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[zfin.org](http://zfin.org)

## Mutant Manifests: Toward a Zebrafish Phenotype Ontology

- ZFIN is developing a Zebrafish Phenotype Ontology in collaboration with the Phenotype Ontology Consortium
- Goal - to annotate zebrafish mutant phenotypes in a way that facilitates cross-species analysis of gene function in embryonic development
- the rising tide of zebrafish mutant data needs to be accommodated in a useful way
  - many mutants, many more coming soon in new mutant screens
  - full genome sequence expected within two years - many new genes expected
  - extensive, systematic morpholino gene- knockdown screens are now beginning - expected to produce thousands of mutant-like phenotypes

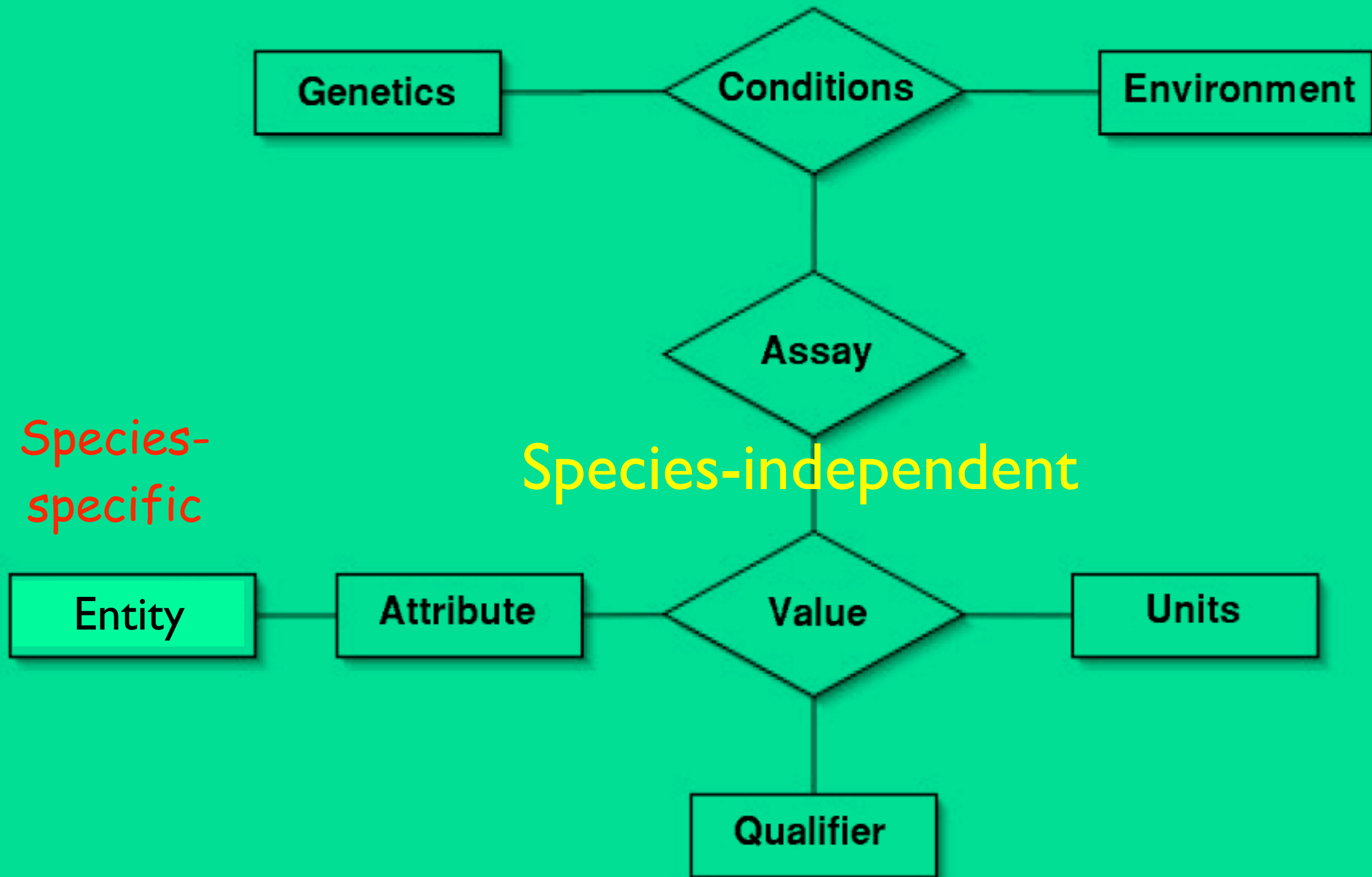
Milwaukee Curator Meeting 28 October 2003

