

## Evolution: *Hox* genes and the cellared wine principle

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**Two *Drosophila Hox* genes involved in segmentation, *fushi tarazu* and *bicoid*, appear to have acquired these roles by functional divergence from classical homeotic genes. Recent results indicate how genes with critical functions in development can evolve completely different functions among species.**

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**Current Biology** 2000, 10:R452–R455

0960-9822/00/\$ – see front matter  
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Things that look the same on the outside can nevertheless change dramatically on the inside, sometimes in a relatively short period of time. Fine examples include renovated downtown warehouses, husbands and wives, and cellared wines. It is becoming increasingly clear that this ‘cellared wine principle’ has a pervasive presence in development and evolution. Many apparently highly conserved biological processes, from spermatogenesis [1] to nematode vulval development [2], from sex determination [3] to arthropod segmentation [4], actually show marked genetic differences among closely related taxa. Two recent studies [5,6] have shown how even members of that paragon of developmental conservatism, the *Hox* family of genes, can contribute to genetic turnover by evolving new functions that in many respects replace functions performed by other genes.

The *fushi tarazu* (*ftz*) gene sits in the middle of the ANTP-C *Hox* complex of *Drosophila melanogaster* [7], flanked by *Sex combs reduced*, which regulates development of the anterior thorax, and *Antennapedia*, which is active in the mid-thorax. Despite the apparently accelerated rate of evolution of its homeodomain-encoding sequence, *ftz* remains closer in sequence to these flanking genes than to any others, but it is not the unambiguous ortholog — that is, homologue related by evolutionary descent of species rather than gene duplication within a lineage — of any vertebrate counterpart [5]. The oddity is that *ftz* is expressed in seven stripes in the fly embryo [8,9], where it is required for establishment of the odd-numbered segments, a function quite distinct from that of specifying the identity of a subset of adjacent segments, as performed by its brethren in the ANTP-C.

Several years ago, a *ftz* homolog was identified in the *Hox* complex of the beetle *Tribolium castaneum* and shown to be expressed in a similar, though considerably broader,

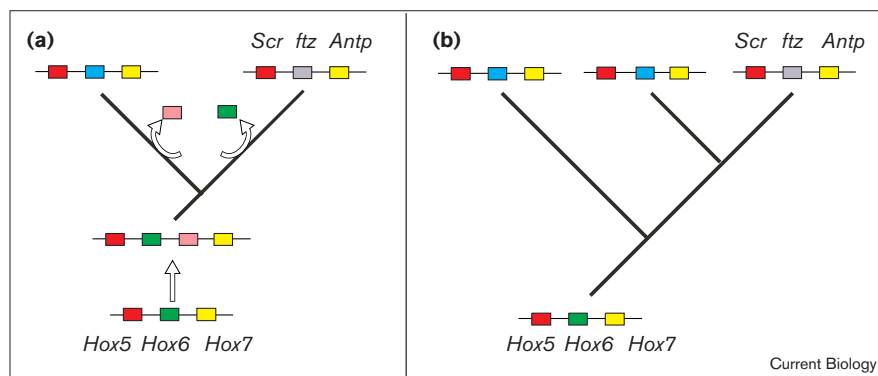
striped pattern in the gastrula [10]. As a deletion of the beetle gene fails to cause a segmentation defect, the role of this expression has not been clear [11]. It is reasonable to suppose that it performs a somewhat redundant function to that of the homeobox gene *even skipped*, which is expressed in every beetle segment but only the even-numbered fly segments. Since then, *ftz*-like sequences have been isolated from several arthropods, including a locust in which it shows no segmental repeat at all [12], as well as from members of each of the major branches of the protostome lineage, but they do not resolve with any confidence as a monophyletic group [13]. Consequently, it has been assumed (Figure 1) that the insect *ftz* appeared by virtue of a relatively recent gene duplication event that gave rise to a new gene with functions in neurogenesis and segmentation.

The alternative hypothesis, that *ftz* was an original member of the protostome *Hox* complex with a homeotic function that has been lost and replaced by the segmentation function, is supported by Max Telford’s recent study [5] of a *ftz* homolog in an outgroup to the arthropods, the mite *Archegozetes longisetosus*. The homeodomain sequence of the protein product of this gene (*Alftz*) is in some respects closer to that of the presumed Lophotrochozoan orthologs than to that present in the segmented arthropods. This is consistent with the notion that it shares a functional constraint that has been shed in the rapidly evolving arthropod *ftz* genes. Tellingly, *Alftz* is expressed in the primordia of the second through fourth legs of the embryonic mite, pretty much where you would expect it to be if it were performing a homeotic function. It is clear that there is no ‘pair-rule’ or other segmental expression of the mite gene, and quite possible that the earliest expression of insect *ftz* in a broad band is a vestige of the ancestral homeotic expression domain.

