Why do geneticists care so much about complementation?
Predicting outcomes of events

news feature

DEAF by design

Employing genetic diagnosis to avoid having a baby with a disability is controversial enough. But a minority of deaf people would consider testing to ensure that they had a deaf child. Carina Dennis finds out why.

John and Karen — not their real names — are both deaf, and desperately wanted a deaf baby. But genetic testing showed that this was extremely unlikely. "They were devastated," recalls Arti Pandya, a clinical geneticist at Virginia Commonwealth University in Richmond, who counselled the couple. It was two years before they got over their disappointment and started trying to conceive their first child.

All together new: deaf culture now encompasses everything from spelling bees (audience shown applauding, above) to Broadway shows (right). The experience," says Gary Kerridge, regional disability liaison officer at the University of Ballarat in Mount Helen, Australia, who lost his hearing as a young child.

For deaf children, the majority of whom are born to hearing parents, even family oath.
Figuring out number of genes represented by a group of mutants:

- basic info necessarily to sort through mutations
- understand gene function (do all mutant alleles look the same?)
- get sense of the complexity of biological process or function – how many different players are involved
- XP complementation groups – in humans looks at complementation via cell fusion
Figure out how many different genes are represented in your collection of mutants.
Xeroderma pigmentosum is genetically heterogeneous
- autosomal recessive
- characterized by
- increased sensitivity to sunlight
- with the development of carcinomas at an early age.

<table>
<thead>
<tr>
<th>Clinical Disorder</th>
<th>Gene Symbol</th>
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<tbody>
<tr>
<td>Xeroderma Pigmentosum Complementation Group A</td>
<td>XPA</td>
</tr>
<tr>
<td>Xeroderma Pigmentosum Complementation Group B</td>
<td>ERCC3</td>
</tr>
<tr>
<td>Xeroderma Pigmentosum Complementation Group C</td>
<td>XPC</td>
</tr>
<tr>
<td>Xeroderma Pigmentosum Complementation Group D</td>
<td>ERCC2</td>
</tr>
<tr>
<td>Xeroderma Pigmentosum Complementation Group E</td>
<td>DDB2</td>
</tr>
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<td>Xeroderma Pigmentosum Complementation Group F</td>
<td>ERCC4</td>
</tr>
<tr>
<td>Xeroderma Pigmentosum Complementation Group G</td>
<td>ERCC5</td>
</tr>
<tr>
<td>Xeroderma Pigmentosum with Normal DNA Repair Rates</td>
<td>POLH</td>
</tr>
<tr>
<td>Cockayne Syndrome Type I</td>
<td>CKN1</td>
</tr>
<tr>
<td>Cockayne Syndrome Type II</td>
<td>ERCC6</td>
</tr>
<tr>
<td>Xerodermic Idiocy of DeSanctis and Cacchione</td>
<td>ERCC6</td>
</tr>
<tr>
<td>Trichothiodystrophy</td>
<td>Undetermined</td>
</tr>
<tr>
<td>Trichothiodystrophy</td>
<td>ERCC2</td>
</tr>
<tr>
<td>Trichothiodystrophy-A</td>
<td>TFB5</td>
</tr>
</tbody>
</table>
Nucleotide excision repair is a DNA repair system that eliminates the large majority of modified nucleotides from DNA by dual incisions on both sides of the lesion in the damaged strand.

In humans, defects in excision repair cause the disease xeroderma pigmentosum (XP), and genetic analyses of cell lines from XP patients defective in repair have identified seven complementation groups, XP-A-XP-G.
The nucleotide excision repair (NER) system consists of a series of reactions by which DNA damage caused by, for example, ultraviolet radiation-induced photoproducts or similar chemically induced products is recognized and repaired\textsuperscript{2, 132}. Damage can occur from external and endogenous sources (shown as a balance in the figure). Photoproducts include cyclobutane pyrimidine dimers (CPDs) and [6–4] photoproducts, which can both involve T and C pyrimidines\textsuperscript{133}. When repair of these photo- or chemical products is faulty owing to mutations in the NER system, replication errors lead to characteristic C to T mutations, especially CC to TT mutations, which are found in TP53, PTCH1 and other oncogenes in sunlight-induced skin cancers of patients with xeroderma pigmentosum (XP) and others\textsuperscript{134, 135, 136, 137}. The damage is endogenous in other systemic disorders and is thought to be caused mainly by reactive oxygen species (ROS). Depending on whether the damage occurs in a transcriptionally active or inactive domain, repair can occur by two pathways: global genomic repair (GGR) or transcription-coupled repair (TCR) (shown in the figure). Damage in transcriptionally active regions is detected through the arrest of transcription by RNA polymerase I (RNAPI; not shown) and RNAPII.
How does our Nasonia experiment relate to this discussion?
Why Nasonia?