## example queries - how, in detail, does a mutant differ from wild type ?

- Mutations in which orthologous genes result in vertebral duplications in mouse, human and zebrafish ?
- Mutations in which genes result in over-production of spinal motoneurons? Which of these genes are normally expressed in the notochord ? Which are kinases ? Same phenotype for orthologues in other vertebrates?
- How frequently do mutations with malformed inner ears also exhibit malformed kidneys ?
- Do zebrafish mutants with increased life-spans have decreased heart-rates ? Is this the case in other vertebrates? Flies?



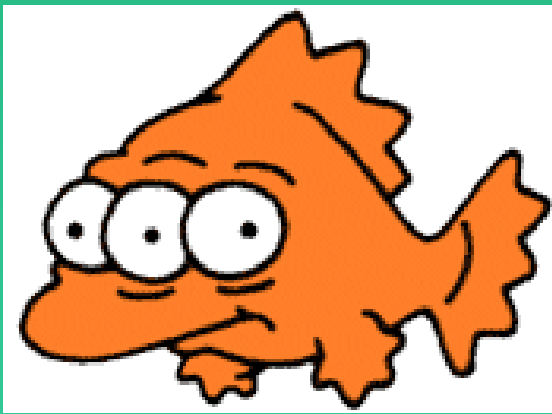
# The PATO way





# basic curation of phenotypes

- 1) select **entity** from zebrafish **AO (Anatomical Ontology)** -
  - The **AO**: ZFIN has developed an ontology of ~1200 anatomical structures and a growing ontology of behavioral and physiological processes. To facilitate cross-species comparisons, as many terms as possible are shared between zebrafish and mouse. Homologous structures like fins and limbs are cross-listed. We continue to add terms as needed to describe gene expression patterns and mutant phenotypes, and encourage input from the research community.
- 2) use **PATO** (Phenotype and Trait Ontology) to find **attribute, value**, etc.



"blinky" mutant, a simple, but non-trivial example

entity

eye



cornea

lens

retina

retinal pigmented epithelium

inner ear

otolith organs

lagena

asteriscus

macula lagena

macula

macula neglecta

sensory hair cells

saccule

sagitta

utricle

lapillus

macula utricule

semicircular canals

caudal vertical

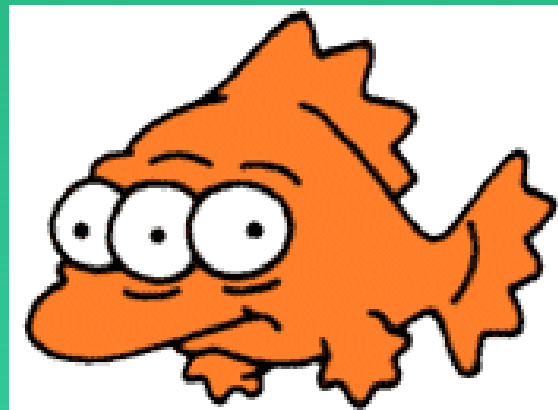
caudal crista

horizontal

lateral crista

rostral vertical

rostral crista



annotated "blinky" phenotype

entity

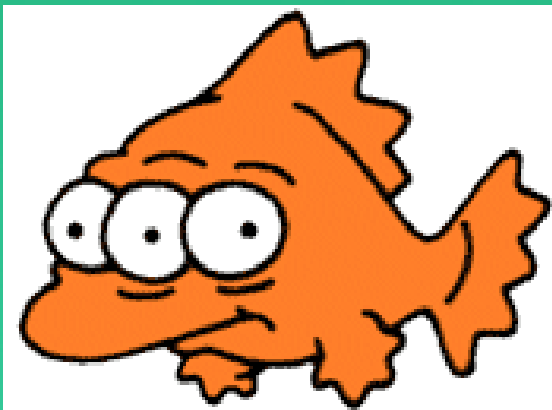
- eye ←
- cornea
- lens
- retina
- retinal pigmented epithelium
- inner ear
- otolith organs
- lagena
- asteriscus
- macula lagena
- macula
- macula neglecta
- sensory hair cells
- sacculle
- sagitta
- utricle
- lapillus
- macula utricule
- semicircular canals
- caudal vertical
- caudal crista
- horizontal
- lateral crista
- rostral vertical
- rostral crista

