11/15/2012 Biol 322 Lecture (first hour of Thursday Lab)

Why do geneticists care so much about complementation?

Predicting outcomes of events

news feature

S. PERLMAN/AP

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.

G. DOMENICO/AP

All together now: deaf culture now encompasses everything from spelling bees (audience shown applauding, above) to Broadway shows (right).

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.

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 gath-

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

OPEN ACCESS Freely available online PLOS GENETICS

Forward Genetic Analysis of Visual Behavior in Zebrafish

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The visual system converts the distribution and wavelengths of photons entering the eye into patterns of neuronal activity, which then drive motor and endocrine behavioral responses. The gene products important for visual processing by a living and behaving vertebrate animal have not been identified in an unbiased fashion. Likewise, the genes that affect development of the nervous system to shape visual function later in life are largely unknown. Here we have set out to close this gap in our understanding by using a forward genetic approach in zebrafish. Moving stimuli evoke two innate reflexes in zebrafish larvae, the optomotor and the optokinetic response, providing two rapid and quantitative tests to assess visual function in wild-type (WT) and mutant animals. These behavioral assays were used in a high-throughput screen, encompassing over half a million fish. In almost 2,000 F2 families mutagenized with ethylnitrosourea, we discovered 53 recessive mutations in 41 genes. These new mutations have generated a broad spectrum of phenotypes, which vary in specificity and severity, but can be placed into only a handful of classes. Developmental phenotypes include complete absence or abnormal morphogenesis of photoreceptors, and deficits in ganglion cell differentiation or axon targeting. Other mutations evidently leave neuronal circuits intact, but disrupt phototransduction, light adaptation, or behavior-specific responses. Almost all of the mutants are morphologically indistinguishable from WT, and many survive to adulthood. Genetic linkage mapping and initial molecular analyses show that our approach was effective in identifying genes with functions specific to the visual system. This collection of zebrafish behavioral mutants provides a novel resource for the study of normal vision and its genetic disorders.

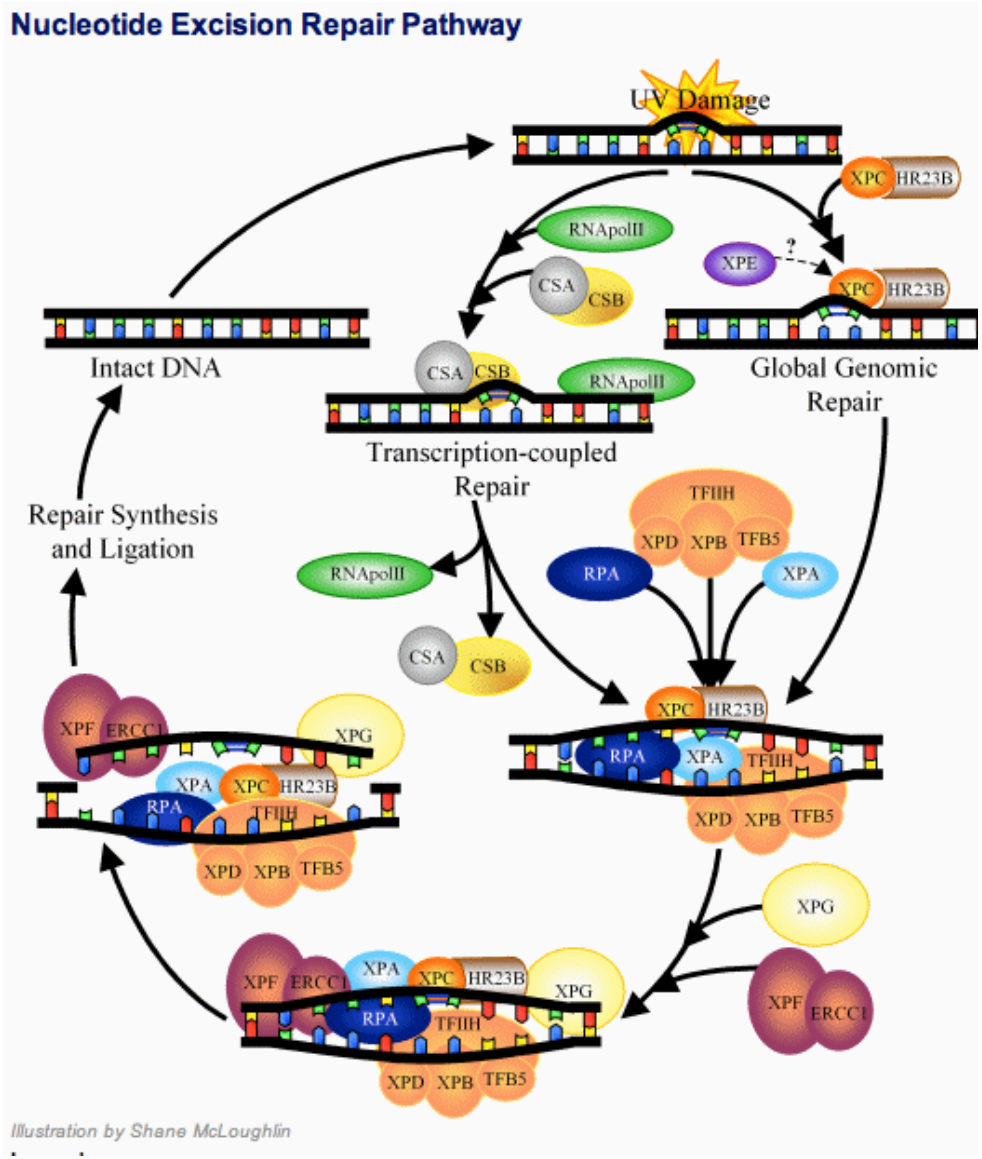
Citation: Muto A, Orger MB, Wehman AM, Smear MC, Kay JN, et al. (2005) Forward genetic analysis of visual behavior in zebrafish. PLoS Genet 1(5): e66.

