Commentary

Postcopulatory sexual selection when a female mates once

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Postcopulatory sexual selection (PCSS) arises via traits that are expressed during and after mating and that increase the likelihood of an individual gaining fertilizations, relative to other members of the same sex (Birkhead & Pizzari, 2002; Pitnick & Hosken, 2010). It can be seen as the combination of selection pressures arising from sperm competition and cryptic female choice. Sperm competition is defined as the competition between the sperm of different males to fertilize the ova of a given female (Parker, 1970; Simmons, 2001). Cryptic female choice is the biasing of paternity by females towards some males over others (Eberhard, 1996; Thornhill, 1983).

In order for inter- or intra-sexual competition to continue after mating there must be a risk that a female will mate more than once before any eggs are fertilized. Therefore, PCSS is commonly said to be a consequence of multiple mating by females (e.g. Birkhead & Pizzari, 2002; Pitnick & Hosken, 2010). While this is true, a distinction needs to be made here between multiple mating at the population level and at the individual level. It is the average risk that a female will remate (or the average number of matings she may be expected to have) that leads to PCSS, and this is dependent on the population level female mating rate (Parker, 1970; Simmons,

2001). At the individual level, some females may remate more than others and at different intervals, so that there will be variation in the mated status of females in the population, and in the number of male ejaculates present in the reproductive tract of a given female at a given time. We note that sperm competition can and does also occur in externally fertilizing species in which competition does not occur inside the female reproductive tract (e.g. Ball & Parker, 1996; Fitzpatrick, Simmons, & Evans, 2012; Smith, Warren, Rouchet, & Reichard, 2014). For the purposes of this discussion we focus on internally fertilizing species. We also note that the focus on multiple matings here is for convenience; again what really matters for PCSS is the number of inseminations the average female receives. This distinction is important as in many species not all matings result in successful insemination (e.g. García-González, 2004; Greenway & Shuker, 2015; Greenway, Dougherty, & Shuker, 2015).

In this commentary, we address the definition of postcopulatory sexual selection as we believe that there is the potential for misinterpretation of PCSS theory with respect to the relationship between PCSS and multiple mating. We use this contribution to outline explicitly the relationship between PCSS and multiple mating in females as we see it. We show how there can be PCSS acting on males or females in either the presence or absence of simultaneously competing ejaculates. We also consider different ways of measuring PCSS acting on male traits, and discuss how PCSS can be measured using either a single-mating or multiple-

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mating experimental design. Finally, we consider how PCSS could be said to occur in a strictly monandrous species.

PCSS DOES NOT REQUIRE THAT EJACULATES COMPETE SIMULTANEOUSLY

PCSS can be separated into its intrasexual (sperm competition) and intersexual (cryptic female choice) components. For both of these processes, selection may arise with or without the simultaneous overlap of ejaculates from different males in the female reproductive tract. We consider each separately below.

Sperm competition is a selective pressure that arises when there is the risk that a female will remate (or has already mated previously) with another male prior to the fertilization of her eggs (Parker, 1970; Simmons, 2001). Although multiple mating by females may frequently lead to the overlap of ejaculates from multiple males in the female reproductive tract (narrow-sense sperm competition), the previous definition highlights the fact that sperm competition may be more rightly considered in a broader sense as the selective pressure acting on a male to reduce the number of fertilizations he loses to other males (Simmons, 2001, 2014). This leads to selection on males in different ways. Most directly, when sperm from a rival male is already present in the female, there will be selection on current male traits that increase paternity share, such as sperm number or quality (e.g. Kelly & Jennions, 2011; Snook, 2005), when sperm compete. However, the risk of sperm competition also selects for male traits that remove the need for direct competition (Simmons, 2001). These can be separated into defensive traits that prevent future inseminations (such as mating plugs: Baer, Morgan, & Schmid-Hempel, 2001; Uhl, Nessler, & Schneider, 2010) or offensive traits that reduce the likelihood that a previous male's sperm will be successful (such as sperm removal organs: Córdoba-Aguilar, Uhía, & Rivera, 2003; Waage, 1979). In some cases these traits may be so effective as to make any female a male mates with subsequently monogamous (Hosken, Stockley, & Tregenza, 2009; Simmons, 2014). Sperm competition can thus be said to drive the evolution of male traits (via PCSS) even when overlapping ejaculates occur very rarely: if there is variation between males in their ability to effectively impose monandry on females, then those that fail to do so will be at a selective disadvantage. We return to this point below.

