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Male-killing *Wolbachia* in a live-bearing arthropod: Brood abortion as a constraint on the spread of a selfish microbe

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Abstract

Maternally inherited, cellular endosymbionts can enhance their fitness by biasing host sex ratio in favor of females. Male killing (MK), an extreme form of sex-ratio manipulation, is selectively advantageous, if the death of males results in increased microbe transmission through female siblings. In live-bearing hosts, females typically produce more embryos than are brought to term, and reproductive compensation through maternal resource reallocation from dead male embryos to female siblings provides a direct, physiological mechanism that could increase the number of daughters born to infected females, thereby promoting MK endosymbiont spread. In this study, a *Wolbachia*-infected line and an uninfected line of the viviparous pseudoscorpion, *Cordylochernes scorpioides* were genetically homogenized for nuclear DNA by repeated backcrossing of the infected line with the uninfected, laboratory population. Photomicroscopy of early-stage embryos demonstrated that female *C. scorpioides* invariably produced an excess of embryos, with *Wolbachia*-infected females producing as many early-stage embryos as uninfected female controls. However, *Wolbachia*-infected females that successfully carried broods to term gave birth to significantly fewer offspring, indicating that the extreme female bias characteristic of their broods results from the killing rather than the feminization of male embryos. Infected females that carried broods to term gave birth to significantly larger nymphs and did produce 10% more female offspring than uninfected females. However, the slight transmission advantage that the MK *Wolbachia* accrued from this reproductive compensation appears to be heavily outweighed by the high rate of spontaneous brood abortion suffered by infected females.

Keywords: *Cordylochernes scorpioides*; Male killing; Pseudoscorpion; Reproductive compensation; Selfish genetic elements; Sex-ratio distortion; Viviparity; *Wolbachia*

1. Introduction

In most plants and animals, females differ from males in transmitting not only nuclear genes but also extra-nuclear genetic elements present in the cytoplasm (Birky, 2001). Males are thus a dead end both for cellular organelles and for the wide range of cellular endosymbionts, including protists, bacteria, and viruses, that may inhabit eukaryotic cells. Selection on cytoplasmic genetic elements should therefore favor variants capable of biasing sex ratio towards females (Cosmides and Tooby, 1981; Eberhard, 1980). Evidence of such sex-ratio distortion is accumulating, with a proliferation of studies demonstrating male killing or feminizing by cellular endosymbionts in arthropods (Hurst and Jiggins, 2000; Majerus, 2003) and by mitochondria in flowering plants (Budar et al., 2003).

In invertebrates, the most intensively studied sex-ratio distorting organisms are members of the bacterial lineage, *Wolbachia*. It has been variously estimated that these obligately, intracellular bacteria infect 16% (Werren et al., 1995), 19% (Werren and Windsor, 2000) or 76% (Jeyaprakash and Hoy, 2000) of insect species. They have also been found in nematodes, amphipods, isopods, mites, and spiders (Charlat et al., 2003; Rowley et al., 2004; Werren, 1997). As a group, *Wolbachia* employ a diversity of mechanisms for manipulating host sex ratio (Bourtzis and

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O'Neill, 1998). In haplodiploid species, *Wolbachia* infection may induce parthenogenesis by converting haploid males into diploid females (Stouthamer et al., 1993). In diploid species, *Wolbachia* may feminize males, as occurs in isopods and amphipods (Bouchon et al., 1998). Alternatively, female bias may be achieved by killing male embryos early in development. Male killing has been demonstrated in beetles (Fialho and Stevens, 2000; Hurst et al., 1999), butterflies (Dyson et al., 2002; Hurst et al., 1999), moths (Kageyama et al., 2003), and fruit flies (Dyer and Jaenike, 2004; Hurst et al., 2000).

