Title: Post-copulatory sexual selection when a female mates once

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1   **Post-copulatory sexual selection and multiple mating**

2

3   Post-copulatory sexual selection (PCSS) arises via traits that are expressed during and after

4   mating that increase the likelihood of an individual gaining fertilisations, relative to other

5   members of the same sex (Birkhead & Pizzari, 2002; Pitnick & Hosken, 2010). It can be seen

6   as the combination of selection pressures arising from sperm competition and cryptic

7   female choice. Sperm competition is defined as the competition between the sperm of

8   different males to fertilise the ova of a given female (Parker, 1970; Simmons, 2001). Cryptic

9   female choice is the biasing of paternity by females towards some males over others

10   (Eberhard, 1996; Thornhill, 1983).

11

12   In order for inter- or intra-sexual competition to continue after mating there must be a risk

13   that a female will mate more than once before any eggs are fertilised. Therefore, PCSS is

14   commonly said to be a consequence of multiple mating by females (e.g. Birkhead & Pizzari,

15   2002; Pitnick & Hosken, 2010). While this is true, a distinction needs to be made here

16   between multiple mating at the population level and at the individual level. It is the average

17   risk that a female will remate (or the average number of matings she may be expected to

18   have) that leads to PCSS, and this is dependent on the population-level female mating rate

19   (Parker, 1970; Simmons, 2001). At the individual level, some females may remate more than

20   others and at different intervals, so that there will be variation in the mated status of

21   females in the population, and in the number of male ejaculates present in the reproductive

22   tract of a given female at a given time. We note that sperm competition can and does also

23   occur in externally-fertilising species in which competition does not occur inside the female

24   reproductive tract (e.g. Ball & Parker, 1996; Fitzpatrick, Simmons, & Evans, 2012; Smith,

25   Warren, Rouchet, & Reichard, 2014). For the purposes of this discussion we focus on

26   internally fertilizing species. We also note that the focus on multiple matings here is for

27   convenience; again what really matters for PCSS is the number of inseminations the average

28   female receives. This distinction is important as in many species not all matings result in

29   successful insemination (e.g García-González, 2004; Greenway & Shuker, 2015; Greenway,

30   Dougherty, & Shuker, 2015).

31

32   In this commentary, we address the definition of post-copulatory sexual selection as we

33   believe that there is the potential for misinterpretation of PCSS theory with respect to the

34   relationship between PCSS and multiple mating. We use this contribution to outline

35   explicitly the relationship between PCSS and multiple mating in females as we see it. We

36   show how there can be PCSS acting on males or females in either the presence or absence

37   of simultaneously competing ejaculates. We also consider different ways of measuring PCSS

38   acting on male traits, and discuss how PCSS can be measured using either a single-mating or

39   multiple-mating experimental design. Finally we consider how PCSS could be said to occur in

40   a strictly monandrous species.

41

42   **PCSS does not require that ejaculates compete simultaneously**

43

44   PCSS can be separated into its intrasexual (sperm competition) and intersexual (cryptic

45   female choice) components. For both of these processes, selection may arise with or

46   without the simultaneous overlap of ejaculates from different males in the female

47   reproductive tract. We consider each separately below.

48

49   Sperm competition is a selective pressure that arises when there is the risk that a female

50   will remate (or has already mated previously) with another male prior to the fertilisation of

51   her eggs (Parker, 1970; Simmons, 2001). Though multiple mating by females may frequently

52   lead to the overlap of ejaculates from multiple males in the female reproductive tract

53   (narrow-sense sperm competition), the previous definition highlights the fact that sperm

54   competition may be more rightly considered in a broader sense as the selective pressure

55   acting on a male to reduce the number of fertilisations he loses to other males (Simmons,

56   2001, 2014). This leads to selection on males in different ways. Most directly, when sperm

57   from a rival male is already present in the female, there will be selection on current male

58   traits that increase paternity share, such as sperm number or quality (e.g. Kelly & Jennions,

59   2011; Snook, 2005), when sperm compete. However, the risk of sperm competition also

60   selects for male traits that remove the need for direct competition (Simmons, 2001). These

61   can be separated into defensive traits that prevent future inseminations (such as mating

62   plugs: Baer, Morgan, & Schmid-Hempel, 2001; Uhl, Nessler, & Schneider, 2010), or offensive

63   traits that reduce the likelihood that a previous males' sperm will be successful (such as

64   sperm removal organs: Córdoba-Aguilar, Uhía, & Rivera, 2003; Waage, 1979). In some cases

65   these traits may be so effective as to make any female a male mates with subsequently

66   monogamous (Hosken, Stockley, & Tregenza, 2009; Simmons, 2014). Sperm competition can

67   thus be said to drive the evolution of male traits (via PCSS) even when overlapping

68   ejaculates occur very rarely: if there is variation between males in their ability to effectively

69   impose monandry on females, then those that fail to do so will be at a selective

70   disadvantage. We will return to this point below.

