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RESISTANCE OF EELS (*GYMNOTHORAX*) TO THE VENOM OF SEA KRAITS (*LATICAUDA COLUBRINA*): A TEST OF COEVOLUTION

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H. Heatwole and J. Powell. Resistance of eels (*Gymnothorax*) to the venom of sea kraits (*Laticauda colubrina*): a test of coevolution. *Toxicon* **36,** 619–625, 1998.—Eels of the genus *Gymnothorax* from the Pacific are selectively preyed upon by banded sea kraits (*Laticauda colubrina*) and have been reported to sustain massive doses of sea krait venom without ill effect. By contrast, the present study found that *Gymnothorax moringa* from the Caribbean, where no sea snakes occur, are sensitive to sea krait venom, with doses as low as 0.01 mg dry wt of venom/kg wet wt of eel resulting in signs of envenomation, and doses as small as 0.1 mg/kg proving to be lethal. These observations suggest that the resistance of Pacific *Gymnothorax* to sea krait venom results from coevolution of predator and prey, rather than from a general hardiness of *Gynmothorax*. This theory is supported further by literature reports of sensitivity to sea snake venom by other taxa of non-coevolved eels that either are allopatric with sea snakes (*Anguilla*), or are sympatric with them but occupy different habitats and are not preyed upon by them (*Heteroconger*). © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Some eels that are favoured prey of sea snakes have unusually high tolerances to the venom of their predators (Heatwole and Poran, 1995) and it has been suggested that such resistance may represent a coevolved adaptation. On the other hand, it could merely be an expression of a general hardiness of those eels. The present study was undertaken to provide information relevant to these alternatives.

The Banded Sea Krait, *Laticauda colubrina*, is a widespread sea snake in southwestern Pacific waters and feeds almost exclusively upon eels (Pernetta, 1977; Voris and Voris, 1983), including members of the genus *Gymnothorax* (Glodek and Voris, 1982; Guinea, 1986). *Gymnothorax* spp. from New Guinea have a remarkable resistance to the venom of sympatric sea kraits, tolerating massive doses with no ill effect other than a temporarily elevated ventilation rate (Heatwole and Poran, 1995). The presence of

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Gymnothorax species in the Caribbean, where no hydrophiids or laticaudids occur, provided an opportunity for comparing the sensitivities to venom by known prey of the sea krait with that of an allopatric congener that clearly did not coevolve with sea snakes.

MATERIALS AND METHODS

Spotted moray eels, *Gymnothorax moringa* were collected between 24 and 27 January 1996 at depths of 11 m or less from patch reefs located 2.5–13 km north to west of Lee Stocking Island, Bahamas. Eels were anesthetized by squirting a jet of Metomidate into the crevices in which they sheltered; after 2–3 min they were winkled out with a hooked wire. They recovered from the anesthesia in about 15 min. They were transported in large plastic buckets of sea water to the Lee Stocking Island Biological Station and maintained there until experimentation began on 31 January 1996. They were kept in aquaria (120 cm × 37 cm × 45 cm high) circulated with aerated sea water at 25° ± 1°C, as measured by maximum–minimum thermometers in the aquaria. Sections of plastic pipe (20 cm long and 9 cm diameter) were provided for each eel to use as shelter. An eel usually occupied a pipe with its head protruding from one end and the posterior part of the body and the tail from the other. There were 2–3 eels per aquarium during the holding period, but eels were housed individually during experimentation. During the holding period the eels were provided with assorted small reef fish and were observed to feed.

On the evening prior to experimentation, the ventilatory movements of each eel were counted for ten episodes of 1–2 min each, and notation made as to whether the animal was inactive or active during the count. The 'active' and 'inactive' counts were averaged separately for each animal individually and used as pre-experimental control values to relate to similar activity levels during experimentation (see Heatwole and Poran, 1995). Then each animal was weighed in a plastic bucket hanging from a spring balance. This weight was a rough estimate because of the activity of the eel and the water clinging to its body. This value was used merely as a guide for determining approximate dose levels. After experimentation, each eel was weighed more accurately and the dosage calculated precisely. During weighing, most eels regurgitated their food and hence were postabsorptive during experimentation. This was confirmed by autopsy after the experiment. There were two exceptions; one had a small amount of partly digested fish remains in its gut at autopsy, and another defecated during the experiment.