The other fly *Hox* gene involved in segmentation, *bicoid* (*bcd*), seems more likely to be the result of a duplication event, followed by functional divergence, that occurred at some point in the arthropod lineage, possibly as late as the origin of the higher diptera [14]. Orthologs of *bcd* have only been cloned from flies, but as the *bcd* gene product plays such a profound role as a graded morphogen in patterning the fate map of the *Drosophila* embryo [15], there has been great reluctance to conclude that it does not exist in outgroups. The gene is also fast-evolving and so may elude screens based on the polymerase chain reaction (PCR), while the absence of a *bcd* ortholog in the heavily screened *Hox* complexes of basal arthropods can be explained by the *ad hoc* postulate that the gene resides elsewhere in the genomes of these organisms. Wolff *et al.* [16] have

Figure 1

Two models for the evolution of *ftz*. In duplication and loss models (a) it is assumed that, at some point in protostome evolution, there was a duplication of a *Hox 6* ortholog that allowed one copy to retain its homeotic function in the Lophotrochozoan clade (left), while the other copy evolved a new segmentation function in some arthropods (right). The timing of the proposed duplication and loss events cannot be established without extensive phylogenetic sampling, and it is possible that such events have occurred numerous times in invertebrate evolution. More parsimonious models of functional transformation (b) posit that *ftz* has simply shed its old homeotic role and in several steps has evolved a new segmentation function. Vestiges of this transformation can be seen by examining expression of *Hox 6* orthologs in basal arthropods such as mites [5].



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presented indirect evidence for the existence of a *bcd*-like function in *Tribolium*, by demonstrating that two beetle genes, *caudal* and *hunchback*, are regulated by *Drosophila bcd* when introduced into transgenic flies.

This approach to dissecting the mechanisms of the evolution of development — cross-specific gene transfer — is a promising complement to descriptive analysis of the presence and expression of orthologs against a phylogenetic framework, but not one without potential pitfalls. The syncytium of a fly embryo is a very different cellular environment to the cellular blastoderm of a beetle, and so long as many of the same gene products are used in the divergent species, it can always be argued that interactions observed between gene products are due to an experimental artifact rather than conservation of function. A different strategy is to try to recreate presumed intermediate steps in the evolution of a novel gene function by genetic manipulation of the *Drosophila* embryo. Wimmer *et al.* [6] recently used this strategy to argue that one of the main functions of *bcd*, patterning of the thorax, can be fulfilled by one of the genes that it activates, namely *hunchback* (*hb*). Their study provides experimental support for the idea that *bcd* is actually a genetic pirate that is in the process of taking over the maternal function of *hb*.

These are clever experiments, for some possibly too clever to allay fears that Wimmer *et al.* [6] have created a deceptively artificial situation. Hunchback protein itself acts as a graded morphogen that helps to pattern the head and thorax of *Drosophila* by activating and/or repressing the expression of other 'gap' genes (segmentation genes required for development of large anteroposterior domains of the embryo). Expression of the *hb* gene shows two phases of autoregulation that are mediated by two

distinct promoters. The early zygotic promoter normally responds to a gradient of Bicoid protein, but it also contains binding sites for maternally provided Hunchback protein. These two transcription factors interact in a cooperative manner to create a threshold of autoactivation that results in a stripe of late *hb* expression in a mid-body region known as parasegment 4 (PS4).

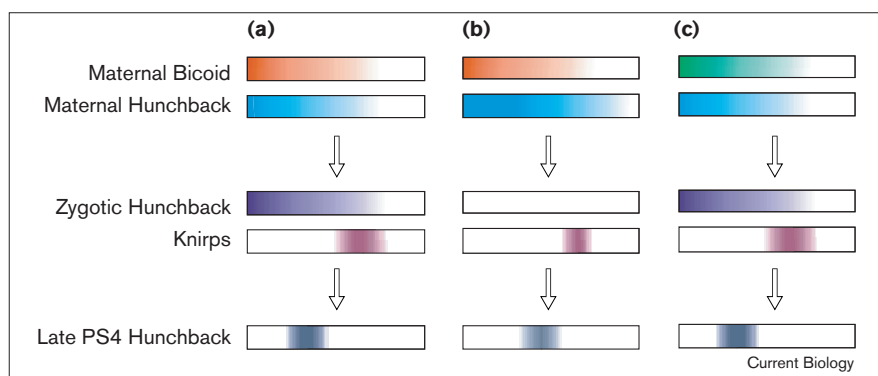
Wimmer *et al.* [6] used two different manipulations (Figure 2) to boost the zygotic expression of *hb* in the PS4 stripe, independently of Bicoid activity. This resulted in at least partial rescue of aspects of thoracic development in a *bcd* mutant, implying that *hb* does not absolutely require Bicoid for this function. Hence, there is nothing magical about *bcd*, rather it has just evolved the capacity to regulate *hb* in dipterans, displacing the function of other genes such as *hb* itself. The intriguing story of where this fly version of *bcd* might have come from, namely a duplicate copy of the dorsal anterior determinant *zerknüllt*, is told in a recent dispatch by Dearden and Akam [17].

