



Potential roles of two *doublesex* genes during embryogenesis in *Phytoseiulus persimilis**

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In arthropods, *doublesex* (*dsx*) is a nexus gene in multiple sex-regulatory pathways, regulating variable downstream genes that further regulate sex differentiation and other reproductive related features. In the present study, we screened two *dsx* genes (*Ppdsx1* and *Ppdsx2*) in *Phytoseiulus persimilis*, investigated their expression, function, and interaction with potential downstream genes, through RNA interference and protein-protein interaction (PPI).

RT-qPCR was conducted to detect relative expression of *Ppdsx1* and *Ppdsx2*. No significant difference was observed in *Ppdsx1* expression between the 1st and 2nd eggs laid by each female, which generally develop into males and females, respectively. In contrast, expression of *Ppdsx2* in the 1st egg was ca. 3 times of that in the 2nd egg. In mated females, expression pattern of the two genes were similar after mating. While peak expression of *Ppdsx1* and *Ppdsx2* observed at 10h after mating, when their expression were 48.9 and 42.1 times than virgin females, respectively. When interfered, expression of *Ppdsx1* and *Ppdsx2* decreased by 66.6% and 34.2%, respectively. About 60% and 75% individuals died when *Ppdsx1* and *Ppdsx2* were interfered. Among survived *P. persimilis*, ca. 17% and 47% became infertile, while fertile individuals had proportions of female offspring decreased by 20% and 6%, respectively. In addition, ca. 4% and 27% of the 1st eggs developed into females, respectively. But only when *Ppdsx2* was interfered, ca. 13% of the 2nd eggs developed into males. These results suggested a role by *dsx* genes in sex determination of *P. persimilis*.

Sequences of *Ppdsx1* and *Ppdsx2* were further cloned to the vector pGBKT7, and used as baits to screen interacting genes, with two *vitellogenin* (*Vg*) genes (*PpVg1* and *PpVg2*) achieved. *Vg* genes are important in reproductive regulation, often considered to be regulated by *dsx*. Our previous studies showed that the *PpVg1* and *PpVg2* are associated with embryogenesis in *P. persimilis*. Interactions between *Ppdsx1* and *PpVg1*, and *Ppdsx2* and *PpVg2*, were further verified herein using the QDO / X / A (SD / - Trp / - Leu / - His / - Ade / X - α -Gal / AbA) yeast hybridization system. These results suggested each *dsx* gene regulates one *Vg* in *P. persimilis*.

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