

# Larval respiratory systems of two anthomyiid flies, *Delia radicum* and *Delia antiqua* (Diptera: Anthomyiidae)<sup>1</sup>

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**Abstract**—The first-instar larvae of *Delia radicum* (L.) and *Delia antiqua* (Meigen) enter host plants to feed in galleries. These galleries can be filled by a liquid resulting from the putrefaction of the host. In this study, we show that *D. radicum* and *D. antiqua* larvae have a metapneustic respiratory system in the first instar and an amphipneustic respiratory system in the second instar, as observed in the majority of cyclorrhaphous Diptera. In addition, we observed four spatulate, ramified structures on the postabdominal spiracles in all three larval instars. We propose that these structures facilitate gas exchange (CO<sub>2</sub> and O<sub>2</sub>), especially in the first-instar larvae when they feed in liquid-filled galleries.

**Résumé**—Les larves de premier stade de *Delia radicum* (L.) et de *Delia antiqua* (Meigen) s'insèrent dans les plantes hôtes et y creusent des galeries pour se nourrir. La putréfaction de l'hôte peut remplir ces galeries de liquide. Notre étude montre que les larves de *D. radicum* et de *D. antiqua* possèdent au premier stade un système respiratoire métapneustique et au second stade un système respiratoire amphipneustique, comme c'est le cas chez la plupart des diptères cyclorrhaphes. De plus, il existe quatre structures ramifiées en forme de spatules sur les stigmates abdominaux postérieurs chez les larves des trois stades. Nous croyons que ces structures facilitent les échanges gazeux (CO<sub>2</sub> et O<sub>2</sub>), particulièrement au premier stade, lorsque les larves s'alimentent dans des galeries remplies de liquide.

[Traduit par la Rédaction]

## Introduction

Larvae of the cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae), feed on

the roots of wild and cultivated crucifers, whereas those of the onion maggot, *Delia antiqua* (Meigen), feed on the roots of *Allium* L. spp. (Alliaceae) (Harris *et al.* 1986; Finch 1989; Ross and Anderson 1992). Both species have three larval instars that spend most of their development within host plants in an aqueous environment of plant sap and plant juice following putrefaction. This aqueous environment can also be acidic because of putrefaction of the host. Consequently, to survive, the larvae of *D. radicum* and *D. antiqua* need a specialized respiratory system.

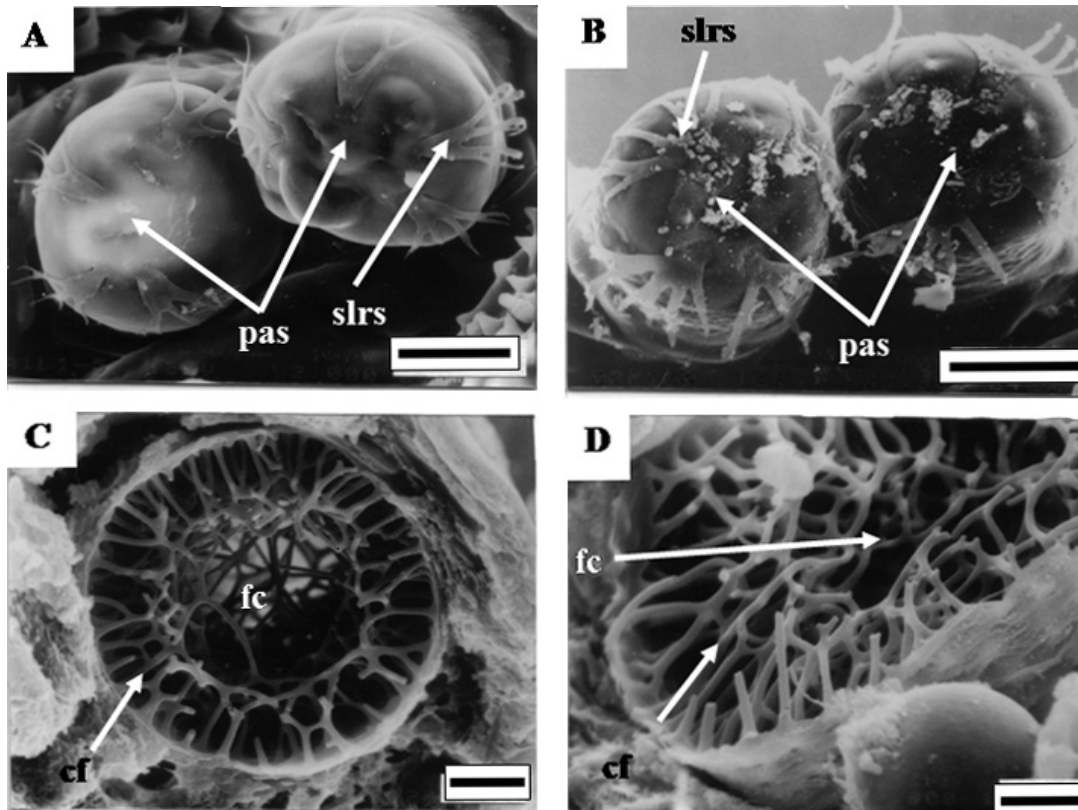
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**Fig. 1.** Respiratory system of first-instar larvae of *Delia radicum* and *Delia antiqua*. (A) Pair of postabdominal spiracles in *D. radicum*; (B) pair of postabdominal spiracles in *D. antiqua*; (C) internal view of one postabdominal spiracle in *D. radicum*; and (D) internal view of one postabdominal spiracle in *D. antiqua*. cf, chitinous fibres; fc, felt chamber; pas, postabdominal spiracle; slrs, spatulate, ramified structures. Scale bars = 10 µm (A, B) and 1 µm (C, D).

