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Genetic differences in the production of male neonates in *Daphnia magna* exposed to juvenile hormone analogs

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Abstract

We studied the susceptibility of three genetically different strains of the cyclical parthenogen *Daphnia magna* (Cladocera, Crustacea) in producing male neonates following exposure to juvenile hormone analogs. In experiment 1, NIES, Clone A, and Belgium A strains were exposed to the insect growth regulators (IGRs) fenoxycarb or epofenonane in a 21-day reproduction experiment. Fenoxycarb exposure decreased the total number of neonates and increased production of male neonates in a concentration-dependent manner in the NIES strain. The decrease in the total number of neonates was so great in Clone A following fenoxycarb exposure that male neonates were not observed, even at the highest concentration, where the total number of neonates was only 2% of the control. In the Belgium A strain, male neonates were observed at a rate of about 20% following exposure to the highest fenoxycarb concentration, but the total number observed was small. Epofenonane did not decrease reproduction in the NIES and Belgium A strains as dramatically as did fenoxycarb, but the neonatal sex ratio changed in a concentration-dependent manner. Although the ratio of males was as low as about 10%, induction of male neonates was also observed in Clone A following epofenonane exposure. In experiment 2, gravid females were exposed to high concentrations (5 or 10 μg/l) of fenoxycarb or pyriproxyfen for 12 h. These treatments induced the production of male neonates in all strains, with a small decrease in the total number of neonates. Although induction of male neonates by juvenile hormones and their analogs was universal among genetically different strains, care is needed in interpreting the results of the 21-day reproduction tests, because decreased numbers of neonates at higher concentrations could obscure the presence of male neonates.

Keywords: Cyclical parthenogenesis; Epofenonane; Fenoxycarb; Insect growth regulator; Offspring sex ratio; Pyriproxyfen

1. Introduction

Small cladoceran crustaceans are among the major constituents of aquatic ecosystems. They are usually

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parthenogenetic, reproducing genetically identical female offspring (Hebert, 1987). Males can appear, triggering sexual reproduction following a change in environmental conditions. Although many aspects of the reproduction of these crustaceans remain unknown, day length, food concentration, and population density are involved in the initiation of the production of males and in the switch to sexual reproduction (Hobaek and Larsson, 1990; Kleiven et al., 1992).

Recently, several studies have demonstrated that exposure to juvenile hormones, or to some pesticides formulated as juvenile hormone mimics, induce the cladoceran crustacean *Daphnia magna* to produce male neonates, and that juvenile hormones and their analogs were involved in sex determination (Olmstead and LeBlanc, 2002, 2003; Tatarazako et al., 2003; Oda et al., in press). The same phenomenon has been observed in other cladoceran taxonomic groups, for example, in the genera *Moina* and *Ceriodaphnia* (Oda et al., 2005). Ten chemicals have been found to be effective in inducing the production of male neonates production in *D. magna* (Tatarazako et al., 2003; Oda et al., in press). All of these chemicals act as juvenile hormones in insects (Nijhout, 1994) or crustaceans (Borst et al., 1987; Laufer et al., 1987a,b, 1993), or their analogs. Thus, although the mechanisms are not clear, the results of such studies suggest that juvenile hormones play a major role in the production of males, not only in *D. magna* but also in other taxonomic groups of the order Cladocera, in which cyclical parthenogenesis occurs (Hebert, 1987).

Due to its relatively short life cycle and ease of handling in the laboratory, *D. magna* has been recommended as a test organism in the Organization for Economic Cooperation and Development (OECD) Test Guidelines 202 and 211 (OECD, 1984, 1998). Although *D. magna* is routinely used in toxicity testing, *D. magna* is not suitable for detecting distortions in the sex ratio caused by endocrine-disrupting chemicals because of its unique reproduction system (i.e., cyclical parthenogenesis). Recent knowledge concerning the induction of male neonates in *D. magna*, however, makes it available for use in screening for chemicals with potential juvenile hormone-disrupting effects and suitable for evaluating endocrine-disruption in crustaceans.

It is commonly known that the total number of neonates in the control group, as well as some additional endpoints, such as EC50 (median effective concentration) for reproductive rate, differ among genetically distinct strains (OECD, 1997). It is plausible that, the detection of changes in neonatal sex ratio in response to disruption of the endocrine system in *D. magna*, would not be recognized because the treatment with juvenile hormone analogs would also decrease the total number of neonates. It is thus, important to know whether there are strain differences in the response to juvenile hormones and their analogs, so that the limitations of the testing using *D. magna* can be clarified. Our goal was to examine the universality of the production of male neonates in response to exposure to juvenile hormones and their analogs, using genetically different strains.

2. Materials and methods

Three genetically distinct strains of the small crustacean *D. magna* (order: Cladocera) were used. The first strain is called "NIES" and originated from the United States Environmental Protection Agency's Environmental Research Laboratory in Duluth, Minnesota, via the Chemicals Evaluation and Research Institute, Japan. It has been maintained for about 20 years at the National Institute for Environmental Studies (NIES) in Tsukuba, Japan. This strain has been used by our group previously to report the production of male neonates in response to exposure to juvenile hormones and their analogs (Tatarazako et al., 2003; Oda et al., in press). The second strain, herein called "Clone A", was obtained from the Chemicals Evaluation and Research Institute, Japan, and has been kept at this institute for more than 10 years. This strain is "Clone A" identified in the OECD Test Guideline 211 (OECD, 1998). The third strain, Belgium A, was established from a resting egg of a Daphtox kit F magna (MicroBioTests, Inc., Nazareth, Belgium) and has been kept for more than a year in our laboratory.

2.1. Acute toxicity experiment with reference toxicants

Acute toxicity experiments were conducted on the basis of OECD Test Guideline 202, "*Daphnia* sp. acute immobilization test" (OECD, 1984). Five chemicals were chosen as reference toxicants; copper (II) sulfate ($CuSO_4$), sodium linear-dodecylbenzenesulfonate (LAS), pentachlorophenol (PCP), zinc chloride ($ZnCl_2$), and iron (II) chloride ($FeCl_2$).

Females, less than 24 h old, were used in these experiments and were exposed to various concentrations of the test substances according to standard OECD test conditions. Experiments were conducted in an incubator at a temperature of 21 ± 1 °C and a photoperiod of 16 h light/8 h dark. Tap water filtered through charcoal was used as a culture medium (control water) after being kept overnight. Six concentrations of each test chemical, including a solvent-only control, were prepared by dilution with culture medium. Four replicate glass jars (100 ml), each containing five *D. magna* neonates in 50 ml of media, were used at each concentration. The jars were covered with Teflon caps to prevent volatilization of the test chemicals. No food was provided during experiments. The measured effect was death, recognized by immobility 48 h after the start of experiments.