at high levels in the distal tips of lepidopteran prolegs (Warren et al. 1994).

Expression of BX-C proteins in sawfly prolegs

In both species of sawfly, Ubx/abd-A was present throughout most of the abdomen for most of embryogenesis

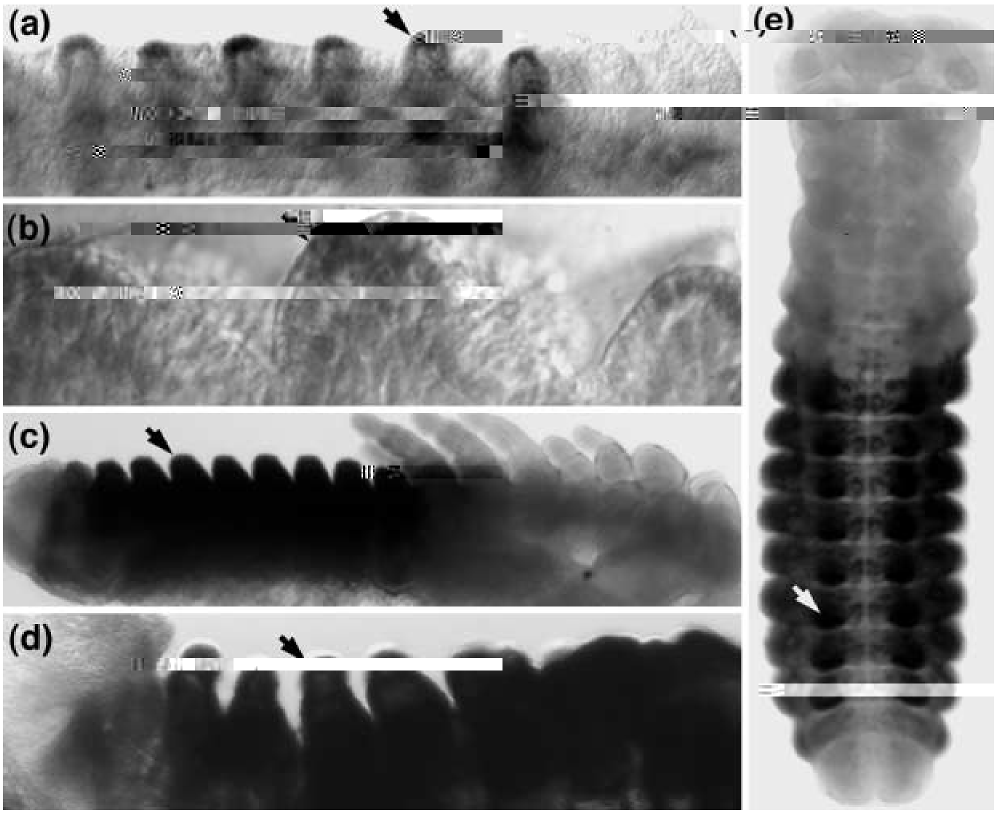


Fig. 3 Ubx/abd-A staining in the abdominal segments of sawflies during early (**a**, **b**), middle (**c**, **e**), and late (**d**) stages of proleg development. Anterior is to the *right* in panels **a**–**d**, and towards the *top* in panel **e**. **a**–**d** Lateral views; **b** is a high-magnification view of the embryo shown in **a**; **e** ventral view. *Arrows* indicate developing prolegs. Ubx/abd-A was expressed to the tips of prolegs throughout the development of the prolegs, and no holes appeared in the domain of Ubx/abd-A expression. **a, b, d** *N. abietis*. **c, e** *D. similis*

