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Ontogeny of the electric organ discharge in two parapatric species of the dwarf stonebasher, *Pollimyrus castelnaui* and *P. marianne* (Mormyridae, Teleostei)

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Abstract

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This is the first description of the ontogenetic development of the electric organ discharge (EOD) in two parapatric mormyrid sibling species, *Pollimyrus castelnaui* (Boulenger, 1911) and *P. marianne* Kramer *et al.* 2003, from the first appearance during the early larval stages at about 0.7 cm total length to the fully developed adult discharge at about 3.0 cm total length. At around day 20 the biphasic larval discharge (which is similar to that of *P. adspersus* larvae) is followed by the emerging triphasic adult discharge with a delay of about 0.7 ms. Both discharge types coexist for about 3 weeks. As the development advances, the amplitude of the adult discharge increases rapidly while that of the larval discharge diminishes. Whereas the adult EOD waveform of *P. marianne* remains triphasic later in life, in *P. castelnaui* an 'initial flourish', in the form of additional small phases, is added and develops at an age of about 40 days, when larval and adult EOD are present. Possible evolutionary scenarios are discussed.

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Introduction

The southern African mormyrid *Pollimyrus marianne* (Teleostei, mormyridae) was discovered only recently (Kramer *et al.* 2003). The morphological differences from *P. castelnaui* (Boulenger, 1911) are quite cryptic but the characteristic electric organ discharge (EOD) waveforms of both species are unmistakable. The EOD is regarded as a valuable tool for taxonomic research (Hopkins 1980; Kramer 1996).

Up to now it was not known exactly when in ontogeny, waveform differences between the EOD of the two sibling species emerge. Almost all we know about the ontogeny of the electric organ regards a single mormyrid species, *P. adspersus*, which had often been confused with *P. isidori* (Crawford 1997). *Pollimyrus adspersus* has reproduced quite easily in several laboratories (Kirschbaum 1975, 1977; Westby and Kirschbaum 1977, 1978; Denizot *et al.* 1978, 1982; Bratton and Kramer 1989; Postner and Kramer 1995; Kramer and Postner 1997).

Data on the ontogenetic development of the EOD are available for very few of the more than 200 mormyrid species known today (Szabo 1960; Heymer and Harder 1975; Schugardt 1997; Werneyer and Kramer, in press). A larval electric organ system, consisting of a primitive electric organ (Kirschbaum 1977) and a set of larval electroreceptor organs (Denizot *et al.* 1998; Bensouilah *et al.* 2002) precede the adult electric system in mormyrid fish. As the sibling species *P. castelnaui* and *P. marianne* reproduced repeatedly in our laboratory, we took the opportunity to document the EOD development from the first larval stages to the occurrence of the adult discharge.

Materials and Methods

All parental animals had been caught in the wild. The *P. marianne* originate from the Upper Zambezi River (caught by F.H. van der Bank and B. Kramer in 1999 at Katima Mulilo, 17°32′31.9″S, 24°26′17.7″E, East Caprivi, Namibia) while the *P. castelnaui* originate from the Okavango Delta (caught by F.H. van der Bank in 2002 at Makwena, 19°03′45.3″S, 22°23′24.5″E, Botswana). Fish of both species reproduced several times in captivity. A close relation between environmental conditions and reproductive behaviour was not obvious because reproduction occurred at temperatures from 23 to 26 °C and at water conductivities ranging from 50 to 200 µS/cm. Reproductive behaviour was always indicated by highly conspicuous behaviours in the male, such as marked territorial behaviour and nest building (Lamml and Kramer 2005), quite similar to that documented in *P. adspersus* (Bratton and Kramer 1989; Crawford 1991). In this study we examined 19 *P. castelnaui* and 19 *P. marianne* larvae or juveniles that hatched in our laboratory.