The venom used in experiments was milked from individuals of the Banded Sea Krait, *Laticauda colubrina*, collected 26 July 1995, at Iriomote Island, Ryukyu Islands, Japan. Snakes were collected from sea caves, transported in cloth bags to a local hotel where venom was expressed into vials. Pooled samples from many snakes were preserved in 5% glacial acetic acid and taken to the Centre d'Etudes Nucleaires Saclay, Gif-sur-Yvette, France, where the venom was lyophilized and stored dry. Appropriate amounts were reconstituted with 0.9% saline prepared from NaCl and distilled water, just before use in experiments.

Experimental animals were treated with venom doses ranging from 0.01 mg venom dry wt/kg fresh wt of eel to 11.0 mg/kg by injecting 0.5 ml of saline containing the requisite amount of venom. In addition to using each animal as its own control for ventilation rate, two eels were employed as a 'handling control' and one as a 'saline control'. The former was treated just like an experimental animal except no fluid was injected and the eel was merely pricked with an empty needle. The saline control was injected with 0.5 ml of saline. In order to inject an eel, it was scooped up with a large-meshed net, while still in its tube, and placed on a table, While the eel was still in the net, a towel was placed over the head end of the tube to prevent the animal escaping forward, and then wrapped around the body protruding from the back end of the tube, leaving the tail exposed. Eels were injected into the muscle of the tail, and then the animal and its tube were returned to its aquarium. This procedure took less than a minute except for one instance when the eel escaped from the tube and bit the net. Freeing it took several minutes.

Immediately following treatment, observations were made on each experimental and control animal and then again at 15-min intervals for the first hour, then half-hourly for the next hour, then hourly for 2 h, and from then on at intervals of 4 h until the animal had either died or 48 h had elapsed. When treated animals began showing signs of severe envenomation, observations were increased in frequency to intervals of 10–15 -min until death occurred. In addition to this schedule, supplementary observations were made opportunistically to enhance the completeness of the data set. At all observation periods, the number of ventilations were counted for a 1-min period, and notes on posture, activity, and behaviour recorded. Any animal that had ceased spontaneous movement and ventilation was prodded and if there was no overt response it was considered dead. One eel that had severe signs of envenomation but thought likely to recover was observed beyond the cut-off time of 48 h to monitor recovery. However, it eventually died. Time of death of all eels was recorded as the time half way between the last observation it was noted to be alive and the time it was first known to be dead.

Ventilation rates are portrayed as percent departure from each animal's own control ventilation rate for the appropriate activity level. In immobile animals, sometimes pulsations of the skin over the heart could be detected and heart rate recorded.

RESULTS

Handling induced an elevation in ventilation rates in all experimental and control animals (Figs 1 and 2). However, the saline medium of injection had no appreciable effect. Following subsidence of ventilation rates after handling, the ventilation rates of the uninjected (handling) control and the saline-injected control did not differ significantly from each other ($P = 0.21$; Wilcoxon Signed Rank Test, paired by observation period; two-tailed; $P = 0.21$), nor did their pooled means differ significantly from pre-experimental values (t-test; $t = -1.39$; $P = 0.19$).

Early signs of envenomation consisted of gaping, elevation of the dorsal fin and slight tremors running along the base of the fin, followed by, or coincident with, laboured ventilation with the mouth continually open. This was followed by lowering of the dorsal fin, convulsions, twitching of other fins, leaning to the side and eventually lying on the side or back. Fluttering of the gill covers was a late phenomenon. Even when ventilation was weak, some twitching of the tip of the tail and/or fins occurred, and sometimes persisted well after no ventilation was evident. Sometimes, when all spontaneous overt signs had disappeared, prodding would induce a movement or twitching.

With severe envenomation, these signs were telescoped and some did not occur at all. The most heavily envenomated animal went into convulsions within half an hour and was lying on its side within an hour with the gill covers fluttering.

