

The wings of *Bombyx mori* develop from larval discs exhibiting an early differentiated state: a preliminary report

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Lepidopteran insects present a complex organization of appendages which develop by various mechanisms. In the mulberry silkworm, *Bombyx mori* a pair of meso- and meta-thoracic discs located on either side in the larvae gives rise to the corresponding fore- and hind-wings of the adult. These discs do not experience massive cell rearrangements during metamorphosis and display the adult wing vein pattern. We have analysed wing development in *B. mori* by two approaches, viz., expression of patterning genes in larval wing discs, and regulatory capacities of larval discs following explantation or perturbation. Expression of Nubbin is seen all over the presumptive wing blade domains unlike in *Drosophila*, where it is confined to the hinge and the wing pouch. Excision of meso- and meta-thoracic discs during the larval stages resulted in emergence of adult moths lacking the corresponding wings without any loss of thoracic tissues suggesting independent origin of wing and thoracic primordia. The expression of wingless and distal-less along the dorsal/ventral margin in wing discs correlated well with their expression profile in adult *Drosophila* wings. Partially excised wing discs did not show *in situ* regeneration or duplication suggesting their early differentiation. The presence of adult wing vein patterns discernible in larval wing discs and the patterns of marker gene expression as well as the inability of these discs to regulate growth suggested that wing differentiation is achieved early in *B. mori*. The timings of morphogenetic events are different and the wing discs behave like presumptive wing buds opening out as wing blades in *B. mori* unlike evagination of only the pouch region as wing blades seen in *Drosophila*.

1. Introduction

Insects display a variety of appendage types in the embryonic, larval or adult developmental phases. The number and segment organization of these appendages vary in different insect orders (Wigglesworth 1973; Williams and Carroll 1993). The lepidopteran insects like the silkmoths (Bombycidae) and the butterflies (Nymphalidae) present an interesting organization where two alternate forms of appendages are formed in the same segments during two different developmental stages. For instance, the embryonic/larval legs and antennae are replaced by the adult legs and antennae respectively, which differ in segment number and morphology (Svacha 1992). However, in most cases the adult wings develop from larval discs during metamorphosis.

The lepidopteran insects have been favourite models for the study of adaptive traits due to the vivid colour patterns found on their wings. These traits are distinctive for every species and show considerable variation in the dorsal and ventral wing surfaces and between the fore- and the hind wings (for details, see Brakefield and French 1999). Extensive investigations on the phenotypic plasticity (Nijhout 1994a), seasonal polyphenism (Rountree and Nijhout 1995) and mimicry in butterfly wings (Nijhout *et al* 1990; Nijhout 1994b; Brakefield *et al* 1996; Jiggins *et al* 1996; Brakefield 1997) have established the “eyespot” as a more tractable model for studying the genetic, developmental and molecular basis of wing patterning in lepidopteran insects (Carroll *et al* 1994; Brakefield *et al* 1996; Keys *et al* 1999). Earlier studies on eyespot development after surgical manipulations (cautery, grafting,

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transplantation) on the signaling foci in the wings of butterflies, *Precis coenia* and *Bicyclus anynana*, resulted either in the absence of eyespots or induction of ectopic eyespots (Nijhout 1985, 1991; Brakefield and French 1995; French and Brakefield 1995). In some wing surfaces, local epidermal damage can also mimic the focal signal and induce ectopic eyespots (French and Brakefield 1992, 1995; Brakefield and French 1995). Extensive surgical experiments on band patterns have been done on the pyralid moth, *Ephestia kuhniella* in which cautery of the early pupal wing can locally deflect a band reminiscent of the changes induced due to altered morphogen gradient (Toussiant and French 1988). Most of the information available from surgical manipulations on the signaling foci for eyespots and the transverse bands, however, were carried out on pupal wings, a stage close to the adult morphogenesis. In this study we have examined some aspects of disc regulation in *B. mori* by either explanting or ablating the wing discs at an earlier developmental stage (third larval instar). Disc explantation resulted in the emergence of healthy moths lacking the explanted wing(s) with no damage on the notum/thorax.

We have also analysed the expression profiles of patterning markers in *B. mori* wing development. Genetic pathways governing wing development in dipterans and lepidopterans appear to be conserved as revealed by the spatial pattern of expression of several wing patterning genes. The complex signaling pathways involving *Distal-less* (*Dll*), *patched* (*ptc*), *cubitus interruptus* (*ci*) and *engrailed* (*en*) are recruited in the butterfly wings, for instance, to specifying the scale colour pattern on the wing surface (Carroll *et al* 1994; Keys *et al* 1999; Brakefield and French 1999). We present here the expression pattern of Nubbin (Nub), Wingless (Wg) and Distal-less (*Dll*) in *B. mori* wing discs using antibodies generated against the corresponding proteins from *Drosophila* or the butterfly *P. coenia*. The expression of Wg and *Dll* as overlapping domains in the distal region in *B. mori* wing discs corresponded to the known pattern of expression of these genes in adult or late pupal wings in *Drosophila* and *P. coenia* (Cohen 1993; Couso *et al* 1993; Carroll *et al* 1994; Panganiban *et al* 1994; Brakefield *et al* 1996).

The gene expression profiles and the explantation experiments revealed that in *B. mori*, the larval wing discs already exhibit the adult wing characteristics and they do not undergo any massive cell movement or rearrangement during larval development and pupal metamorphosis to the adult moth.

2. Materials and methods

Multivoltine races of mulberry silkworm *B. mori*, strains *Nistari* and Pure Mysore were used for these studies. All the cultures were maintained at 25°C at 70–80% relative humidity.

2.1 Antibody staining

Antibody staining was done according to the protocol by G Panganiban (personal communication) with some minor modifications. Antibodies against *Drosophila* Wg, *Dll* and Nub proteins were used as heterologous probes for immuno histochemistry of *B. mori* wing discs. We have established that heterologous antibodies against *Drosophila* proteins (e.g. PCNA, Wg, *Dll* and En) work well in the *B. mori* system and pick up the appropriate size protein bands in Western blots (Singh and Gopinathan 1997; Dhawan and Gopinathan 2000; S Dhawan and K P Gopinathan, unpublished observations). The antibodies to Nub and *Dll* employed in the present study have been shown by others also to cross react across species (Panganiban *et al* 1994, 1995, 1997; Averoff and Cohen 1997; Abzhanov and Kaufman 2000).

