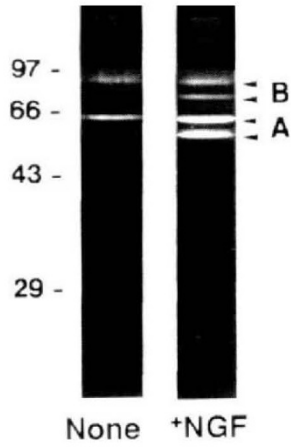

Secretion of gelatinases A and B by PC-12 cells in the absence (none) and presence (+NGF) of nerve growth factor. Arrows A and B, gelatinases A and B, respectively. In each enzyme, the upper band is the proenzyme, and the lower is the mature enzyme. The levels of the proenzymes increased about 16-fold for gelatinase A and about 4-fold for gelatinase B by the NGF treatment.

METHODS. PC-12 cells were grown to semi-confluence in 10 ml RPMI-1640 medium supplemented with 10% horse serum and 5% FCS on collagen-coated 10-cm plastic dishes. The cultures were washed with serum-free RPMI medium and then incubated in the serum-free medium in the absence or presence of 50 ng ml^{-1} NGF at 37 °C for 2 days. Resultant conditioned media were collected and concentrated 30-fold by ammonium sulphate precipitation, and then subjected to zymography on a gelatin-containing polyacrylamide gel as described previously[5].

cells[4], most of the mature membrane-bound APP is intracellularly cleaved by α-secretase in the trans-Golgi network or in a post-Golgi compartment rather than at the plasma membrane. Therefore, we expect that the addition of TIMP-1 into culture medium would not affect the intracellular action of α-secretase, even if gelatinase A is α-secretase. Taken together, these data show that the possibility that gelatinase A is α-type APP secretase cannot yet be excluded.

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1. Miyazaki, K., Hasegawa, M., Funahashi, K. & Umeda, M. *Nature* **362**, 839–841 (1993).
2. Walsh, D. M., Williams, C. H., Kennedy, H. E., Allsop, D. & Murphy, G. *Nature* **367**, 27–28 (1994).
3. Sambamurti, K., Shioi, J., Anderson, J. P., Pappolla, M. A. & Robakis, N. K. *J. Neurosci. Res.* **33**, 319–329 (1992).
4. Kuentzel, S. L., Ali, S. M., Altman, R. A., Greenberg, B. D. & Raub, T. J. *Biochem. J.* **295**, 367–378 (1993).
5. Kato, Y., Nakayama, Y., Umeda, M. & Miyazaki, K. *J. biol. Chem.* **267**, 11424–11430 (1992).

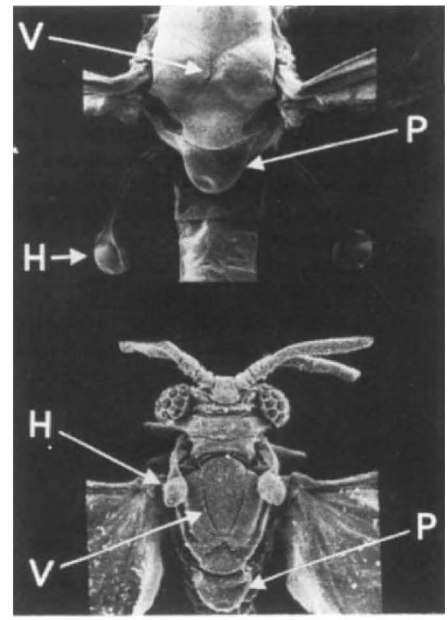
Insect homeotic transformation

SIR — Strepsiptera is a small order of insects with unusual morphological, physiological and behavioural characteristics. Molecular and morphological data support Strepsiptera as sister-group to Diptera. We suggest that a homeotic mutation resulting in ectopic expression of *Ultrabithorax* (*Ubx*) protein transformed the second thoracic segment (T2) to a metathorax and the third (T3) to a mesothorax in the ancestral strepsipteran lineage.