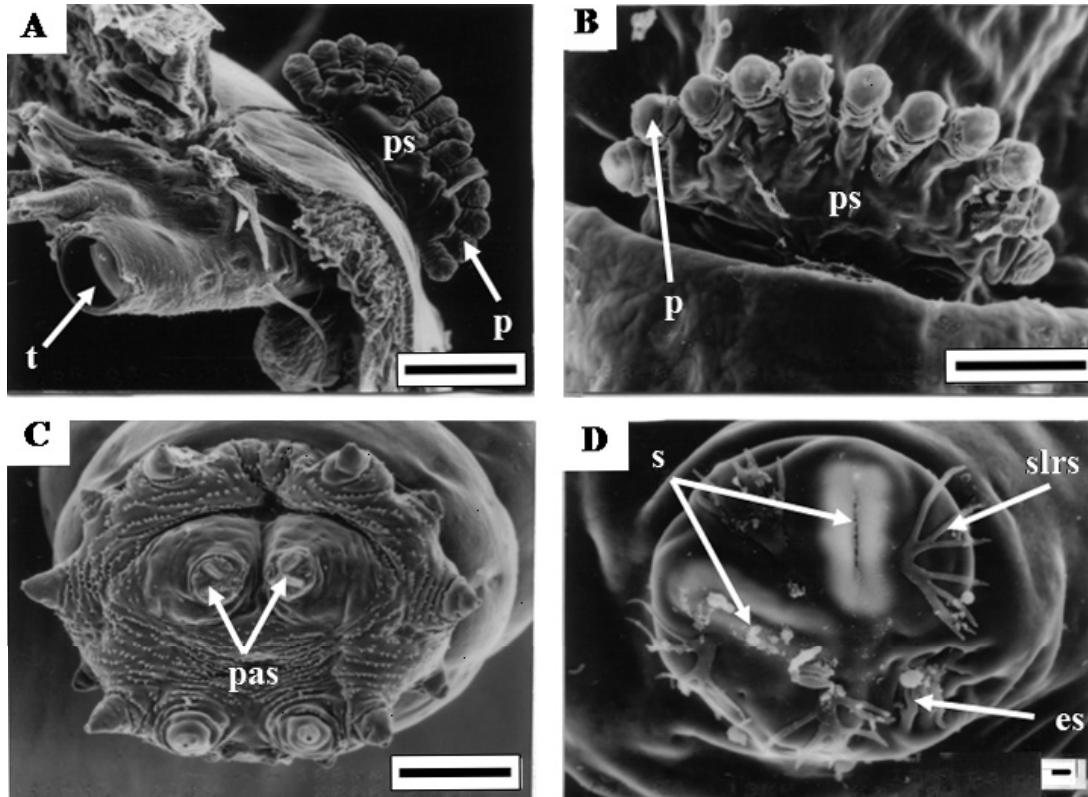


Dipteran larvae are found in various habitats, including streams, rivers, waterfalls, lakes, hot mineral springs, water reservoirs of terrestrial and epiphytic plants, sand, and living or decaying plants and animals (Keilin 1944). This diversity of conditions is reflected in the high diversity of dipteran larval respiratory systems. Seven types of respiratory systems have been described in dipterous larvae: holopneustic (one prothoracic, one metathoracic, and eight abdominal pairs of spiracles), peripneustic (one prothoracic and eight abdominal pairs of spiracles), hemipneustic (one prothoracic and seven abdominal pairs of spiracles), amphipneustic (one prothoracic and one postabdominal pair of spiracles), metapneustic (one postabdominal pair of spiracles), propneustic (one prothoracic pair of spiracles), and apneustic (no functional pair of spiracles) (Keilin 1944; Teskey 1981).

In cyclorrhaphous Diptera, first-instar larvae usually have a metapneustic respiratory system,

and the knob-like posterior spiracles are brownish yellow and heavily sclerotized (Smith 1927; Keilin 1944; Teskey 1981). In the second instar, the respiratory system is generally amphipneustic. Anterior spiracles are present and consist of a pair of fan-shaped organs located laterally on the prothoracic segment and bearing several processes with a slit (Smith 1927; Keilin 1944). The posterior spiracles are more developed and bear two slits (Smith 1927; Keilin 1944). The respiratory system of the third instar is also