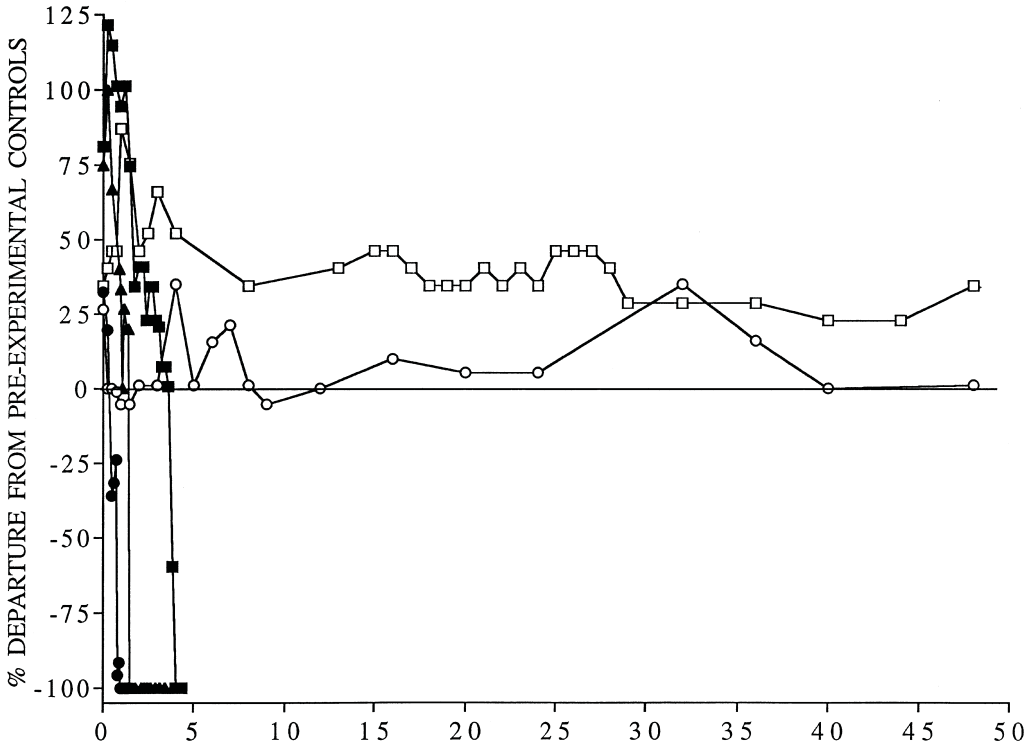

Fig. 1. Change in ventilation rates of five *Gymnothorax moringa* injected with venom of *Laticauda colubrina*. Black symbols indicate animals that died suring the 48 h experimental period; white symbols indicate animals that survived 48 h (although one died later). Dosages were 11 mg/kg (dots), 5 mg/kg (black squares), 4 mg/kg (triangles), 0.1 mg/kg (open squares),

