Feeding by mandibular raking in a snake

The tiny threadsnake has a unique way of devouring ants before they can strike back.

Most snakes transport prey through the mouth by using asynchronous ratcheting movements of their upper jaws[1,2]. In contrast, we have found that threadsnakes (members of the basal snake clade Scolecophidia) have a unique feeding mechanism in which the tooth-bearing elements of the lower jaw rotate synchronously in and out of the mouth, dragging prey into the oesophagus. This mechanism, which we call 'mandibular raking', is the only vertebrate feeding mechanism known in which prey is transported exclusively by movements of the lower jaw.

Threadsnakes (family Leptotyphlopidae) are tiny, burrowing serpents that feed predominantly on the larvae, pupae and adults of social insects[3] (Fig. 1a). They are rarely encountered in the wild and, because of their specialized diet, are difficult to maintain in captivity. As a result, little is known about their natural history or about how they feed[4,5]: their extremely small size and ventrally placed mouths (Fig. 1a–c) also make it difficult to study their feeding mechanics.

We used an inverted dissecting microscope coupled to a high-speed video system to study ingestion and prey transport in the threadsnake *Leptotyphlops dulcis*. The imaging apparatus was positioned beneath a clear Plexiglas feeding chamber to record an unobstructed, highly magnified view of the snake's subterminal mouth.

This technique revealed that these snakes ingest prey by rotating the anterior, tooth-bearing halves of the lower jaw rami rapidly in and out of the mouth like a pair of swinging doors (Fig. 1d). This mechanism is made possible by the triple-jointed lower jaw of threadsnakes. In addition to the typical vertebrate jaw joints, which connect the mandible to the rest of the skull and allow the mouth to open like a trapdoor, threadsnakes also have extremely flexible interramal and intramandibular joints (Fig. 1c,d). The interramal joint allows movement between the tips of the mandibular rami, and the intramandibular joints allow the distal halves of the lower jaw rami to rotate backwards into the mouth.

In most other snakes, the interramal and intramandibular joints work passively, allowing the lower jaw to conform more closely to the shape of large vertebrate prey, thereby maximizing the potential gape[1,2]. In contrast, mandibular raking in *Leptotyphlops* involves active flexion of the intramandibular joints (Fig. 1d). Furthermore, the structure of these joints allows a much greater degree of mandibular flexion than is possible in other snakes. In most snakes, the anterior and posterior segments of each mandibular ramus fit closely together in an interdigitating fashion, and flexion at the intramandibular joint is limited by ligaments along the lateral surface of the lower jaw. In *Leptotyphlops*, however, there is a wide gap dorsally between the anterior and posterior mandibular segments, and the ventral articulation between the anterior splenial and posterior angular bones is highly mobile[6,7] (Fig. 1c). These features make the lower jaw of threadsnakes extremely mobile and allow the transversely oriented mandibular tooth rows (the only teeth in the skull) to be rotated backwards, raking prey into the oesophagus.

Mandibular raking allows threadsnakes to transport prey more rapidly than typical snakes, with cycles of mandibular protraction and retraction often occurring at frequencies exceeding 3 Hz (Fig. 1d). The speed of mandibular raking may be related to the hazardous foraging strategy of threadsnakes. To obtain sufficient quantities of ant brood, the snakes must invade nests that are tenaciously defended by worker ants. Large, aggressive ants can seriously injure or even kill these small snakes[8], which as adults are usually less than 2.5 mm in diameter and 1.5 g in weight. Although the anal-gland secretions of *Leptotyphlops* can repel some species of ant[9], this effect is variable, and selection is likely to favour individuals that can feed quickly, minimizing the time spent exposed to attack.

In contrast, most other snakes engulf their relatively large vertebrate prey by using a 'pterygoid walk', in which the highly mobile, tooth-bearing elements of the upper jaws are alternately ratcheted over the surface of the prey, thereby 'walking' the snake over and around its prey[2]. It was thought that understanding the feeding mechanisms of scolecophidian snakes (Leptotyphlopidae and two other families of small snakes that have similar feeding habits) may shed light on the origins of the pterygoid walk, as Scolecophidia occupies a phylogenetic position between 'lizards' and the clade containing all other living snakes (Alethinophidia)[5,10]. However, the mandibular raking used by *Leptotyphlops* is unlike any feeding mechanism known among lizards or snakes, with prey transport entirely dependent on active flexion of the lower jaw.