Wing discs were dissected out from staged larvae, transferred to *Drosophila* Ringer's and fixed for 30 min in 4% paraformaldehyde in PEM buffer [1XPBS (phosphate buffered saline, pH 7.4), 1 mM MgSO₄ and 2 mM EGTA], and mixed with equal volumes of heptane. The discs were washed three times for 10 min each in PBST (1X PBS and 0.1% Triton X-100) and transferred to 1% bovine serum albumin (BSA) in PBST for blocking. Primary antibodies were added at the following dilutions: rabbit anti-Wg 1 : 25; rabbit anti-*Dll* 1 : 100 and rabbit anti-Nub 1:10 and the discs were incubated overnight at 4°C. The samples were washed in PBST twice for 10 min and were incubated for 2 h at room temperature in blocking solution with 1 : 200 secondary anti-rabbit antibody conjugated to horse radish peroxidase (HRP). The discs were washed and diaminobenzidine (DAB) reaction was carried out on ice. The reaction was stopped with PBST after optimum colour development, the samples were mounted in 50% glycerol and photodocumented on an Olympus microscope and camera.

2.2 Explantation and excision

These experiments were performed on wing discs from the third-, fourth-, and fifth instar larvae reared from staged egg collections. Water-anaesthetized *B. mori* larvae recover and resume normal feeding and undergo normal development to adulthood. A small incision was made on the dorsal epidermis of the water-anaesthetized larvae (from third instar onwards) using a surgical blade. The discs were explanted from these incisions and a small amount of phenylthiourea was applied to the epidermal opening to prevent oxidation of the hemolymph. When the wing discs from the right side of the larvae were explanted, the contra-lateral wing discs acted as controls. In a few larvae both the left and right, fore- or hind-wing discs were explanted. The adult moths which emerged from these larvae were observed for wing development.

In excision experiments the discs were exposed through small incisions on the dorsal epidermis taking care not to break or damage the tracheal connectives. The excisions were carried out surgically by removing either the anterior (aa'), posterior (pp') or the lateral (ll') fragments from the late third- or early fourth instar wing discs (figure 5). Following excision the discs were eased back into the haemocoel of the same larva to provide the natural growth condition and pharate adults were recovered at 50–60% frequency.

3. Results

3.1 Larval and adult appendages in *B. mori*

A pair of meso- and meta-thoracic discs located on either side of the larvae gives rise to the corresponding fore- and hind-wings of the adult in *B. mori*. The adult wings bear uniformly coloured scales lacking eye-spots or transverse (para-focal) elements. Consequently, the fore- and the hind-wings differ only in terms of shape and size.

The wing discs are specified during embryogenesis at 5 days after egg laying (AEL). The discs are present as epithelial sacs, near the lateral spine just below the dorsal epidermis in the meso- and meta-thoracic segments of the larva. They are attached to the surface by a thin pedicel and held in position by tracheal connectives. By the end of the third larval instar, shape and size differences appear between the mesothoracic (fore-wing) and meta-thoracic

(hind-wing) discs (figure 1a, f). Venation patterns are initiated on both the wing discs by mid fourth instar (figure 1b, g) and these become distinct and complete by the end of the fifth larval instar (figure 1c, h). The wing discs display almost four-fold increase in their size from the third instar to the end of larval life. They continue to grow in size even after cocoon formation and during metamorphosis (figure 1d, i). Evidently, the wing discs unlike the external appendages, viz., antenna and legs, are not directly affected by larval moulting. The adult moths emerge with the fore- and the hind-wings (figure 1e, j) on the meso- and meta-thoracic segments respectively, and the wings do not have any colour pattern but a uniform distribution of yellowish scales. Unlike *Drosophila* wing imaginal discs, the *B. mori* discs do not display complex patterns of cell migration as evidenced by the presence of a landmark venation pattern that is conserved throughout larval life and metamorphosis. In the absence of evagination, the proximal-distal spatial relationships are retained throughout development. Adult wing formation in *Drosophila*, involves coordinated cell movements, cell shape changes, cell adhesion and cell–cell signalling modifications (Fristrom *et al* 1993; Nardi 1994).

3.2 Patterns of *Nub*, *Dll* and *Wg* expression in the *B. mori* wing discs

The organization of *B. mori* wing disc was probed using markers which are highly conserved in terms of their