PATO attributes

- [-] **ISA** pato\_ontology
  - [-] **ISA** attribute:qualitative
    - [-] **ISA** attribute:specific\_qualitative
      - [+] **ISA** attribute:activity
      - [+] **ISA** attribute:aspect\_of\_mass
      - [+] **ISA** attribute:color
      - [+] **ISA** attribute:compatability
      - [+] **ISA** attribute:composition
      - [+] **ISA** attribute:concentration
      - [+] **ISA** attribute:consistency
      - [+] **ISA** attribute:disease\_sensitivity
      - ISA** attribute:flavor
      - [+] **ISA** attribute:function
      - [+] **ISA** attribute:hardness
      - ISA** attribute:life\_span
      - [+] **ISA** attribute:morphology
      - [+] **ISA** attribute:number
      - [+] **ISA** attribute:nutritional
      - [+] **ISA** attribute:occurence
      - ISA** attribute:odor
      - [+] **ISA** attribute:pattern
      - [+] **ISA** attribute:pilosity
      - [+] **ISA** attribute:quality
      - [+] **ISA** attribute:sensitivity
      - [+] **ISA** attribute:spatial
      - [-] **ISA** attribute:structure ←

PATO viewed with  
DAG-Edit 1.320





annotated "blinky" phenotype

## entity

eye ←

- cornea
- lens
- retina
- retinal pigmented epithelium
- inner ear
- otolith organs
  - lagena
    - asteriscus
    - macula lagena
  - macula
  - macula neglecta
    - sensory hair cells
  - saccule
  - sagitta
  - utricle
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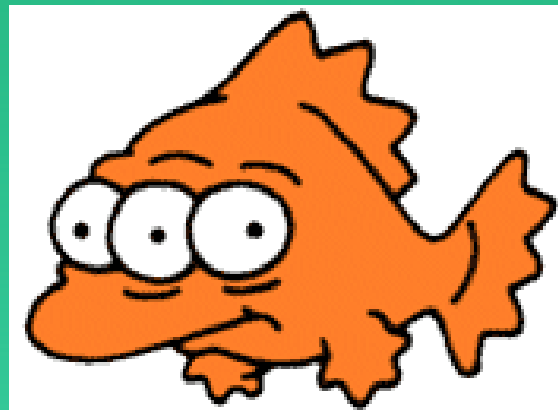
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```

## PATO values

```
[-] isa attribute:structure
  [PARTOF] value:apoptotic
  [PARTOF] value:degenerate
  [PARTOF] value:deposition_defective
  [PARTOF] value:dysplastic
  [PARTOF] value:ectopic ←
  [PARTOF] value:fused
  [PARTOF] value:herniated
  [PARTOF] value:hyperplastic
  [PARTOF] value:hypertrophied
  [PARTOF] value:hypoplastic
  [PARTOF] value:hypotrophied
  [PARTOF] value:malformed
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  [PARTOF] value:obstructed
  [PARTOF] value:perforated
  [PARTOF] value:supernumerary
  [PARTOF] value:unfused
```

PATO viewed with  
DAG-Edit 1.320



annotated "blinky" phenotype

## entity

- eye** ←
- [cornea](#)
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Both values hold -  
illustrates need for  
consistent curation  
guidelines and smart  
search algorithms