amphipneustic but there are three slits on the posterior spiracles and more processes on the anterior spiracles. This developmental sequence of the three larval instars is the most frequent in cyclorrhaphous dipterous larvae. Some species, such as *Thrixion halidayanum* Rondani (Tachinidae) and *Panzeria rudis* Fallén (Tachinidae), are metapneustic during their first and second larval instars and become amphipneustic in the third

**Fig. 2.** Respiratory system of second-instar larvae of *Delia radicum* and *Delia antiqua*. (A) Internal and external view of a prothoracic spiracle in *D. radicum*; (B) prothoracic spiracle in *D. antiqua*; (C) end view of postabdominal segment in *D. radicum*; and (D) postabdominal spiracle in *D. antiqua*. es, external scar; p, process; pas, postabdominal spiracle; ps, prothoracic spiracle; s, slit; slrs, spatulate, ramified structure; t, trachea. Scale bars = 10  $\mu$ m (A–C) and 1  $\mu$ m (D).



instar, whereas others, such as *Ocyptera brassicaria* Fabr. (Tachinidae) and *Hydrellia modesta* Loew (Ephyridae), are metapneustic throughout their larval development (Keilin 1944; Teskey 1981). Finally, species such as *Mydaea urbana* (Meigen) (Muscidae) and *Myospila mediatunda* (Fabr.) (Muscidae) are amphipneustic in all three larval instars, whereas *Ateleneura spuria* Meigen (Pipunculidae) is apneustic in the first instar and amphipneustic in the second and third instars (Keilin 1944). In this paper we report on the development of spiracles of two anthomyiid flies, *D. radicum* and *D. antiqua*.

