Insect Gene Transformations and DNA Vaccines

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Why the topic fits in here: mechanisms of action of a vaccine

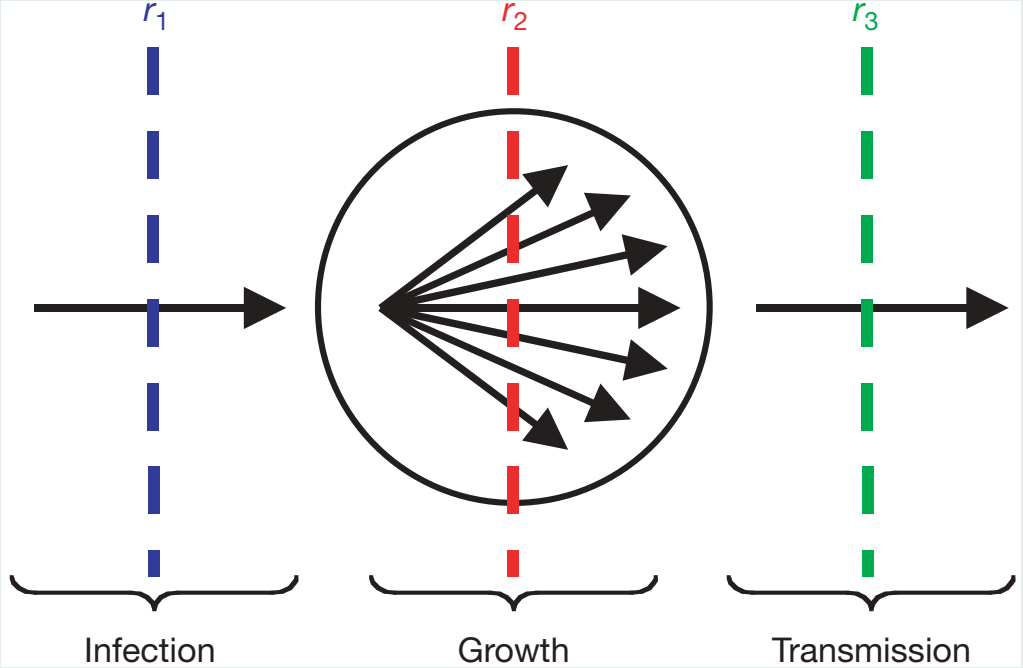


Figure 1: r1, anti-infection resistance; r2, anti-growth rate resistance; r3, transmission-blocking resistance. A fourth type of resistance-antitoxin resistance, r4 is not shown because it only acts upon host death (Gandon et al. 2001)