The real thing - a zebrafish embryo at a late embryonic stage

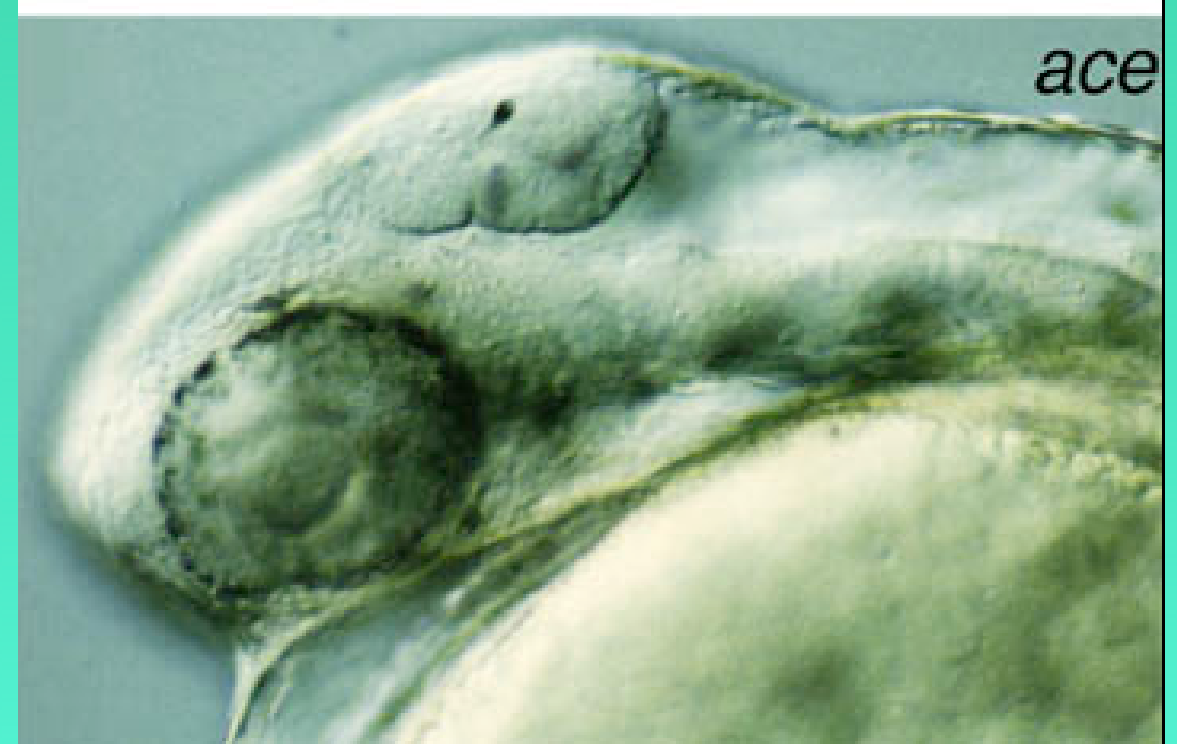
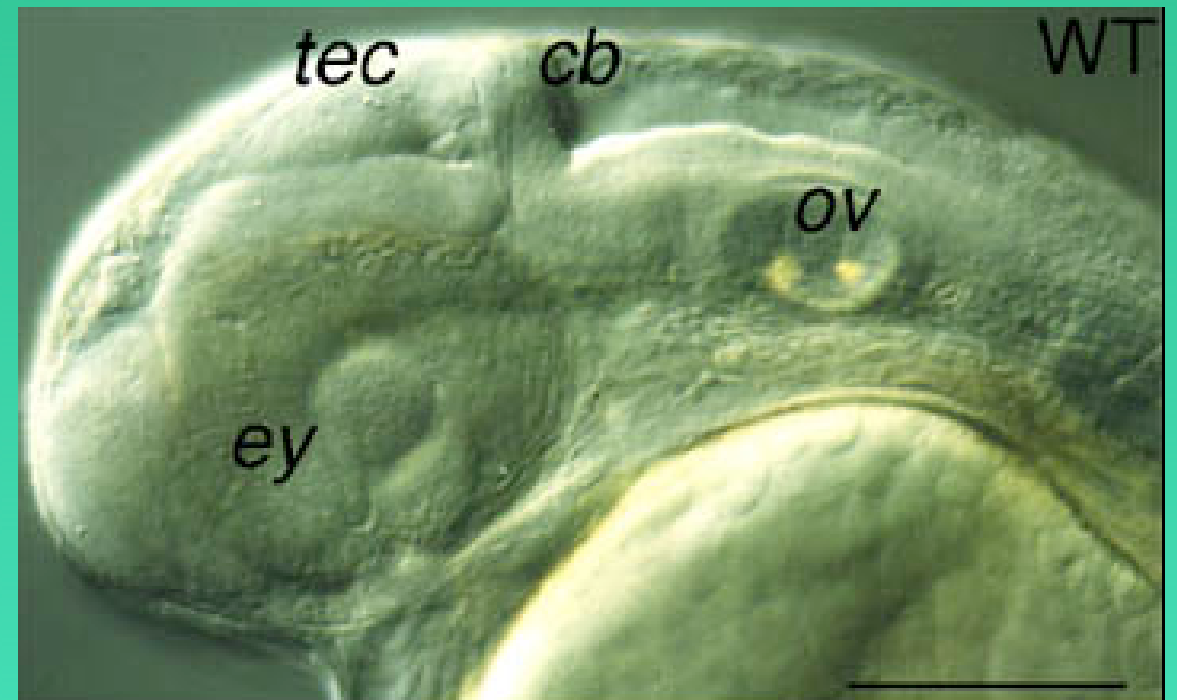




