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Differential Courtship Activity of Competing Guppy Males (*Poecilia reticulata* Peters; Pisces: Poeciliidae) as an Indicator for Low Concentrations of Aquatic Pollutants

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There is a strong need of test systems suitable for monitoring low concentrations of water-borne pollutants. Behavioral assays have been highly sensitive to sublethal levels of contaminants but they usually require highly trained personnel and long observation periods (e.g., Lemly and Smith 1986). To score behavior activities of the guppy (Poecilia reticulata Peters), however, does not require highly trained personnel, long observation periods nor large numbers of test fish. The experimental equipment necessary for this kind of behavioral investigation is easy to handle and affordable. Since all fish survive when exposed to sublethal doses of pollution, this type of toxicological experimentation will fulfil the requirements of animal protection regulations.

For several reasons the guppy was chosen for the present experiments. Many well-established and highly inbred strains of this species are available, the genetics and behavior of which were already quantitatively analyzed (reviewed by Schröder 1983). This versatile and modest live-bearing guppy is one of the smallest vertebrates and therefore may be kept in relatively small aquaria. The sexual activities of guppy males are also easy to score. In streams and rivers in northeastern South America and adjacent Caribbean islands (Rosen and Bailey 1963), male guppies court females up to 13 times in 5 min (Farr 1975). In addition, this courting is a primary determinant of reproductive success; more active males have a greater chance of encountering receptive females and are preferred by females (Farr 1980a). When males compete for the insemination of the same females, their reproductive success roughly corresponds to the relative amount of courtship activity previously exhibited (Farr 1980a). Both quantitative male sexual behavior patterns and intermale aggressiveness are patri-

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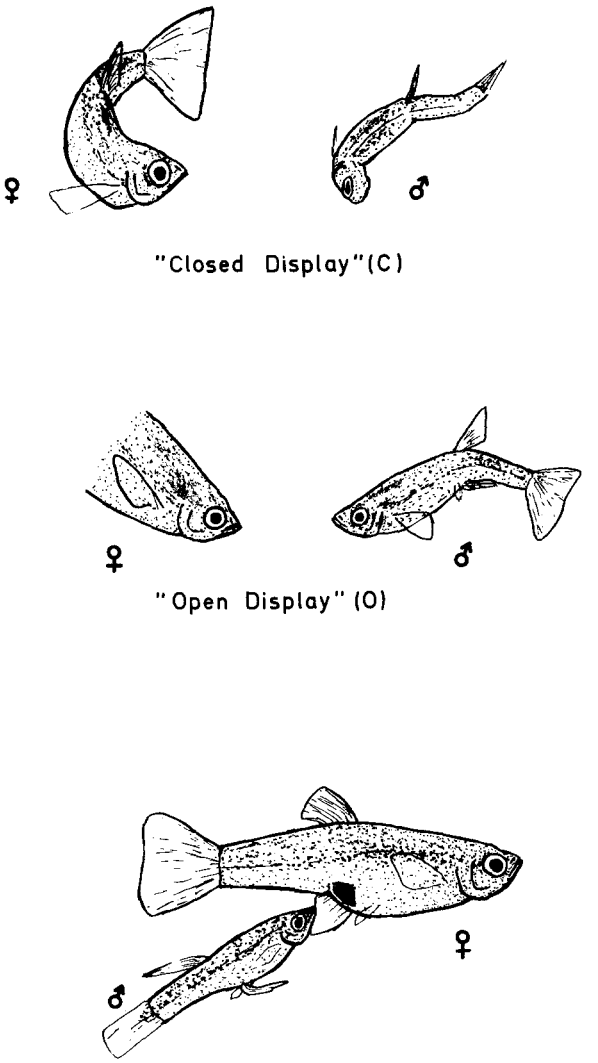

Figure 1. Sigmoid courtship display with fins either folded ("Closed Display", C) or fully spread ("Open Display", O) and "Gonopodial Thrusting", T) as defined by Baerends et al. 1955.

cliniously inherited, autosomal factors modifying the primary influence of Y-chromosomal genes (Farr 1983; Pohla 1986).

Because guppy males maximize their courtship activity only in the presence of a competitor, the courtship activity of two competing males was scored simultaneously. The difference between the courtship activities of the two males then determines the relative fitness of the male in question (Farr 1980a).

