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Morphological Diversity: Taking the Spine out of Three-Spine Stickleback

Dispatch

Cheryll Tickle[1*] and Nicholas J. Cole[1,2]

A gene already known to play a crucial developmental role in chick and mouse embryos has been fingered as a candidate for naturally occurring variation in three-spine stickleback anatomy.

One of the most challenging problems in biology is to understand the basis for the marvellous morphological diversity of plants and animals. Three-spine stickleback come in a wide range of phenotypes with many anatomical variations [1,2]. Two recent papers have now reported the mapping of a major gene locus [3,4] that is associated with loss of pelvic spines and girdle — equivalent to loss of hindlimbs — and one of these papers [3] goes on to report the identification of a candidate gene in this locus. This gene, *Pitx1*, is already known from studies in chick and mouse embryos to play a crucial role in hindlimb development [5]. Sequence data [3] and gene expression studies [3,6] are consistent with the idea that a change in a regulatory region controlling *Pitx1* expression in the developing pelvis is responsible for spine and girdle deficiency. Loss of pelvic structures has occurred, independently, in several natural populations of stickleback. Remarkably, these new data from Shapiro *et al.* [3] and Cresko *et al.* [4] implicate the same genetic locus in different populations. In fact, other aspects of stickleback anatomy are also now coming under the spotlight [4,7]. The flurry of publications on the genetics and embryology of three-spine stickleback has established this fish as a premier model for studying variation in vertebrates.

The skeleton of three-spine stickleback, including pelvic spines and associated pelvic girdle, is reduced to varying extents in different populations [1]. Various explanations for the loss of spines have been suggested including lack of predator pressure and reduced levels of calcium. At one end of the spectrum are marine stickleback — very spiny fish, with a pair of pelvic spines articulating with a robust pelvic girdle (Figure 1A–C); at the other end, freshwater stickleback totally lacking pelvic spines and girdle (Figure 1D–G); and in between, a range of phenotypes showing varying degrees of pelvic reduction.

Shapiro *et al.* [3] and Cresko *et al.* [4] crossed fully spined female stickleback with male stickleback completely lacking pelvic structures. Genetic mapping of offspring of both crosses located a single locus with a major influence on pelvic reduction. In order to identify candidate genes, Shapiro *et al.* [3] took note of the gene cascade involved in the initiation of hindlimb development that had been identified in chick and mouse embryos [5]. At least some of these genes are known to be conserved in paired fin development in zebrafish [8]. They then tested whether any of these genes map to the region responsible for pelvic reduction. Quite remarkably, the *Pitx1* gene is located in this region and is tightly linked with pelvic reduction.

Pitx1 was first identified in screens for novel homeodomain factors in mouse and human [9] and was called *backfoot* because it is expressed in hindlimbs but not forelimbs (Figure 2). Other genes encoding transcription factors of the *Tbx* family are also expressed in limb-type specific patterns, with *Tbx4* specifically in hindlimbs, and the related gene *Tbx5* specifically in forelimbs. *Tbx4* appears to be downstream of *Pitx1* [5] (Figure 2). An intriguing feature of hindlimbs of *Pitx1*-deficient mice is that they are frequently asymmetrical with right femur being shorter than left [10]. This characteristic resonates with the fact that stickleback pelvic reduction is also frequently asymmetrical, showing enhanced reduction on the right (Figure 1G), and indeed strengthens the candidacy of *Pitx1*.

Asymmetry in hindlimb development in *Pitx1*-deficient mice has been ascribed to compensation by the related gene *Pitx2*, which is involved in establishing left–right asymmetry and is expressed preferentially on the left of early embryos (Figure 2). When the dosage of both *Pitx* genes is reduced in mice, the limbs are more severely affected [10]. The fact that *Pitx* gene dosage in mice leads to a range of different phenotypes fits with the varying degrees of pelvic reduction seen in stickleback populations. In stickleback, however, *Pitx2* does not appear to be expressed in pelvic regions at the time girdle and spines are developing and thus seems unlikely to compensate for *Pitx1* [6] (Figure 2).

Shapiro *et al.* [3] sequenced *Pitx1* in both spined and pelvic-reduced fish and found that the coding sequence is the same in fish of both phenotypes. Furthermore, although *Pitx1* expression cannot be detected in the pelvic region of spineless fish (Figure 2), *Pitx1* is expressed in other sites, such as thymus and lips, as it is in spined stickleback. One explanation is that different *cis*-regulatory elements govern *Pitx1* expression in different anatomical sites and that pelvic reduction is due to a mutation in the element that drives *Pitx1* expression specifically in this region. The simplicity of this model is attractive. It will be important to identify the predicted regulatory element and determine how it acts.

One of the fascinating features of pelvic reduction is that the same anatomical change has evolved independently in different populations of stickleback — in Canada, Iceland, Alaska and Outer Hebrides. So is the genetic basis the same in these different fish? There are strong hints that the answer is yes. Shapiro *et al.* [3] and Cresko *et al.* [4] used pelvic-reduced fish from different locations for their crosses but nevertheless pinpointed the same major genetic locus. Classical genetic tests, crossing pelvic-reduced fish from different populations,

[1]Division of Cell and Developmental Biology, Wellcome Trust Biocentre, University of Dundee, Dundee DD1 5EH, Scotland, UK. *E-mail: c.a.tickle@dundee.ac.uk [2]Victor Chang Cardiac Research Institute, Darlinghurst, Sydney, NSW 2010, Australia.

