Paternal Transmission of a Secondary Symbiont during Mating in the Viviparous Tsetse Fly

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Abstract

Sodalis glossinidius, a maternally inherited secondary symbiont of the tsetse fly, is a bacterium in the early/intermediate state of the transition toward symbiosis, representing an important model for investigating establishment and evolution of insect–bacteria symbiosis. The absence of phylogenetic congruence in tsetse-Sodalis coevolution and the existence of Sodalis genotypic diversity in field flies are suggestive for a horizontal transmission route. However, to date no natural mechanism for the horizontal transfer of this symbiont has been identified. Using novel methodologies for the stable fluorescent-labeling and introduction of modified Sodalis in tsetse flies, we unambiguously show that male-borne Sodalis is 1) horizontally transferred to females during mating and 2) subsequently vertically transmitted to the progeny, that is, paternal transmission. This mixed mode of transmission has major consequences regarding Sodalis’ genome evolution as it can lead to coinfections creating opportunities for lateral gene transfer which in turn could affect the interaction with the tsetse host.

Key words: symbiont, paternal transmission, genetic diversity, Sodalis glossinidius, Glossina.

Main Text

To ensure successful symbiont inheritance and dissemination within insect populations, mechanisms have evolved within different insect-symbiont associations that optimize the transmission of bacterial symbionts from one generation to another (Bright and Bulgheresi 2010). The mode of transmission plays a key role in the evolutionary trajectory of these associations where strict vertical symbiont transmission results in congruent host–symbiont phylogenies whereas horizontal transfer tends to eliminate congruence with partner phylogenetic trees (reviewed in Ebert [2013]). The obligate blood-feeding tsetse fly, the sole vector of human pathogenic African trypanosomes, harbors several bacterial symbionts that influence important aspects of its physiology and ecology. Tsetse flies display a unique viviparous mode of reproduction where the offspring develops in utero where it is nourished by maternal milk gland secretions containing tsetse’s two main symbiotic bacteria, that is, an anciently associated obligate mutualist Wigglesworthia glossinidia and a more recently established commensal Sodalis glossinidius. Tsetse’s third symbiont, Wolbachia, has a transovarial transmission route. It is assumed that these three symbionts are strictly vertically transferred from the mother fly to the intrauterine progeny. However, the absence of phylogenetic congruence in the tsetse-Sodalis coevolution (Toh et al. 2006) and the ability to experimentally establish a stable heritable symbiosis in tsetse flies cleared of their native Sodalis symbionts with a Sodalis species isolated from a different tsetse fly species (Weiss et al. 2016) are indicators that horizontal transmission of Sodalis symbionts could occur in tsetse populations. To date, no natural mechanism for the horizontal transfer of this symbiont has been identified. The presence of Sodalis in male testes (Balmand et al. 2013) and female spermathecae (Cheng and Aksoy 1999) has led us to hypothesize that transfer of Sodalis might occur during copulation. Such transfer would be biologically relevant in terms of host–symbiont coevolution if the symbionts are subsequently transmitted maternally to the progeny, that is, paternal transmission.

Experimental evidence for the latter is currently lacking due to the absence of an appropriate methodology to reliably trace this alternative transmission route. To date, only a few studies have utilized genetic techniques to explore the nature of host–symbiont interactions due to the fastidious nature of these bacteria.

In this study, we adapted and optimized the Tn7 transposition technology for the genetic manipulation of Sodalis. Furthermore, we developed novel techniques for introducing genetically modified Sodalis in tsetse allowing us to explore sexual transmission and ensuing vertical transmission of sexually acquired Sodalis to the offspring, that is, paternal transmission. Although paternal transmission has been shown to have important implications regarding the coevolution of hosts and symbionts, it has been reported in only a few...
insect-symbiont associations to date (Moran and Dunbar 2006; Damiani et al. 2008). In this study, we show unambiguously that male-borne green fluorescent protein (GFP)-tagged Sodalis is horizontally transferred to females during mating and subsequently vertically transmitted to the progeny.