MATERIAL AND METHODS

The courtship display of the male guppy (Fig. 1) consists of a sigmoid arching of the body with the unpaired fins either fully spread ("Open Display", O) or closed ("Closed Display", C; Baerends et al. 1955; Liley 1966). Rates of closed and open displays vary with changes in female fertility and receptivity (Liley 1966; Farr 1980a). During a copulation attempt the male brings his gonopodium (which consists of the anal fin modified into a copulatory organ) into a forward position and attempts to insert the gonopodial tip into the female's genital pore. This behavior called "Gonopodial Thrusting" (T) occurs more often when females are not in the receptive portion of their brood cycle (Liley 1966; Farr 1980a). In the present study, no true copulation with transfer of sperm was observed. Since only non-virgin females were used which normally do not respond to the activities of courting males and therefore are considered as being non-receptive (Farr 1980b), female response behavior could be neglected.

Each pair of competing males was tested three times a day for a 20-min observation period during 2 - 3 consecutive days thus producing 6 - 9 replicates for each pair. To score both frequency and duration of the three male sexual activities, an event recorder ('Peiseler Stopprechner') was used. Each pair of male competitors consisted of one male belonging to the Wild Maculatus (Wild Mac) strain while the other was a Blue Iridescense (Blue Ir) male. While Wild Mac males represent the guppy strain which exhibits the highest rate of displays (69.67 $\pm$ 6.32 mean total courtship displays per 50 min observation time), Blue Ir strain males court at the lowest known intensity (24.20 $\pm$ 3.44 mean total courtship display per 50 min; Farr 1980a). However, the mean numbers of gonopodial thrusts per 50 min observation time were 0.73 $\pm$ 0.34 for Wild Mac and 13.53 $\pm$ 1.92 for Blue Ir, respectively (Farr 1980a). Because both strains have been used to analyze the inheritance of quantitative fitness traits in the guppy (Farr 1983), these strains are the behaviorally best-known guppy

strains and therefore were used for the present investigation. Three pairs of competing males were used for the Lindane experiment (Fig. 2), while nine pairs of guppy males were exposed to Gross-Lappen (GL) wastewater (Fig. 3).

To prevent habituation of courting males to the wild-type females, males were only exposed to two females immediately before the onset of an observation period. Newly introduced females are preferred by courting males over residing females for at least 15 min (Heinrich and Schröder 1986). Consequently, after each 20-min observation both females were removed and males remained separated from females until the beginning of the next observation.

With the exception of pure inbred strains, all our guppy strains are routinely maintained in collective breeding aquaria where fish mate at random. It can be assumed that maintenance of the fish in small population sizes, with many generations and occasional "bottle-necking" of the populations over the years, have resulted in a reduction in genetic variability in the stocks and a subsequent high degree of homozygosity among the individuals of a given stock. A detailed description of the Neuherberg guppy stocks is given by Farr (1980a) and Schröder (1983).

The present experiments were conducted in 37,5-L aquaria filled with Munich tap water or wastewater, respectively, at a mean temperature of 25°C. During the first week of observation, each pair of male competitors was maintained in tap water and thus served as the control to the second week during which the same pair of males was kept in wastewater either containing lindane or Gross-Lappen water. All experimental aquaria were illuminated with flourescent lights on a 12L:12D photooeriod. The fish were fed daily with TetraMin® dried flake food supplemented occasionally with Tubifex worms, Artemia nauplia, Daphnia, and mosquito larvae.

For the first experiment, 1 mg lindane (hexachlorocyclohexane; benzene hexachloride) was dissolved in 1 L Munich tap water (5.8 mval total hardness; 5.1 mval temporary hardness; 537 µs/cm conductance; 10.4 mg O_2/L oxygen content; pH 7.30; 22.4°C) and diluted with Munich tap water to a concentration of 1 ppb lindane (1 µg/L). To prepare the wastewater for the second experiment, a 10% dilution of Gross-Lappen (GL) water was made using Munich tap water. The GL-water stemmed from the last clearing basin of a Munich sewerage plant at Gross-Lappen near Munich, West Germany. This water is normally collected in a collection tank before being released directly into the Isar river.