71

72   Cryptic female choice also does not require the strict condition that a female chooses

73   between two simultaneous ejaculates, only that certain male phenotypes are better able to

74   overcome female anatomical and physiological barriers to fertilisation (e.g. Arnqvist, 2014;

75   García-Gonzaléz & Simmons, 2007; Rönn, Katvala, & Arnqvist, 2007). Eberhard (1996) lists

76   more than twenty ways in which females may select the sperm of some males over others,

77   many of which do not require the presence of ejaculates from multiple males inside the

78   female. This is most obvious for those species in which females can actively control whether

79   or not to allow sperm to enter and/or remain in her reproductive tract during or after

80   copulation (Eberhard, 1996; Pizzari & Birkhead, 2000; Tallamy, Powell, & McClafferty, 2002).

81   For example, in Orthoptera, females may exert strong choice by removing the male

82   spermatophore almost immediately following mating, potentially preventing any sperm

83   from entering the reproductive tract (Sakaluk & Eggert, 1996; Simmons, 1987), or by

84   preventing any sperm that does enter the reproductive tract from entering the sperm

85   storage organs (Hall, Bussiere, Demont, Ward, & Brooks, 2010; Tuni, Beveridge, & Simmons,

86   2013). These are all forms of sequential cryptic female choice, which will lead to PCSS on

87   male traits in the same way that simultaneous choice will. An analogy can be made to pre-

88   copulatory mate choice: in the same way that the decision to mate or not is a form of choice

89   (Dougherty & Shuker, 2015; Edward, 2015; Kokko & Mappes, 2005), so too is the decision to

90   allow insemination and sperm storage during or after mating. PCSS will only favour the

91   selective use (or even total rejection) of a male's sperm if females typically have the

92 opportunity to mate with several males, and thus cryptic female choice is expected to occur

93 only when females have the potential to remate.

94

95 In summary, both forms of PCSS arise due to actual or potential multiple mating by females.

96 This does not mean, however, that selection only occurs when there is simultaneous overlap

97 of competing ejaculates. It also doesn't mean that selection only occurs in females that

98 mate more than once. Even in a highly polyandrous species the number of times an

99 individual mates is variable, and some females may be monandrous by chance, perhaps

100 because they die early or do not encounter multiple males (Kokko & Mappes, 2013;

101 Rhainds, 2010). The important point is that post-copulatory selection will act on males,

102 regardless of whether a given female with which he mates remains monandrous or not.

103

104 **Measuring PCSS**

105

106 A consideration of the origin of PCSS is important when we want to measure the strength of

107 selection acting on individuals or on specific phenotypic traits. Most often we are concerned

108 with male post-copulatory reproductive success and PCSS on male phenotypes (PCSS acting

109 on female traits has received less attention: Ah-King, Barron, & Herberstein, 2014; Arnqvist,

110 2014; Eberhard, 1996). How should we go about measuring PCSS? Specifically, should we

111 use a single or multiple-mating experimental design? The answer to this depends both on

112 the specific question we are asking and the type of selection we are interested in.

113

114   Many studies attempt to quantify the strength of PCSS acting on male traits by correlating

115   these traits with a measure of reproductive success. A frequently used method in these

116   studies is a multiple-mating (or competitive fertilisation) experimental design, in which two

117   or more males are mated to the same female. In this context male post-copulatory

118   reproductive success is determined by his paternity share, which can be assessed using

119   genetic or phenotypic markers or sterile male techniques (Simmons, 2001). Thus for species

120   in which females frequently mate multiply, a measurement of paternity share can be used

121   to assess how PCSS is acting on males or male traits.

122

123   However, there are other measures of male post-copulatory success that can be used in

124   non-competitive mating situations. For example, as mentioned above males of many species

125   may sometimes fail to fertilise a female (García-González, 2004; Greenway & Shuker, 2015;

126   Greenway et al., 2015), and so PCSS may arise from the differential insemination or

127   fertilisation success of males (e.g. Dougherty, Rahman, Burdfield-Steel, Greenway, & Shuker,

128   2015; Holwell, Winnick, Tregenza, & Herberstein, 2010; Tadler, 1999). A single-mating

129   design can thus be sufficient to detect PCSS, as there will be selection on any male trait that

130   is correlated with either of these measures of non-competitive fertilization success.