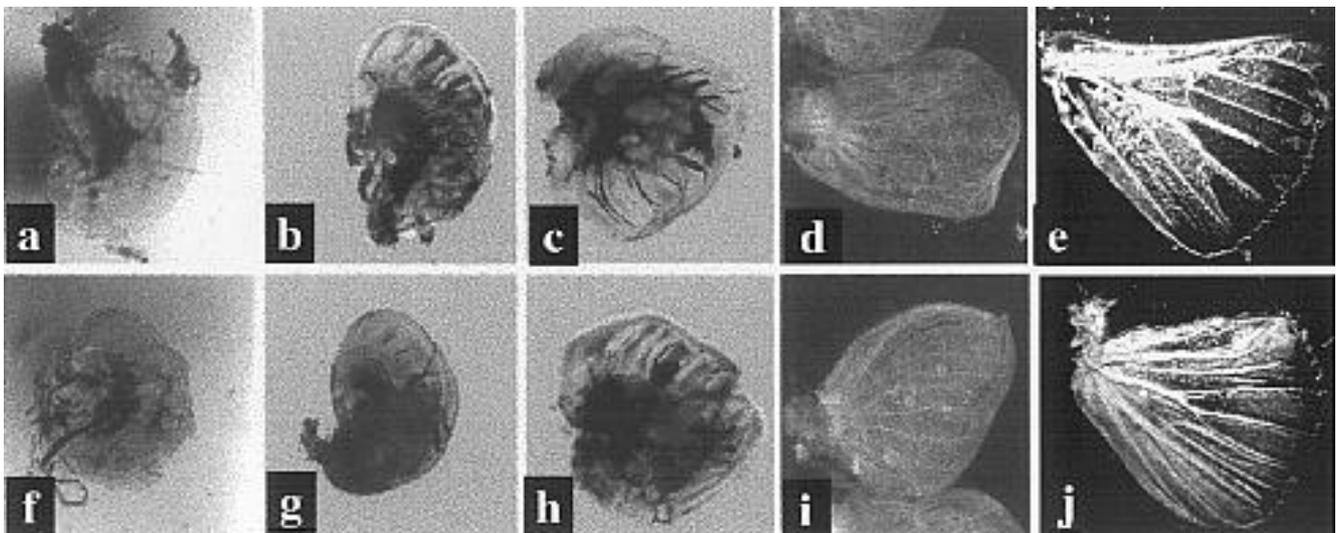


Figure 1. Wing development in *B. mori*. Developmental profiles of meso-thoracic (a–d) and meta-thoracic (f–i) wing discs' from third, fourth and fifth larval instars and pupal stages, as well as the adult fore (e) and hind (j) wings are presented. Both the meso- and meta-thoracic discs continue to grow in size with larval age and do not display any massive cell rearrangement during metamorphosis (compare c with d, and h with i) during the larval to pupal transition. The venation patterns appear at the end of the fourth larval instar and are maintained to pupal and adult development. The meso- and meta-thoracic discs form the fore- and the hind-wings in the adult moth. (a–c and f–h $\times 15$; d, e, i and j $\times 3.3$)

expression and function (Patel *et al* 1989; Carroll *et al* 1994; Panganiban *et al* 1994, 1995, 1997; Brakefield *et al* 1996; Keys *et al* 1999; Abzhanov and Kaufman 2000). In *Drosophila*, Nub (or *pdm1*) specifically marks the hinge and the wing pouch region of the disc which corresponds to the

adult wing blade. Expression of Nub in *B. mori* wing disc was seen over the entire meso- and meta-thoracic discs and not just the pouch region (figure 2a, b) suggesting that these discs contribute primarily to the wing blade and may not specify any body wall structures of the adult.