Figure out how many different genes are represented in your collection of mutants

Clinical Disorder	Gene Symbol
Xeroderma Pigmentosum Complementation Group A	XPA
Xeroderma Pigmentosum Complementation Group B	ERCC3
Xeroderma Pigmentosum Complementation Group C	XPC
Xeroderma Pigmentosum Complementation Group D	ERCC2
Xeroderma Pigmentosum Complementation Group E	DDB2
Xeroderma Pigmentosum Complementation Group F	ERCC4
Xeroderma Pigmentosum Complementation Group G	ERCC5
Xeroderma Pigmentosum with Normal DNA Repair Rates	POLH
Cockayne Syndrome Type I	CKN1
Cockayne Syndrome Type II	ERCC6
Xerodermic Idiocy of DeSanctis and Cacchione	ERCC6
Trichothiodystrophy	Undetermined
Trichothiodystrophy	ERCC2
Trichothiodystrophy-A	TFB5

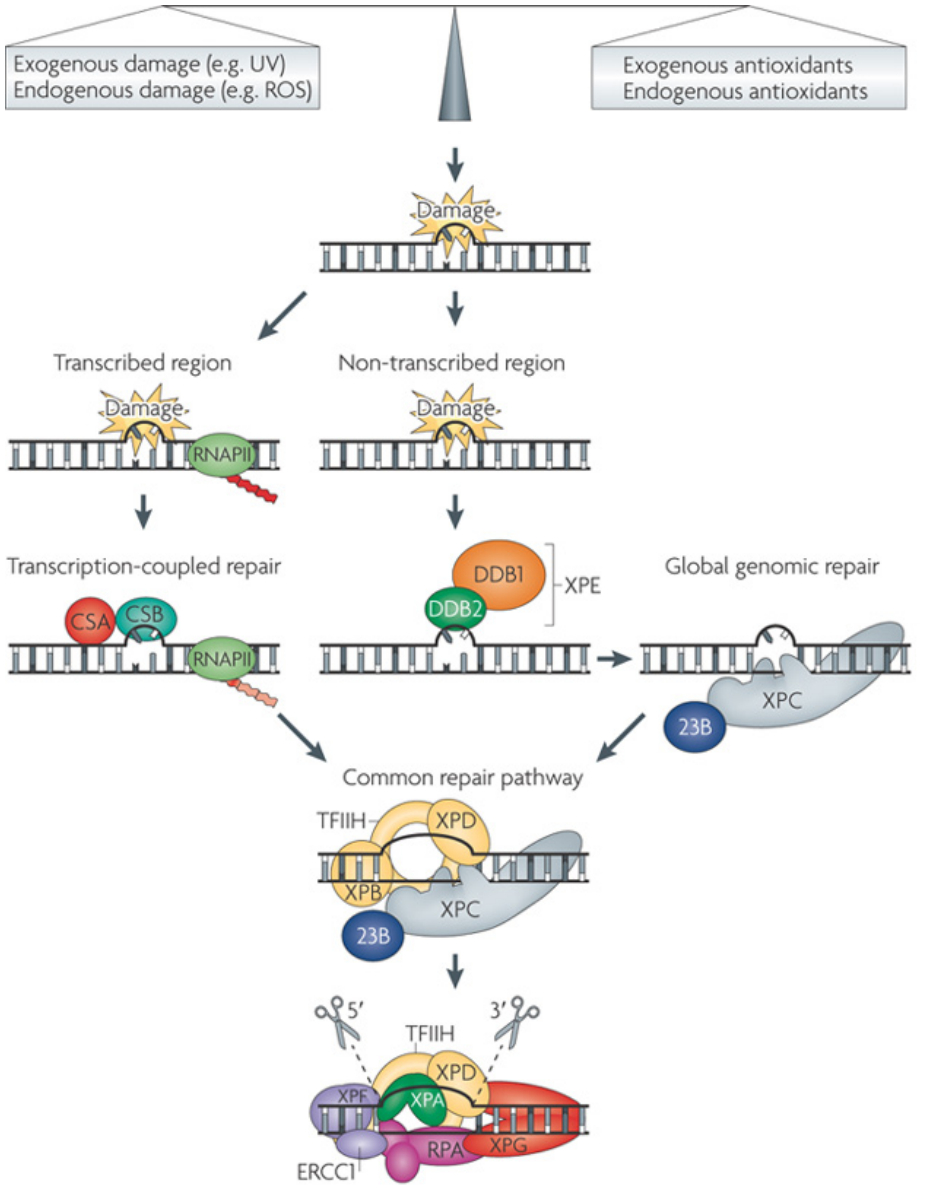
Xeroderma pigmentosum is genetically heterogeneous

