

As spawning events have never been witnessed for 'skin-storing' squids (including *Architeuthis*), it is not known how females access sperm to fertilize their eggs. The suckers or beak may be used to peel open the skin covering the spermatophores, or the sperm may migrate to the surface on hormonal or chemical cues. Alternatively, the female's skin might degrade on spawning, exposing the embedded sperm stores.

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## Homeotic transformation in *Drosophila*

Fossil records indicate that insect wings are pleural appendages, primitively present on three thoracic and the nine abdominal segments as wings and winglets, respectively<sup>1</sup>. Homeotic proteins are generally considered to be the ontogenic switches governing the choice of developmental pathway of such appendages<sup>2</sup>. Here we provide examples of regulatory changes in developmental genes leading to a homeotic transformation in the ectopic wing tissue of prothoracic or metathoracic segments in *Drosophila melanogaster*, which lead to the development of wings rather than halteres.

Of the two homeotic clusters in *Drosophila* (*Antennapedia* and *Bithorax*), only the *Antennapedia* (*Antp*) gene is expressed in segments from which the wings and halteres arise. However, *Antp* is not required to form thoracic wing primordia or the adult wings<sup>3</sup>, indicating that ancestrally, wings probably arose in all segments without homeotic gene involvement (the homeotic ground state)<sup>3,4</sup>. Furthermore, regulatory interactions between homeotic proteins and the developmental genes involved in appendage formation seem to have been important evolutionary innovations in the diversification of arthropod body plans and appendages<sup>3</sup>. If this is true for the evolution of insect wings, then changes in homeotic or developmental genes should result in

homeotic transformations.

The *engrailed* (*en*) gene encodes a homeodomain-containing transcription factor which establishes the posterior identity of embryonic and adult segments<sup>5</sup>. The *wingless* (*wg*) gene encodes a member of the Wnt family of signalling molecules which regulates several developmental pathways in embryos and imaginal discs<sup>6</sup>. We ectopically expressed *en* from a constitutive promoter in cell clones generated by the *flp*-out cassette<sup>7</sup>.

We identified clones expressing *en* on the wing and haltere by the presence of forked (*f*) bristles. As expected, those in the anterior compartment of wings showed characteristics of the posterior compartment and organized new anterior compartments in front of them (see also ref. 7). But all of the 65 clones that we observed induced transformations of halteres to wing in anterior haltere compartments, whereas the posterior haltere compartments maintained their normal structure and remained associated with transformed wing (Fig. 1a). Because *Ultrabithorax* (*Ubx*) mutations transform haltere primordia to wing primordia<sup>8</sup>, it is likely that this transformation of halteres into wings, or of wings into halteres by the absence of *en* function<sup>9</sup>, may be

due to negative regulation of *Ubx* by *en*<sup>10</sup>.

To examine the role of *wg*, we used different hypomorphic alleles, such as *wg<sup>P</sup>*, *wg<sup>J</sup>*, *wg<sup>CX3</sup>*, *wg<sup>CX4</sup>* and *wg<sup>JacZ</sup>* (refs 11,12), some of which are regulatory mutations and, except for *wg<sup>J</sup>*, are homozygous lethal at different developmental stages. Several heteroallelic combinations are viable and we analysed these for defects or transformations at emergence or at pharate adult stages. Heteroallelic combination of *wg<sup>P</sup>* with *wg<sup>J</sup>*, *wg<sup>CX3</sup>*, *wg<sup>JacZ</sup>* or *wg<sup>CX4</sup>* induced the development of wings in the prothoracic (T1) segment. About 30% of the 200 *wg<sup>P</sup>/wg<sup>JacZ</sup>* flies examined had new T1 wing-like structures, ranging from rudimentary to well-formed (Fig. 1b).

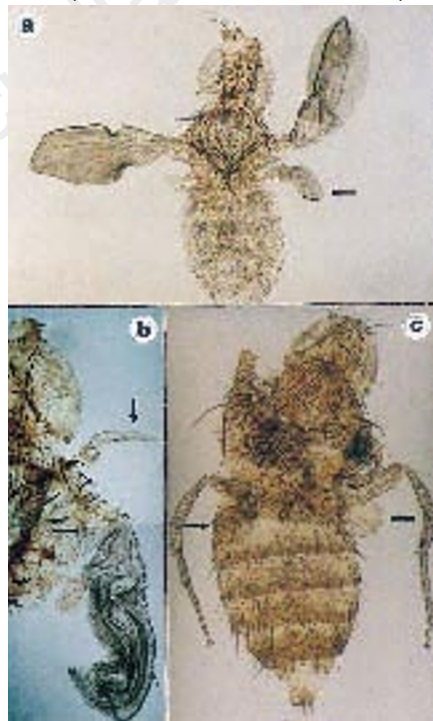
In the *wg<sup>P</sup>/wg<sup>J</sup>* heteroallelic combination, T1 wings were seen in 25% of individuals whereas the mesothoracic (T2) wings and metathoracic (T3) halteres developed variably. In other heteroallelic combinations such as *wg<sup>P</sup>/wg<sup>CX3</sup>* or *wg<sup>P</sup>/wg<sup>CX4</sup>*, 25% showed T1 wings but the T2 wings and T3 halteres were always absent. Presence of T1 wings even when normal T2 wings and T3 halteres were absent confirms a segment-specific differential expression and regulation between the homeotic and developmental genes.

The heteroallelic combination *wg<sup>P</sup>/wg<sup>CX3</sup>* also showed a varying degree of transformation of T3 legs into halteres in 10% of individuals (Fig. 1c), ranging from graded removal of the distal segments (tibia and tarsus), through distortion of the femur and graded removal of leg bristles, to transformation of the coxa and trochanter into the first and second segments of the haltere.

These homeotic transformations in *Drosophila* thoracic discs caused by the mis-expression of developmental genes like *wg* or *en* indicate that altered regulation of developmental genes was important in the evolution of body plans and the elaboration of new structures.

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**Figure 1** Homeotic transformations in *Drosophila*. **a**, Ectopic expression of *En* in a *Tuba1 > en* somatic clone in the anterior compartment of the right haltere (arrow) caused its transformation into a wing. The left haltere is normal. **b**, A prothoracic wing (arrow) in a *wg<sup>P</sup>/wg<sup>JacZ</sup>* pharate adult fly. Normal T2 wings and T3 halteres are also seen. **c**, Transformation of a metathoracic leg to a haltere (arrows) in *wg<sup>P</sup>/wg<sup>CX3</sup>* pharate adult fly. The common phenotype of *wg<sup>CX3</sup>* mutants, absence of T2 wings and T3 halteres, and duplication in the thoracic segments, is also seen.

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