Why the topic fits in here: malaria paradigm

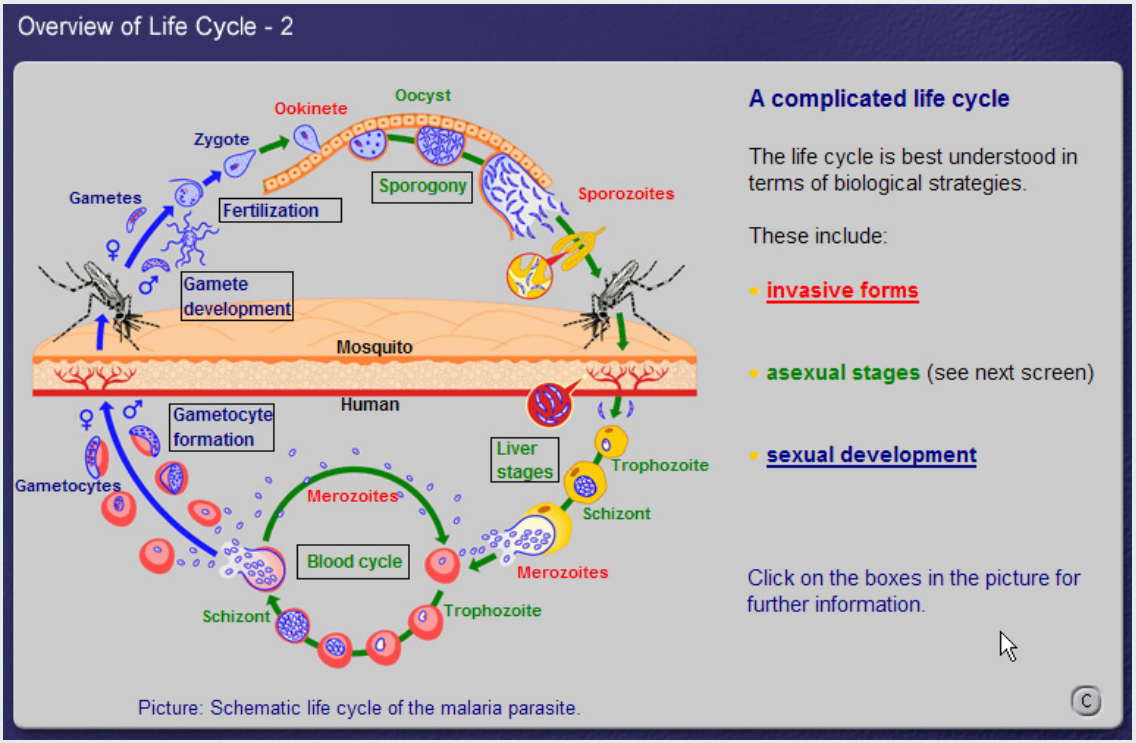


Figure 2: Malaria life cycle (picture from The Wellcome Trust)

Why the topic fits in here: impact on immune profile

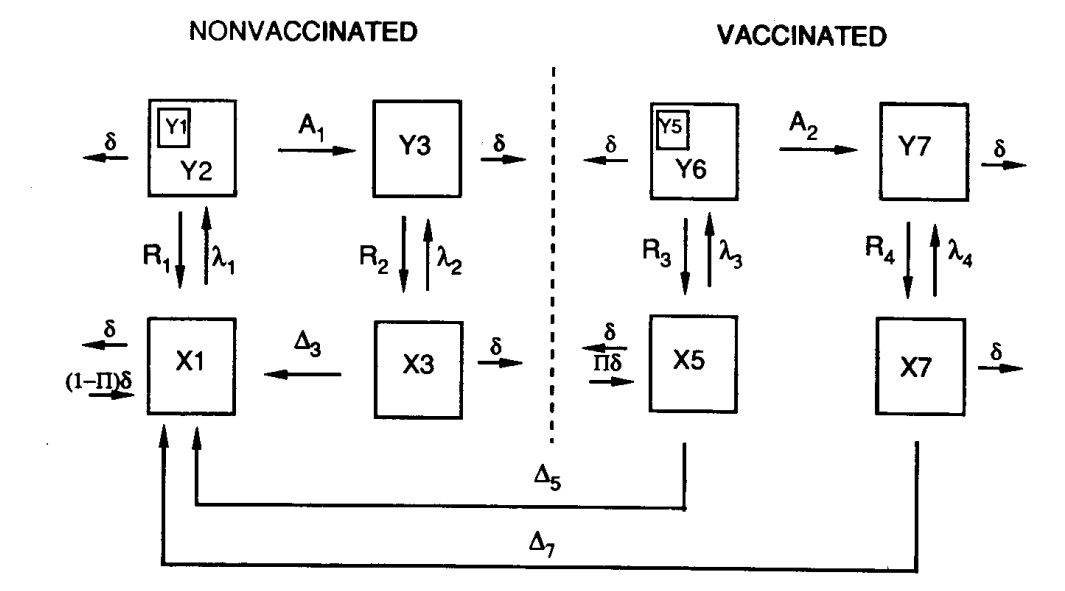


Figure 3: Epidemiological compartments and immune profile rearrangement after vaccination under immune boosting, parasite load dependent acquisition of immunity, and differential morbidity. (Struchiner et al. 1989, Halloran et al. 1989)

