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The hinge phenotype of *In(2L)wg^{P}* in *Drosophila melanogaster*.

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Despite the extensive studies of the *Drosophila* wing and notum, the developmental biology of the wing hinge remains largely uncharacterized. The *wingless* (*wg*) gene plays an important role in the development of several structures in the proximal and distal dorsal and ventral hinges (Neumann and Cohen, 1996; Buratovich and Wilder, 2001). One particular *wg* allele, *In(2L)wg^{P}*, shows a dominant outstretched wing phenotype for reasons that remain undetermined. We have examined wing hinges from *In(2L)wg^{P}* animals to determine if any detectable hinge abnormality exists and to correlate such findings with the mechanism of *wg* activity in the hinge.

The mutation that originally defined the *wg* gene was a viable allele that eliminated the wing blade and converted the hinge region into notum (Sharma and Chopra, 1976). Later mutagenesis screens isolated embryonic-lethal *wg* alleles that showed a distinctive segment polarity phenotype and other alleles that caused pupal lethality while affecting the development of all imaginal derivatives (Nusslein-Volhard *et al.*, 1984; Baker, 1988a, b). Several of these x-ray-induced alleles of *wg* contain severe lesions in the *wg*-coding region (van den Heuvel *et al.*, 1993), but one allele in particular, *In(2L)wg^{P}*, is a regulatory mutation that results from an inversion with breakpoints at 28A1-3 and 32E-F (Baker, 1988b). The distal breakpoint of *In(2L)wg^{P}* (hereafter referred to as *wg^{P}*) at 28A1-3 maps 9-11 kilobases downstream the transcription termination site of the *wg* gene, presumably within a transcriptional enhancer that controls the imaginal-specific expression of *wg* (van den Heuvel *et al.*, 1993). Indeed the designation "*wg^{P}*" comes from the pupal lethality of this allele in combination with null alleles. Strangely, *wg^{P}* homozygotes show embryonic lethality and express *wg* transcripts in seven stripes rather the usual 14 stripes, but *wg^{P}* heterozygotes also show a dominant adult phenotype that consists of outstretched wings (Baker, 1988b). Because mutations that affect the structure of the wing hinge often cause flies to hold their wings in the outstretched position at rest (Neumann and Cohen, 1996; Russell, 2000; Buratovich and Wilder, 2001), an investigation of the wing hinge of *wg^{P}* heterozygotes might reveal a hinge defect that elucidates the role of Wg in the development of the wing hinge.

Light microscope preparations of hinge hinges were made as previously reported (Buratovich and Wilder, 2001). In these preparations we used *wg^{P}* / *CyO* animals and flies of the same genotype that were outcrossed to *Canton S* to generate *wg^{P}* / + heterozygotes. We used these flies with *wg^{P}* in combination with different chromosomes to eliminate any potential variations in hinge structure due to differing genetic backgrounds. Both stocks showed the outstretched wing phenotype to a great extent. Our wing hinge preparations showed no detectable defects in the dorsal wing hinge (n = 27, data not shown). However, the same preparations of the ventral hinge showed a consistent absence of a structure called the yellow club (YC; data not shown).

The YC is one of the most conspicuous structures in the ventral wing hinge (Bryant, 1978). It extends from the cuticle in an anterior direction and lies just proximal to the proximal ventral radius