The fry were kept in different sized tanks. Young larvae were fed live and defrosted rotifers and, at a later stage of development, chopped defrosted chironomid larvae. For the recordings of the first larval discharges a special glass chamber (3 cm^3), fitted with two platinum electrodes (separation 2.4 cm), was used. Recordings of juveniles were made either in a small circular tank (diameter 5 cm), fitted with four disk-shaped carbon electrodes (diameter 5 mm), or directly in the keeping tanks using carbon rod electrodes (diameter 2.5 mm or 5 mm). The electrodes were positioned just in front of the head and behind the tail, with the positive electrode at the head.

The EODs of the fish were differentially amplified (variable amplifier 1× to 100×, electronics workshop of the University of Regensburg), captured by a digital oscilloscope (Tektronix 420, bandwidth 100 MHz, 12-bit vertical resolution), and stored on the hard disk of a PC. For analysis we used the software FAMOS 4.0 (IMC, Berlin, Germany). The amplification of the signal was selected to optimize the use of the vertical resolution of the oscilloscope.

To determine the length of the larvae and of the juvenile fish we took photographs in a glass cuvette (5 × 1 × 5 cm) fitted with a scale. For larvae we measured the total length (TL, from the tip of the snout to the end of the tail fin, Fig. 1A) and for juvenile fish the standard length (SL, from the tip of the snout to the midbase of the caudal fin, Fig. 1B) was measured. Measurements are reported with an accuracy of 0.1 cm.