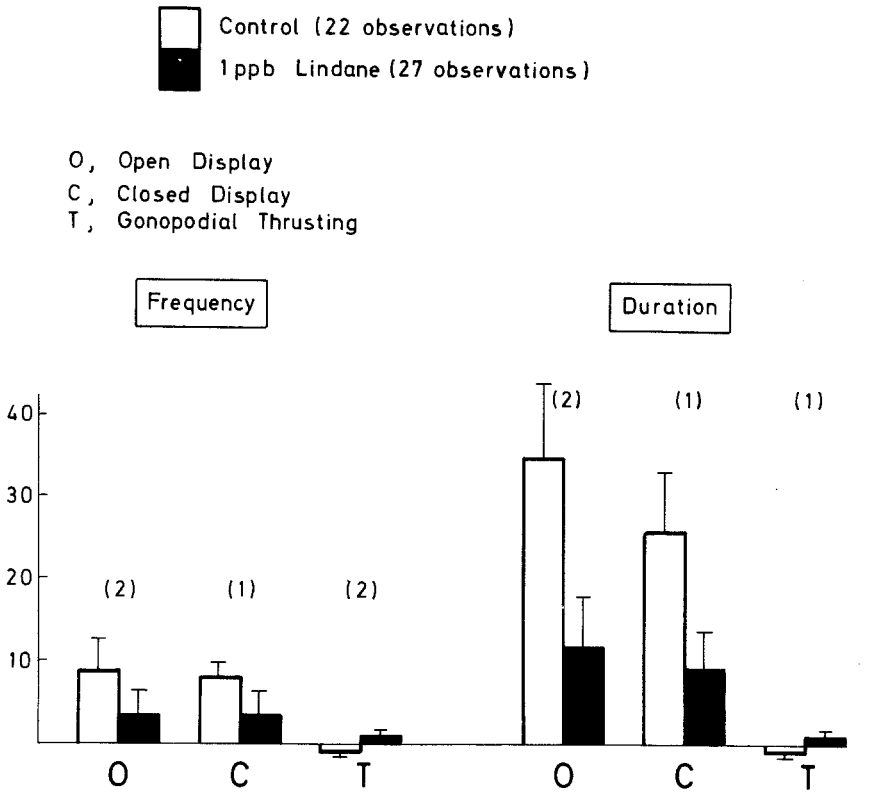

Figure 2. Change of mean differential courtship activity (mean ± standard error of 3 pairs of male competitors) as determined by the differences between ♂ Wild Mac - ♂ Blue Ir after treatment with 1 µg of lindane/L H_2O. All differences between control and lindane treatment were found to be significant (t test; $P < 0.0002$). Parantheses indicate number of pairs that differed significantly between control and Lindane ($P < 0.05$).

RESULTS AND DISCUSSION

The pooled data of the male pairs were weighed to prepare the graphic presentation of Figs. 2 and 3.

Both lindane and GL water addition caused a decrease of the mean differential courtship activities Open and Closed Display and an increase in Gonopodial Thrusting as compared to the control values. In the case of lindane treatment (Fig. 2), the combined mean differential male sexual activities were significantly different between control and exposure to lindane. However, not