We determined the sequence of regions of nuclear 18S ribosomal DNA for representatives of Strepsiptera, all holometabolous insect orders, and outgroups. Details of data acquisition and phylogenetic analysis will be presented elsewhere. Relationships resulting from the molecular data are largely concordant with the most recent morphologically based phylogeny[1], except for the placement of Strepsiptera as sister-group to Diptera. We have found new morphological characters which support this hypothesis. Strepsiptera possess some ground-plan characters of supra-ordinal groups to which Diptera belong (loss of ovipositor, absence of labial endite lobes and spermatophore)[2]. Moreover, in all male Strepsiptera, Mecoptera (scorpionflies) and basal Diptera[3], abdominal segment 9 is enlarged and ring-like.

Similar morphological modifications of T2 and T3 occur on opposite segments in Diptera and Strepsiptera (see figure). We postulate that thoracic modifications derived in the immediate ancestor of these taxa were subsequently transformed to opposite thoraces in the ancestral strepsipteran lineage. This transformation might be caused by a homeotic mutation resulting in ectopic expression of *Ubx* in T2 and suppression in T3 in strepsipteran embryonic or larval stages. *Ubx* product in *Drosophila* is associated with specialization of T3 into a metathorax[4]. When suppressed by the mutations *bithorax* and *postbithorax*, the haltere is transformed into another full-sized wing and T3 is transformed into another mesothorax[5,6]. Conversely, *Contrabithorax* mutations causing ectopic expression of *Ubx* in T2 result in a transformation of the T2 wing to a haltere (occasionally with some veins extant — as is found in Strepsiptera) and may transform T2 into a metathorax[7].

Are Strepsiptera *Cbx Ubx/pbx bx*? Over-expression of *Ubx* in T2 and suppression in T3 would account for some of the unusual morphology of this group. We favour the transformation hypothesis because it more parsimoniously explains character distribution than to postulate parallelism, and despite potential lethality, has a plausible genetic basis. This



Scanning electron micrographs, dorsal view. Top, Diptera (*Tipula sp.*, × 24) and (Bottom) Strepsiptera (*Caenocholax fenyesi* Pierce, × 51). H, halteres; V, V-shaped notal suture; P, expanded postnotum. In Diptera, V and P are on T2, H on T3; in Strepsiptera, V and P are on T3, H on T2. Halteres in Strepsiptera and Diptera are functionally identical (used for gyroscopic equilibrium during flight)[8] and morphologically similar. In both groups the halteres are club shaped, filled with haemolymph, attached to the pleuron, and possess alula, tegula, and ventral chordotonal and sense organs[2]. Because primitive wasps have a putatively homologous V-shaped suture on T2[9], T2 placement of V and P and T3 placement of H are the primitive character states. Hence the hypothesized transformation would have occurred in the strepsipteran rather than the dipteran lineage.

hypothesis could be further tested by *in situ* hybridization of *Ubx* probes in strepsipteran embryos and instars. Strepsiptera may be one of the best examples of homeosis occurring in nature and causing drastic changes in the morphology of a group, which has led in part to its subsequent specialization and diversification.

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1. Kristensen, N. P. in *Insects of Australia* 2nd edn (ed. CSIRO) 125–140 (Melbourne Univ. Press, 1991).
2. Kathirithamby, J. *Syst. Ent.* **14**, 41–92 (1989).
3. Wood, D. M. & Borkent, A. in *Manual of Nearctic Diptera* (ed. McAlpine, J. F.) Vol. 3, Ch. 114 (Res. Branch Agr. Can., Monogr. No. 32, 1989).
4. Lewis, E. B. *Cold Spring Harb. Symp. quant. Biol.* **50**, 155–164 (1985).
5. Lawrence, P. A. *The Making of a Fly* (Blackwell, Oxford, 1992).
6. Lewis, E. B. *Nature* **276**, 565–570 (1978).
7. Gonzalez-Gaitan, M. A. *et al. Genetics* **126**, 139 (1990).
8. Pix, W., Nalbach, G. & Zeil, J. *Naturwissenschaften* **80**, 371–374 (1993).
9. Gould, I. & Bolton, B. *The Hymenoptera* (BMNH & Oxford Univ. Press, 1988).