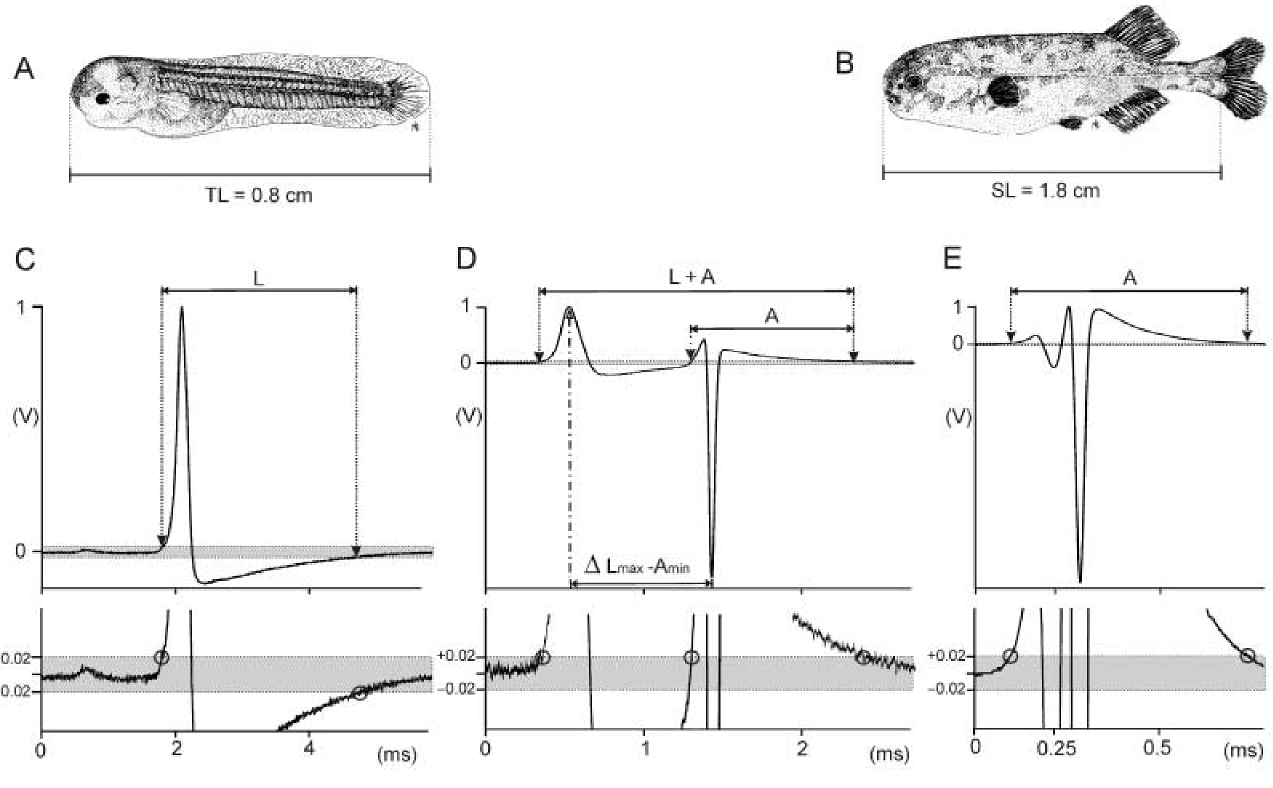

Fig. 1—**A**. A 10-day-old larva of *Pollimyrus castelnaui*, showing how the total length (TL) was measured. —**B**. A juvenile *P. castelnaui* about 3 months old at developmental stage IV, with standard length (SL). —**C**. Larval EOD, stage I. —**D**. Larval EOD followed by the emerging adult EOD, stage II. EOD duration estimated as indicated. —**E**. Adult EOD, stage IV. C–E. Illustrations of how the threshold criterion of ± 2% peak amplitude was applied for estimating EOD duration. Upper panels: the shaded areas indicate the ± 2% level of the normalized positive peak; lower panels: as upper panels, but magnified ×3 in C, ×8 in D and ×20 in E, for clarity. The circles mark the threshold crossings used for estimating EOD durations. L = larval discharge, L + A = larval and adult discharge; A = adult discharge; $\Delta L_{max} - A_{min}$ = separation of larval positive peak from adult negative peak.

To determine the duration of the waveforms we normalized the EODs to a positive peak equal to 1 and used a ± 2% threshold criterion from baseline to determine the start and termination points of the discharge waveform. For an explanation of how the EOD duration was measured in larvae and in juveniles of different stages, see Fig. 1.

We compared the recorded larval and juvenile EODs of *P. castelnaui* and *P. marianne* with those of *P. adspersus*. Therefore we used earlier EOD recordings from B. Kramer's laboratory (Kramer 1996; Fig. 5.16).

Results

When the first discharges were recorded on day 8 after hatching, they were indistinguishable between the two species *P. castelnaui* and *P. marianne* (Fig. 2A,B stage I) and similar to those of *P. adspersus* (Fig. 3). The larval EOD was a strong, head-positive pulse that was terminated by a weaker negative potential. Already at the age of 16 days the adult discharge was detected riding on the rising slope of the negative phase of the larval discharge (Fig. 2A,B stage II). After several days the adult discharge was much stronger than the larval discharge.

The larval discharge became weaker and weaker while the adult discharge increased in strength every day (stage III). In both species, the adult discharge already showed the three distinctive phases of adult *P. marianne* EODs: i.e. the leading positive phase (P1), the negative main phase (N) and a second positive phase (P2) with the longest duration (Fig. 2A stage III early compare with Fig. 2B stage III early). Up to this stage, EOD development did not differ between *P. marianne* and *P. castelnaui*, and was similar to that of *P. adspersus*, apart from the later emergence of the adult discharge in *P. adspersus* at an age of 30–40 days (Westby and Kirschbaum 1978).

In *P. marianne* the larval EOD grew weaker still, while the adult EOD increased in strength with little change in waveform (Fig. 2 stage IV). At a later stage, some individuals developed a small indentation on top of the P1 phase (see Kramer *et al.* 2003; Fig. 4; and Markowski *et al.* submitted for publication).