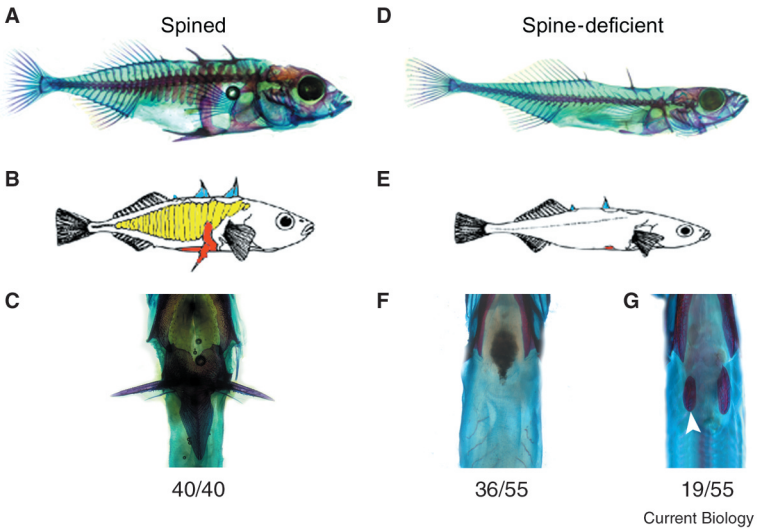

Figure 1. Extreme stickleback phenotypes. (A–C) Spined fish with pelvic spines and girdle from estuary in Kent. (A) Side-view of whole mount stained with Alcian Blue (cartilage) and Alizarin Red (bone) to show skeleton. (B) Diagram highlighting pelvic spine and girdle (red), lateral plates (yellow), and dorsal spines (blue). (C) Ventral view of pelvic girdle showing spines and girdle. All 40 fish examined had this phenotype. (D–G) Spine-deficient fish with pelvic reduction from Outer Hebrides. (D) Side-view of whole mount stained as in (A). (E) Diagram highlighting pelvic structures (red), complete absence of lateral plates, and dorsal spines (blue, two). (F,G) Ventral views with complete absence of girdle (F) and remnants of girdle (G). Note asymmetry in pelvic reduction, which is more marked on the right (arrowhead). Numbers of fish with phenotype/total number of fish from the population examined are shown in (C,F,G).

performed by both groups, also failed to restore pelvic development strongly suggesting that the same gene is involved. Furthermore, *Pitx1* expression (and, presumably as a direct consequence, *Tbx4* expression) is undetectable in stickleback with reduced pelvic structures collected from yet another separate population (Figure 2). A key question is why this particular gene in the pathway appears to have been the instrument of anatomical variation rather than say *Tbx4*, which is also expressed in hindlimb but not forelimb.

The recent focus on three-spine stickleback is leading to insights into other anatomical variations. Marine stickleback, in addition to having robust pelvic structures, have extensive body armour, consisting of lateral plates down each side of the body, which are also absent in pelvic-reduced freshwater relatives (Figure 1). Recent papers report progress in mapping a major gene locus affecting lateral plates [4,7]. The locus is in a different linkage group to *Pitx1*, so another gene must be involved. Again, as with pelvic reduction, the data suggest that the same gene locus is involved in loss of lateral plates in different populations of fish. Stickleback have many other variable traits including those affecting morphology — body size, snout shape, number of gill rakers, physiology and behaviour-learning, personality, and sexual selection. Stickleback spines are just the tip of exploring a wealth of diversity.

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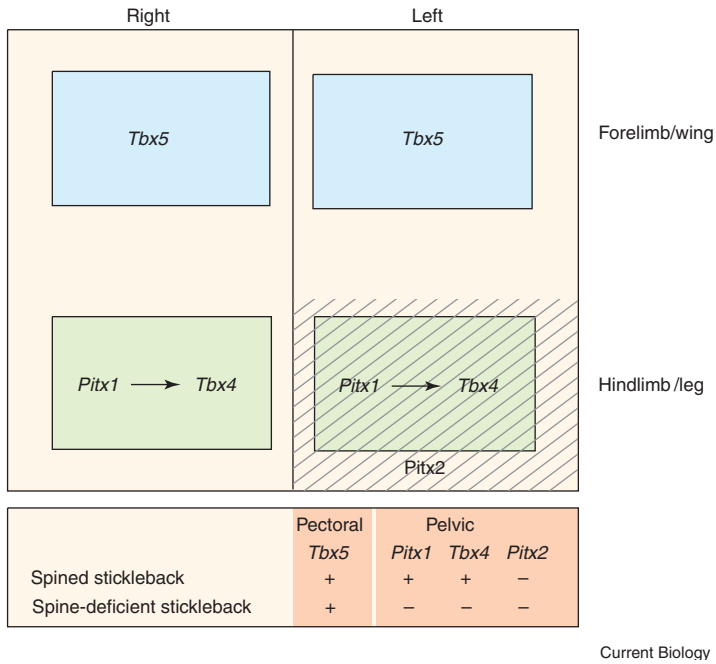

Figure 2. Ventral view schematic diagram showing expression of genes in forelimb/wing and hindlimb/leg regions of mouse and chick embryos, respectively, and a table showing the expression of these genes in fin-forming regions of larvae of spined and spine-deficient (pelvic-reduced) stickleback shown in Figure 1. Note that *Pitx2* is expressed widely in the posterior left of both mouse and chick embryos. The table shows that *Tbx5* is expressed in pectoral fin buds in both spined and spine-deficient fish. *Pitx1* and *Tbx4* are expressed in pelvic fin buds in spined stickleback but there is no trace of expression of these genes in the pelvic regions in spine-deficient fish. *Pitx2* expression is not detected in either pelvic fin buds of spined fish or in pelvic regions of pelvic-reduced stickleback.

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