- autosomal recessive
- characterized by
- increased sensitivity to sunlight
- with the development of carcinomas at an early age.



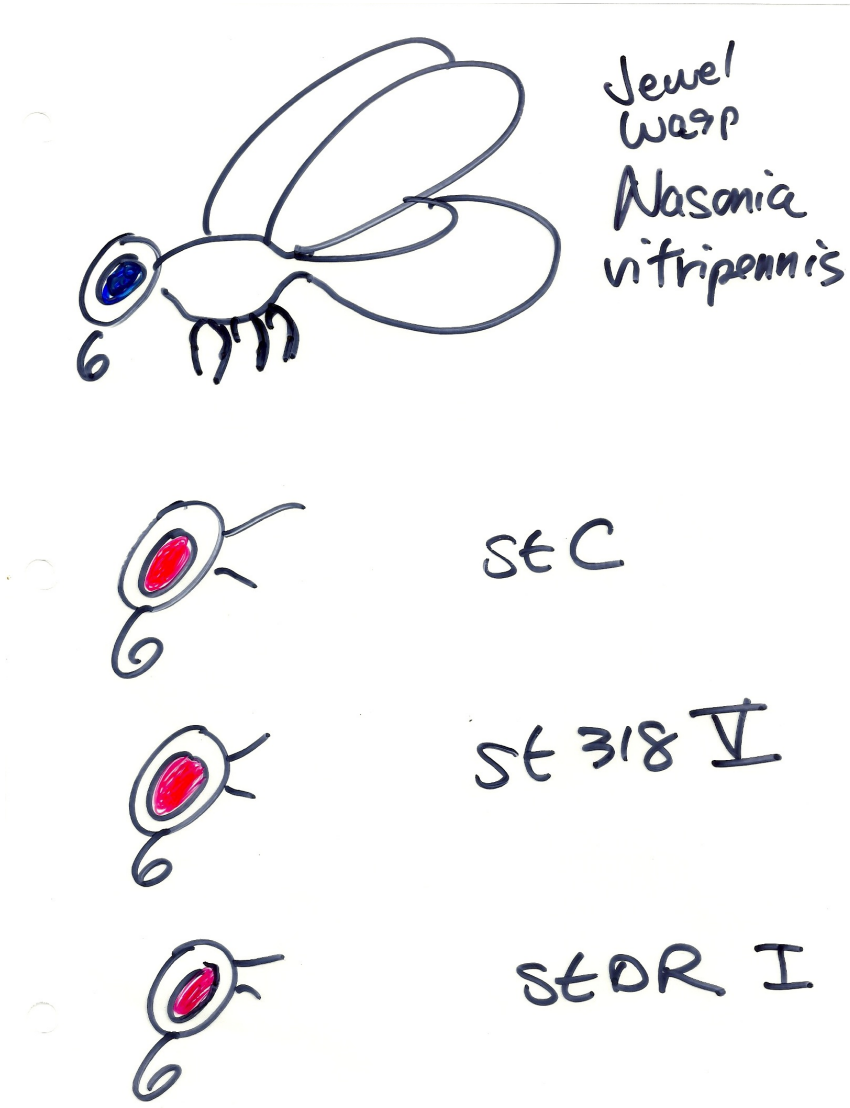
Nucleotide excision repair is a DNA repair system that eliminates the large majority of modified nucleotides from DNA bydual incisions on both sides of the lesion in the damaged strand.