### Materials and methods

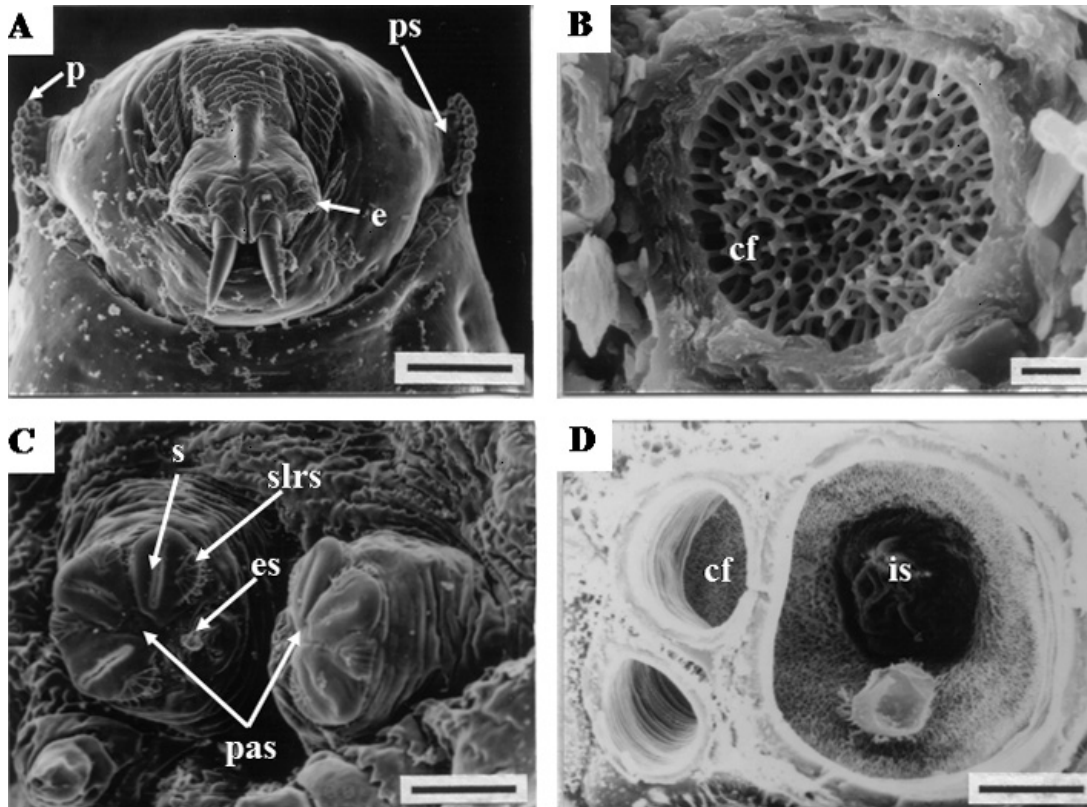
Larvae used for this study were taken from our rearings of *D. radicum* and *D. antiqua* originating from Le Rheu, France (48°10'N,

01°04'W). Rearing procedures were based on Whistlecraft *et al.* (1985).

At least 10 viable eggs of *D. radicum* and *D. antiqua* (*i.e.*, white and turgid) (Mukerji and Harcourt 1970), less than 12 h old, were placed in a petri dish containing moist sand and half of a rutabaga or onion, respectively. At least 20 petri dishes were prepared. All petri dishes were held at  $19 \pm 1$  °C,  $65\% \pm 5\%$  RH, and 16L:8D.

Every second day for 20 days, two petri dishes, one each of *D. radicum* and *D. antiqua*, were randomly chosen. The living larvae were placed for 6 h in a petri dish containing 2% paraformaldehyde – 2% glutaraldehyde solution, pH 7.4. Larvae were dehydrated through successive baths (10 min each) of ethanol (70, 80, 90, 95, and 100%) and then placed in a 100% acetone solution. They were critical-point dried in a Balzers CPD 010 critical point dryer

**Fig. 3.** Respiratory system of third-instar larvae of *Delia radicum* and *Delia antiqua*. (A) Head of *D. radicum*; (B) internal view of a process of a prothoracic spiracle of *D. radicum*; (C) pair of postabdominal spiracles of *D. radicum*; and (D) felt chamber of a postabdominal spiracle of *D. radicum*. cf, chitinous fibre; e, eye; es, external scar; is, internal scar; p, process; pas, postabdominal spiracle; ps, prothoracic spiracle; s, slit; slrs, spatulate, ramified structures. Scale bars = 100  $\mu$ m (A, C), 10  $\mu$ m (D), and 1  $\mu$ m (B).



with liquid CO<sub>2</sub> and then gold-palladium coated using a JEOL JFC-1100 fine coat ion sputter. The samples were observed using a JEOL JSM 6400 scanning electron microscope.