While conversion of dead-end males to microbe-transmitting females by feminization or parthenogenesis induction clearly provides a direct benefit to the infecting *Wolbachia*, the fitness gain to be derived from male killing is less apparent. For male killing to be favored, kin selection theory requires that the death of male embryos increases the transmission of clonally-related *Wolbachia* lineages in female siblings (Hurst, 1991; Werren, 1987). This fitness benefit, known as fitness compensation, may accrue, in egg-laying hosts, through cannibalism, lowered risk of inbreeding or reduced sibling competition for environmental resources (Hurst and Majerus, 1993; Hurst, 1991; Werren, 1987). In live-bearing hosts, reallocation of maternal resources from dead male embryos to their female siblings provides an additional, physiological mechanism through which kin selection could favor the evolution of male killing. In human and mammalian genetics, the term "reproductive compensation" is frequently used to denote a more restricted form of fitness compensation in which dead embryos are replaced by potentially viable ones (Charlesworth, 1994; Hastings, 2001). If females typically produce more embryos than they can carry to term and reproductive compensation occurs, an elevated rate of fetal mortality need not translate into a proportional decrease in the number of offspring born. In this paper, we use reproductive compensation to indicate this restricted form of fitness compensation.

The recent discovery of *Wolbachia* and apparent male killing in the harlequin beetle riding pseudoscorpion, *Cordylochernes scorpioides*, has identified an ideal system for investigating the importance of reproductive compensation in the spread of male-killing (MK) *Wolbachia* in a live-bearing species (Zeh et al., 2005). Unlike most terrestrial arthropods (Zeh and Smith, 1985), pseudoscorpions are viviparous, with embryos developing in a translucent brood sac, overlying the female's genital aperture (Weygoldt, 1969). Using a specialized mouth precursor (the pumping organ), individual embryos actively draw nutritive fluid, consisting of phospholipids, proteins, and polysaccharides (Makioka, 1968), from the mother's reproductive tract (Weygoldt, 1969). This "external-womb" form of viviparity facilitates visual assessment of the post-fertilization consequences of endosymbiont infection for embryonic development. In an investigation that combined inheritance studies, antibiotic treatment and molecular assays, a highly female-biased *C. scorpioides* line (the "FB line") was found to be infected with a novel strain of *Wolbachia*. Sex-ratio distortion was maternally inherited and associated with lower than average reproductive success (number of nymphs born). Antibiotic treatment cured females of the *Wolbachia* infection, restored offspring sex ratio to 1:1, lowered rate of spontaneous brood abortion and significantly improved female reproductive success. Although, the study implicated male killing as the most likely mechanism of sex-ratio distortion, we could not eliminate the alternative hypothesis that low brood size and extreme female bias resulted from a bacterium that not only feminized males but also reduced the number of eggs ovulated (Zeh et al., 2005).

Here, we report the results of a study that combined photomicroscopy of early-stage embryos in *Wolbachia*-infected, FB-line females and in uninfected *C. scorpioides* controls with assessment of female reproductive success. The two lines had previously been genetically homogenized for nuclear DNA by back-crossing the *Wolbachia*-infected line to the uninfected laboratory population for 12 generations. Our results indicate that: (1) male killing is the mechanism responsible for extreme female bias in the FB line, and (2) the potential for reproductive compensation to promote the spread of the *C. scorpioides* MK *Wolbachia* is likely to be constrained by the high rate of spontaneous brood abortion suffered by infected females.

2. Materials and methods

2.1. Pseudoscorpions

Virgin FB females were randomly selected from the *Wolbachia*-infected FB line established from a collection made in the vicinity of Llano Cartí, Republic of Panamá (09°18′N, 78°58′W; see Zeh et al., 2005). A large population of this line (≈500 individuals per generation) has been maintained in the laboratory for 15 generations by outcrossing FB females to males from uninfected families (for laboratory rearing methods, see Zeh et al., 2005). Spontaneous brood abortion rate and extreme female bias has been consistent in this line across all generations (Zeh et al., 2005). For the control treatment, virgin females were randomly selected from a laboratory population of *C. scorpioides* families with sex ratios not significantly different from 1:1. The uninfected laboratory population has been maintained in the laboratory for 12 generations (800–1000 individuals per generation), and was established from 82 field-inseminated females collected in Panamá from 12 decaying *Ficus* spp. trees. This laboratory population provided the males for the last 12 generations of outcrossing of the FB-line, as well as the males used in both the FB and control replications of the experiment.

Before mating, each female was held flat under a glass slide and a digital image (approximately 30×) was recorded, using an Olympus SZ60 stereomicroscope equipped with a DP12 digital camera. NIH Image (version 1.62) was then used to measure cephalothorax length, the trait most close-