Mandibular raking therefore seems to be a uniquely derived feeding mechanism in

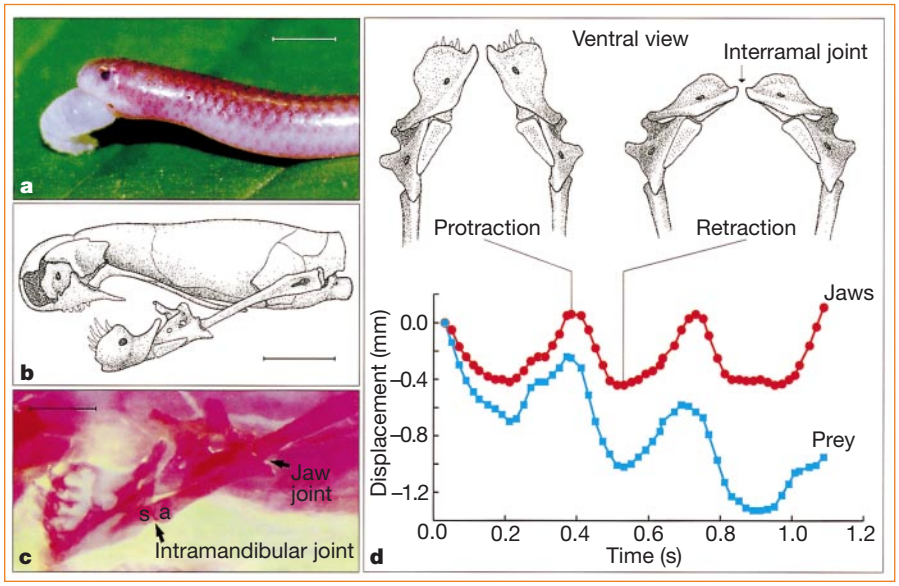

Figure 1 Morphology and function of the feeding apparatus in *Leptotyphlops dulcis*. **a,** An adult swallowing an ant larva. **b,** Left lateral view of the skull. **c,** Left lateral view of the lower jaw of a cleared and alizarin-stained specimen, showing the well developed intramandibular joint formed by the contact of the splenial (s) and angular (a) bones. Note the division between the anterior and posterior mandibular segments. **d,** Kinematic plots of jaw (red) and prey (blue) movements for an adult ingesting a large ant pupa, showing the rapid protraction–retraction cycles of the jaws and resultant movement of the prey. Although each jaw protraction results in slight slippage of the prey, the prey movements associated with jaw retraction are greater, resulting in net transport to the oesophagus. The lower jaw is drawn in ventral aspect to show the mechanics of jaw protraction and retraction. Positions of mandibular elements during retraction were estimated from high-speed video sequences (see Supplementary Information) and drawn from cleared and stained specimens. Scale bars: **a,** 2.5 mm; **b,** 1 mm; **c,** 0.5 mm.

Leptotyphlopidae, and is unlikely to represent the primitive feeding mode in snakes, despite the phylogenetically basal position of Scolecophidia.

N. J. Kley, E. L. Brainerd
Organismic and Evolutionary Biology Program and Department of Biology, University of Massachusetts, Amherst, Massachusetts 01003-5810, USA
e-mail: kley@bio.umass.edu

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Supplementary information is available on *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of *Nature*.

Genetic techniques

A universal marker for transgenic insects

Genetic manipulation of insects and other arthropods may enable better control strategies to be developed against agricultural pests and disease vectors. Transposon-based transformation techniques have been implemented in *Drosophila*[1] and other insects[2] such as medflies[3,4] and mosquitoes[5,6]. A major obstacle in the use of these transposons, however, has been the difficulty in obtaining marker genes that will allow easy and reliable identification of transgenic animals. Here we describe a marker system that is suitable for following gene transfer in most arthropods and in many other phyla.

Species-specific transformation markers can be generated by isolating visible mutations in the species of interest, cloning the corresponding gene, and then rescuing the mutant phenotype by incorporating a wild-type copy of the gene through transformation. But this procedure is laborious, with every species needing the same investment.

A universal marker that could be used to follow gene transfer in any species is the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*, as it is active across the animal and plant kingdoms[7]. However, GFP requires a strong promoter to enable single-copy insertions to be detected. Activation should be in a spatial pattern so the transgene signal can be distinguished from common autofluorescence.

We find that an artificial promoter containing three binding sites for Pax-6 homodimers in front of a TATA box (3xP3)[8] is hyperactive, regionally restricted and universal. This promoter can drive expression of an enhanced GFP variant (EGFP)[7] in the eye of the fruitfly *Drosophila melanogaster* (Fig. 1a) and in the flour beetle *Tribolium castaneum* (Fig. 1b), which are from lineages that separated about 250 million years ago. The evolutionary conservation and the 'master regulatory' function of Pax-6 in the eye development of insects and vertebrates[9] means that the 3xP3 promoter should be active in any photoreceptor cell. The small size of the marker gene (1.3 kilobases) allows for small transposon constructs, resulting in high transformation rates.