From stage III (late) onwards, the EOD of *P. castelnaui* began to diverge from that of *P. marianne*. Usually before the larval discharge had vanished completely, a small biphasic potential on the rising slope of the P1 phase became visible (Fig. 2 stage III late, see inset). This biphasic potential increased in strength, finally crossing the zero line, and

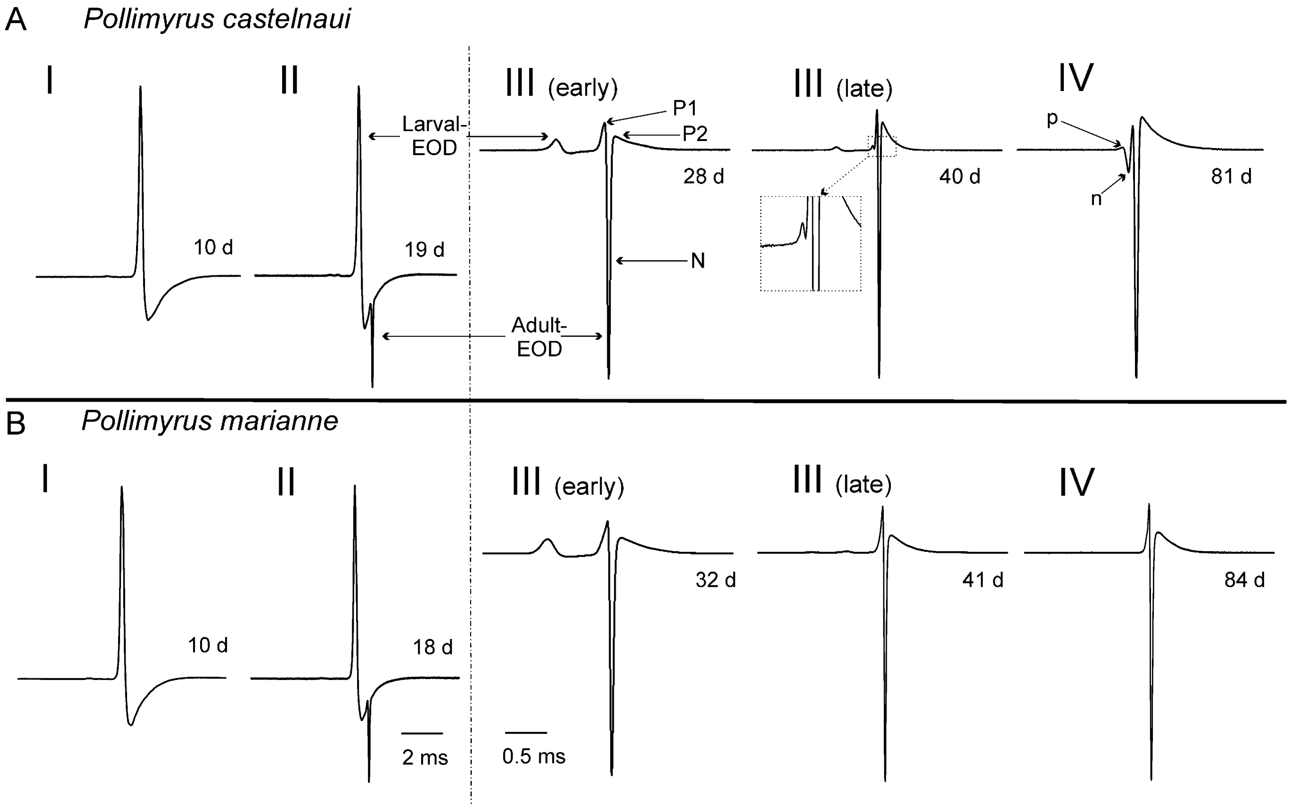

Fig. 2—**A**. EOD ontogeny of *Pollimyrus castelnaui* and —**B**. EOD ontogeny of *P. marianne* at developmental stages I–IV. Amplitudes are normalized to the positive peak of the waveform. Roman numerals indicate developmental stages, arabic numerals give the age in days. The time-scale of 2 ms holds for stages I and II (to the left of the vertical broken line), and a scale of 0.5 ms holds for the rest of the Figure.