Why the topic fits in here: impact on parasite virulence

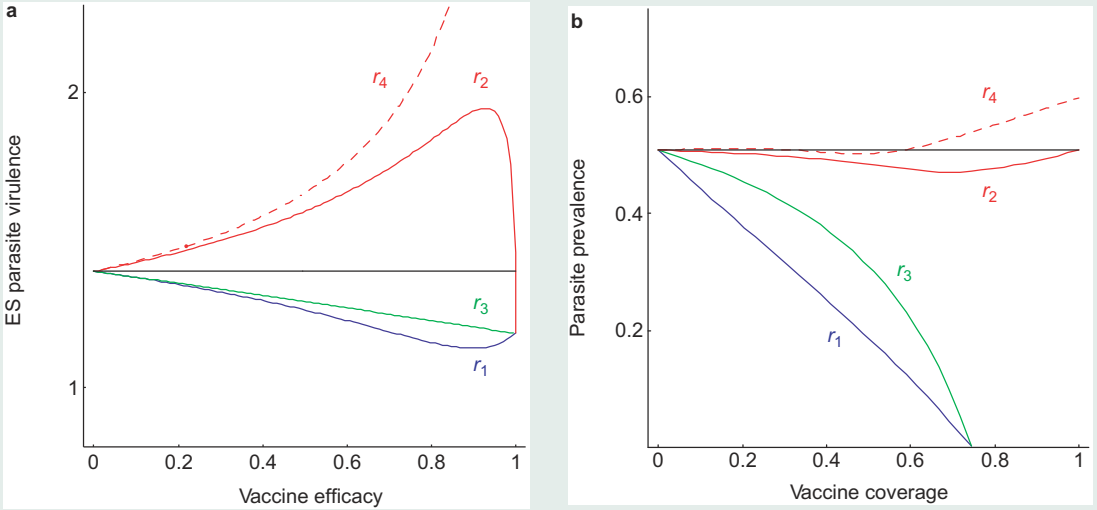
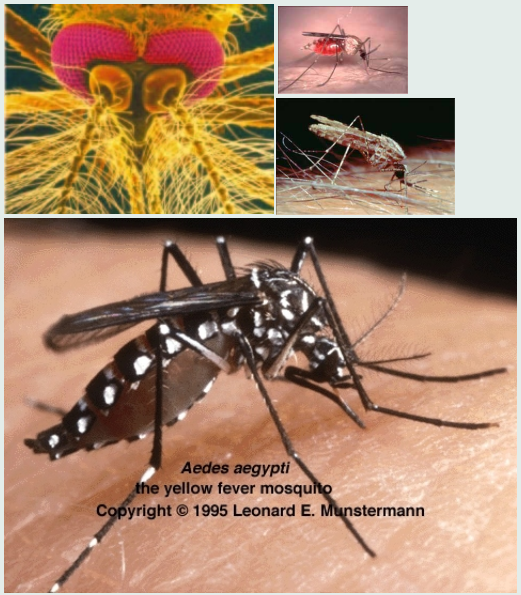


Figure 4: a. Evolutionarily stable (ES) parasite virulence (on susceptible hosts) vs efficacy; b. Parasite prevalence (fraction of infected hosts) against coverage. Horizontal black lines show the outcome in the absence of vaccination (Gandon et al. 2001)