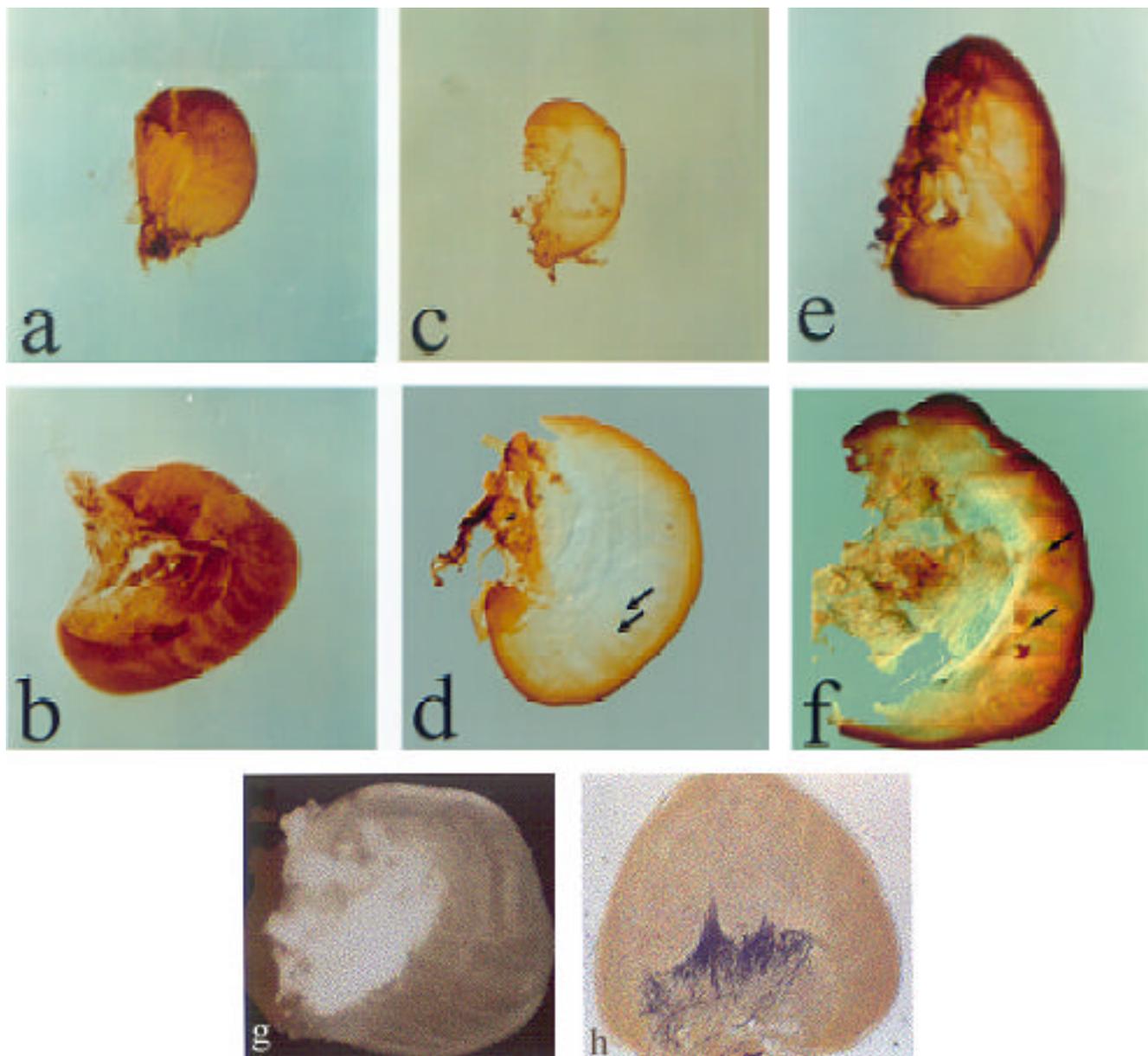


Figure 2. Patterns of gene expression in the meso-thoracic wing discs of *B. mori*. The upper and lower panels display patterns of gene expression mesothoracic discs of third and fifth instar larvae, respectively. The wing discs were dissected out from water-anesthetized larvae and processed for antibody staining for protein expression as described in § 2. Heterologous antibodies against *Drosophila* or *P. coenia* counterparts were used. The heterologous antibodies used here have been established to specifically interact with their counterparts from *B. mori*. (a, b) Nub expression (in the entire disc). (c, d) Dll expression (along the wing margin). (e, f) Wg expression (seen in the wing margin in overlapping domains) ($\times 13$). The specificity of staining was established by including appropriate controls where the discs were subjected to the same treatments except that the primary antibodies were left out (h) or were not treated with either antibodies (g). The somewhat diffused appearance of the staining is genuine to the system because *Drosophila* tissues were processed in parallel to ensure our staining protocols and served as controls.

Dll expression in the early third and fifth instar meso-thoracic discs is presented in figure 2c, d. Both the meso- and meta-thoracic discs exhibited Dll expression along the periphery of the wing blade. This pattern was retained in the wing disc throughout its development from larva to early pupa. Adult wings could not be stained as they are highly cuticularized.

Wg expression was observed in the wing margin, which represents the dorsal/ventral (D/V) boundary of the wing blade, in both the meso- and meta-thoracic discs (figure 2e, f). This pattern of Wg expression was maintained throughout larval to the early pupal wing discs. Although the domain of Wg expression in *B. mori* wing discs appear relatively diffused when compared to that of D/V margin in *Drosophila* (Couso *et al* 1993), the pattern of expression observed was consistent. The Wg expression pattern in *B. mori* discs was in strong contrast to the pattern in *Drosophila* where it appears as a stripe across the D/V boundary that marks the presumptive wing margin and in a circular ring along the wing hinge during the third larval instar. The latter region forms the fly wing and the remaining disc develops into ventral pleura that is lateral thorax.

The controls for antibody staining where the primary antibodies have been left out are presented in figure 2g, h. No background signals were seen, which clearly authenticated the signals generated in the antibody reactions.

The striped pattern of expression of Dll and Wg in *B. mori* protrude inside towards the wing blade in each wing cells (marked by arrow). The overlap in the patterns of Wg and Dll expression in *B. mori* expression confirmed that the presumptive margin is the distal-most structure specified in the wing discs throughout development.

3.3 Explantation of wing imaginal discs

The possible absence of notal and pleural precursors in the wing disc epithelia, as indicated from the above observations was confirmed by analysing the effect of disc explantation on normal development in pupae and adult (figure 3a, b). When the meso-thoracic or the meta-thoracic discs were individually explanted, the pupae and the moths which emerged from such pupae, did not possess the corresponding fore- (figure 3e, f) or hind wings (figure 3c, d). Explantation of both the meso- and meta-

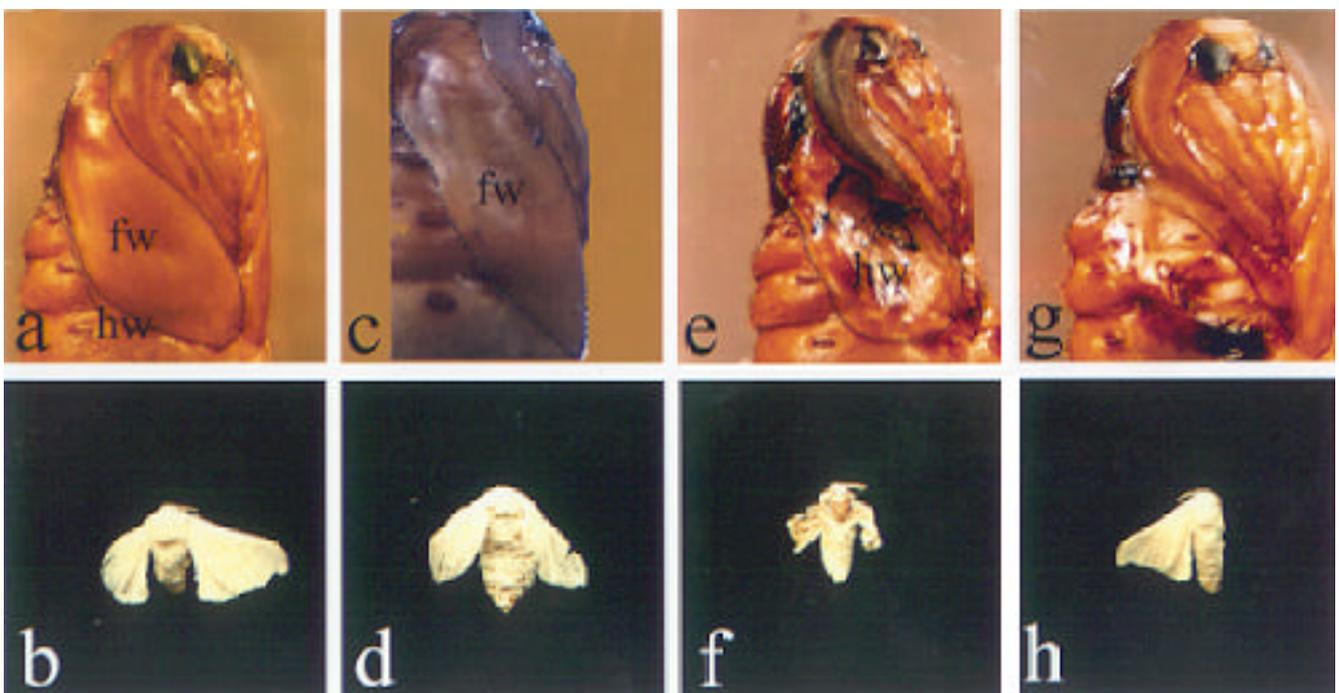


Figure 3. Explantation of the wing imaginal discs. (a) Wild type pharate depicting the size and shape of the forewing (fw) and hindwing (hw) which form the normal four-winged moth (b). Explantations and excisions were carried out as described in § 2. Explantation of the meta-thoracic wing discs from a third instar larva resulted in pharates and adult moths carrying only the two forewings (c and d respectively). Explantation of the mesothoracic wing imaginal discs resulted in the development of pharates and emergent moths carrying only the pair of hind wings (e and f respectively). The explantation of both the meso- and meta-thoracic discs from the right side of the larvae resulted in the emergence of pupae and moths lacking the wings on their right side (g, h).

