

GENETICAL ANALYSIS OF TONIC IMMOBILITY IN TWO  
SUBSPECIES OF *MACROPODUS OPERCULARIS*

SHORT COMMUNICATION

P. KABAI and V. CSÁNYI

DEPARTMENT OF BEHAVIOUR GENETICS, L. EÖTVÖS UNIVERSITY,  
HUNGARY

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Tonic immobility (TI) has recently been discussed as a species-specific antipredator behaviour [5]. Its biological function and possible survival value is now being investigated by some authors [2, 6]. Physiological and biochemical processes involved in this behavioural phenomenon are scarcely known (for review see [1]). Few attempts have been made to study genetical influence on TI [3, 4, 7], though data provided by genetical analysis would help to understand better the underlying mechanism of escape behaviour. This paper presents a preliminary experiment on the genetical differences in TI between two subspecies of the paradise fish (*M. o. opercularis* and *M. o. concolor*).

*Subjects*

Our two laboratory strains: "Y" (*M. o. opercularis*) and "F" (*M. o. concolor*) were chosen as parent populations ( $P_1$  and  $P_2$ , respectively). The strains (both originated from a dealer in Florida, Gossington, USA) were bred in our laboratory by sibmating: at the time of the experiment strain "Y" ( $P_1$ ) was at the 8th, and strain "F" ( $P_2$ ) at the 6th generation of inbreeding. The two parent populations ( $N_{P_1} = 21$  and  $N_{P_2} = 28$  fish), their hybrids ( $N_{F_1} = 27$  fish) and the two back-cross populations ( $N_{B_1} = 26$  and  $N_{E_2} = 39$  fish) were used for the experiment. The fish were kept in 60 l homing tanks, then moved into 4 l individual tanks one day before measurement.

*Apparatus and Procedure*

A simple glass cylinder, 50 cm high ( $d = 6.5$  cm), was filled with day-old tap water, which was changed before each measurement. The outer part of the glass was marked by blue circles at heights of 12.5, 25, and 37.5 cm. The apparatus was illuminated by diffuse light. When the fish were put into the water one by one, they sink within 3–10 seconds to the bottom, where they generally stayed immobile in unusual postures (body parallel with the

bottom, and fins fully erected). Motion of the eyes could be observed during TI. TI was often interrupted for a few seconds by short intensive tail beats, followed by another period of immobility. This sequence was repeated several times until there was a return to normal motions, defined in this experiment as rising to 15 cm in height. The duration of TI here refers to the whole period until normal swimming was resumed. During swimming locomotor activity was measured by the number of circles the fish passed. Number of air gulps was also recorded for the entire 10 min of the test.

### Results and Discussion

Mean values of locomotor activity and number of air gulps for each population are shown in Table 1. The hybrids exceeded the parents in both

**Table 1**  
Mean values of locomotor activity and number of air gulp

Locomotor activity		Number of air gulp	
$P_1 \bar{x} = 220$ $s = 64$	$P_2 \bar{x} = 103$ $s = 84$	$P_1 \bar{x} = 19$ $s = 5$	$P_2 \bar{x} = 5$ $s = 4.5$
$F_1 \bar{x} = 273$ $s = 86$		$F_1 \bar{x} = 27$ $s = 7$	
$B_1 \bar{x} = 195$ $s = 85$	$B_2 \bar{x} = 142$ $s = 80$	$B_1 \bar{x} = 13$ $s = 7$	$B_2 \bar{x} = 10$ $s = 7$

of these two parameters. Heritability and number of allelic differences could not be estimated for locomotor activity and air gulping frequency because transformations of the scale failed to bring about the means and variances fulfilling Mather's two criteria.

Distribution of data of TI is shown in Fig. 1. Applying a logarithmic scale transformation, Mather's first criterion was fulfilled. Variances of the data of the parent populations remained still significantly different ( $F = 6.48$ ,  $P < 0.001$ ). *Macropodus o. opercularis* ( $P_1$ ) had a high dominance ( $D = 0.70$ ) in TI over *M. o. concolor* ( $P_2$ ). Heritabilities ( $H^2$ ) of TI in this experiment were 31% and 33% calculated from the variances of the transformed data of the first and second back-cross populations, respectively. The minimal number of gene differences ( $k$ ) between the two strains was estimated as 0.06 from the data of the first back-cross (toward the dominant parent) population, and as

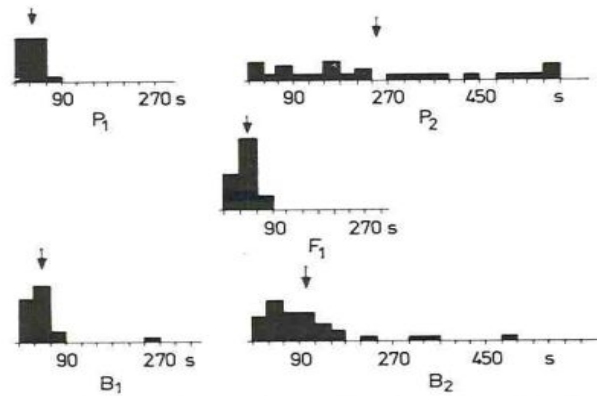


Fig. 1. Distributions of tonic immobility durations of each population

1.78 from the second back-cross (toward the recessive parent) population. The average number of  $k$  being  $0.91 \approx 1$  is a rough estimation of the number of major genes responsible for the differences in this behavioural phenomenon between the two subspecies. Our results suggest that the genetical factors influencing the duration of TI in our test situation are not very complex.

Table 2  
Duration of tonic immobility

Original data (sec)		Transformed data (ln sec)	
$P_1$ $\bar{x} = 33$ $s = 13$ $N = 21$	$P_2$ $\bar{x} = 251$ $s = 199$ $N = 28$	$P_1$ $\bar{x} = 3.4$ $s^2 = 0.20$	$P_2$ $\bar{x} = 5.1$ $s^2 = 1.35$
$F_1$ $\bar{x} = 41$ $s = 15$ $N = 27$		$F_1$ $\bar{x} = 3.65$ $s^2 = 0.14$	
$B_1$ $\bar{x} = 43$ $s = 46$ $N = 26$	$B_2$ $\bar{x} = 101$ $s = 99$ $N = 39$	$B_1$ $\bar{x} = 3.4$ $s^2 = 0.81$	$B_2$ $\bar{x} = 4.2$ $s^2 = 0.85$

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PÉTER KABAI }  
VILMOS CSÁNYI } H-2131 Göd, Jávorka S. u. 14.