Glossina morsitans morsitans (Westwood) from the colony at the Institute of Tropical Medicine (Antwerp, Belgium) were used in all experiments. Flies, maintained at 26°C and 65% relative humidity, were fed 3 days per week with defibrinated bovine blood using an artificial membrane system. A chromosomally GFP-tagged Sodalis strain (Sod:GFPi) was constructed by Tn7-mediated transposition. Briefly, the lacZ promoter_gfp fusion cassette was cloned into the multiple cloning site of the pGRG25 plasmid (McKenzie and Craig 2006) encoding the Tn7 transposition machinery which allows for the site-specific transposition at the attTn7 locus of Sodalis (fig. 1A; additional details are provided as supplementary material, Supplementary Material online). We found that the lacZ promoter drives strong expression of GFP (fig. 1B) in a constitutive manner due to the absence of a Sodalis lac repressor.

Subsequently, a novel technique for the introduction of the GFP-labeled endosymbionts into tsetse flies was developed. Third-instar larvae, collected immediately after larviposition, were microinjected with 10^6 CFU of Sod:GFPi using a 5 μl Hamilton 75RN microsyringe with gauge 34 removable electrotapered needles and allowed to pupate resulting in efficiently colonized male flies upon emergence 30 days later (fig. 2). Microscopic observations of the male reproductive organs of Sod:GFPi colonized flies revealed that Sod:GFPi was found predominantly in the testes (fig. 3A), although not apparent within the sperm heads themselves. Interestingly, Sod:GFPi could not be detected in the accessory glands.

Prior to being mated with wild-type (WT) virgin female flies from the colony, Sod:GFPi containing males flies were given three blood meals. During mating, male tsetse deposit a "spermatophore" (i.e., a capsule consisting of male accessory gland secretions and sperm) in the uterus of female flies which can be isolated from the uterus of the female tsetse by dissection immediately after the mating event (fig. 3B). Using confocal microscopy Sod:GFPi was found to be present in low densities (up to 20 Sod:GFPi cells/spermatophore) in

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spermatophores collected from WT females immediately after mating (fig. 3B). Polymerase chain reaction (PCR)-analysis revealed a transmission efficiency of 27.5% (8 of 29 spermatophores). Furthermore, in 18.7% of WT females (3 of 16) mated with Sod:GFPi colonized males, Sod:GFPi was found to remain present within the reproductive organs for up to 4 weeks postmating, indicating that Sod:GFPi bacteria were able to persist within the host upon sexual acquisition. Finally, a proportion of females (n = 23) mated with Sod:GFPi colonized males were allowed to give birth to fully developed third-instar larva to evaluate the presence of Sod:GFPi in the F_1 progeny. The percentage of F_1 individuals carrying the recombinant bacteria was found to be 17.4% using PCR analysis (4 of 23 individuals). These results indicate that transmission of only a few Sodalis cells, residing within the spermatophore, is sufficient for establishment in the female “receiving” fly and subsequent transfer to the offspring.

In this study we have demonstrated unequivocally that paternal transmission through sexual reproduction is an additional route for S. glossinidius symbiont transmission to the progeny, besides maternal vertical transmission. This mixed mode of transmission could strongly impact Sodalis’ genome evolution as transfer between tsetse fly individuals infected with different symbiont strains can lead to coinfections creating opportunities for lateral gene transfer or exchanging phage or phage genes increasing symbiont genetic diversity.
within the host population. The importance of genetic diversity in *Sodalis* is emphasized by the fact that different genotypes of *Sodalis* harbored by *G. palpalis gambiensis* have been shown to correlate with the tsetse fly ability to transmit trypanosomes including the human-pathogenic *Trypanosoma brucei gambiense* (Geiger et al. 2007). Although the paternal transfer of *Sodalis* in tsetse is a low to moderately frequent type of transmission, it has been demonstrated for other symbionts that even very low rates of paternal transmission can be consequential especially when particular combinations of host and symbiont genotypes have a direct effect on host fitness (Oliver et al. 2005). We propose that paternal gene transfer could be an important evolutionary factor that should be taken into account to explain the mechanisms and consequences of genetic diversity of *S. glossinidius*. However, assessing the importance of paternal transmission for the dynamics of *Sodalis* symbionts with a particular trait will require an understanding of the relative importance of the different natural transmission routes and transmission rates through these routes in natural tsetse populations.

Finally, in the context of a *Sodalis*-based paratransgenic approach (i.e., genetic manipulation of symbiotic microorganisms to interfere with pathogen development in the host) to control African trypanosomiasis (De Vooght et al. 2014), paternal transfer could provide an additional route for introducing genetically modified symbionts into populations as a single infected male can transfer symbionts to the progeny of several females.

**Supplementary Material**

Supplementary material is available at Molecular Biology and Evolution online (http://www.mbe.oxfordjournals.org/).

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