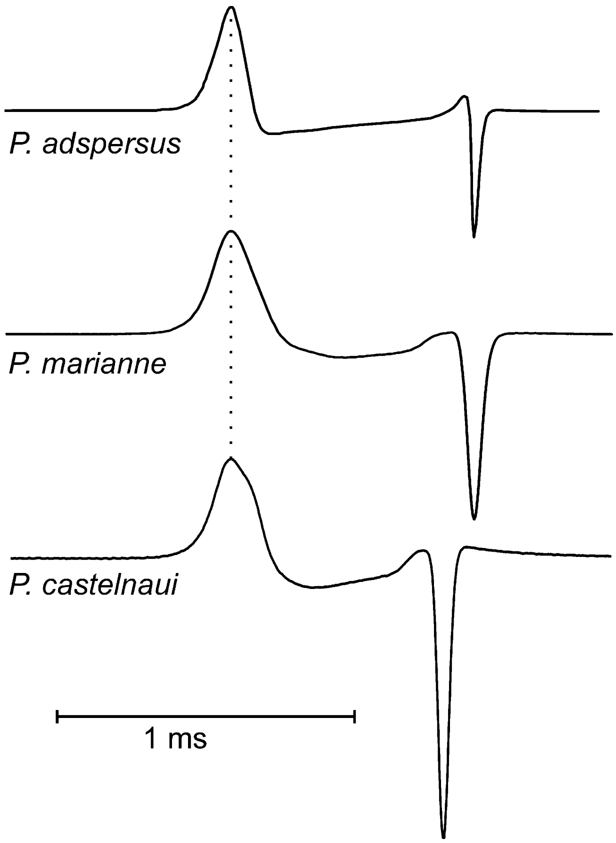

Fig. 3—EODs of juveniles from three *Pollimyrus* species at developmental stage II: *P. adspersus* (53 days), *P. marianne* (19 days), *P. castelnaui* (21 days). The dotted line marks a common reference point at the positive peak of all three waveforms. Note the strong resemblance in EOD waveform of these three species.

became the new *p* and *n* potentials that preceded the main discharge. At that early stage, P1 represented the positive maximum of the discharge. At a later stage P1 became weaker 'pulled down' by an *n* phase that was increasing in strength), and P2 formed the positive peak amplitude in adult animals (Fig. 2A stage III late).

Table 1 Length (in cm; TL for stage I, SL for stages II, III and IV) of *Pollimyrus marianne* and *P. castelnaui* larvae and juveniles at different developmental stages

	Pollimyrus marianne				***Pollimyrus castelnaui***			
Stage	Min	Max	Mean	*N*	Min	Max	Mean	*N*
I	0.70	1.00	0.86	6	0.70	1.20	0.92	11
II	1.25	1.40	1.3	4	1.10	1.80	1.31	11
III	1.60	1.80	1.69	4	1.35	1.95	1.63	4
IV	2.25	3.00	2.73	9	1.90	2.40	2.48	7

For further explanation see Materials and Methods and Fig. 1. Min, minimum length; Max, maximum length; Mean, average length; *N*, number of individuals studied. The different stages were reached at an average age of: 8–10 days for stage I, 15–30 days for stage II, 25–40 days for stage III and 45–75 days for stage IV.

All recordings were easily attributed to developmental stages I–IV (Tables 1 and 2). Differences in temperature and feeding conditions caused considerable differences in growth rate; days given in Fig. 2 represent an average.

Discussion

Although a considerable number of mormyrid species have been kept in captivity already, very few have also reproduced, and in even fewer species has the ontogenetic development of the EOD waveform been studied (*P. adspersus*: Westby and Kirschbaum 1977, 1978; *Mormyrus rume*, *Campylomormyrus cassaicus*: Schugardt 1997; *Marcusenis macrolepidotus*: Werneyer and Kramer, in press).