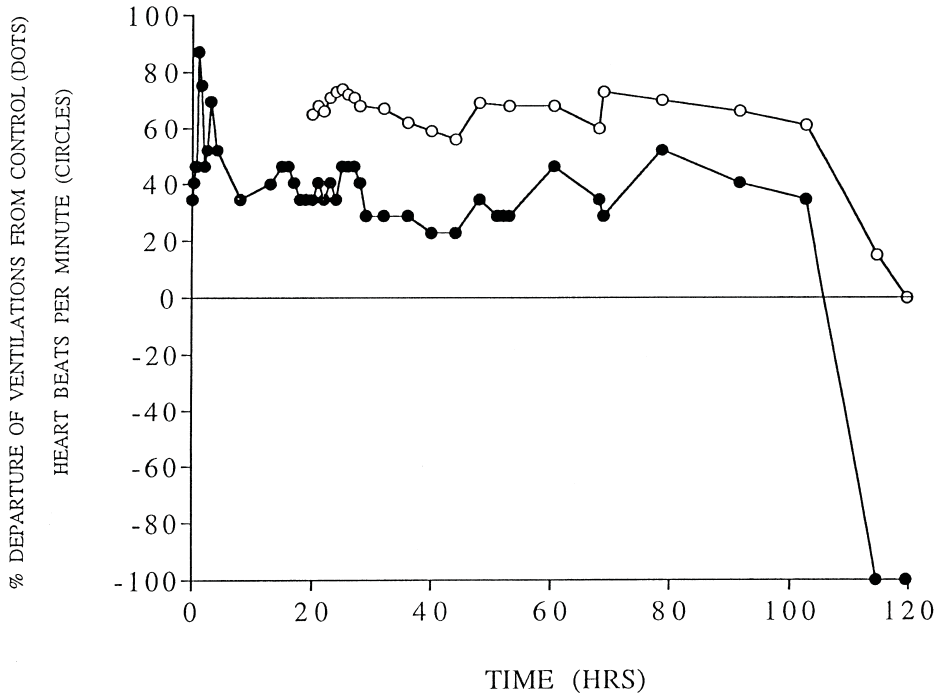

Fig. 2. Heart rate (circles) and change in ventilation rate (dots) of a *Gymnothorax moringa* that survived a dose of *Laticauda colubrina* venom of 0.1 mg/kg for 117 h. Horizontal line represents zero departure from control values of ventilation.

The animal surviving a dose of 0.01 mg/kg merely gaped several times and exhibited tremors along the base of the dorsal fin, but showed no other overt signs except for an elevated ventilatory rate (see below).

One animal showed signs of severe envenomation from a dose of 0.1 mg/kg, and although surviving the prescribed 48 h period (Fig. 2), never recovered mobility and eventually died. It began gaping and developed tremors within the first hour, laboured breathing by 8 h and was lying on its side by 13 h; thereafter, except for a regular and sustained ventilation and heart rate and slight movement of the tip of the tail, it was immobile until its death at 117 h (Fig. 2). It did not give a muscular response to prodding during that time. The skin became covered with a white mucous.

Envenomation stimulated an elevation of ventilation rate above control levels, with the extent and duration of that effect varying with dosage. After subsidence of the handling effect, the animal injected with 0.01 mg/kg had ventilation rates that averaged slightly, but significantly, higher than control levels ($P = 0.01$; Wilcoxon Signed Rank Test; paired by observation period; two-tailed) over the course of the experimental period. This effect had disappeared by 40 h and ventilation rates had returned to control levels (Fig. 1). At that time the animal appeared normal in all respects and eventually was released into the sea.

Animals that succumbed to the venom showed an initial, handling-induced elevation in ventilation, but thereafter a marked decline until, or some time before, death (Fig. 1).

The decline was steepest in the most heavily envenomated animal (11 mg/kg). An animal injected with 4 mg/kg had a sharper decline in ventilation rate and earlier cessation of ventilation than did one receiving a slightly larger dosage (5 mg/kg). However, the former survived for some time after ventilation as evidenced by occasional heart beats, twitching of body muscles and quivering of the gill covers; both animals had similar survival times (Fig. 3).

The animal that survived 117 h had an initial handling-induced increase in ventilation, after which the ventilation rate remained elevated for several days even though the animal was immobile; ventilation rate only dropped a few hours before death, but then did so precipitously (Fig. 2). During the time this animal was immobile, its heart rate cycled around a mean of 68 beats per minute, with highs at about midday (Fig. 2), until a precipitous decline at about the time ventilation ceased.

Survival time was related to venom dosage (Fig. 3). A dose of 0.01 mg/kg was not lethal and the animal recovered completely. The animal injected with 0.1 mg/kg died

