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The sterile male technique: Irradiation negatively affects male fertility but not male courtship

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ABSTRACT

The sterile male technique is a common method to assign paternity, widely adopted due to its relative simplicity and low cost. Male sterility is induced by exposure to sub lethal doses of chemosterilants or irradiation, the dosage of which has to be calibrated for every species to provide successful male sterilisation, without affecting male physiology and behaviour. While the physiological effects of sterilisation are usually assessed for each study, the behavioural ones are rarely analysed in detail. Using the orb web spider *Argiope keyserlingi* as a model we first tested (1) the validity of the thread assay, which simulates male courtship behaviour in a standardised context, as a proxy representing courtship on a female web. We then investigated (2) the effectiveness of male sterilisation via irradiation and (3) its consequences on male courtship behaviour. Our results validate the thread assay and the sterile male technique as legitimate tools for the study of male courtship behaviour and fertilisation success. We show that these techniques are time and cost effective and reduce undesirable variation, thereby creating opportunities to study and understand the mechanisms underlying sexual selection.

1. Introduction

Polyandry is common and widespread among animal taxa (Simmons, 2005; Taylor et al., 2014). This phenomenon has selected for multiple post-copulatory mechanisms based on optimising the number of ova fertilised by preferred males. Post-copulatory mechanisms can be classified as sperm competition (Parker, 1970), or cryptic female choice, both of which can result in differential fertilisation success. The proposed mechanisms under sperm competition include displacement, inactivation or mere dilution of rival sperm (Simmons, 2005). Under cryptic female choice, females allocate sperm from preferred males based on differences in male characteristics or behaviour (Eberhard, 1996).

Studying post-copulatory selection requires experimental techniques that quantify the paternity shares of different males. Two of the most common techniques currently used are genetic markers (e.g., Achmann et al., 1992; Simmons and Achmann, 2000) and the sterile male technique (e.g., Parker, 1970). The genetic marker technique requires the development of microsatellite markers, and hence can become relatively expensive and time consuming, particularly in taxa in which many eggs are produced. As a consequence, one of the most widely adopted techniques to assign paternity to individual males is the sterile male technique because of its simplicity and low-cost. Males are sterilised by exposure to sub lethal doses of chemosterilants or radiation from X-ray or γ sources. The doses are ideally optimised so that they do not affect condition, sperm viability or fertilisation capacity, but do induce complete sterility through chromosomal mutations that result in the early death of embryos sired by treated males (Parker, 1970). Females are mated to one male that is fertile and one male that is sterile. Paternity of the offspring from double matings is assigned on the basis of egg development (Boorman and Parker, 1976).

Despite the advantages associated with the sterile male technique, there remain some significant issues to be resolved. The sterilisation treatment, dosage and type, has to be calibrated for every species to ensure successful male sterilisation, while also limiting the potential effects of irradiation on male behaviour. Limiting the side-effects of irradiation is particularly important as irradiation may alter male behaviours, such as courtship performance, on which cryptic female choice might be based. However, while the effects of irradiation on male fertility are often assessed on a study-by-study basis, the effects of irradiation on male behaviour are rarely assessed in detail (but see Schneider et al., 2006; Schneider and Lesmono, 2009).

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Argiope keyserlingi (Karsch 1878) is an excellent model for studying how male courtship performance influences male reproductive success. *A. keyserlingi* females are polyandrous and exhibit cryptic female choice via controlling copulation duration (Elgar et al., 2000). Moreover male *A. keyserlingi* courtship is long, complex and primarily vibrational, including elements such as shuddering (comprising of several antero-posterior rocks), abdominal wags (comprising of several dorso-ventral abdominal pumps) and mating thread dances (comprising of plucks and bounces on the mating thread) (Robinson and Robinson, 1980; Wignall and Herberstein, 2013a). The male enters the female's web and spends the initial phase wandering in the web periphery performing shudders and abdominal wags. The male then approaches the hub at the centre of the web where the female is located, performing frequent shudders. After reaching the female, the male spends from several minutes up to over 2 h at the hub touching her legs and abdomen. This tactile courtship is regularly interspersed with shudders, abdominal wags, grooming sessions and rests. The male then builds a mating thread. Hanging upside down from it he then starts generating vibratory signals known as the mating thread dance (Wignall and Herberstein, 2013a). The female responds by moving onto the mating thread and entering a characteristic "acceptance posture" that allows copulation to take place (Wignall and Herberstein, 2013a).

In *A. keyserlingi*, the shudders performed during the approach towards the female are important as a means for the male to reduce the risk of being attacked by the female (Wignall and Herberstein, 2013a,b). Females can also recognise differences in male courtship quality and express their preference by responding faster to preferred males and by reducing the incidence of post-copulatory sexual cannibalism (Wignall and Herberstein, 2013a). The importance of male shuddering behaviour for female mating decisions, and the ease with which it can be measured makes this system ideal for examining the effects of irradiation on male behaviour. We previously developed a simulated courtship thread assay for *Argiope radon* (Wignall et al., 2014). The thread assay consists of males walking and courting (shuddering) on a silk dragline collected from an adult virgin female. This assay simulates the male's approach from the periphery of the web to a female located at the hub. Male performance in this assay is highly consistent in *Argiope* (Wignall et al., 2014). This suggests that the thread assay is a good indicator of the intrinsic courtship quality of males.