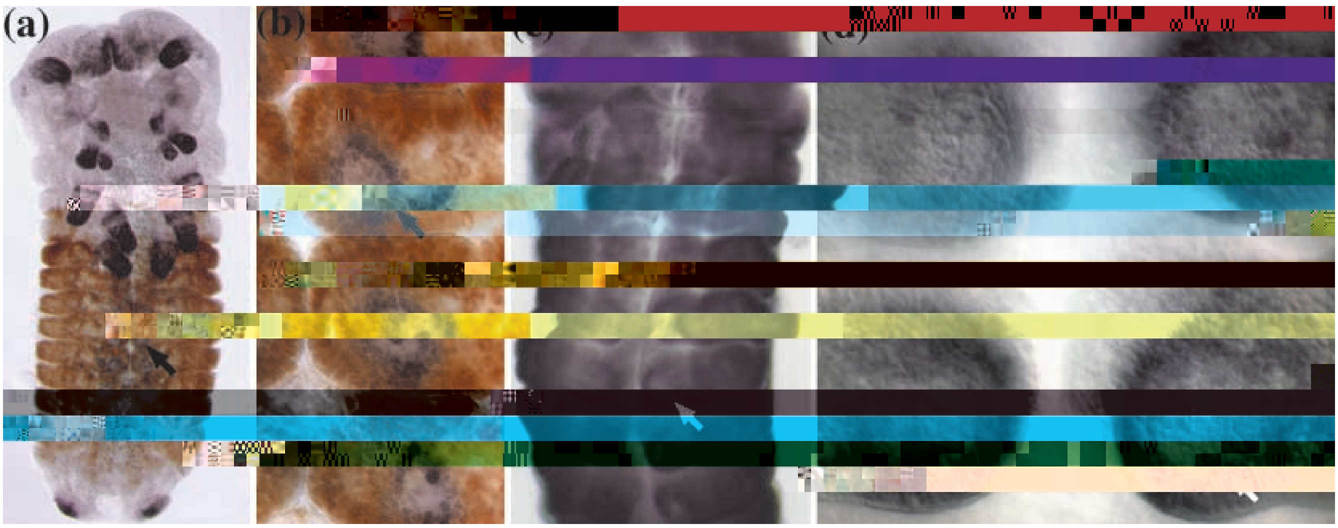
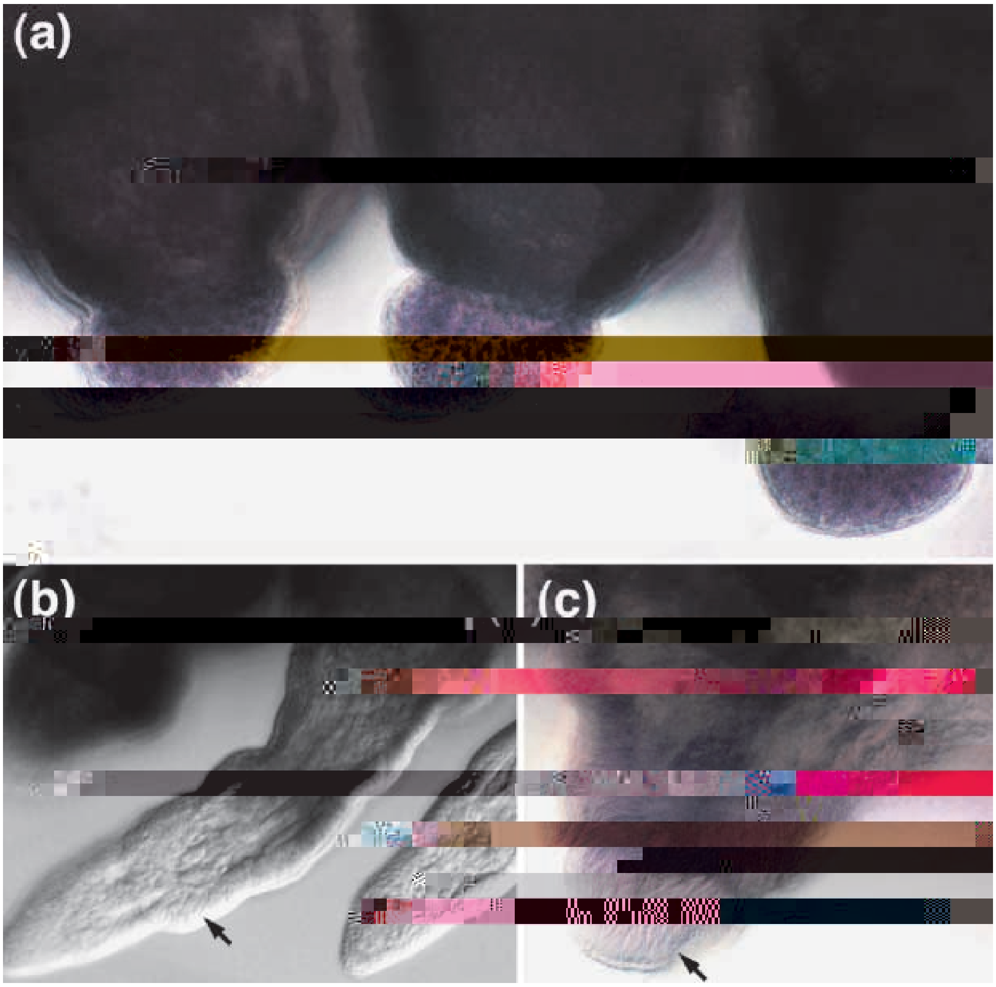