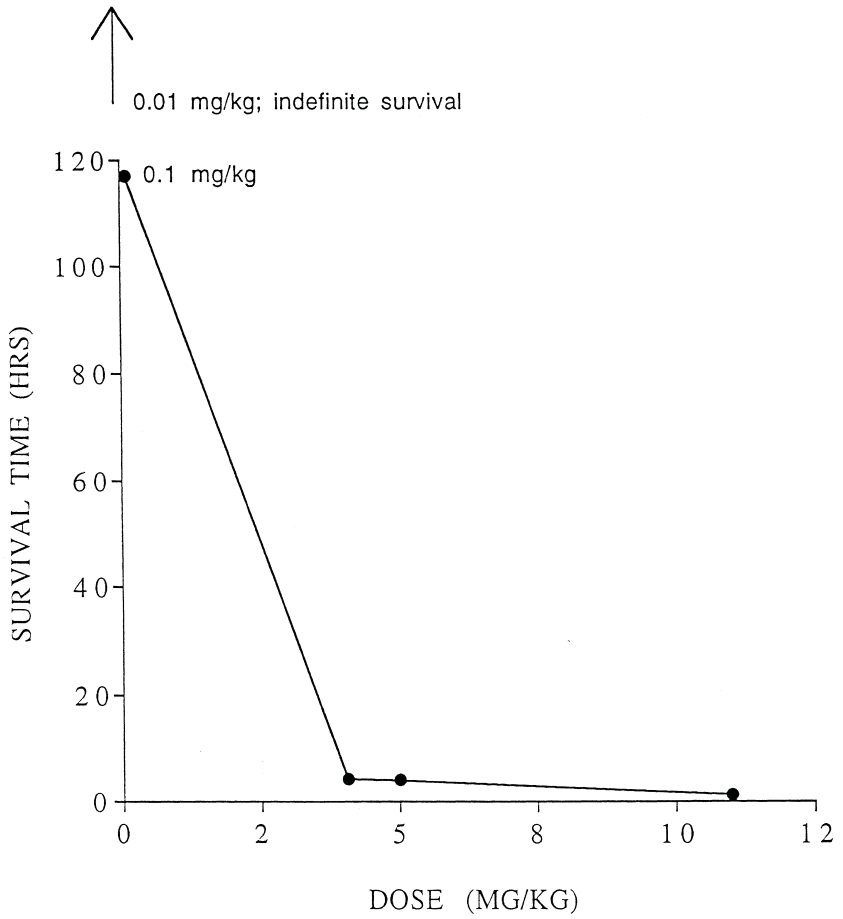

Fig. 3. Effect of dosage of *Laticauda colubrina* venom on survival time of five envenomated *Gymnothorax moringa*.

but only after a prolonged period. Doses of 4 mg/kg or larger were all lethal within less than 5 h (Fig. 3).

DISCUSSION

Elevated ventilatory rate, as observed in the present study, seems to be a common response by eels to envenomation by sea snakes. For example, Heatwole and Poran (1995) found increased frequency of ventilation in resistant eels that otherwise showed no overt response to envenomation.

Gymnothorax from the Caribbean are highly sensitive to sea krait venom. Whereas *Gymnothorax hepaticus* and *G. undulatus* from New Guinea tolerated massive dosages of sea krait venom (75 mg/kg and 42.5 mg/kg, respectively) without severe symptoms of envenomation (Heatwole and Poran, 1995), the Caribbean *G. moringa* studied in the present investigation died at dosages several orders of magnitude lower (0.1 mg/kg). The Atlantic eel, *Anguilla rostrata*, is also sensitive to sea snake venom, having an LD_{50} of 0.08 mg/kg to *Aipysurus laevis* venom (Heatwole and Poran, 1995). Garden eels (*Heteroconger hassi*) from New Guinea are sympatric with sea kraits but are not syntopic with them and are not known to be preyed upon by snakes; they are much more sensitive to *Laticauda colubrina* venom (underwent spasms at a dose of 3.0 mg/kg) than are New Guinean *Gymnothorax*, but are more resistant than Caribbean *Gymnothorax* (killed by doses of only 0.1 mg/kg). Thus, it appears that the degree of resistance to sea krait venom is not correlated taxonomically (species of *Gymnothorax* from the Caribbean and New Guinea differ greatly in their sensitivity to sea krait venom), but rather correlates with the extent of potential contact with the snakes. Species that are allopatric with the snakes (*Anguilla* and Caribbean *Gymnothorax*) or are sympatric but not syntopic and unlikely to be prey of snakes (*Heteroconger*), are sensitive to the venom, whereas species that are syntopic and known to be prey (New Guinean *Gymnothorax*) are resistant. All of these observations converge to support the hypothesis that resistance to sea krait venom is coevolved.

Zimmerman *et al.* (1992) proposed that the mechanism of resistance might be related to degree of cutaneous respiration because several kinds of fish showed differential sensitivities to the venom of *Aipysurus laevis* (Zimmerman *et al.*, 1990; Zimmerman and Heatwole, 1992) that corresponded to their relative dependence upon cutaneous respiration. However, this idea was not substantiated by later experimentation that showed that *Anguilla* was sensitive to sea snake venom (Heatwole and Poran, 1995) despite having highly developed cutaneous respiration (Berg and Steen, 1965). It is likely that the various species of *Gymnothorax* have similar extents of cutaneous respiration and yet they differ greatly in resistance to sea krait venom. It appears that the main mechanism of tolerance to venom is not dependent upon cutaneous respiration.

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