thoracic wing discs from the right side of a fourth instar larva resulted in the development of adults carrying only one fore- and hind-wing on the left side of the pharate and adult moth (figure 3g, h). Note that the pharates or adults did not show any loss of notal and thoracic structures following explantation. The explantation of either of these discs from the larvae resulted in three-winged moths lacking the wing corresponding to the explanted disc (not shown). However, when all four wing discs were explanted the larvae did not survive for long, possibly due to excessive loss of haemolymph during explantation.

A closer examination of the body wall damage, if any, following extirpation of the wing discs is presented in figure 4. There were minimal cuticular damages but wound healing marks were visible. The ventral (figure 4a, b, c) and dorsal (figure 4d, e, f) views of the pharate as

well as the dorsal (figure 4g) and ventral (figure 4h) views of the adult moths emerging from such larvae from which both the wing discs from one side were extirpated are shown. The absence of wing blades without any notable cuticular deformities other than wound healing marks, further implied the absence of any role for the wing discs in body wall formation. This conclusion, however, is entirely based on morphological criteria in the absence of detailed fate maps or cuticular genetic markers in *B. mori*.

The above results also suggested that the *B. mori* imaginal discs are simple in organization in terms of the cell types they specify, viz., only the wing cells. The larval wing discs showed a one-to-one correspondence with the adult wings as evident from the conservation of the venation pattern in the adult wings. We, therefore, analysed the correspondence at the level of domains within

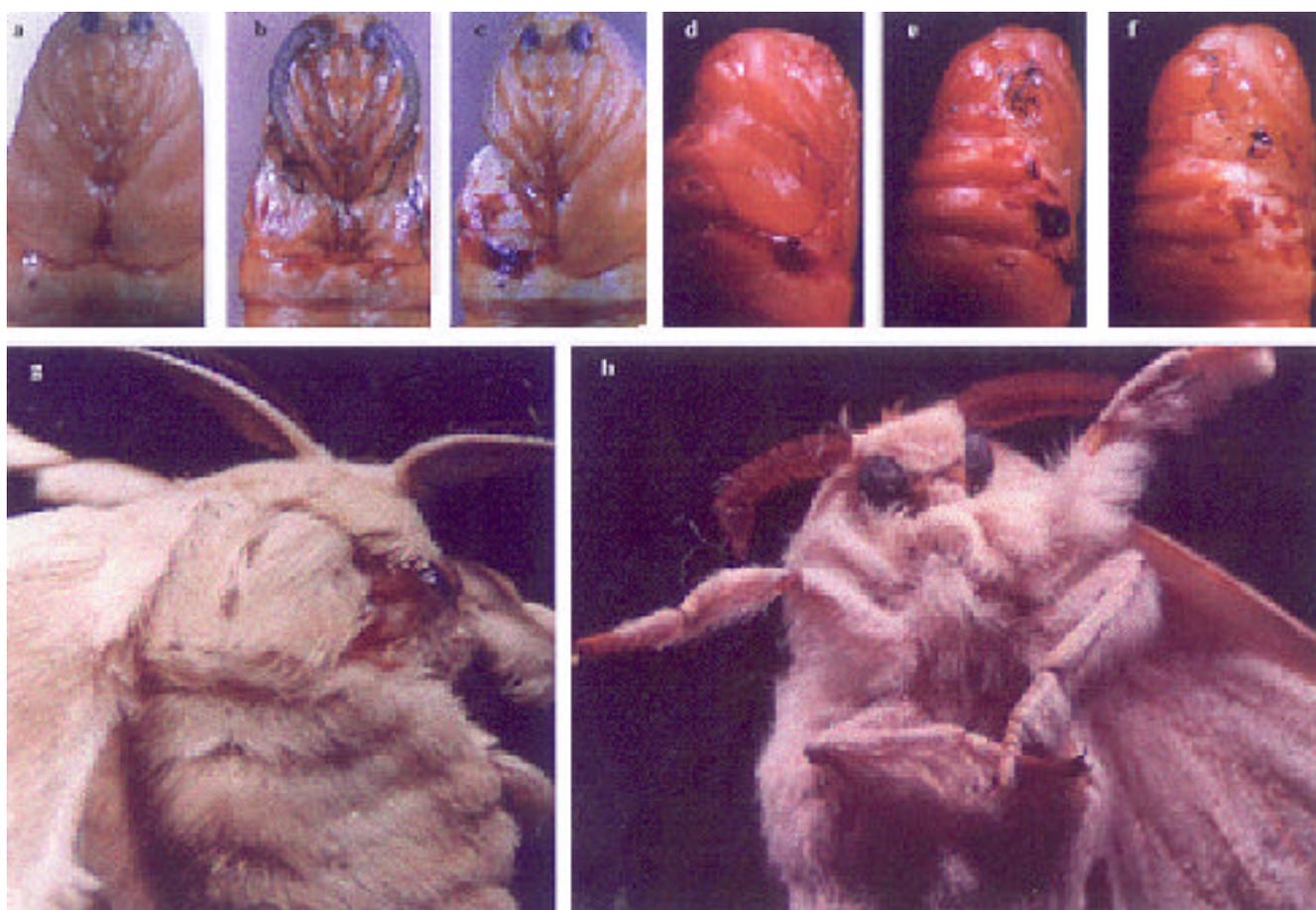


Figure 4. Minimal cuticular damage on surgical excision of larval wing discs. The wing discs were removed surgically at the larval stages following water-anaesthesia. The surviving larvae were reared to the adult stages. External cuticular damages, if any due to wing disc excision was examined in pharates and adult moths. The wound healing marks are clearly visible. (a–c) Ventral view: (a) control pharate, (b) pharate from which meso-thoracic discs were removed from both sides and (c) pharate from which both discs were removed from one side. (d–f) Dorsal side view: (d) control pharate after mock surgery (without removal of discs), (e) and (f) pharate from which both discs from one side or one disc completely and the other partially excised, respectively. Dorsal (g) and ventral (h) views of the adult moth emerging from larvae subjected to surgery. The conspicuous absence of wing blades and presence of wound healing marks on one side are evident.

