

Stiff-legged behaviour in the green and black poison frog *Dendrobates auratus* in response to simulated predation

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Predation pressure has led to the evolution of a diversity of antipredator strategies in nature. Stiff-legged behaviour is a defensive strategy that has been described in several neotropical, leaf-litter frog species of the families Brachycephalidae, Bufonidae, Craugastoridae, Cycloramphidae, Leptodactylidae, Microhylidae and Odontophrynidae (Sazima, 1978; Rocha et al, 1998; García, 1999; Toledo & Zina, 2004; Bertoluci et al., 2007; Menin & Rodrigues, 2007; Toledo et al., 2011; Mira-Mendes et al, 2016). The behaviour is usually preceded by short leaps and is characterised by frogs flattening their body and fully extending their fore and back limbs into a stiff (or rigid) position and remaining motionless. Given that this behaviour has only been described in cryptically-coloured frogs, remaining motionless is thought to reduce or prevent predator detection (Toledo et al., 2011); however, it is also possible that (1) remaining motionless would inhibit predators that use movement to detect prey or require live prey, and/or (2) the extended and rigid position would make it more difficult for the predator to handle and consume the prey. Although both possibilities are similar to the presumed functions of thanatosis (death-feigning), Toledo et al. (2011) distinguishes stiff-legged and thanatosis from each other mainly on the basis of limb extension and position. Anurans exhibiting thanatosis do not fully extend their limbs into a rigid position as in stiff-legged behaviour, but instead keep them in a loose (or relaxed) position (Toledo et al., 2010; 2011). In following Toledo et al. (2011), herein, we report the presence of stiff-legged behaviour in naïve, captive-raised, conspicuously-coloured *Dendrobates auratus* (Dendrobatidae) from a Panamanian lineage.

The natural range of the green and black poison frog *D. auratus* is from southern Nicaragua to Colombia (Savage, 2002). The species is characterised by its variable colours and patterns that, to predators with colour-vision, signal the presence of alkaloid defenses (Daly et al., 1992; Saporito et al., 2012; Stuckert et al., 2019). Body raising behaviour has been reported in captive and wild-caught adults of *D. auratus* from Costa Rica following simulated predation trials (Blanchette & Saporito, 2016; 2017).

We undertook laboratory tests of the defensive behaviour of juvenile *D. auratus* (5 months post-metamorphosis) in response to low and high rates of simulated predation; no frogs were harmed during these tests. To simulate



Figure 1. *Dendrobates auratus* exhibiting stiff-legged behaviour in response to simulated predation (the brown particles present on the body of the frog are small pieces of moss from the experimental enclosures)

predation, we disturbed eight frogs by picking them up and then releasing them using 7.5 cm pressure-sensitive forceps for three consecutive days, followed by one day of no such disturbance. The tests lasted for 23 days (25 September to 17 October of 2022). Once a frog displayed the stiff-legged behaviour, it was removed from the experiment. The three days in which there was simulated predation were referred to as ‘predation periods’. Four frogs were subjected to low disturbance rates, in which they were picked up and released 12 times daily in each predation period (36 times) and four frogs were subjected to high disturbance rates, in which they were picked up and released 24 times daily in each predation period (72 times). Of the individuals exposed to the high disturbance rate one exhibited stiff-legged behaviour five days after the start of the experiment, two individuals displayed this behaviour after six days and the fourth individual after eleven days. Frogs exposed to the low disturbance rate also exhibited stiff-legged behaviour, but its appearance took a longer exposure to the simulated predation treatment. One frog began stiff-legged behaviour six days after the start of the experiment, while the other

three individuals exhibited the behaviour after 22 days. Stiff-legged behaviour was identical in all *D. auratus* and consisted of frogs keeping their eyes open and assuming a stiff-and-cross-legged position (Fig. 1). All individuals held the position even when further manipulated with forceps. On average, the behaviour lasted for five minutes, after which the frog returned to a normal, relaxed posture and behaviour.

Our results demonstrate that stiff-legged behaviour is not restricted to cryptically-coloured frogs and may be a more widespread defensive adaptation than previously thought. Its presence in conspicuously-coloured *D. auratus* also suggests that its defensive function extends beyond detectability (or camouflage), especially with respect to colour-visioned predators. Among conspicuously-coloured frogs, stiff-legged behaviour could function more similarly to thanatosis and limit predation to predators that require live prey and/or make it more difficult for certain predators to handle and consume the frogs. Stiff-legged behaviour in *D. auratus* may act as a supplementary defense, and in particular against colour-visioned birds, which are thought to be common predators of poison frogs (Saporito et al., 2007; Noonan & Comeault, 2009; Maan & Cummings, 2012; Rojas, 2017). Further, stiff-legged behaviour was induced in response to simulated predation, suggesting that its presence in wild populations of *D. auratus* (and other species) may depend upon the extent of predation pressure. All of the individuals in the present study were juveniles, and although it is possible that they express different defensive adaptations when compared to adults, Blanchette et al. (2016) reported body raising in both juveniles and adults of *D. auratus*. Surprisingly, none of the individuals in the present study exhibited body raising behaviour, which has been described in Costa Rican lineages of *D. auratus* exposed to similar predation treatments in both the lab and field (Blanchette & Saporito, 2016; 2017; Saporito unpublished data). The unexpected absence of body raising and presence of stiff-legged behaviour in this Panamanian lineage of *D. auratus* needs further study. Finally, field studies are required to determine if stiff-legged behaviour also occurs in wild individuals, and if so, its potential defensive function.

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