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28. The standard deviations in Fig. 1 are due to variations between experiments. (A) Eighteen different experiments with TA-1–ricin over 11 months were pooled to yield the averages (one experiment was omitted because of exceptionally low numbers of CFU-GEMM colonies). (B) Two different experiments with T101-ricin were pooled. (C) Three experiments performed with UCHT1-ricin. One experiment that was different is not shown in (C) since both the PHA and CFU-GEMM experiments were very sensitive to immunotoxin, with both responses showing a tenfold decrease. Possible explanations are large donor variations or an undetected error. For each experiment in (B) and (C) we used the same donor for peripheral blood and bone marrow cells. Only two experiments in (A) used the same donor cells. The experimental error between experiments (shown) is of the same magnitude as the error within replicates of one experiment (not shown). In control experiments, ricin with or without lactose displayed similar toxicity toward T cells and stem cells measured in these assays, showing that the selectivity of the immunotoxin was not due to cell type differences in ricin sensitivity.
29. We thank C. C. B. Soderling, K.-E. Kim, S. Azemove, and R. Quinones for technical assistance and suggestions, and Hybritech, Inc., for providing T101. Supported in part by NCI grants CA-31618, CA-31685, CA-25097, CA-23021, CA-36725, AM-24027, and Minnesota Medical Foundation grant SMF-320-82. D.A.V. is a Scholar of the Leukemia Society of America.

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Predatory Capture of Toads by Fly Larvae

Abstract. *A natural occurrence of predation upon toads (*Scaphiopus multiplicatus*) by fly larvae* (Tabanus punctifer*) is described. The larvae lie buried in mud, seize the toads with hooked mandibles, pull them partly into the mud, and kill them by feeding on their body fluids. The larvae may ordinarily subsist mostly on invertebrates and take Amphibia only opportunistically.*

On the evening of 27 August 1982, by a small pond near Portal, Chochise County, Arizona, we observed thousands of spadefoot toads (*Scaphiopus multiplicatus*) which, having metamorphosed in close synchrony from the aquatic tadpole stage, were emerging from the water and congregating on the muddy shores. Spaced only centimeters apart in places, they were all of minimal adult size (body length, 1.5 to 2 cm). Conspicuous among them were toads that were dead or dying, apparently having been seized by a predator in the mud and drawn partly into the substrate, until only their head, or head and trunk, projected above ground. We counted dozens of such semisubmerged toads (Fig. 1E) along a stretch of several meters of shoreline. Most were near the edge of the water, where the mud was soft and wet. There were also remains of toads killed on some previous night and dried by daytime exposure.

When we attempted to pull fresh carcasses from the mud with forceps, we always felt a counterpull, which persisted until almost the moment of extrication of the toads. The predator remained concealed below the surface and seemed capable of quick evasive burrowing. By sifting through mud from around captive toads we found it to be a large grublike insect larva, subsequently identified as that of the horsefly *Tabanus punctifer*. Roughly equal in size to the toad itself (Fig. 1F), it occurred with fresh carcasses only, and always singly.

Placed in mud in aquariums, the larvae buried themselves (Fig. 1, A to D) in seconds by forcing themselves into the soil rear-end first, until their front end, which bears the mouthparts (Fig. 1G), was flush or nearly flush with the surface (Fig. 1D). They remained thus for hours or repositioned themselves by moving about underground periodically.

Scaphiopus that we offered to the larvae singly were all eventually caught when they came to rest upon the mouthparts of semisubmerged larvae. The larvae caught them with their pointed hinged mandibles and dragged them partway into the mud within minutes. Of 20 toads that were captured by the 12 larvae