# A realistic example of phenotype annotation - the acerebellar (*ace*) mutant

## Some of the major phenotypes

- cerebellum is absent
- midbrain-hindbrain boundary is absent
- enlarged tectum
- small ear, eye
- cardiac ventricle is severely reduced



ZFIN ID: ZDB-FISH-980202-822

[Add/Update this Record](#)[Delete or Merge this record](#)[Updated: Sep 3, 2003](#)Name: [acerebellar](#)

Your Input Welcome

Abbreviation: *ace*<sup>ti282a</sup>

(Record Owner is Driever, Wolfgang)

Previous names:

Affected Gene:

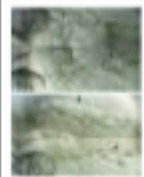
**Cloned Gene:** [fibroblast growth factor 8 \(fgf8\)](#) has been shown to correspond to the *acerebellar* locus. ( [Reifers et al, 1998](#) )

## Images:

Dev. Stage(s): [Segmentation](#) : [5-somite](#)

Preparation: live

Orientation: side view, anterior to left

Dev. Stage(s): [Pharyngula](#) : [Prim-15](#)

Preparation: live

Orientation: dorsal, anterior to left

Dev. Stage(s): [Pharyngula](#) : [Prim-25](#)

Preparation: live

Orientation: side view, anterior to left

Keywords: nervous system;brain;midbrain;midbrain hindbrain boundary;eyes / ears;ear;organs;

## ORIGINS AND AVAILABILITY



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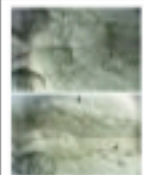
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Orientation: dorsal, anterior to left

Dev. Stage(s): [Pharyngula](#) : [Prim-25](#)

Preparation: live

Orientation: side view, anterior to left

Mutant phenotypes are currently annotated using a small, un-staged subset of the anatomical ontology - (this is a serious limitation)

Keywords: nervous system;brain;midbrain;midbrain hindbrain boundary;eyes / ears;ear;organs;

## ORIGINS AND AVAILABILITY

# Annotating affected structures using the full zebrafish Anatomical Ontology

prim-25 stage embryo (36-42 hrs)

nervous system

central nervous system

brain

forebrain

diencephalon

epiphysis

floor plate diencephalon

hypophysis

hypothalamus

lateral wall diencephalon

optic stalk

postoptic commissure

telencephalon

floor plate telencephalon

lateral wall telencephalon

optic recess

third ventricle

hindbrain

cerebellum ←

fourth ventricle

rhombomeres

rhombomere 1

rhombomere 2

rhombomere 3

rhombomere 4

rhombomere 5

rhombomere 6

rhombomere 7

rhombomere 8

midbrain

floor plate midbrain

lateral wall midbrain

roof plate midbrain

tectal ventricle

tectum ←

tegmentum

midbrain hindbrain boundary ←

floor plate

ganglia

cranial ganglia

lateral line ganglia

select cerebellum  
as entity

prim-25 stage embryo (36-42 hrs)