the wing disc by perturbation of discs in the third instar larvae and looking at the pattern of the adult wing in the pharates.

3.4 Perturbation of wing discs

In *B. mori*, the large disc sizes permitted their excision *in situ*. The excisions attempted are shown in the cartoon (figure 5). The phenotypes resulting from wing disc excisions were observed in the pharate adults. The wings were sufficiently everted and immobile at this stage to assess the pattern lost as a result of perturbation (also seen in *P. coenia* and *B. anynana*; for review, see Brakefield and French 1999). The pharate wings presented a picture of the region lost due to excision of discs by comparing the landmark venation pattern of the wings from the 'disc-ablated' moths to the wild-type wings (figure 6a). A range of pattern losses were inflicted on the late third instar larval discs by removing fragments indicated in figure 5 (the area between the solid and the dotted lines) to examine the concomitant pattern loss in the pharate wings.

(i) The excision of the aa' fragment resulted in emergence of pharates with wings lacking the anterior wing margin (figure 6b; compare to figure 6a). The severity of the loss

could be correlated to the extent of the cut made on the wing disc (figure 6b) where the fore-wing (fw) became curved/bent down towards the hind-wing due to the loss of structures from the anterior wing blade regions. The hind-wings (hw) appeared reduced with the concomitant loss of anterior margin structures.

(ii) The pp' excisions resulted in the loss of the posterior wing margin and wing blade structures (figure 6c) depending on where the cut was made. All other structures in the wing blade, however, were unaffected by the pattern loss in the region of posterior margin. Both the fore-wing (fw) and the hind-wing (hw) displayed a loss of posterior margin upon excision, which was more severe in the forewing.

(iii) The ll' excisions resulted in the emergence of pharates lacking the distal most regions of the wing blade (figure 6d). These regions corresponded to the presumptive submarginal bands on the adult wings. Therefore, the veins appeared as though cut-off abruptly and the wing flattened considerably in the lateral region.

(iv) Simultaneous aa' and pp' excisions resulted in the loss of corresponding structures on the pupal wings (figure 6e). Both the fore- and hind-wings emerged were very small with only about one vein running through the remaining wing blade.

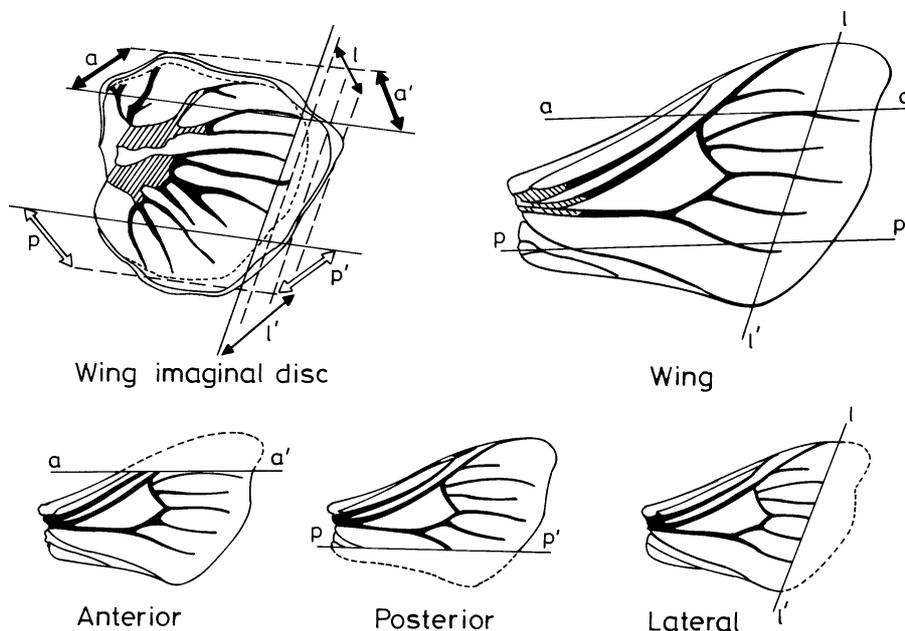


Figure 5. Excision of the larval wing discs and the development of wings. Approximate area of excision in the wing discs along the anterior (a, a'), posterior (p, p') and lateral (l, l') axes and the predicted loss of pattern in the adult wing. The larger arrows are associated with the areas excised along each axes which mark the range of excision along any given axis. The panel on the right presents a fully developed normal pupal wing. The panels shown in the bottom row present the outcome in the wing pattern upon excision.

(v) Excision along all three co-ordinates resulted in extremely reduced wings (figure 6f) lacking anterior, posterior and lateral margin structures.

These observations indicated that the developing wings tend to lose the fragments that were excised/explanted. In no examples of the excision classes, we could see any partial regeneration or duplication of pattern (see also figure 4, showing closer views of wound healing marks but no signs of regeneration). The cells of the ablated wing discs, therefore, were not respecified in the period following excision and the only response was possibly that of wound healing as evident from the morphology of pupal wing.