Whatever the times of origin of *ftz* and *bcd* turn out to be, they provide incontrovertible evidence that the earliest elements of developmental regulatory pathways are evolutionarily labile. Within the arthropods, alternating stripes of 'segment polarity' genes — those segmentation genes expressed with a one-segment periodicity — come to mark segmental boundaries by a variety of different genetic mechanisms. So long as the external form does not change, the internal genetic elements are free to change.

Insight into how genetic turnover is tolerated at the level of the regulation of gene expression has come from an ongoing dissection of the stripe 2 enhancer of the 'pair-rule' gene *even-skipped* (*eve*) by Ludwig, Kreitman and co-workers [18].

**Figure 2**

Demonstration of the ability of Hunchback (Hb) to organize thoracic pattern. Each box represents the anteroposterior axis of a *Drosophila* embryo (anterior to the left), with colored gradients indicating domains of protein expression. In wild-type embryos (a), maternal Bicoid (Bcd) and Hb gradients combine to drive expression of Hb from a zygotic promoter. This expression then contributes to the activation of a late stripe of Hb in parasegment 4 (PS4), the posterior limit of which is set by Knirps protein. After eliminating the zygotic Hb promoter (b), Wimmer *et al.* [6] still managed to generate weak late PS4 expression by increasing the dosage of maternal Hb and reducing the dosage of Knirps. This resulted in partial rescue of thoracic segments, showing that much of the role



of Bcd in the thorax is mediated through Hb. They achieved even stronger rescue of thoracic segmentation after replacing

Bicoid (c) with a GAL4 driver that was used to express zygotic Hb in the anterior half of the embryo.

The pair-rule segmentation genes are expressed with a two-segment periodicity, and specific regulatory elements have been defined that confer their expression in specific stripes. The core of the *eve* stripe 2 enhancer is less than a kilobase in length [19], yet it directs expression of a *lacZ* reporter gene in the blastoderm of transgenic flies precisely in the position of the second of seven stripes. The homologous sequence isolated from a wide variety of other *Drosophila* species generates more or less the same pattern, albeit with subtle variation in timing, intensity and stripe resolution [20]. The binding sites for numerous *trans*-acting transcription factors in the *eve* enhancer region, including Hunchback, show a surprisingly high level of polymorphism within and between species, although the pattern of variation fits models of neutral molecular evolution [21].

Strikingly, Ludwig *et al.* [18] found that chimeric *eve* stripe 2 enhancers, made from alternate halves of the *D. melanogaster* and *D. pseudoobscura* enhancers, failed to generate normal stripes. One combination led to expansion of the stripe on both sides, while the other shifted it two cells to the posterior end of the embryo, so that it abutted the normal stripe 3. These observations imply that mutations have accumulated in the 40–60 million years since these species diverged that have little combined effect on function of the enhancer, but in the absence of compensatory substitutions elsewhere in the enhancer render the element mis-functional [18]. Stabilizing selection appears to act to retain the phenotype, but allows for substitution of tolerated individual sites.

The fascinating question that must be addressed if micro- and macro-evolutionary studies are to be connected is ‘what is the driving force for such hidden renovation?’ Developmental biologists often take their cue from a history of adaptationist evolutionary thinking, in supposing that there must be some advantage to observed

changes, perhaps in relation to developmental rates and/or yolk content and embryonic size. In general, though, selection should only be invoked when the null hypothesis of neutrality cannot explain the data. As with cellared wines, this internal change may be inevitable. Given variation in a system, change should occur simply by random assortment of the variation [22], and in fact phenotypic change is often observed to occur more slowly than predicted given levels of intraspecific variation [23].

The balance of mutation pressure and genetic drift in the context of genetic networks as complex as those that orchestrate early development has barely been explored, either by theory or empirical study, and cannot be excluded as the predominant mode of renovation. Nor should a compromise position be ignored, namely that selection on pleiotropic functions of regulatory genes may drive the internal evolution of embryonic patterning mechanisms. Dorsal-ventral determinants are known to be reused in the *Drosophila* immune system; signal transduction mechanisms affect wing shape and eye development; gap and pair-rule genes are involved in neurogenesis and many other processes. The demonstration that there is uncoupling of phenotypic and genetic evolution presents one of the most profound challenges to neo-Darwinian thought since the development of the neutral theory of molecular evolution in the early 1970s. Once again, *Hox* genes are being found to have far-reaching ramifications.