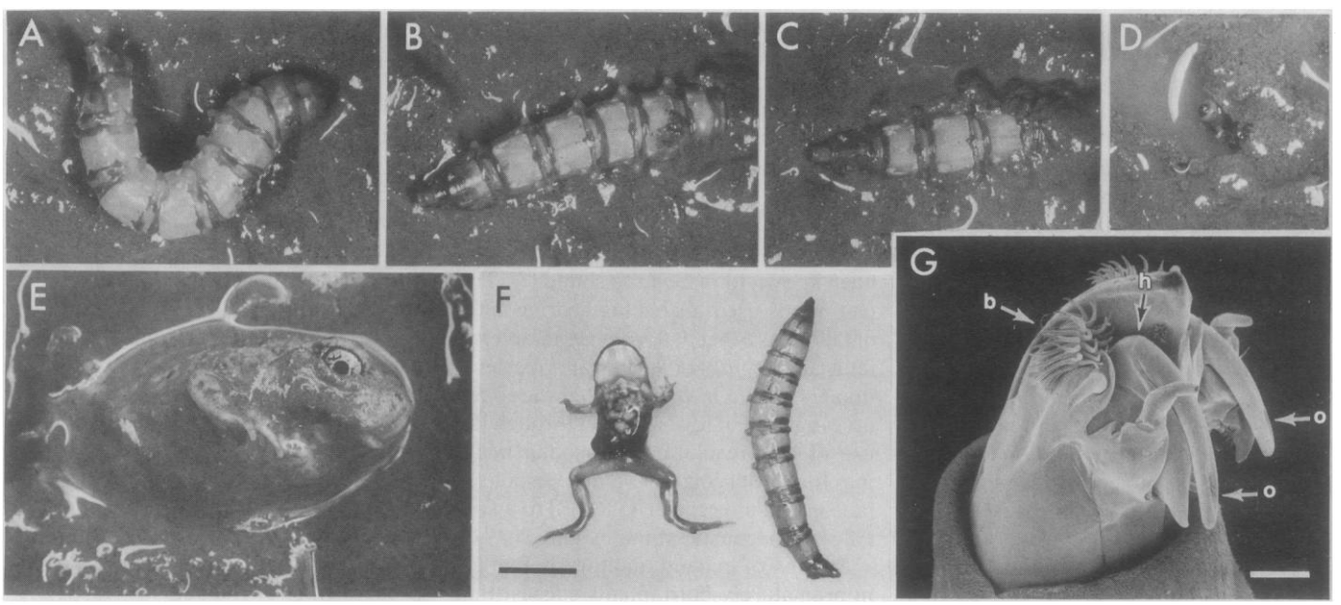

Fig. 1. (A to D) *Tabanus punctifer* larva, burying itself in mud. The larva withdraws until only the mouthparts (D) project from the surface. (E) Moribund toad (*Scaphiopus multiplicatus*) being fed upon by a *T. punctifer* larva that caught it and pulled it partway into the mud. (F) *Tobanus punctifer* larva, beside the toad upon which it was feeding; the abdominal bulge at the base of the right hind leg of the toad marks the site through which the larva was imbibing the body fluids of its prey. (G) Scanning electronmicrograph of head capsule of *T. punctifer* larva showing the hooked mandibles by which the prey are caught (*h*, hinge of mandible). The cephalic brushes (*b*) are presumed to help the larva anchor itself to its prey. The orifices (*o*) on the mandibles denote the openings of the presumed venom glands [morphological interpretation based on Teskey (*2*)]. Scale bars: (F) 1 cm and (G) 0.2 mm.

that we had available for experimentation, some were taken by a hind leg, others by the flank or midsection of the belly. Toads that we pulled away from larvae as they were being drawn into the mud had only small cuts in the skin to mark the place of larval attachment.

Our laboratory tests indicate that *T. punctifer* larvae invariably kill the toads that they capture. They abandon the prey within hours, after ingesting blood and body fluids only. Tabanid larvae are generally fluid feeders (*1*), and *T. punctifer* is not unusual in that respect.

Since toads still showed some responsiveness to poking half an hour after capture by larvae, larval venom (*2, 3*), if injected at all into the toads, must be slow-acting at best. When we handled the larvae, we often felt mildly painful (and eventually itchy) skin punctures, which they visibly inflicted with their mouthparts. Quick immobilization of insect prey, presumably caused by envenomization, and painful "bites" to humans, have been reported for other tabanid larvae (*2, 3*). The single orifices at the tip of the larval mandibles (Fig. 1G) are presumed to be the openings of the venom glands (*3*).