Our study had three aims: first, we tested whether male courtship performance in the thread assay could be used as a reliable proxy for male courtship performance when interacting with a female in her web. Second, we tested whether a dosage of 40 Gy (16 Gy/min for 2.5 min) from a cobalt γ-emitter is sufficient to induce complete sterilisation in *A. keyserlingi*. Third, we tested whether sterilisation through irradiation affects male courtship behaviours in the thread essay. A dosage of 40 Gy (0.8 Gy/min for 50 min) has been used in related species (*Argiope lobata*: Welke and Schneider, 2009; *Argiope bruennichi*: Schneider et al., 2006; Schneider and Lesmono, 2009) in which it has been shown to be sufficient to induce complete sterilisation of males, while not affecting sperm viability (Schneider et al., 2006), courtship duration, copulation duration or cannibalism (Schneider and Lesmono, 2009).

2. Materials and methods

2.1. Study animals: Collection and care

The animals were collected between November 2012 and January 2013 from several suburban populations of metropolitan Sydney, Australia. All individuals were collected as juveniles and reared in the laboratory to ensure they were virgin and to standardise developmental conditions. This limited differences in experience and body condition, factors that are known to affect reproductive decision-making and aggression in some spiders (Wilder et al., 2009; Gibson and Uetz, 2012).

Spiders were housed individually in 250 ml upturned plastic cups with a mesh floor for airflow and maintained in a temperature controlled room (26 °C) on a 12:12 h light:dark cycle. Males were fed with vinegar flies (*Drosophila melanogaster*) twice a week, females were fed with vinegar flies, sheep blowflies *Lucilia cuprina* (Calliphoridae) or house flies (*Musca domestica*) twice a week (see Zschokke and Herberstein, 2005 for rearing techniques). All spiders were sprayed with water every day. Individuals were checked for moults daily until they reached the adult stage, which can easily be recognised because of the differentiated secondary mating organs of males (the paired pedipalps) and the sclerotised epigynum of the females. Before the mating trials adult females were transferred into Perspex frames (50 × 50 × 10 cm), to build webs.

2.2. Experimental design

2.2.1. Validity of the thread assay as a model for natural courtship

2.2.1.1. The thread assay. We tested whether male courtship during a controlled courtship assay (a thread assay) is a suitable proxy for male courtship in a female's web. A silk dragline thread was pulled from a mature virgin female's spinnerets and fixed to a wooden frame across a 30 cm span (Fig. 1) angled at ~45° (Wignall et al., 2014). The female on her web was placed beside the wooden frame to allow passive flow of volatile female pheromones, which, together with the contact chemicals present on the silk dragline, induce male courtship behaviour (Gaskett et al., 2004; Gaskett, 2007). A virgin male ($n = 12$) was weighed before the assay then placed in a plastic vial (30 ml). The male in the open vial was placed beside the female in her web to allow him to receive pheromones. The vial was then closed for 2 min and attached to the lower left side of the span for the male to acclimate. The vial lid was then removed, and the male was allowed to make his own way out and up onto the span. During a typical trial, a male walked up the dragline, performing the typical shudders observed during natural courtship along the way (Supplementary Video 1). The assay was considered over when the male first touched the wooden frame at the end of the dragline. Between every trial the dragline was removed, the frame was washed with fresh water and a

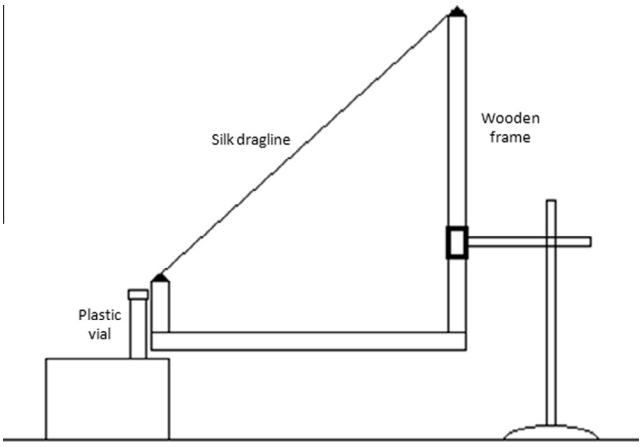

Fig. 1. Thread essay frame. A silken dragline thread pulled from a female's spinnerets was fixed to the wooden frame. The female on her web was placed at the right side of the wooden frame. The male was released from a plastic vial at the lower left side of the frame and allowed to make his own way out and up onto the span.