131

132   Additionally, single-mating designs can be used to investigate proximate post-copulatory

133   processes that affect competitive fertilization success, such as factors influencing how many

134   sperm reach the female sperm-storage organ following a mating (e.g. Holwell et al., 2010;

135   Tadler, 1999). Such proximate outcomes may be influenced by purely male effects (such as

136   the size of the ejaculate), female effects (such as the number of sperm transported to

137   storage), or the interaction between males and females (such as the ability of male genitalic

138 structures to stimulate the female during mating). A single-mating experimental design thus

139 allows us to assess post-copulatory processes in the absence of the ejaculate of a previous

140 male (whether or not this is ecologically realistic), as long as the absence of a rival male or

141 ejaculate does not alter the selective process. These processes can equally be investigated

142 using a multiple-mating design in conjunction with a method of identifying sperm from

143 different males (e.g. Hall et al., 2010; Lüpold, Manier, Ala-Honkola, Belote, & Pitnick, 2010;

144 Tuni et al., 2013). Proximate outcomes can then be used to infer how PCSS may act if they

145 can be convincingly shown to influence male paternity, though only studies recording actual

146 fitness outcomes can show this conclusively.

147

PCSS in strictly monandrous species

149

150 The fact that PCSS is driven by multiple mating suggests that it cannot occur in a species in

151 which females always mate once. This is true for species that are monandrous due to life

152 history or biological constraints (e.g. in mayflies that have such a short adult lifespan that

153 the opportunity for multiple mating is very low). However, in other species monandry may

154 be imposed on females by males (Hosken et al., 2009; Simmons, 2001; Wedell, 2005). For

155 example, males may physically block the female reproductive tract using mating plugs (e.g.

156 Baer et al., 2001; Uhl et al., 2010), manipulate female physiology to reduce female

157 receptivity (e.g. Avila, Sirot, LaFlamme, Rubinstein, & Wolfner, 2011; Chapman, 2001; Craig,

158 1967), or deposit chemicals which make females unattractive to rival males (e.g. Andersson

159 et al., 2000). In some cases such manipulations are able to make females permanently

160 unreceptive following mating (e.g. Craig, 1967; Riemann & Thorson, 1969). In such species,

161   there may be PCSS acting on males if some males are more effective than others at

162   imposing monandry. There will be strong selection against any males that fail to impose

163   monandry on a female due to loss of fitness via sperm competition, and so in this way PCSS

164   will also maintain these traits in the population. The result is a population in which almost

165   all females are monandrous, except for a small minority that mate with males of low

166   effectiveness.

167

168   Additionally, male-induced monandry may be in conflict with the fitness optima of females

169   (Hosken et al., 2009), and may thus lead to selection on females to resist male imposition. In

170   turn, this may drive selection on males to evolve more effective suppressing mechanisms,

171   resulting in a familiar 'arms race' for control of mating (Arnqvist & Rowe, 2005). Again, in

172   such a population the maintenance of monandry (if males 'win' the arms race), or the

173   reversion to polyandry (if females win), will be driven by PCSS.

174

175   Finally, if males are so effective as to make all females monandrous, and there is no

176   variation in the ability of males to impose monandry, then PCSS cannot act any further. In a

177   population such as this, monandry can be seen as an evolved response to strong PCSS in the

178   past (even if extant females do not remate), and so PCSS is currently absent (Simmons,

179   2014). Nonetheless, such a situation is expected to be unstable, because selection on male

180   adaptations that impose monandry will be relaxed when females are monandrous. If these

181   adaptations are costly, selection would then favour the loss of the male adaptations and a

182   return to low levels of polyandry, once again imposing selection on those male traits. Thus,

183   episodes of PCSS may maintain monandry in otherwise strictly monandrous species, and so

184   may never be truly absent. One way to measure the strength of PCSS acting on males in

185 such a monandrous population would be to experimentally increase the mating rate of

186 females, and then observe the fitness cost to males that this generates. In most cases a

187 suitable manipulation (one that induces a female to become fully receptive without other

188 side-effects) may be difficult to develop, though we suggest it may be possible in some

189 cases (e.g. experimental removal of anti-aphrodisiac pheromones following mating).

190 Nevertheless, doing this should show strong PCSS on those male traits that act to impose

191 monandry (such as mating plugs), but no PCSS on male traits that increase fertilisation

192 success relative to other males (such as sperm removal organs). In such a monandrous

193 species, any selection acting on a male trait that increases female fecundity is best

194 considered a form of natural selection, as there is no post-copulatory competition between

195 males (Shuker, 2014).

196

Conclusion

198

199 In this contribution, we have outlined the relationship between female multiple mating and

200 post-copulatory sexual selection. We emphasise that PCSS arises due to the average risk

201 that a female in a population will remate, and that selection may occur in the absence of

202 simultaneously competing ejaculates. Depending on the questions being asked and the

203 processes being considered, PCSS can be measured using either single or multiple mating

204 experiments. Finally, PCSS may lead to the evolution and maintenance of male-imposed

205 monandry, and in such cases can be said to be acting to *prevent* females from mating more

206 than once.

207

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