We observed the development of the external respiratory system of the three larval instars of *D. radicum* and *D. antiqua* (first instar, days 0 to 3; second instar, days 4 to 7; third instar, days 8 to 20) to determine the number of functional pairs of spiracles and the type of spiracle present for each instar. We also compared the respiratory system of *D. radicum* and *D. antiqua* larvae for each instar.

## Results

The first-instar larvae of *D. radicum* and *D. antiqua* have only one functional pair of postabdominal spiracles, located in the centre of the caudal disc; each spiracle has four

spatulate, ramified structures and no slit (Figs. 1A, 1B). The spiracular chambers (felt chambers) of the *D. radicum* and *D. antiqua* larvae are filled with chitinous fibres forming a filter (Figs. 1C, 1D).

The second instars of both species have a functional pair of anterior spiracles (Figs. 2A, 2B) and a functional pair of postabdominal spiracles (Figs. 2C, 2D). The anterior spiracles are located on the lateral sides of the prothoracic segment. The anterior spiracles of the two species consist of a pair of fan-shaped organs bearing  $12 \pm 2$  processes for *D. radicum* and  $11 \pm 2$  processes for *D. antiqua*. Each process has a slit and is filled with chitinous fibres. The postabdominal spiracles of both species are located in the centre of the caudal disc (Fig. 2C) and bear four spatulate, ramified structures, two slits, and an external scar (Fig. 2D). The external scar indicates the position of the postabdominal spiracle

in the previous instar. The postabdominal spiracular chambers are also filled with chitinous fibres.

The third larval instars of *D. radicum* and *D. antiqua* have functional pairs of anterior (Fig. 3A) and postabdominal spiracles (Fig. 3C) similar to those observed in the second instar. Each process has a slit and is filled with chitinous fibres (Fig. 3B). The postabdominal spiracles of both species have three slits, rather than two as in the second instars (Figs. 2D, 3C). The external and internal scars of the posterior spiracles of the third instars of both species (Figs. 3B, 3D) were observed.

### Discussion

Our data show that *D. radicum* and *D. antiqua* larvae have a metapneustic respiratory system in the first larval instar and an amphipneustic respiratory system in the second and third instars, as observed in the majority of cyclorrhaphous dipteran species (Keilin 1944; Teskey 1981). We did not observe functional slits on the postabdominal spiracles of first-instar larvae. The slits on the postabdominal spiracles of the second- and third-instar larvae of both species are linear, unlike those of other species in which pyriform (genus *Muscina* Robineau-Desvoidy; Muscidae) or sinuous (*Musca domestica* L.; Muscidae) slits are observed (Keilin 1944). All larval instars of *D. radicum* and *D. antiqua* have four spatulate, ramified structures on each postabdominal spiracle (Figs. 1A, 1B, 2D, 3C).

Several earlier studies reported the presence of similar spatulate, ramified structures, but their function has not yet been experimentally demonstrated (Keilin 1944; Bursell 1956; Teskey 1981). Recently, these structures have been observed in the larvae of several Diptera living in various habitats: *Bactrocera cucurbitae* (Coquillett) (Tephritidae) (J.P. Nénon *et al.*, unpublished data), *Ceratitis capitata* (Wiedemann) (Tephritidae) (J.P. Nénon *et al.*, unpublished data), and *Dermatobia hominis* (L.) (Oestridae) (Filippis and Leite 1997). They also seem to be present on the pupae of subantarctic dipteran species (G. Chauvin, unpublished data). Huff (1925) suggested that they act as a mechanism to prevent entry of fluid into the tracheal lumen, whereas Khole (1977) proposed that they provide extra stiffness to the surrounding membrane, which is necessary to elevate the

spiracular plate. This feature would help attain a larger angle of contact to facilitate oxygen consumption. Thus, the spatulate, ramified structures may facilitate gas exchange, especially in the first-instar larvae when they feed in liquid-filled galleries. This hypothesis, however, remains to be examined experimentally.

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