• For this lab it is a convenience

What other types of biological questions can be addressed in this organism?
What does haplodiploidy mean?

Sex Determination

- What is the molecular regulatory circuitry underlying haplodiploidy?
- Do all Hymenopterans share the same underlying sex determination circuitry?
- Is one or two genome copies really the primary signal?
- Are sex-determining genes known to be important in other insects or animals also found in bees, wasps and ants?
**Life History and Sex ratios**

- Ultimate system for female choice: females mate and store sperm and then “decide” whether or not to fertilize eggs
- how do environmental conditions influence sex ratio

**EvoDevo** -- evolution of developmental processes

- why do Nasonia vitripennis males have short stubby wings while closely males of closely related species have long wings?
Tracking progress of wasp experiment:

Recall in the Mendel Revisited lab, the F1 progeny addressed the question of sex linkage and the F2 progeny addressed the issue of linkage and independent assortment.

How many generations do we need to track to determine whether two scarlet mutations are alleles of the same gene or different genes?

**Thursday Nov 15**
- check fly pupae to see if females have laid eggs and drooled all over them
- If yes, remove females and discard in morgue
- If no or not sure, leave females in vial until next Tuesday
**GOAL:** Progeny ready to score no later than Thursday 11/29 (when we will score/collect class data and figure out genotypes)

- **MONITOR the development of the wasp progeny with this goal in mind**
- Consult developmental timetable in handout and note effect of temperature on larval and pupal development
- track development of progeny and peek inside fly pupal case when you first think that you might have yellow pupae or before if you want to see larvae
- track crosses until you see females at the vested or black pupal stage* (why females?) → transfer to 4°C (put in bin marked 322 wasps in frig with clear glass doors)
  - *yellow pupal stage is too young because wildtype eyes are red at that stage – follow animals until they are vested and/or black but don’t let them eclose (crawl out of their pupal case) as we are not set up to knock them out for scoring
- if necessary shift between 28°C and 18°C so you catch the animals at the correct stage.
Fig. 1 Life cycle of *N. vitripennis* at a temperature of 25 °C
FIG. 2. STAGES IN THE DEVELOPMENT OF Mormoniella
(a) Diapausing larva. (b) Defecating larva. (c) Early prepupa. (d) Pink pupa.
(e) Red eyes. (f) Black head and thorax. (g) All black. (h) Adult male. (i) Adult female. (j) Sarcophaga puparium broken open to reveal enclosed diapausing larvae and pupae of Mormoniella. The size of the larva is 2.2 mm. (From Schneiderman and Horwitz, 1958).
Figure 1 | Examples of pupal stages of *N. vitripennis*. (a) Yellow stage. Pupae in this stage are ideal for pRNAi experiments. (b) Red-eyed stage. This is the latest stage recommended for injection in pRNAi experiments focusing on embryonic patterning genes. (c) Half-pigmented stage. This stage is not recommended for injection. (d) Fully pigmented stage. This stage is also not recommended for injection in pRNAi experiments focusing on embryonic patterning genes. (e) Eclosed adult stage.
### Table 1

From the following article

**A method for parental RNA interference in the wasp *Nasonia vitripennis***

Jeremy A Lynch and Claude Desplan

doi:10.1038/nprot.2006.70

**Table 1. Pupal stages of *N. vitripennis*.

<table>
<thead>
<tr>
<th>Pupal stage</th>
<th>Yellow stage</th>
<th>Red-eyed stage</th>
<th>Half-pigmented stage</th>
<th>Fully pigmented stage</th>
<th>Eclosed adult stage</th>
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<tbody>
<tr>
<td>Time after egg lay at 18 °C (d)</td>
<td>18–22</td>
<td>23–26</td>
<td>26–28</td>
<td>28–30</td>
<td>30</td>
</tr>
<tr>
<td>Time after egg lay at 25 °C (d)</td>
<td>7–9</td>
<td>9–11</td>
<td>11–12</td>
<td>12–14</td>
<td>14</td>
</tr>
<tr>
<td>Time after egg lay at 28 °C (d)</td>
<td>5.5–7</td>
<td>7–8</td>
<td>8–9 9–10</td>
<td>9–10</td>
<td>10</td>
</tr>
<tr>
<td>Maximum time can be stored at 4 °C</td>
<td>2 months</td>
<td>2 months</td>
<td>0</td>
<td>2 weeks</td>
<td>Several days</td>
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<tr>
<td>Appropriate for RNAi against embryonic patterning genes?</td>
<td>Yes, ideal</td>
<td>Yes, latest recommended</td>
<td>No</td>
<td>No</td>
<td>Not tested</td>
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</table>