"Vaccine" Target



How: migratory routes and developmental sites

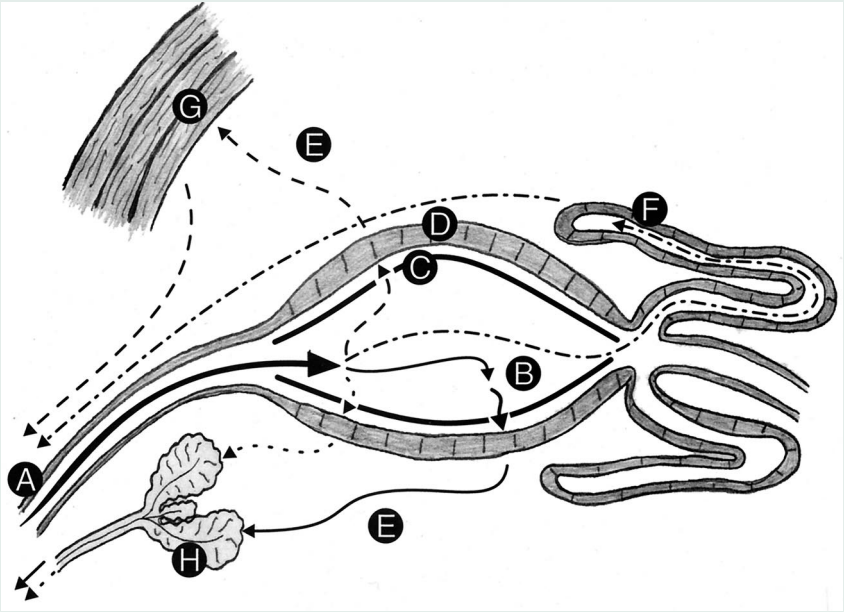


Figure 5: Developmental sites within the mosquito for viruses, malaria parasites, and filarial worms are defined by the letters A to H, and migratory routes are represented by lines. Blood meal (A), midgut (B), peritrophic matrix (C), midgut epithelial cells (D), hemolymph-filled hemocoel (E), Malpighian tubules (F), thoracic musculature (G), salivary glands (H). Viruses (—), malaria parasites (. . .), filarial worms of humans (- - -) and dog heartworm (-.-.-) (Beerntsen et al. 2000)

How: immune responses

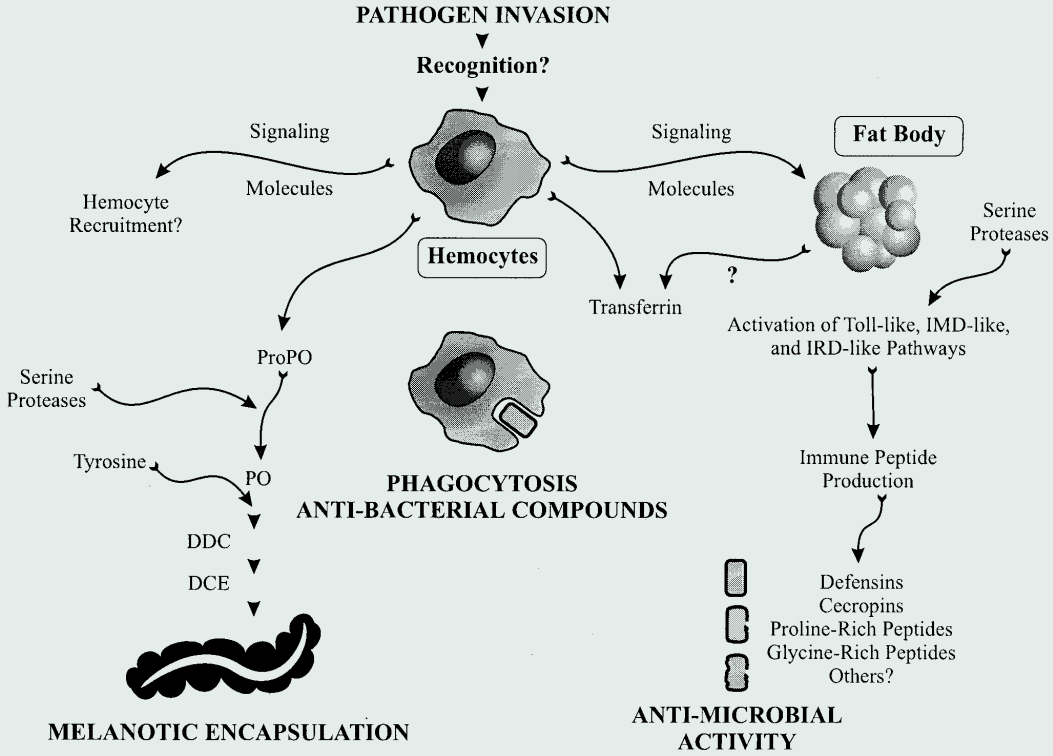


Figure 6: Mosquito immune responses to pathogens include melanotic encapsulation, phagocytosis, and production of antibacterial compounds and immune peptides (Beerntsen et al. 2000)

