has not been unambiguously related to male genital morphology in any species. To establish such a relationship would be the first necessary step in a test of the sexual selection hypothesis (Table 1). However, this will not suffice in order to distinguish between the different sexual selection hypotheses: it will also be absolutely crucial to determine the mechanism by which such non-random male fertilization success is brought about (Alexander *et al.*, 1996). This will undoubtedly prove extremely difficult. The question of why sperm of males with certain genitalia are more successful in fertilizing eggs than those of others will have to be addressed. Can non-random fertilization success among males based on their genitalic morphology be unambiguously assigned to active female control of sperm use (cryptic female choice), or is such a pattern caused by differing male ability to coerce their reproductive/fertilization interests on females (sexual conflict)? Determinations of male and female interests as well as of which sex controls sperm use, or rather to which degree the sexes control fertilization events, will be necessary (cf. Walker, 1980; Alexander *et al.*, 1996). Further, such a pattern would arise if genitalic variation among males affected, directly or indirectly, their ability to compete with sperm of other males (sperm competition). Covariation between genital morphology and, for example, sperm displacement/dislocation ability (Waage, 1979, 1984), sperm quality or sperm quantity would in itself cause sexual selection on male genitalia. Finally, to further complicate matters, these three different mechanisms are not mutually exclusive and could grade into one another. For example, distinguishing between male genital stimulation of female sperm use on one hand, and male coercion on the other, will not be relevant if the reproductive interests of the sexes coincide. Also, irrespective of whether male sperm compete directly via genital morphology, or whether males compete only indirectly via their ability to stimulate/coerce female sperm use, there will always be an element of sperm competition. Clearly, innovative experimental studies, using novel methods such as radioactive labelling of sperm and genetic paternity determination of offspring, linking male genitalic morphology to the mechanisms of female sperm use, are required.

Female choice and genitalia

As mentioned above, there is currently little direct empirical support for the hypothesis that animal genitalia evolve via cryptic female choice. However, *if* cryptic female choice proves to be generally responsible for genitalic evolution, intramittent genitalia may offer a new and exciting adaptational domain in which to test and assess the relative importance of various processes of female choice for the evolution of sexually dimorphic traits (Eberhard, 1985, 1990, 1993a), and to possibly resolve the controversy between various models of female choice (notably the good-genes, Fisherian runaway and sensory exploitation models) (Ryan, 1990; Ryan & Rand, 1990; Kirkpatrick & Ryan, 1991; Eberhard, 1993a; Andersson, 1994; Johnstone, 1995). The extent to which the evolutionary processes that are responsible for genitalic evolution will prove to be generalizable to other sexual traits is questionable at this point, however. One difference between genital and classic dimorphic traits (such as coloration) could be a relatively low survival cost of genitalic elaboration, as suggested by Eberhard (1993a). This, however, has not yet been empirically

demonstrated. Actually, there are good grounds to assume that genitalia are indeed costly in some sense: any trait evolving by directional sexual selection (especially by a Fisherian run-away scenario) will eventually be brought to a halt by antagonistic/conflicting selection.

Distinguishing between different hypotheses of female choice has proven extremely difficult (Andersson, 1994; Johnstone, 1995; Andersson & Iwasa, 1996), and animal genitalia provides no exception to this empirical dilemma. Studies focusing on male genitalia alone, such as studies of developmental or other costs of genitalic elaboration and condition dependence (Andersson, 1982, 1994; Nur & Hasson, 1984; Grafen, 1990a, b; Price *et al.*, 1993; Arnqvist, 1994; Johnstone, 1995), phenotypic correlations between male genitalia and offspring growth/survival (Andersson, 1994; Kirkpatrick & Ryan, 1991) and genetic correlations between male genitalia and female genitalia/sperm use (Kirkpatrick & Ryan, 1991; Andersson, 1994; Breden, Gerhardt & Butlin, 1994; Pomiankowski & Sheridan, 1994; Bakker & Pomiankowski, 1995), would be illuminating. However, such studies are clearly not sufficient to unambiguously distinguish among female choice hypotheses (Andersson & Iwasa, 1996). Additional insight would be gained from studies on the evolution of the presumed mechanism of female preference itself (Kirkpatrick, 1987; Kirkpatrick & Ryan, 1991; Johnstone, 1995): cryptic female choice. This, again, calls for studies that not only demonstrate how non-random fertilization success among males is related to their genital morphology (i.e. demonstrate sexual selection on genitalia), but which focus on the mechanisms by which such a pattern is brought about (see above). If sexual selection proves to be acting on genitalic traits, such studies will be key to our understanding of which mode of sexual selection is operating and how non-random fertilization success among males has evolved.

To conclude, species specificity and rapid evolution of genitalia is one of the most striking patterns of morphological divergence in animals, and at the same time one of the least understood (Eberhard, 1985; Edwards, 1993). There are still very few direct empirical data on the evolutionary patterns and processes of genitalic evolution. Thorough intra-specific studies are badly needed, since such studies are necessary for a conclusive discrimination between the different main hypotheses for genitalic evolution.

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