Cryptic female choice also does not require the strict condition that a female chooses between two simultaneous ejaculates, only that certain male phenotypes are better able to overcome female anatomical and physiological barriers to fertilization (e.g. Arnqvist, 2014; García-González & Simmons, 2007; Rönn, Katvala, & Arnqvist, 2007). Eberhard (1996) listed more than 20 ways in which females may select the sperm of some males over others, many of which do not require the presence of ejaculates from multiple males inside the female. This is most obvious for those species in which females can actively control whether or not to allow sperm to enter and/or remain in their reproductive tract during or after copulation (Eberhard, 1996; Pizzari & Birkhead, 2000; Tallamy, Powell, & McClafferty, 2002). For example, in Orthoptera, females may exert strong choice by removing the male spermatophore almost immediately following mating, potentially preventing any sperm from entering the reproductive tract (Sakaluk & Eggert, 1996; Simmons, 1987), or by preventing any sperm that does enter the reproductive tract from entering the sperm storage organs (Hall, Bussiere, Demont, Ward, & Brooks, 2010; Tuni, Beveridge, & Simmons, 2013). These are all forms of sequential cryptic female choice, which will lead to PCSS on male traits in the same way that simultaneous choice will. An analogy can be made to precopulatory mate choice: in the same way that the decision to mate or not is a form of choice (Dougherty & Shuker,

2015; Edward, 2015; Kokko & Mappes, 2005), so too is the decision to allow insemination and sperm storage during or after mating. PCSS will only favour the selective use (or even total rejection) of a male's sperm if females typically have the opportunity to mate with several males, and thus cryptic female choice is expected to occur only when females have the potential to remate.

In summary, both forms of PCSS arise due to actual or potential multiple mating by females. This does not mean, however, that selection only occurs when there is simultaneous overlap of competing ejaculates. It also does not mean that selection only occurs in females that mate more than once. Even in a highly polyandrous species the number of times an individual mates is variable, and some females may be monandrous by chance, perhaps because they die early or do not encounter multiple males (Kokko & Mappes, 2013; Rhainds, 2010). The important point is that postcopulatory selection will act on males, regardless of whether a given female with which he mates remains monandrous or not.

MEASURING PCSS

A consideration of the origin of PCSS is important when we want to measure the strength of selection acting on individuals or on specific phenotypic traits. Most often we are concerned with male postcopulatory reproductive success and PCSS on male phenotypes (PCSS acting on female traits has received less attention: Ah-King, Barron, & Herberstein, 2014; Arnqvist, 2014; Eberhard, 1996). How should we go about measuring PCSS? Specifically, should we use a single- or multiple-mating experimental design? The answer to this depends both on the specific question we are asking and the type of selection we are interested in.

Many studies attempt to quantify the strength of PCSS acting on male traits by correlating these traits with a measure of reproductive success. A frequently used method in these studies is a multiple-mating (or competitive fertilization) experimental design, in which two or more males are mated to the same female. In this context a male's postcopulatory reproductive success is determined by his paternity share, which can be assessed using genetic or phenotypic markers or sterile male techniques (Simmons, 2001). Thus for species in which females frequently mate multiply, a measurement of paternity share can be used to assess how PCSS is acting on males or male traits.

However, there are other measures of male postcopulatory success that can be used in noncompetitive mating situations. For example, as mentioned above, males of many species may sometimes fail to fertilize a female (García-González, 2004; Greenway & Shuker, 2015; Greenway et al., 2015), and so PCSS may arise from the differential insemination or fertilization success of males (e.g. Dougherty, Rahman, Burdfield-Steel, Greenway, & Shuker, 2015; Holwell, Winnick, Tregenza, & Herberstein, 2010; Tadler, 1999). A single-mating design can thus be sufficient to detect PCSS, as there will be selection on any male trait that is correlated with either of these measures of noncompetitive fertilization success.