How: TE driver (molecular biology)

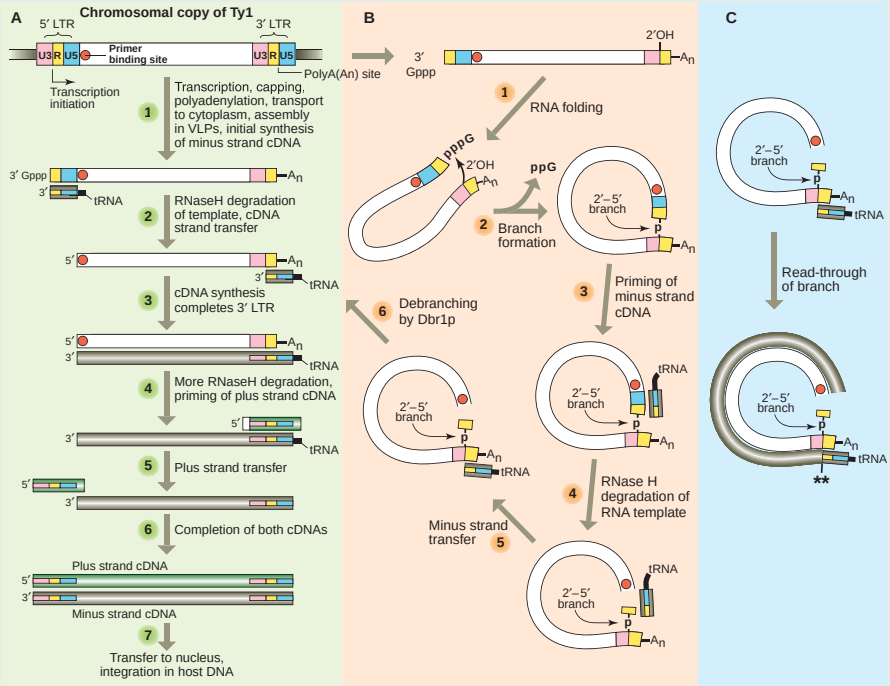


Figure 7: Reverse transcription of the Ty1RNA element of yeast: (A) Depicted is the conversion of single-stranded Ty1 RNA into a double-stranded cDNA copy; (B) Model for RNA branching during the replication cycle of Ty1; (C) A model for cDNA synthesis in yeast cells lacking debranching activity (Perlman & Boeke 2004)

How: Transgene

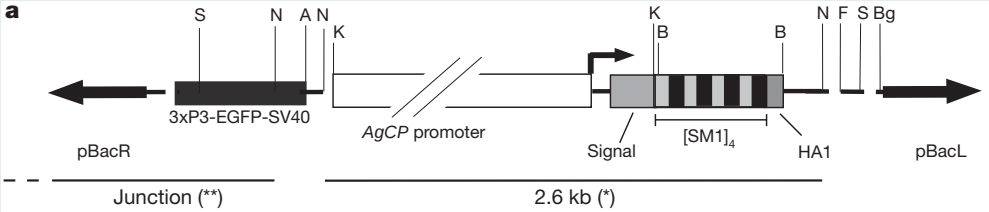


Figure 8: Loaded Transposon: Schematic diagram of the AgCP[SM1]4 gene that was transformed into the A. stephensi germ line. The construct consists of the A. gambiae carboxypeptidase (AgCP) promoter (the bent arrow indicates the transcription initiation site), the AgCP 50 UTR (line to the right of the promoter), the AgCP signal sequence, four units of the SM1 repeat (hatched boxes are the linker amino acids, black boxes are the SM1 peptides), the haemagglutinin epitope (HA1) and the AgCP 30 UTR (line to the right of HA1). 3xP3-EGFPSV40 is the gene that expresses GFP from an eye-specific promoter13. The arrows at the end of the construct represent the piggyBac arms. Dashed lines represent flanking plasmid sequences. Restriction sites: S, Sal I; N, Not I; A, Asc I; K, Kpn I; B, BamHI; F, Fse I; Bg, Bgl II. The size of the junction fragment is variable and depends on the site of integration in the A. stephensi genome. (Ito et al. 2002)