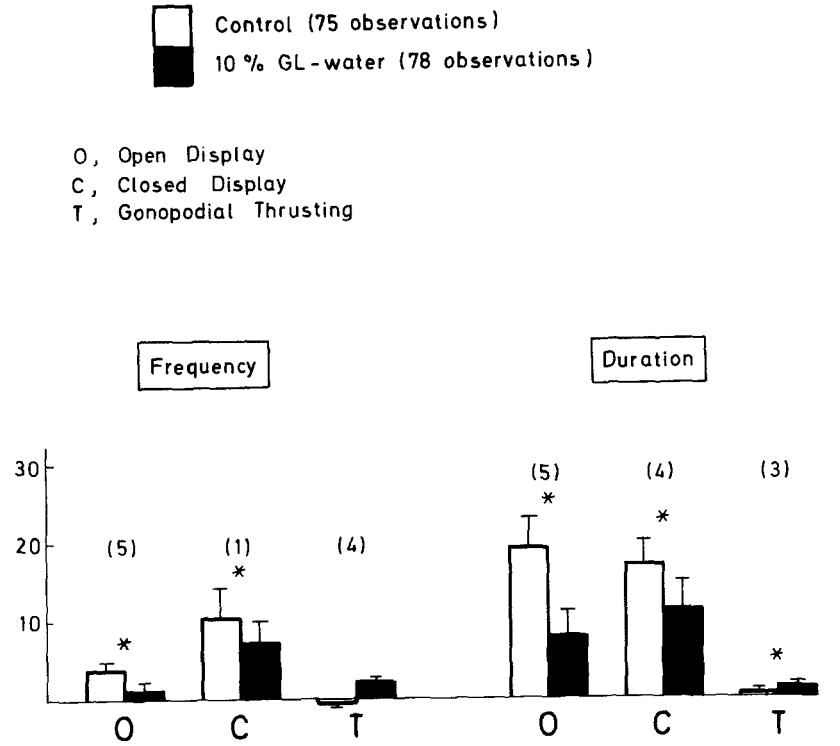

Figure 3. Change of mean differential courtship activity (mean ± standard error of 9 pairs of male competitors) as determined by the differences between ♂ Wild Mac - ♂ Blue Ir after addition of 10% wastewater drawn from the last clearing basin of a Munich purification plant (Gross-Lappen, GL). *, significant differences between control and 10% GL-water (t test; $P < 0.05$). Parantheses indicate the number of pairs that differed significantly between control and GL ($P < 0.05$).

all pairs were affected in each activity. The same tendency holds true for the treatment with GL-water (Fig. 3). If one compares the nine pairs of male competitors of this experiment, GL-water treatment provoked at least one significant change of either frequency or duration of the three sexual activities for each of the nine male pairs. Since all the changes of mean courtship activities tended to shift towards the same direction, weighing of the pooled data again improved the significance between control and treatment (Fig. 3).

With the exception of the frequency of Gonopodial Thrusting, all differences of differential activities between control and GL-water treatment were statistically significant. Both treatments thus caused a decrease of the mean differential display activities as exhibited by Open and Closed Displays and an increase in mean differential Gonopodial Thrusting.

For the sake of simplicity and of sparing words, the original data of the competing males, viz. those of Wild Mac and Blue Ir males were not included in the present paper. Consequently, the reader cannot check that the decrease of mean differential display activities and increase in mean differential thrusting rate was caused by an excess of decrease of Open and Closed Display activities and an excessive increase of Thrusting of Wild Mac males as compared to their Blue Ir competitors. In other words, there was a genotype-dependent influence of the comtaminants which perhaps was due to metabolic differences between Wild Mac and Blue Ir guppy males. However, not all the males of the competing pairs responded differently in like manner to the contaminants. Only 11% up to two-third of the competing male pairs showed significant differences for the individual courtship activities as given by the numbers in parentheses of Figs. 2 and 3.

Since the relative reproductive success of Wild Mac males as compared to that of Blue Ir males was found to be 2.5 times higher in the competition experiment (Farr 1980a), the excessive reduction of Wild Mac display rates indicates a corresponding reduction of the relative fitness by both kinds of aquatic pollution. The rôle of increasing Thrusting activities in the present study, however, is not yet quite clear and therefore requires further research efforts. As far as we know from Farr's extensive studies (1980a, 1980b, 1983), gonopodial thrusts hardly enhance the chance of effective inseminations and thus cannot compensate the decrease in courtship display rate. Because the male offspring sired by one of the competing males can be easily recognized by their Y-linked sexual coloration, the relative reproductive success of two competing males may be determined by the use of virgin females. Such kind of experimentation giving direct evidence for the relative fitness is now in progress.

That aquatic pollution really affects the survival of fish under natural conditions was shown by Brown et al. (1985) who studied the predator-avoidance behavior of guppies after pollution by different concentrations of pentachlorophenol. They found a decrease of fitness in so far as the guppies revealed a slower response to predator attacks at the higher dosages.

However, the main purpose of the present study was to demonstrate that toxic effects of low concentrations of water-borne pollutants may easily be checked by scoring social behavior activities of the guppy. Because behavioral assays using the guppy courtship activities are not only highly sensitive to extremely low concentrations of aquatic contaminants but also fulfil all requirements of modern animal protection regulations, such endpoints should replace all LC-50 procedures. Furthermore, the present study with a water contamination of only 1 μgL^{-1} lindane as well as the use of a 10-percent addition of wastewater normally being drained into a live river without further cleaning clearly demonstrates the need for stronger regulations concerning environmental protection. Otherwise the differential response of Wild Mac and Blue Ir to contamination shows the genotype-dependent influence of aquatic contaminants to changes of fitness traits. It therefore requires the examination of more than one genetically defined animal stock to check the possible toxic effects of environmental pollution on social behavior activities.

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