The larval electric organ appears to be a plesiomorphic character for the genus *Pollimyrus* (Kirschbaum 1995). This is possibly true for the whole family of mormyridae (Heymer and Harder 1975; Schugardt 1997). In the two species studied here, the early development of the EOD resembles that in *P. adspersus* (Kirschbaum 1977; Denizot *et al.* 1978), supporting the presence of a larval electric organ as a common feature for the whole genus *Pollimyrus*. The ontogenetically early emergence of the additional *p* and *n* phases in the adult

Table 2 EOD duration (ms) in *Pollimyrus marianne* and *P. castelnaui* larvae and juveniles at different developmental stages

	Pollimyrus marianne				***Pollimyrus castelnaui***			
Stage	L*	L + A*	A*	$\Delta L_{max} - A_{min}$	L*	L + A*	A*	$\Delta L_{max} - A_{min}$
I	2.08 ± 0.17	–	–	–	2.38 ± 0.50	–	–	–
II	–	1.65 ± 0.24	–	0.75 ± 0.07	–	1.62 ± 0.21	–	0.71 ± 0.08
III	–	1.75 ± 0.15	1.21 ± 0.24	0.77 ± 0.08	–	1.63 ± 0.11	0.92 ± 0.07	0.75 ± 0.10
IV	–	–	1.12 ± 0.12	–	–	–	0.87 ± 0.12	–

* Duration determined using a ± 2% peak amplitude criterion; temperature, 25 ± 1 °C.
L = larval discharge, L + A = larval and adult discharge; A = adult discharge; $\Delta L_{max} - A_{min}$ = separation of larval positive peak from adult negative peak.
All means are given with standard deviation. For further explanation, see Materials and Methods and Fig. 1.

discharge of *P. castelnaui* raises the question of their phylogenetic origin.

The seemingly most parsimonious hypothesis states that the relatively simple triphasic waveform of *P. marianne* – occurring early in the ontogeny of both species examined – represents the ancestral condition from which the complex pentaphasic waveform of *P. castelnaui* is derived. However, this hypothesis is in conflict with the phylogenetic tree of Kramer *et al.* (2003) who reported that *P. castelnaui* is ancestral to *P. marianne*.

Therefore, we favour the alternative hypothesis that the common ancestor of *P. castelnaui* and *P. marianne* already possessed a pentaphasic discharge and that *P. marianne* has lost this feature during speciation. This hypothesis is supported by small indentations in the EOD waveforms of some *P. marianne* individuals being more prominent and encountered commonly in *P. marianne* taken from a presumed hybrid zone (the Kwando River; Kramer *et al.* 2003). A simple mathematical model accounts for the whole range of variability (Markowski *et al.*, submitted for publication). The model supports the notion that the small indentations are attenuated versions of the additional EOD phases of *P. castelnaui*; that is, they probably represent historical remnants, inherited from common, presumably pentaphasic, ancestors.

Species-typical differences in mormyrid EOD waveforms are explained by variations in electrocyte stalk morphology and innervation (Bennett 1971). Unfortunately no data on the morphology of the electric organ of *P. castelnaui* and *P. marianne* are available but Teugels and Hopkins (1998) reported on the anatomy of the electrocytes of four *Pollimyrus* species, three of which have tri- or polyphasic waveforms resembling those of our southern African *Pollimyrus* species. The electrocyte stalks of these three species are innervated posteriorly. However, some of these stalks are also innervated posteriorly but penetrate the cell body twice, and only then fuse with the caudal cell face. The striking differences in the EOD of *P. castelnaui* and *P. marianne* could be caused by variation of the ratio of penetrating to non-penetrating stalks, or by density differences in ion channels and electrical excitability.

As the interrelation of the electric organ's morphology and the EOD waveform is not definitely resolved, histological reconstruction of the two dwarf stonebasher species' electric organs at different developmental stages might yield insights into the mechanism of EOD generation and differentation of waveforms.

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