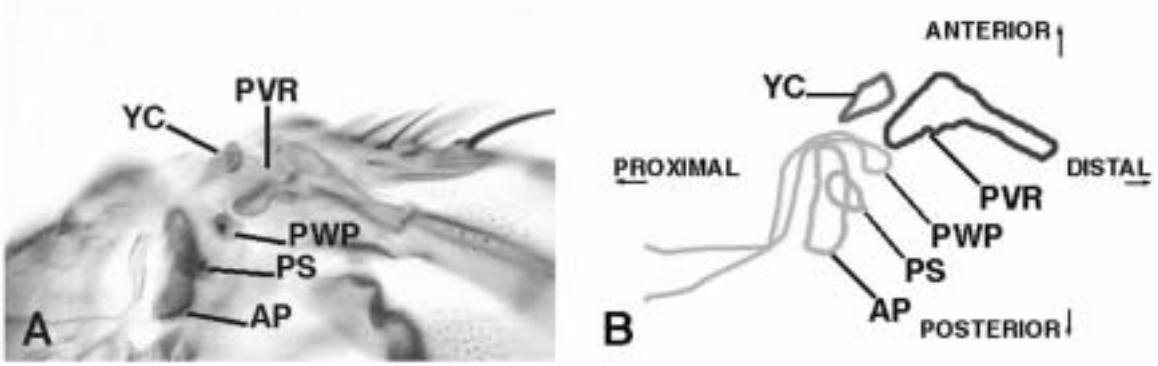

Figure 1. (A) Ventral hinge from a wild-type adult. Ventrally, the confluence of wing veins 1-3 terminates in the Proximal Ventral Radius (PVR). Just proximal to the PVR is the Yellow Club (YC), which is the most conspicuous ventral hinge structure. Posterior to the YC is a series of plates and a pouch. The Axillary Pouch (AP) is an out pocket of the ventral hinge cuticle. Just anterior to the AP lies the Pleural Wing Process (PWP). The PWP arches around the AP to form the pleural suture, which separates the pleura into the mesopleura and the pteropleura. The head of the PWP articulates with the ventral side of Axillary Sclerite 2 on the dorsal hinge. Posterior to the PWP is the Pleural Sclerite (PS). The PS is hidden from view in some preparations by the AP, which overlies and obscures it (Bryant, 1978). (B) A cartoon tracing of Figure 1A.

(PVR). The PVR lies at the confluence of the first three wing veins and this thickened flap of wing cuticle points posteriorly. Below the PVR is the pleural wing process (PWP). Extending from the cuticle, toward the PVR, the PWP probably acts with the PVR as a doorstop to prevent the wing from hyperextending in the ventral direction (Dickinson and Tu, 1997). The PWP extends around an extra pouch of tissue called the axillary pouch (AP), and adjacent to the AP is another sclerite called the pleural sclerite (PS), which is often obscured from view by the AP (Figure 1A-B).

Because of the lack of resolution of light microscopy, we decided to check our light microscopy results with the scanning electron microscope (SEM). We outcrossed wg^{P} / *In(2LR)Gla,* $wg^{Gla\text{-}1}$ animals to *Oregon R* to generate wg^{P} / + heterozygotes and fixed these adult animals for SEM, according to the procedure of Idle (1970). Observation of wing hinges from wg^{P} / + heterozygotes (n = 13) with a JEOL JSM T300 scanning electron microscope revealed no detectable defects in the dorsal hinges of these animals (data not shown), but the ventral hinges showed a variety of abnormalities. Some ventral hinges showed deformation of the proximal ventral radius (PVR) and pleural wing process (PWP), even though these structures were always present (data not shown). The YC was consistently missing in these hinges (Figure 2A-B), but in one hinge, the YC was quite small (data not shown). Such diminution could certainly render the YC undetectable during light microscopy.

The absence of the YC in wg^{P} heterozygotes does not correlate well with the observed effects of *wg* in the wing hinge. Ectopic expression or overexpression of *wg* in the dorsal hinge replaces the Unnamed Plate (UP), Axillary Sclerite 1 (AS1), and Axillary Sclerite 2 (AS2) with a duplicated copy of Axillary Sclerite 3 (AS3). Loss of Wg activity in the dorsal hinge deletes AS3, which suggests that Wg is necessary for the formation of AS3 in the dorsal hinge (Buratovich and Wilder, 2001). In the distal dorsal hinge, removal of Wg activity from this region, as in the case of the regulatory *spd* allele of *wg*, causes deletion of the distal wing hinge (Neumann and Cohen, 1996). In the ventral hinge, ectopic or overexpression of *wg* removes the AP, but inactivation of Wg causes a duplication of the AP, again

suggesting that Wg is required for the establishment of the AP in the ventral hinge (Buratovich and Wilder, 2001). In all of these cases, neither ectopic nor overexpression of *wg* nor diminution of Wg activity in the ventral hinge affect the formation of the YC.

