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Although it has been hypothesised that cis-regulatory mutations contribute significantly to natural evolution, clear demonstration of a causative mutation has proven to be challenging. Here we demonstrate how cis-regulatory mutations can contribute to pelvic reduction in three-spined sticklebacks. Pelvic reduction is a major skeletal alteration that has evolved repeatedly in different animals, and is genetically tractable in sticklebacks. Previous genetic and expression studies have linked pelvic reduction to unknown regulatory changes near the major developmental control gene Pitx1. Here we have characterised its molecular basis using both laboratory crosses and natural stickleback populations. We conducted association mapping in a natural population segregating pelvic reduction using microsatellite markers flanking Pitx1. We identified a conserved non-coding region consistently associated with pelvic reduction. Functional transgenic assays demonstrated tissue-specific enhancer function: it drives reporter gene expression specifically in the developing pelvis of stickleback larvae. Introduction of a transgene containing the pelvic-complete enhancer driving a Pitx1 coding region restores pelvic formation in pelvic-reduced sticklebacks. Identification of this key region allows detailed comparison of the molecular basis of pelvic reduction in different populations. Genotyping and sequencing studies revealed distinct deletions occurring on different pelvic-reduced haplotypes. Together, these haplotypes defined a 481bp interval and show patterns consistent with fixation following selective sweeps in the same region. The current work identifies how parallel mutations in cis-regulatory sequences can contribute to evolution through inactivation of a tissue specific enhancer of the Pitx1 gene, giving rise to a major adaptive alteration in the vertebrate skeleton.

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## S14-04

Pecking at the origin of vertebrate diversity: Insights from the beak of the Finch <u>Arkhat Abzhanov</u>

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The faces of vertebrates are often readily recognizable as they display a number of species-specific characteristics. It is likely that this stunning diversity of cranial morphology in vertebrates was generated by alterations in craniofacial development. We are employing a combination of genetic, genomic, molecular, bioinformatic, 2D and 3D imaging and modeling approaches to understand evolution of craniofacial structures, such as highly adaptive beak morphologies in such species as Darwin's Finches (a classic example of species multiplication and diversification caused by natural selection) and their relatives, the African Seedcrackers Pyrenestes ostrinus (textbook example of adaptive polymorphism), and other avian and reptilian species. The major goal of these studies is to use both novel approaches on well-studied evolutionary systems to address some of the long-standing questions in animal development and evolution.

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## S14-05

Genetic dissection of pollination syndromes in Petunia <u>Cris</u> <u>Kuhlemeier</u>, Ulrich Klahre, Alexandre Dell'Olivo, Julien Venail, Eligio Bossolini

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Animal-mediated pollination is essential in the reproductive biology of many flowering plants and tends to be associated with pollination syndromes, sets of floral traits that are adapted to particular groups of pollinators. The complexity and functional convergence of various traits within pollination syndromes are outstanding examples of biological adaptation, raising questions about their mechanisms and origins. Elucidation of the molecular-genetic basis of this interesting reproductive biology requires a model system that combines distinct pollination syndromes with excellent molecular and classical genetics. In the genus Petunia, complex pollination syndromes are found for nocturnal hawkmoths (P. axillaris), diurnal bees (P. integrifolia) and hummingbirds (P. exserta), with characteristic differences in petal color, corolla shape, reproductive organ morphology, nectar quantity, nectar quality and fragrance. We dissected the Petunia syndromes into their most important phenotypic and genetic components. Several quantitative trait loci were identified for each syndrome component. Using petal color as an example, we show that a polymorphism in a single gene can have a major effect on pollinator preference. The combined data provide a framework for a detailed understanding of floral syndromes, from their developmental and molecular basis to their impact on animal behavior.

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