How: TE driver (transgene expression)

Figure 9: Eye color phenotypes in Hermes-transformed adult A. aegypti. Transformation of the kh^w (white-eye) strain of Aedes aegypti with a Hermes transposon carrying a wild-type copy of the D. melanogaster cinnabar gene (encoding kynurenine hydroxylase) restores eye color. Counterclockwise from the top left: head of a wild-type mosquito showing deep purple eyes; head of a kh^w/kh^w mosquito showing white eyes; three heads of transformed mosquitoes from independent Hermes insertions showing different eye colors. The variability in the eye color among transformed lines presumably results from insertion site effects that modulate the expression of the transgene. (Beerntsen et al. 2000)

How: TE driver (transgene expression)

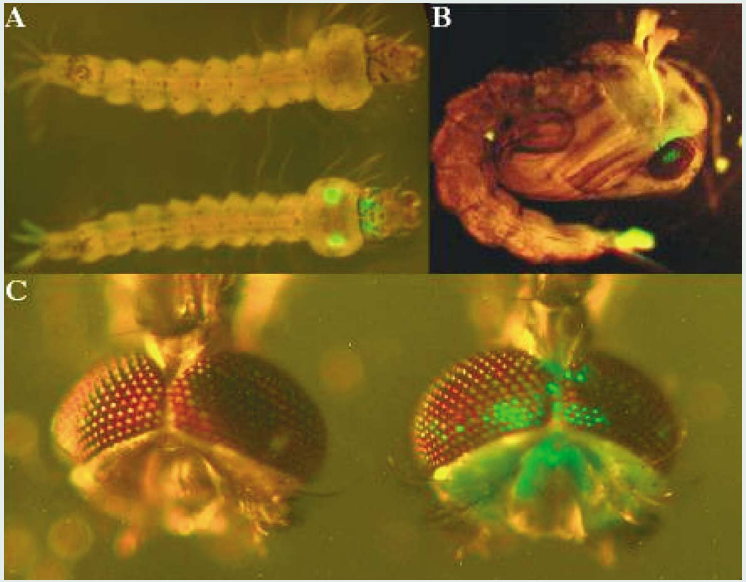


Figure 10: Pattern of green fluorescent protein (GFP) expression in transgenic Anopheles stephensi mosquitoes transformed with a piggyBac vector (Horn et al., 2000). The GFP gene was under the control of the eye-specific 3XP3 promoter. (A) Two larvae: transgenic (bottom) and non-transgenic (top). GFP is visible in the ocelli and salivary glands of the transgenic larva. (B) Transgenic pupa. Note GFP fluorescence in some of the eye ommatidia. (C) Eyes of a non-transgenic (left) and transgenic (right) mosquito. Note that while all eye ommatidia of the transgenic mosquito express GFP, the pattern of fluorescence depends on the angle of incident light. (Moreira et al. 2002)

How: TE driver (genetics)