In situ hybridization of third-instar wing imaginal discs from wg^P heterozygotes with *wg* probes revealed no detectable differences in *wg* expression when compared to wild-type discs (data not shown). The absence of the YC might have to do with the distal breakpoint of the inversion and nothing to do with the *wg* gene. A molecular analysis of wg^P revertants that no longer show the outstretched wing phenotype should provide some insight into this question.

Also, why animals that lack the YC should hold their wings outstretched is difficult to explain. None of the main direct flight muscles that are responsible for wing positioning are definitively known to attach to the YC. Therefore, there is no apparent reason why flies without YCs in their ventral hinges should hold their wings out. There are three possibilities as to why these flies hold their wings out. First of all, the absence of the YC might weaken nearby muscle attachment sites. For example the prealar apophysis (PAA), which lies very close to the YC, provides attachment sites for direct flight muscles 49, 51 and 52 (Miller, 1994). The absence of the YC could conceivably destabilize the PAA or some other nearby muscle attachment site. A second possibility is that the YC is an attachment site for specific flight muscles whose attachment sites have yet to be determined. Finally, the outstretched wing phenotype of wg^P heterozygotes and the absence of the YC might also be completely unrelated and reside in muscle problems that have yet to be detected. In this case a more detailed analysis of the configuration of the direct flight muscles in wg^P heterozygotes could also provide insight into why these animals hold their wings out.

In conclusion the wg^P allele of wg causes a dominant wings outstretched adult phenotype that is highly correlated with the elimination or diminution of the Yellow Club in the ventral wing hinge. The significance of this with respect to the role of *wg* in hinge development or the functional integrity of the wing hinge is not understood at this time.