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



Nature Reviews | Genetics

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[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[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[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

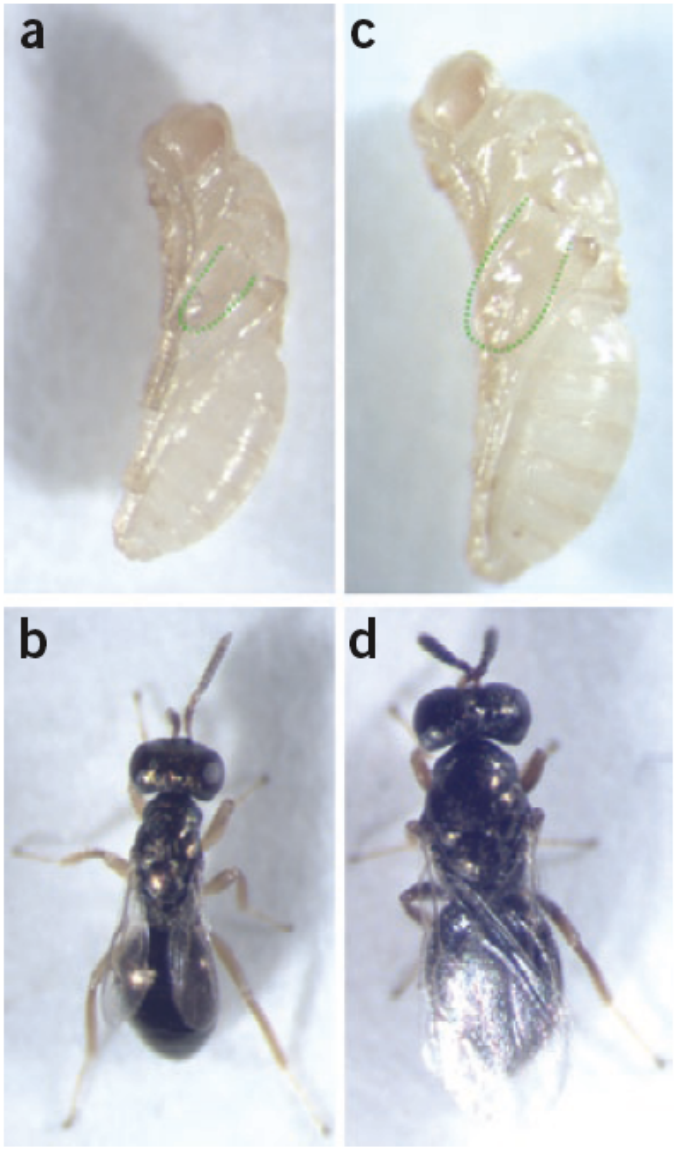


How does our Nasonia experiment relate to this discussion?

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

- Utimate 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 muations 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 4ºC (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 28ºC and 18ºC so you catch the animals at the correct stage.