nervous system

central nervous system

brain

forebrain

diencephalon

epiphysis

floor plate diencephalon

hypophysis

hypothalamus

lateral wall diencephalon

optic stalk

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optic recess

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rhombomeres

rhombomere 1

rhombomere 2

rhombomere 3

rhombomere 4

rhombomere 5

rhombomere 6

rhombomere 7

rhombomere 8

midbrain

floor plate midbrain

lateral wall midbrain

roof plate midbrain

tectal ventricle

tectum ←

tegmentum

midbrain hindbrain boundary ←

floor plate

ganglia

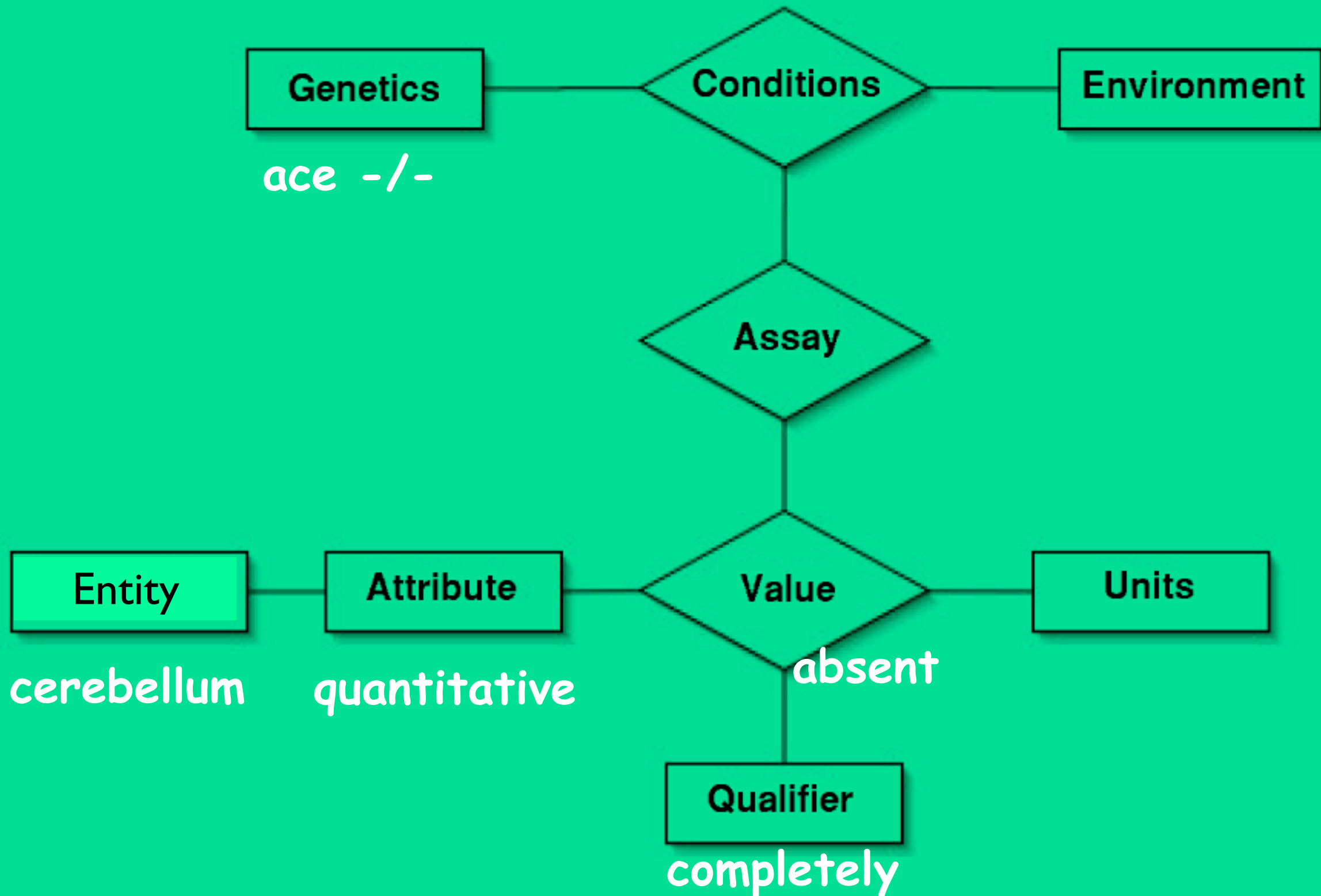
cranial ganglia

lateral line ganglia

annotate the cerebellar  
phenotype using PATO  
attributes and values

- [-] **ISA** pato\_ontology
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  - [-] **ISA** attribute:quantitative
    - ISA** attribute:absolute\_drug\_sensitivity
    - [+] **ISA** attribute:absolute\_enzyme\_function
    - ISA** attribute:absolute\_height
    - ISA** attribute:absolute\_length
    - ISA** attribute:absolute\_mass
    - ISA** attribute:absolute\_number
    - ISA** attribute:absolute\_orientation
    - ISA** attribute:absolute\_photosensitivity
    - ISA** attribute:absolute\_sensitivity
    - ISA** attribute:absolute\_size
    - ISA** attribute:absolute\_speed
    - ISA** attribute:absolute\_temperature
    - ISA** attribute:absolute\_temporal
    - ISA** attribute:absolute\_thermosensitivity
    - ISA** attribute:absolute\_thickness
    - ISA** attribute:absolute\_volume
    - ISA** attribute:absolute\_weight
    - ISA** attribute:absolute\_width
    - ISA** attribute:absolute\_yield
    - PARTOF** value:absent ←
    - PARTOF** value:present

"cerebellum is completely absent in ace -/- mutant"





# major phenotypes of theocerebellar (ace) mutant in PATO format

Phenotype description	Entity	Attribute	Value
cerebellum is absent	cerebellum	quantitative	absent
midbrain-hindbrain boundary is absent	midbrain-hindbrain boundary	quantitative	absent
cardiac ventricle is severely reduced	cardiac ventricle	size	reduced
small ear	otic vesicle	size	reduced
enlarged tectum	tectum	size	enlarged