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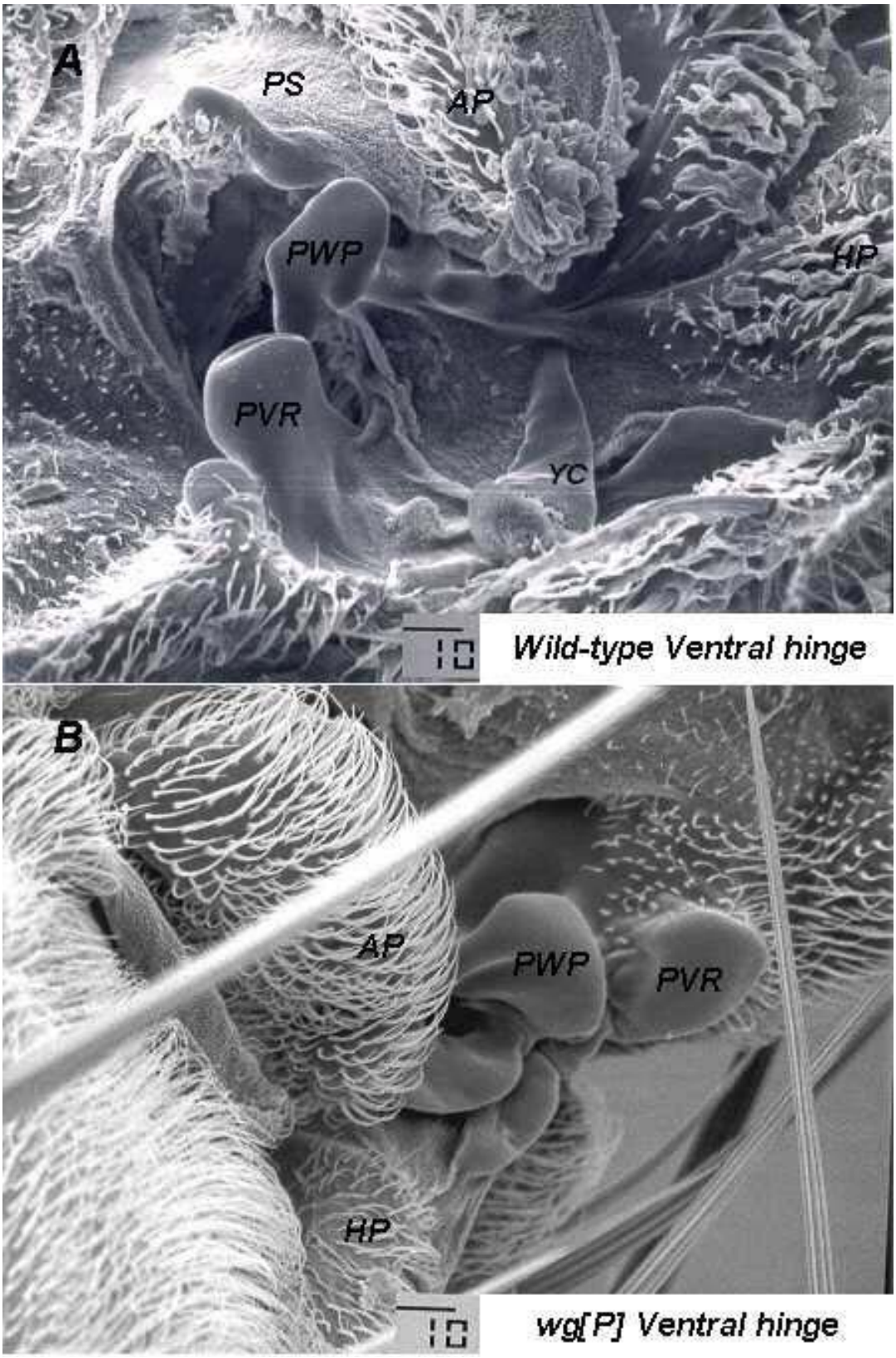
Figure 2 (next Page). (A) SEM photomicrograph of a ventral hinge from a wild-type Oregon R adult *Drosophila*. The abbreviations used to label the hinge structures are the same as those used in Figure 1, with the exception of the Humeral Plate (HP), which is a marginal, anterior cuticular plate that spans the anterior margin of the ventral and dorsal wing hinges. The HP lies just distal to the tegula (not shown). (B) SEM photomicrograph of a ventral hinge from a wg^P / + adult *Drosophila*. The YC is missing and the PWP and the PVR are flatter and expanded in appearance.


A
PS
AP
PWP
HP
PVR
YC
10
Wild-type Ventral hinge
B
AP
PWP
PVR
HP
10
wg[P] Ventral hinge

References: Baker, N.E., 1988a, Development 102: 489-497; Baker, N.E., 1988b, Dev Biol 125: 96-108; Bryant, P.J., 1978, Pattern Formation in Imaginal Discs. *In: The Genetics and Biology of* Drosophila (M. Ashburner and T.R.F. Wright, eds.), Vol. 2C, pp. 229-335. Academic Press, New York; Buratovich, M.A., and E.A. Wilder 2001, Dros. Inf. Serv. 84: 145-157; Dickinson, M.H., and M.S. Tu 1997, Comparative Biochemistry and Physiology 116A: 223-238; Idle, D.B., 1970, Journal of Microscopy 93: 77-79; Miller, A., 1994, The internal anatomy and histology of the imago of *Drosophila melanogaster*. *In: Biology of* Drosophila (M. Demerec, ed.), pp. 420-534. Cold Spring Harbor Press, Cold Spring Harbor, NY; Neumann, C.J., and S.M. Cohen 1996, Development 122: 1781-1789; Nusslein-Volhard, C., E. Wieschaus, and H. Kluding 1984, Roux's Archives of Developmental Biology 193: 267-282; Russell, S., 2000, Mol. Gen. Genet. 263: 690-701; Sharma, R.P., and V.L. Chopra 1976, Dev. Biol. 48: 461-465; van den Heuvel, M., C. Harryman-Samos, J. Klingensmith, N. Perrimon, and R. Nusse 1993, EMBO J. 12: 5293-5302.