To measure the forces that larvae exert when pulling on toads, we fastened toads that were partly submerged and dead or nearly dead (no muscular response to poking) to an electronic force transducer by means of a wire harness strapped around their front ends. The transducer was pulled vertically upward at a constant rate (0.72 cm per minute) by a motorized lift, to which the larvae offered a resistance that was registered graphically (force as a function of time) on an oscilloscope screen. Three larvae were pulled upward in this fashion until they released the toads. One oscilloscope tracing, typical for all three, is shown in Fig. 2. The larvae held onto the prey for several minutes (7.4 ± 0.7 minutes). Maximum resistance forces (12.9 ± 1.4 g) were 20 to 30 times the larval body weight (0.56 ± 0.08 g) and 10 to 16 times the toad body weight (0.97 ± 0.03 g) (*4*).

Larvae caught feeding on toads in the field were all full grown (*5*). We do not know whether younger larvae might also feed on *Scaphiopus*, but we suspect larval *T. punctifer* of all stages to subsist primarily on insects, as other tabanid larvae apparently do (*1, 6*). Our captive larvae ate crickets and were capable even of catching bombardier beetles, against whose defensive spray they were shielded by the mud (*7*). Because mass emergences of *Scaphiopus* occur only at lengthy intervals (*8*), it seems likely that

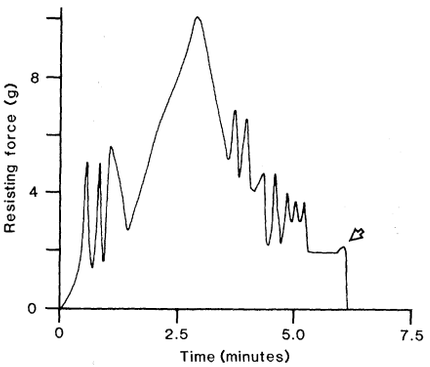

Fig. 2. Resisting force exerted by *T. punctifer* larva as the toad it was grasping was slowly pulled away by vertical lift. Oscillations were caused by sporadic body contractions of the larva. The arrow denotes point where larva released the toad.

T. punctifer feeds on this toad (as well as possibly other anurans) on an opportunistic basis only.

While we know of no previous records of tabanid larvae feeding on Amphibia, cases have been cited of insects feeding on vertebrates. A number of aquatic insects (beetles, Hemiptera, and nymphal dragonflies) have been observed to take fish, tadpoles, and frogs (*9*). Frogs, as well as small birds and a mouse, have been seen to be eaten by preying mantids (*10*). The case we report is a reversal of the usual toad-eats-fly paradigm, although in the case of *Scaphiopus* and *Tabanus* the paradigm may also prevail in its conventional form. Adult *Scaphiopus* might well on occasions have predatory access to the very *Tabanus* flies that as larvae preyed on their conspecifics.

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4. Values are the means ± standard error of the mean.
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The Development of Human Fetal Hearing

Abstract. *Blink-startle responses to vibroacoustic stimulation were monitored ultrasonically in human fetuses of known gestational age. Responses were first elicited between 24 and 25 weeks of gestational age and were present consistently after 28 weeks. Defining the developmental sequence for audition provides a foundation for diagnosing deafness and recognizing aberrant responses antenatally.*

The late third-trimester fetus has long been known to respond to sound (*1*). The end points used for testing hearing antenatally have been a change in overall fetal activity (*2*) or heart rate acceleration (*1, 3*). We have attempted to reopen investigation of fetal audition through the use of high-resolution ultrasound imaging for observing eye-blink responses [auropalpebral reflex (APR) (*4*)] to a specific vibroacoustic stimulus pattern.

Studies of evoked auditory potentials in prematurely born infants suggest that the auditory system is functional by the start of the third trimester (*5*). We studied 236 fetuses between 16 and 32 weeks of gestational age. Their mothers were women from the general population who had been referred for ultrasound studies; gestational age had been established by known date of conception, ultrasonic staging before 22 weeks of gestational age, or both. Infants (singletons) were subsequently born in good health at term. None of the mothers had taken any medication other than iron and vitamin supplements, none had consumed any alcohol or smoked within 24 hours of testing. Mothers with diabetes, hypertension, rhesus factor sensitization, premature labor, or any systemic medical process were excluded.

Each fetus was stimulated with a hand-held, battery-powered, vibroacoustic noise source (Electrolarynx, model 5B, Western Electric, 16-mm circular disk contact surface) applied firmly to the maternal abdomen directly overly-