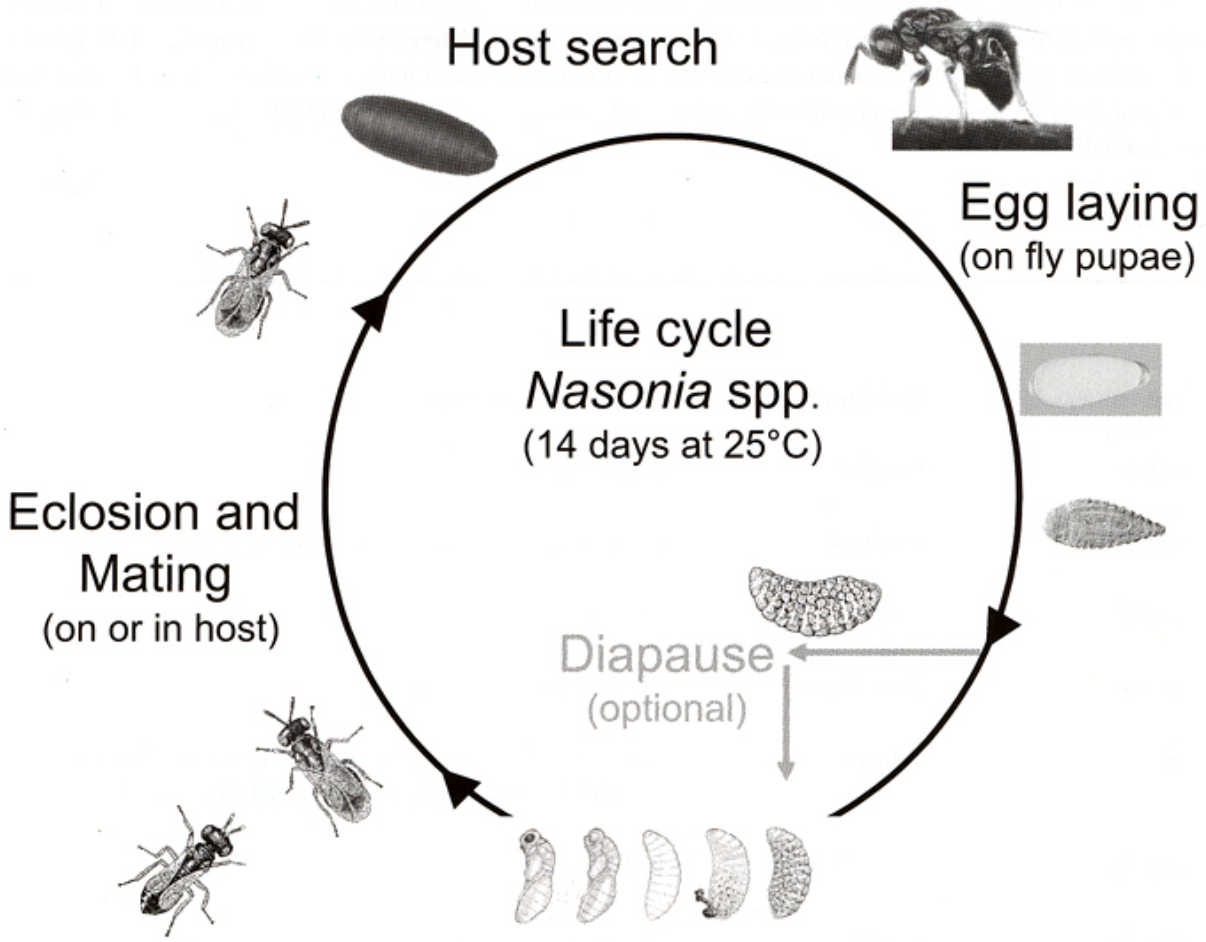


Fig. 1 Life cycle of *N. vitripennis* at a temperature of 25 °C

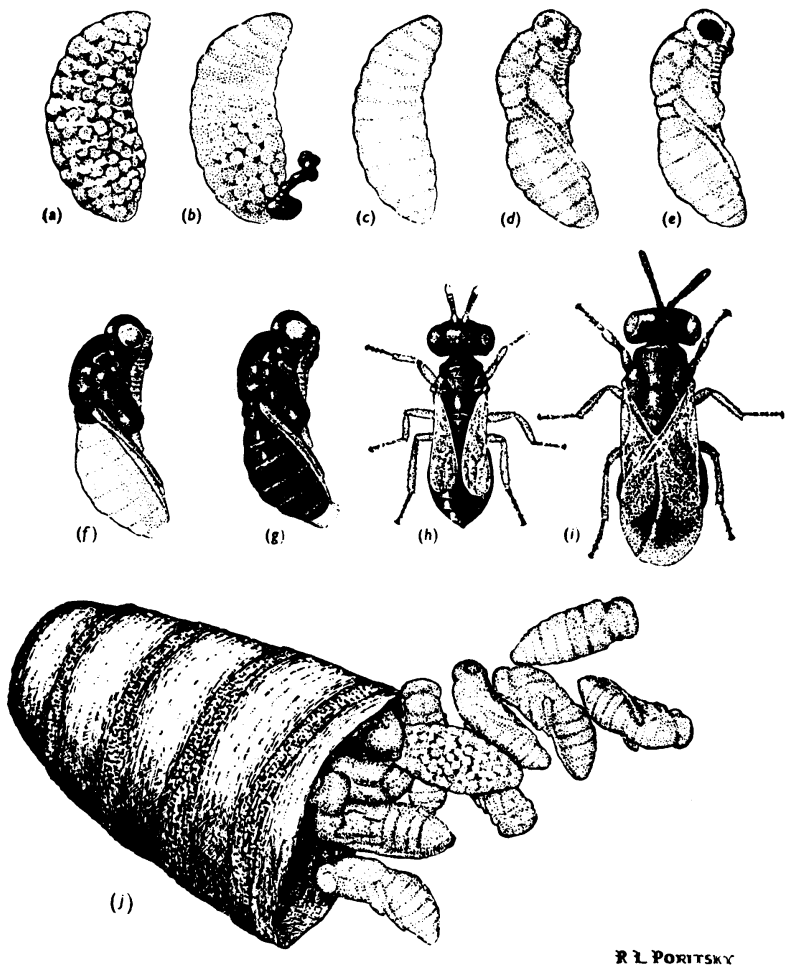


FIG. 2. STAGES IN THE DEVELOPMENT OF *Mormoniella*

(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).

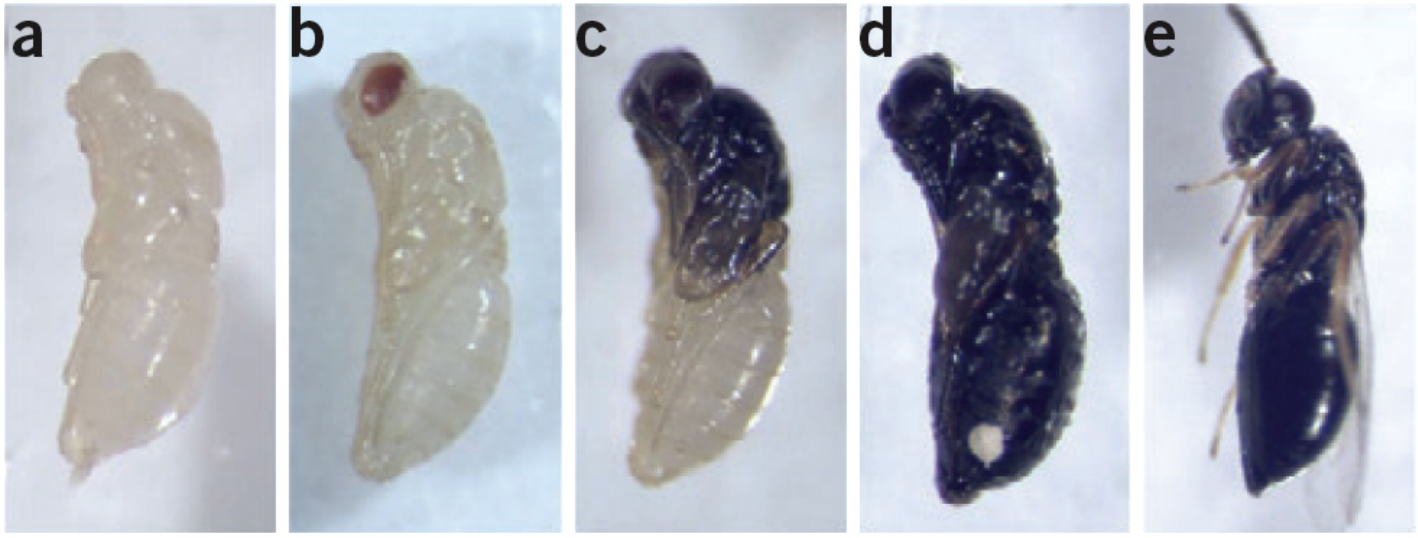


Figure 1 | Examples of pupal stages of *N. vitripennis*. (**a**) Yellow stage. Pupae in this stage are ideal for pRNAi experiments. (**b**) Red-eyed stage. This is the latest stage recommended for injection in pRNAi experiments focusing on embryonic patterning genes. (**c**) Half-pigmented stage. This 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
Nature Protocols **1**, 486 - 494 (2006)
doi:10.1038/nprot.2006.70

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

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

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