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Neurogenetics of birdsong

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Songbirds are a productive model organism to study the neural basis of auditory-guided vocal motor learning. Like human babies, juvenile songbirds learn many of their vocalizations by imitating an adult conspecific. This process is a product of genetic predispositions and the individual's life experience and has been investigated mainly by neuroanatomical, physiological and behavioral methods. Results have revealed general principles governing vertebrate motor behavior, sensitive periods, sexual dimorphism, social behavior regulation and adult neurogenesis. More recently, the emerging field of birdsong neurogenetics has advanced the way we think about genetic contributions to communication, mechanistically and conceptually.

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Introduction

The power of genetic model organisms, foremost *Drosophila melanogaster*, *Caenorhabditis elegans*, *Danio rerio* and *Mus musculus* is unsurpassed when it comes to linking the activity and inheritance of genes to neural physiology and behavior. However, learning how to produce communication sounds, a behavior that humans, parrots, songbirds, and hummingbirds excel at, is apparently not part of the behavioral repertoire of fruitflies, roundworms, zebrafish and lab mice.

Songbirds have provided mechanistic insights into how a sender codes complex sound sequences as behaviorally relevant signals, how a receiver decodes these, and how young songbirds develop these skills [1,2]. Prerequisite for these discoveries was extensive knowledge about the underlying neural circuits, collectively called 'song system', pioneered by Nottebohm and Konishi [3,4]. Less is known about the molecular substrates needed for perception, production and learning of song [1], about the genetics of natural variation in song behavior [5••,6,7], and about the genetic changes during the evolution of the song system [8,9•]. Neurogenetic information in these areas will help to decipher complex traits including language and music and their pathologies.

Here we summarize genetic resources in songbirds, discuss descriptive gene expression and genome wide association studies and highlight the first gene-function studies that point toward future promising open research questions.

Suitability of songbirds as genetic model organism

The neurogenetics of birdsong is primarily studied in zebra finches (*Taeniopygia guttata*), because they reproduce year-round in captivity with 3–5 chicks per clutch and a short generation time. In their natural Australian habitat they form large flocks [10] and in captivity mixed sex groups maintain a rich social behavior repertoire [11]. They have not been selectively bred for particular behaviors, but many colormorphs exist with potentially linked neural and behavioral differences [12].

Advances in DNA sequence information

Reports of the first songbird cDNA sequences in the mid-nineties [13–15] were followed by EST-sequence (Box 1) information [16] and development of other genetic tools [16]. Microarrays yielded the first global gene expression studies [7,16–20,21••,22,23], revealing genes differentially expressed in the song system, either seasonally, sex-specifically, or as a consequence of singing. Through a significant community research effort the zebra finch genome was sequenced [24•], followed by the parrot genome [25]. Since molecular phylogenetic evidence supports parrots as a sister group of songbirds [26,27], the comparison of zebra finch and budgerigar genomes will add resolution to the genetic dissection of traits common to both species, including vocal learning. Continued annotation, filling of the gaps and describing the epigenetic landscape of these genomes are important current efforts.

Population genetic approaches can also uncover behaviorally relevant candidate genes. Basically a set of DNA-markers (typically microsatellites or Short Nucleotide Polymorphisms, SNPs (Box 1)) is investigated in many individuals that vary phenotypically in a particular trait. If a region of the genome is not linked to the behavior in question, the alleles are expected to show a random distribution, whereas genomic regions linked to

Box 1

BAC-transgenesis:

Bacterial Artificial Chromosomes consist of large (100–200 kb) pieces of DNA containing both regulatory and coding region(s) of genes. After introduction into a host organism (e.g. via homologous recombination) the regulatory regions usually restrict the introduced 'trans-' gene expression to specific cell types.

ChIP:

Chromatin Immunoprecipitation (ChIP) is a method to identify DNA fragments bound by a particular transcription factor.

EST-sequences:

Expressed Sequence Tag. Short (500–800 bp) partial sequence of a cDNA sequence, usually reflecting (part of) a particular transcript of a unique gene.

Forward and reverse genetics:

Forward genetics refers to an experimental approach that seeks to identify the genetic basis of a particular phenotype. Here, phenotypes are altered experimentally (e.g. via mutagenesis or selective breeding) to map the underlying genetic basis.

Reverse genetics refers to the inverse experimental approach that analyzes the effects of manipulating specific genes on a phenotype.

HVC:

HVC is used as proper name for a forebrain nucleus of the passerine song system. HVC is part of the descending motor pathway (including but not limited to song nuclei HVC, RA and motorneurons of the vocal organ) and the anterior forebrain pathway (including HVC, Area X, DLM, lMAN and RA).

In situ hybridization:

Method to visualize the spatial distribution of transcripts in a tissue. RNA probes are hybridized to the tissue and either detected via radioactive labeling or via an enzymatic reaction.

iPSCs:

Induced Pluripotent Stem Cells that are derived from somatic cells via reprogramming. This is usually achieved by artificially inducing the expression of certain genes, often transcription factors.

Lentiviral vectors:

Lentiviruses are a genus of the *Retroviridae*. They are commonly used to insert a transgene into nondividing cells. Their capacity (i.e. the size of the RNA that can be packaged) is limited to approximately 10 kb.

Microsatellites:

They consist of 2–6 base pairs that naturally occur repeatedly at irregular intervals throughout the genome. In population genetics these stretches are used as markers to analyze the heritability of a trait.

PGCs:

Primordial Germ Cells. PGCs are the cells during embryonic development that give rise to the germline.

QTL:

Quantitative trait loci are stretches on the DNA of an organism that code for the genes underlying a certain trait that varies in degree in different individuals of the same species. Identification of the genes in such a region is the first step to uncover genes affecting the trait (e.g. a behavior).

RADSeq:

Restriction-site associated DNA-sequencing. Genomic DNA is digested with a restriction enzyme of choice and the resulting fragments are sequenced using Next Generation Sequencing. Each fragment represents one potential marker for population genetic studies.

siRNA:

Small interfering RNAs are 21nt long double stranded pieces of RNA that promote sequence specific degradation of their target RNA.

SNPs:

Single Nucleotide Polymorphisms are DNA-sequence variations where a single nucleotide differs between alleles. SNPs are useful to conduct population genetic studies.

a behavior should show association of certain alleles with trait characteristics. Candidate genes, probably influencing the trait, cluster around the marker. Several aspects of zebra finch song (e.g. mean frequency) show heritabilities [5••], but population genetic studies investigating song learning are still lacking. The power of the population genetic approach is exemplified by a study that used 1404 informative SNP in zebra finches and a recently published linkage map [28] to identify quantitative trait loci (QTL, Box 1) for beak morphology. Many genes known to affect