4. Discussion

4.1 Patterning of wing appendages in *B. mori*

Genes such as *Distal-less (Dll)*, *wingless (wg)* and *nubbin (nub)* have displayed considerable conservation in many insect species both in terms of genetic function and regulation (Carroll *et al* 1994; Panganiban *et al* 1994; Brakefield *et al* 1996; Averof and Cohen 1997). The cognates of *Drosophila* genes *wg*, *engrailed (en)*, *Dll*, *apterous (ap)*, *scalloped (sd)*, *hedgehog (hh)*, *patched (ptc)* and *cubitus interruptus (ci)* have been cloned from the butterfly *P. coenia* (Carroll *et al* 1994; Keys *et al* 1999). Their

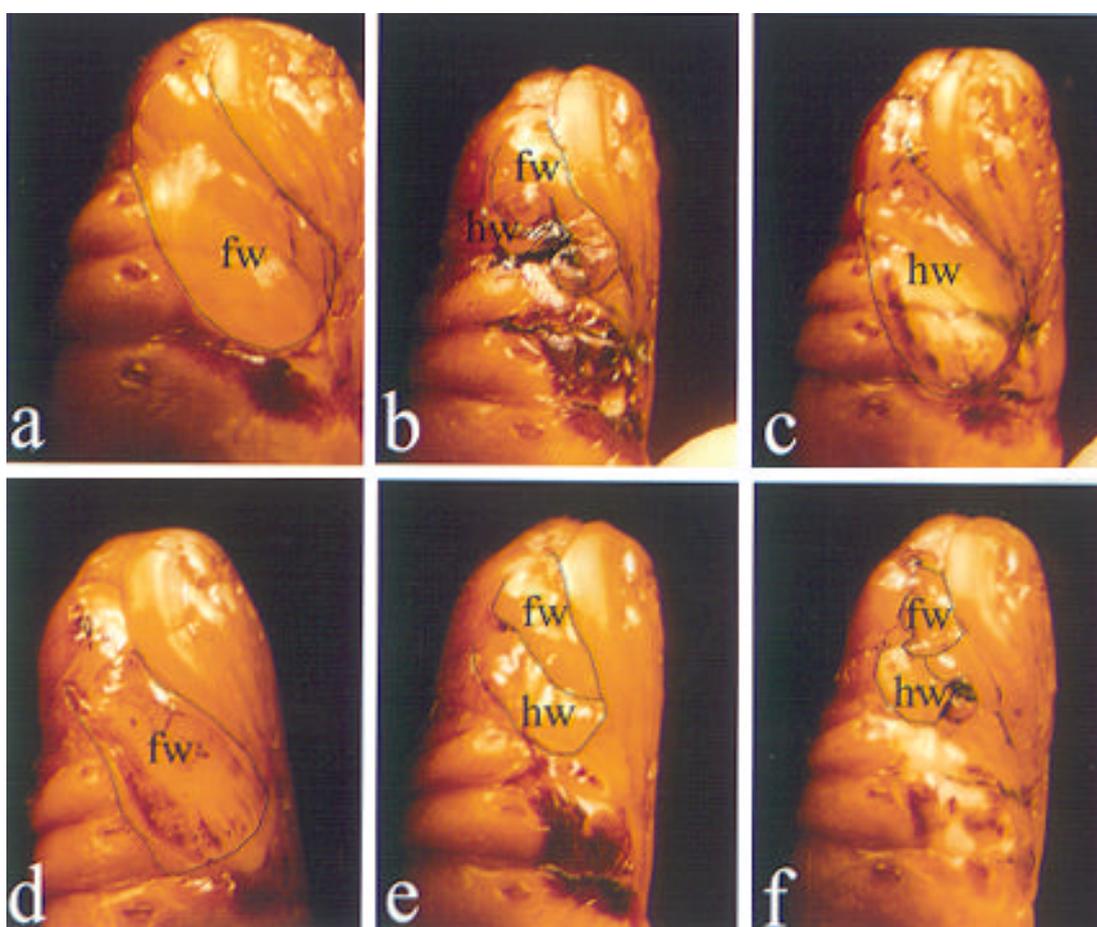


Figure 6. Wing patterns following wing disc excision. (a, b) Excisions along the anterior wing margin represented by lines along the (a, a'). Minor excisions resulted in a smaller fore-wing where the arch of the fore-wing is lost (a) whereas a severe excision from near the wing vein resulted in a forewing with reduced wing blade area (b). Bending of this wing occurred due to tissue loss only on one side of the wing. A single vein is observed in the ablated wing. (c) Posterior excisions along the (p, p'). These excisions resulted in the loss of wing blade tissue from the posterior side. The loss was more extensive in the forewing whereas only a very small portion of the posterior wing margin was lost upon excision of the hind-wing. (d) Lateral excision represented by the lines along (l, l') resulted in a straightening of the lateral wing margin. (e) Simultaneous excision along the anterior and the posterior axes resulted in extremely small wings lacking the anterior and posterior wing blade structures, but the shape of the lateral wing margin was retained. (f) Simultaneous excision along all three axes also resulted in the emergence of pharates with extremely small wings where only the central wing blade structures were retained and all marginal pattern was lost. Note that outlines of the fore- and the hind-wings are marked by hashed line in each panel.

expression patterns in the wing imaginal discs of butterfly are comparable to those in the *Drosophila* wing imaginal discs, suggesting a gross conservation in the domains of gene expression and possibly gene function.

In *B. mori*, a set of mutants which cause a no-wing or very small wing phenotype in the adult moth has been identified (Doira 1983). *Flugellos* (*fl*) belongs to this group, which result in eclosion of wingless moths (Fujiwara and Hojyo 1997). Developmental profiles of the mutant wing discs from *fl* suggested that its product is involved specifically in wing development. The gene has not been isolated or characterized so far. A novel gene *Urbain*, inducible by 20-hydroxyecdysone, was shown to be expressed in the *B. mori* prepupal wing discs and at the end of every larval instar (Besson *et al* 1996) but its role in wing morphogenesis is not known.