# Goals

- current phenotype annotation at ZFIN uses a very limited # of anatomical terms, no stage information
- next - use full anatomical ontology with stage information to list affected structures
- integration of *GO* terms into entity list
- anatomical ontology annotated with *PATO* terms
  - interface design - make streamlined *PATO* curation interface possibly displaying a convenient subset of attributes and values generally applicable for particular entities
- ongoing expansion of embryonic processes list
- non-genetic phenotypes - toxicology?

# Phenotype Curation Issues

- values of structural attributes not in PATO :
  - "sickle-cell like", "resembling a raw prawn"
- sometimes difficult to translate text description into PATO format :
  - retinal axons grow halfway to their normal target in the optic tectum, then turn and make ectopic synapses in the epiphysis
- choice of entity: structure AND/OR process that forms structure ?
  - disorganized fin stripes
    - entity : fin stripes
    - entity : processes that generate organized fin stripes
- uniformity of curation? minimize TMTOWTDI through formal guidelines

# Acknowledgements & partial list of PATO people

- many thanks to Erik Segerdell and Monte Westerfield (ZFIN)
- Raymond Lee, Eric Schwarz, David Hall and Zeynep Altun (*C. elegans*)
- Michael Ashburner and Rachel Drysdale (*Drosophila*)
- Duncan Davidson, Jonathan Bard, Janan Eppig and Martin Ringwald (mouse)
- Albert Burger, Bonnie Webber and Stuart Aitken (Edinburgh/XSPAN)
- Simon Twigger (RGD)
- Pete White (Oncogenic database)
- Robin Winter (human dysmorphology database)

# Useful sites

- <http://obo.sourceforge.net/>
- <http://www.geneontology.org/>
- [http://www.informatics.jax.org/searches/MP\\_form.shtml](http://www.informatics.jax.org/searches/MP_form.shtml)
- [http://rgd.mcw.edu/tools/ontology/ont\\_view.cgi?term\\_key=61127&ontology=po](http://rgd.mcw.edu/tools/ontology/ont_view.cgi?term_key=61127&ontology=po)
- [http://www.gramene.org/plant\\_ontology/index.html#to](http://www.gramene.org/plant_ontology/index.html#to)
- <http://flybase.bio.indiana.edu/genes/fbalquery.hform>