Fig. 4 Circular clearings appeared in the expression domain of Ubx/abd-A in segments A3–A6 of the butterfly *Precis coenia* (**a**, **b**), and the moth *Manduca sexta* (**c**, **d**). Anterior is towards the *top* in all panels. **b** A high-magnification view of the embryo shown in **a**; **e** a high-magnification view of the embryo shown in **d**. In **a** and **b**, the *brown* staining represents Ubx/abd-A expression and *black* staining represents Dll expression. In **c** and **d**, the *black* staining represents Ubx/abd-A expression. In all panels, *arrows* indicate circular clearings in Ubx/abd-A expression

(Gonzàlez-Crespo and Morata 1996; Aspland and White 1997; Abu-Shaar and Mann 1998). In the distal portion of the limb, termed the telopodite, exd protein remains in the cytoplasm, and patterning is controlled by Dll; in the proximal portion of the limb, termed the coxopodite, exd protein is transported into the nucleus and controls patterning in the absence of Dll. If sawfly prolegs are equivalent to coxopodites, then exd protein should be nuclear-localized all of the way to the tip of each proleg. Antibody staining confirmed this prediction (Fig. 5a). This pattern of exd staining can be contrasted to that of all insect thoracic limbs that have been examined (e.g. sawfly

Fig. 5



thoracic limbs are shown in Fig. 5b) and to developing prolegs in Lepidoptera (Fig. 5c), where exd protein was not apparent in the nuclei of cells near the distal tips.

Evolution of prolegs

The development of sawfly prolegs without the expression of *Dll*, combined with the nuclear localization of exd protein all of the way to the proleg tips, suggests that sawfly prolegs may be limb bases, like the insect mandibles (Popadic et al. 1998). In *Drosophila*, mutations in the *Dll* gene can result in the development of a stump-like structure that appears to correspond to the coxal segment of a normal leg (Campbell and Tomlinson 1998). Based on our results, we propose that sawfly prolegs are roughly equivalent to the stump-like structure that is developed by these *Dll* mutants, corresponding to the coxal segment of a thoracic leg.

In contrast to sawflies, lepidopteran prolegs appear to have both proximal and distal portions. Morphologically, it is clear that lepidopteran prolegs have cuticular structures at the distal tips that are absent in sawfly prolegs (data not shown). *Dll* expression in lepidopteran prolegs may be required for the formation of these distal structures.

Based upon the phylogenetic distribution of prolegs among the holometabolous insects, Nagy and Grbic (1999) hypothesized that prolegs evolved independently in different lineages. According to this model, although the prolegs in different orders may share homologies at some basic levels (e.g. shared mechanisms of limb development), the particular mechanisms by which the derepression of appendage development occurs in the abdomen are likely to be evolutionary novelties. Our results are consistent with a model of evolutionary convergence, in which derepression of abdominal appendage development has occurred independently in various insect lineages. Future studies on additional holometabolous insect species whose larvae develop prolegs should allow us to model proleg evolution with greater confidence.

Interestingly, sawflies also differ from Lepidoptera in the way the larvae hang onto branches. To grasp onto things, Lepidoptera use both the coxa and the distal cuticular structure of each proleg (Snodgrass 1935). Sawflies hold onto pine needles between each stubby pair of limb bases, but this apparently does not provide adequate

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