We observed that the expression pattern Wg, Dll and Nub remain unchanged throughout postembryonic development. The comparable profiles of Dll expression in *B. mori* wing discs and of *Drosophila* adult wings suggested that the cells of *B. mori* wing discs have already attained distal identity and do not possess the capacity to respecify or reallocate this positional information following perturbation. Unlike the butterflies where a spatio-temporal evolution in Dll expression has been described due to its additional roles in eye spot development (Carroll *et al* 1994; Jiggins *et al* 1996), in *B. mori* Dll expression is in the distal wing margin throughout development similar to the pattern in butterflies lacking eyespots (Jiggins *et al* 1996). The absence of colourful eyespots or transverse bands on the adult wings in *B. mori* may also represent "simpler wing organization" where all the cells in the wing disc carry similar patterning information towards the terminal differentiation into yellowish scales.

The expression pattern of Wg in *B. mori* wing discs contrasted with that reported in *Drosophila* where Wg expression evolves in spatio-temporal manner during larval development, from ventral sector to the wing margin during larval life and to only the anterior triple row cells of the pharate wings (Couso *et al* 1993). In case of *B. mori* Wg distribution is seen along the wing margin of the disc throughout larval life. Thus, the wings appear to be patterned along the proximo-distal axis quite early in development. In *Drosophila*, Nub specifically marks the wing pouch of the disc which corresponds to the wing blade of the adult fly and not the notum which forms the body wall (Averof and Cohen 1997). In *B. mori* wing disc, Nub expression is seen all over the disc suggesting that the entire disc is responsible for formation of only the wing blade. The stereotyped expression pattern of Wg, Dll and Nub from early larval to early pupal wing discs of *B. mori* also indicated that there was no major cellular rearrangement or migration during metamorphosis in wing development.

4.2 *B. mori* wing discs are differentiated in larval stages

The explanation of the *B. mori* wing discs revealed the absence of the presumptive body wall structure in them. Therefore, unlike the diptera where imaginal discs also formed a complement of the ventral pleura and the lateral thorax and the wing hinge, in *B. mori* and possibly in other lepidoptera these structures are laid down presumably by the thoracic cells around the tracheal connectives. The preservation of expression patterns of developmental markers such as Wg, Nub and Dll during larval and pupal development in *B. mori* suggested that the wing disc does not undergo cell rearrangement and cell movement. In *Drosophila* the wing disc undergoes massive cell movements as evidenced by the dynamic expression profiles of these markers to attain the final differentiated state of the pupal wing. The correspondence of the larval expression patterns of these markers in *B. mori* wing discs to that of the differentiated wing stage of *Drosophila* suggests that the timings of morphogenetic events are different and they attain a differentiated state early in development. The wing disc is already folded in *B. mori* larvae so that its presumptive dorsal and ventral surfaces are apposed to one another, whereas in *Drosophila* the disc is not folded until it evaginates and therefore the presumptive dorsal and ventral surfaces are adjacent to one another in the larval disc.

4.3 *B. mori* wing discs lack regulation by duplication/regeneration

Many epithelia undergo growth regulation. For instance, the *Drosophila* imaginal discs and the cockroach legs when cultured after fragmentation tend to regenerate or duplicate the lost portions by intercalation (Bryant 1975; French *et al* 1976; Anderson and French 1985). The paradigms of growth regulation are most elegantly explained by the polar co-ordinate model (French *et al* 1976) which specifies a co-ordinate system and the rules for pattern regulation in differentiating fields. Growth regulation in *Drosophila* imaginal discs is manifested either by regeneration or duplication of the lost structures (Bryant 1975). Besides, it also involves the generation of new cells by mitosis and a reprogramming of the positional values of existing cells (Adler 1981, 1984). Only recently, efforts have been made to understand the molecular mechanisms for disc regulation (Maves and Schubiger 1999; Gibson and Schubiger 1999).

The lepidopteran models have shown a variable response to excision or cautery (Nijhout 1985, 1991; Tousiant and French 1988; Brakefield and French 1995; French and Brakefield 1995). In *B. anynana* transplanta-

tion or epidermal damage revealed the capacity of cells to form ectopic eyespots or position dependent differences in signalling (Brakefield and French 1995; French and Brakefield 1995; French 1997). Modest or no regeneration was seen in cauterized discs of *P. coenia*, *Philosomia cynthia ricini* and *Papilio machaon*, in contrast to *Lymantria dispar* and *Vanessa* (Nijhout and Grunett 1988). In insects lacking the colourful eyespots (e.g., *E. kuhniella*) the transverse colour bands responded to micro cautery in the pupal wings by either local or global modification of pattern (Toussiant and French 1988). The ablated discs do not exhibit any obvious growth regulation. The adult wings displayed a one-to-one correspondence with the larval discs in *B. mori* indicating that the discs did not experience massive cell rearrangements during metamorphosis. Secondly, the positions of the cells forming the wing margins appeared to be fixed from a very early stage in development. Besides, the cells seemed to be incapable of reallocating positional information of the lost structures to the ones adjacent to the site of injury. Although a fate map of the *B. mori* imaginal discs is not available at present, their large size permitted us to ensure the extent of extirpation in each case. The conservation of expression patterns in the discs and the inability to regulate growth indicated that these cell types are differentiated early in *B. mori*. The results presented here provide the first documentation on wing development in *B. mori*.

The imaginal disc mode of development for appendages based on *Drosophila* model, accepted today as a paradigm may not hold good for most lepidopterans. The antennal structures in *B. mori* have been shown to be developed from their larval counterparts and not through imaginal disc like structures (Svacha 1992). We have seen a similar type of developmental mechanism in *B. mori* leg appendages also (A Singh and K P Gopinathan, unpublished results). In this instance the larval thoracic legs develop as adult legs whereas the larval abdominal legs are lost during metamorphosis. The wing discs, on the other hand, are set aside during embryonic development but stay in a quiescent state in the larvae. They behave like presumptive wing buds that open out as wing blades rather than evagination of only the pouch region as wing blades while leaving the notum to form the body wall as seen in *Drosophila*. *B. mori* wing development, thus follows a mechanism that is different from the fruit fly.

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