We constructed three vectors based on the *Hermes*[10], *piggyBac*[11] and *mariner*[12] transposons, each carrying the 3xP3-EGFP marker. Together with helper plasmids to provide the respective transposases[4–6], these vectors were microinjected into *Drosophila* eggs of a strain mutant for the *white* gene (vector and helper plasmids at 500 and 300 ng μl^{-1}, respectively). We obtained transgenic lines displaying strong fluorescence with transformation efficiencies of 4% for *mariner*, 50% for *Hermes* and 35% for *piggyBac* (the percentage of fertile injection survivors producing fluorescent offspring). In parallel, we microinjected the *Hermes* and *piggyBac* vectors into the posterior pole of *Tribolium* eggs from a strain lacking eye pigmentation (*pearl* mutants). We obtained transgenic beetle lines with frequencies of 1% for *Hermes* and 60% for *piggyBac*.

The transgenes seem to be stably integrated into the genome as they have been inherited over at least six generations. We detected strong fluorescence for both species, even after outcrossing to wild-type strains (Fig. 1c,d). This result shows that our marker can also be detected in the presence of eye pigments. In both species, all photoreceptor cells express EGFP, including cells in the eyes of larvae (Fig. 1e, f), pupae and adults, and in the ocelli of *Drosophila*.

The transposon-mediated generation of transgenic beetles provides a powerful new technique for studying a large group of pest species. Moreover, owing to its artificial origin[8], 3xP3-EGFP probably does not require any other host-specific factors and is therefore a universal marker that should function in all animals that have eyes. With a set of promiscuous transposon vectors, such a system can be used to study almost any species, not just established model organisms, in comparative biological and functional evolutionary studies.

Expression in the eyes allows the signal to be seen in animals that do not have transparent cuticle, and transgenic animals can be identified as larvae, pupae and adults. The system can be applied to competitive wild-type strains, rather than just potentially labile mutant lines, making it suitable for pest-management programmes. The use of arthropod transgenics might become so widespread that new regulatory efforts may be needed to oversee the number of species that can be transformed.

Andreas J. Berghammer*, Martin Klingler*, Ernst A. Wimmer†
**Zoologisches Institut, Universität München, Luisenstrasse 14, 80333 München, Germany*
†Lehrstuhl für Genetik, Universität Bayreuth, Universitätsstrasse 30, 95447 Bayreuth, Germany
e-mail: ernst.wimmer@uni-bayreuth.de

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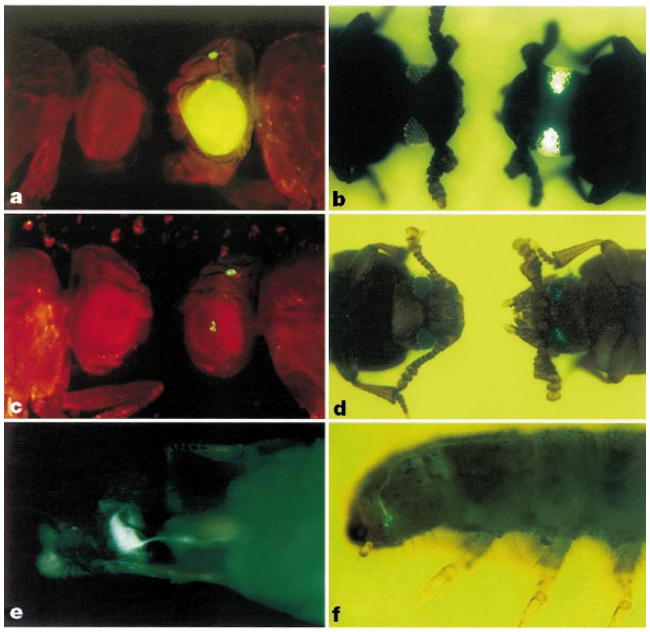

Figure 1 Transgenic fruitflies and flour beetles identified by the universal marker 3xP3-EGFP. **a–d,** Left, non-transgenic controls; right, transgenic individuals marked by 3xP3-EGFP. **a,** In a *Drosophila white* mutant background, the complete compound eye and the ocelli fluoresce. **b,** In a *Tribolium pearl* mutant background, all ommatidia fluoresce. **c,** In a *Drosophila white*/+ background, fluorescence is seen in the ocelli and the pseudopupil of the compound eye. **d,** In a *Tribolium pearl*/+ background, fluorescence can be seen only in the ommatidia that point straight towards the observer. **e, f,** In *Drosophila* (**e**) and *Tribolium* (**f**) larvae, EGFP can be seen in the photoreceptors of the larval eyes and in the optic nerve.