Additionally, single-mating designs can be used to investigate proximate postcopulatory processes that affect competitive fertilization success, such as factors influencing how many sperm reach the female sperm storage organ following a mating (e.g. Holwell et al., 2010; Tadler, 1999). Such proximate outcomes may be influenced by purely male effects (such as the size of the ejaculate), female effects (such as the number of sperm transported to storage), or the interaction between males and females (such as the ability of male genitalic structures to stimulate the female during mating). A single-mating experimental design thus allows us to assess postcopulatory processes in the absence of the ejaculate of a previous male (whether or not this is ecologically realistic), as long as the absence of a rival male or ejaculate does not alter the

selective process. These processes can equally be investigated using a multiple-mating design in conjunction with a method of identifying sperm from different males (e.g. Hall et al., 2010; Lüpold, Manier, Ala-Honkola, Belote, & Pitnick, 2010; Tuni et al., 2013). Proximate outcomes can then be used to infer how PCSS may act if they can be convincingly shown to influence male paternity, although only studies recording actual fitness outcomes can show this conclusively.

PCSS IN STRICTLY MONANDROUS SPECIES

The fact that PCSS is driven by multiple mating suggests that it cannot occur in a species in which females always mate once. This is true for species that are monandrous due to life history or biological constraints (e.g. in mayflies that have such a short adult life span that the opportunity for multiple mating is very low). However, in other species monandry may be imposed on females by males (Hosken, Stockley, Tregenza, & Wedell, 2009; Simmons, 2001; Wedell, 2005). For example, males may physically block the female reproductive tract using mating plugs (e.g. Baer et al., 2001; Uhl et al., 2010), manipulate female physiology to reduce female receptivity (e.g. Avila, Sirot, LaFlamme, Rubinstein, & Wolfner, 2011; Chapman, 2001; Craig, 1967) or deposit chemicals which make females unattractive to rival males (e.g. Andersson, Borg-Karlson, & Wiklund, 2000). In some cases such manipulations are able to make females permanently unreceptive following mating (e.g. Craig, 1967; Riemann & Thorson, 1969). In such species, there may be PCSS acting on males if some males are more effective than others at imposing monandry. There will be strong selection against any males that fail to impose monandry on a female due to loss of fitness via sperm competition, and so in this way PCSS will also maintain these traits in the population. The result is a population in which almost all females are monandrous, except for a small minority that mate with males of low effectiveness.

Additionally, male-induced monandry may be in conflict with the fitness optima of females (Hosken et al., 2009), and may thus lead to selection on females to resist male imposition. In turn, this may drive selection on males to evolve more effective suppressing mechanisms, resulting in a familiar 'arms race' for control of mating (Arnqvist & Rowe, 2005). Again, in such a population the maintenance of monandry (if males 'win' the arms race), or the reversion to polyandry (if females win), will be driven by PCSS.

Finally, if males are so effective as to make all females monandrous, and there is no variation in the ability of males to impose monandry, then PCSS cannot act any further. In a population such as this, monandry can be seen as an evolved response to strong PCSS in the past (even if extant females do not remate), and so PCSS is currently absent (Simmons, 2014). None the less, such a situation is expected to be unstable, because selection on male adaptations that impose monandry will be relaxed when females are monandrous. If these adaptations are costly, selection would then favour the loss of the male adaptations and a return to low levels of polyandry, once again imposing selection on those male traits. Thus, episodes of PCSS may maintain monandry in otherwise strictly monandrous species, and so may never be truly absent. One way to measure the strength of PCSS acting on males in such a monandrous population would be to experimentally increase the mating rate of females, and then observe the fitness cost to males that this generates. In most cases a suitable manipulation (one that induces a female to become fully receptive without other side-effects) may be difficult to develop, although we suggest it may be possible in some cases (e.g. experimental removal of antiaphrodisiac pheromones following mating). Nevertheless, doing this should show strong PCSS on those male traits that act to impose monandry (such as mating plugs), but no PCSS on male traits that increase fertilization success relative to other males (such as sperm removal organs). In such a monandrous species, any selection acting on a male trait that increases female fecundity is best considered a form of natural selection, as there is no postcopulatory competition between males (Shuker, 2014).

Conclusion

In this commentary, we have outlined the relationship between female multiple mating and postcopulatory sexual selection. We emphasize that PCSS arises due to the average risk that a female in a population will remate, and that selection may occur in the absence of simultaneously competing ejaculates. Depending on the questions being asked and the processes being considered, PCSS can be measured using either single- or multiple-mating experiments. Finally, PCSS may lead to the evolution and maintenance of male-imposed monandry, and in such cases can be said to be acting to prevent females from mating more than once.

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