## References

- Palopoli MF, Wu C-I: **Genetics of hybrid male sterility between *Drosophila* sibling species: a complex web of epistasis is revealed in interspecific studies.** *Genetics* 1994, **138**:329-341.
- Félix M-A: **Evolution of developmental mechanisms in nematodes.** *J Exp Zool (MDE)* 1999, **285**:3-18.
- Marin I, Baker BS: **The evolutionary dynamics of sex determination.** *Science* 1998, **281**:1990-1994.
- Patel NH: **Developmental evolution: insights from studies of insect segmentation.** *Science* 1998, **266**:581-590.

5. Telford M: Evidence for the derivation of the *Drosophila fushi tarazu* gene from a Hox gene orthologous to lophotrochozoan *Lox5*. *Curr Biol* 2000, **10**:349-352.
6. Wimmer E, Carleton A, Harjes P, Turner T, Desplan C: *bicoid*-independent formation of thoracic segments in *Drosophila*. *Science* 2000, **287**:2476-2479.
7. Kuroiwa A, Hafen E, Gehring WJ: Cloning and transcriptional analysis of the segmentation gene *fushi tarazu* of *Drosophila*. *Cell* 1984, **37**:825-831.
8. Hafen E, Kuroiwa A, Gehring WJ: Spatial distribution of transcripts from the segmentation gene *fushi tarazu* during *Drosophila* embryonic development. *Cell* 1984, **37**:833-841.
9. Weiner AJ, Scott MP, Kaufman TC: A molecular analysis of *fushi tarazu*, a gene in *Drosophila melanogaster* that encodes a product affecting embryonic segment number and cell fate. *Cell* 1984, **37**:843-851.
10. Brown SJ, Hilgenfeld RB, Denell RE: The beetle *Tribolium castaneum* has a *fushi tarazu* homolog expressed in stripes during segmentation. *Proc Natl Acad Sci USA* 1994, **91**:12922-12926.
11. Beeman RW, Stuart JJ, Haas MS, Denell RE: Genetic analysis of the homeotic gene complex (HOM-C) in the beetle *Tribolium castaneum*. *Dev Biol* 1989, **133**:196-209.
12. Dawes R, Dawson I, Falciani F, Tear G, Akam M: *Dax*, a locust Hox gene related to *fushi tarazu* but showing no pair-rule expression. *Development* 1994, **120**:1561-1572.
13. de Rosa R, Grenier JK, Andreeva T, Cook CE, Adoutte A, Akam M, Carroll SB, Balavoine G: Hox genes in brachiopods and priapulids: implications for protostome evolution. *Nature* 1999, **399**:772-776.
14. Stauber M, Jäckle H, Schmidt-Ott U: The anterior determinant *bicoid* of *Drosophila* is a derived Hox class 3 gene. *Proc Natl Acad Sci USA* 1999, **96**:3786-3789.
15. Driever W, Nusslein-Volhard C: The bicoid protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell* 1988, **54**:95-104.
16. Wolff C, Schröder R, Schult C, Tautz D, Klingler M: Regulation of the *Tribolium* homologues of *caudal* and *hunchback* in *Drosophila*: evidence for maternal gradient systems in a short germ embryo. *Development* 1998, **125**:3645-3654.
17. Dearden P, Akam M: Developmental evolution: Axial patterning in insects. *Curr Biol* 1999, **9**:R591-R594.
18. Ludwig MZ, Bergman C, Patel NH, Kreitman M: Evidence for stabilizing selection in a eukaryotic enhancer element. *Nature* 2000, **403**:564-567.
19. Stanojevic D, Small S, Levine M: Regulation of a segmentation stripe by overlapping activators and repressors in the *Drosophila* embryo. *Science* 1991, **254**:1385-1387.
20. Ludwig MZ, Patel NH, Kreitman M: Functional analysis of eve stripe 2 enhancer evolution in *Drosophila*: rules governing conservation and change. *Development* 1998, **125**:949-958.
21. Ludwig MZ, Kreitman M: Evolutionary dynamics of the enhancer region of *even-skipped* in *Drosophila*. *Mol Biol Evol* 1995, **12**:1002-1011.
22. Lande R: Expected time for random genetic drift of a population between stable phenotypic states. *Proc Natl Acad Sci USA* 1985, **82**:7641-7645.
23. Spicer GS: Morphological evolution of the *Drosophila virilis* species group as assessed by rate tests for natural selection on quantitative characters. *Evolution* 1993, **47**:1240-1254.

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