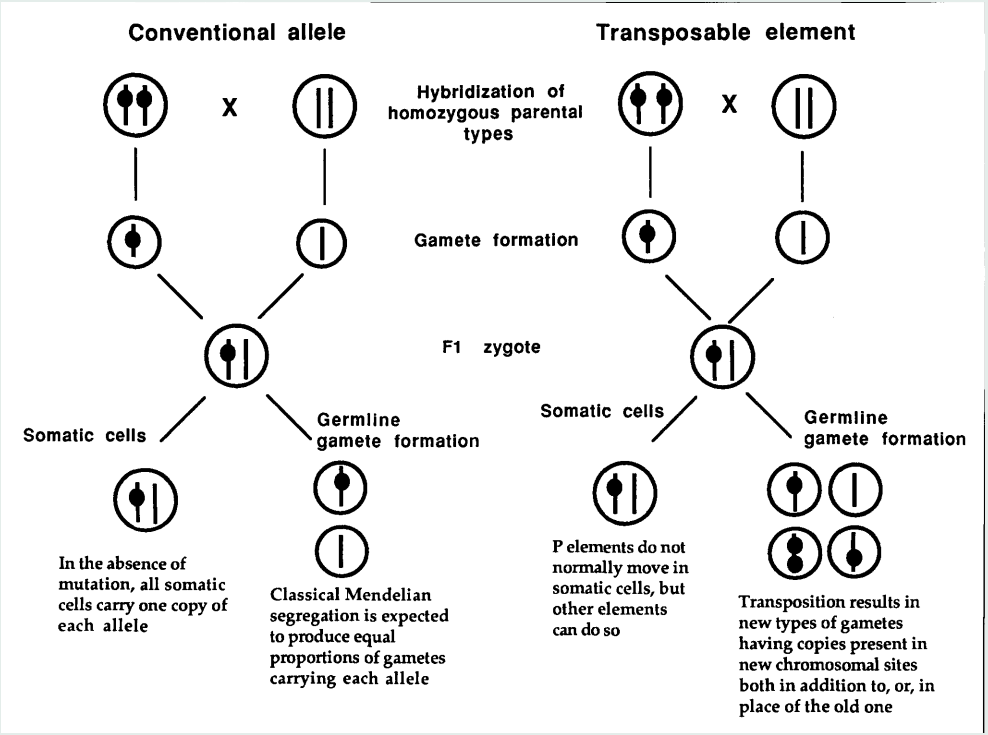


Figure 11: Transmission of a conventional allele compared with that of an active transposable element such as the P element Note that, in the case shown, 75% of the gametes contain transposable elements, and thus this element could afford to kill up to 25% of its offspring and still become fixed in the population. (Kidwell & Ribeiro 1992)

How: TE driver (infectivity)

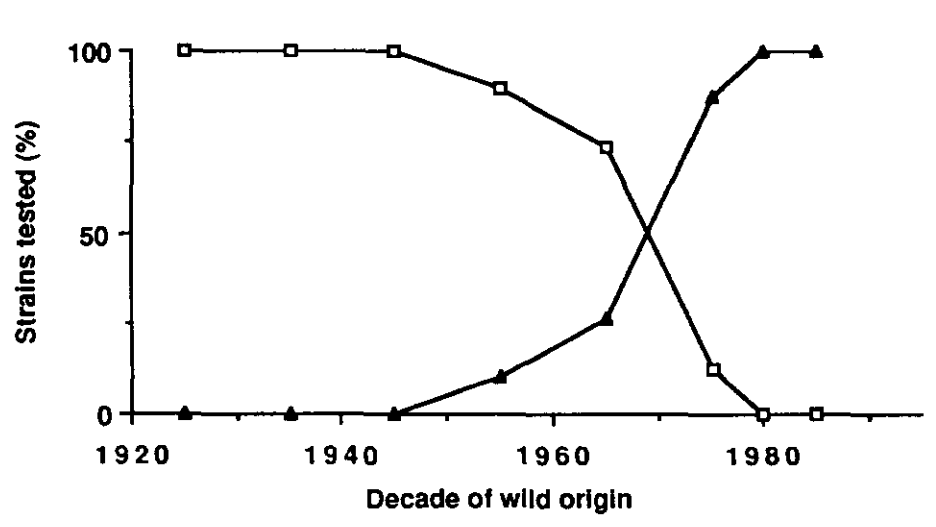
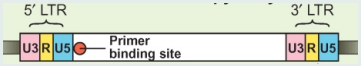
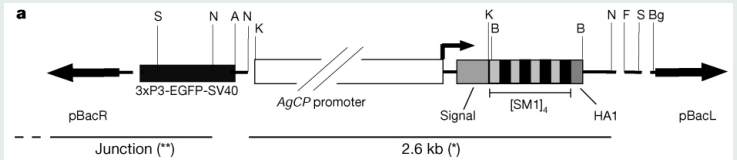


Figure 12: Graph showing the rapid spread of P elements in populations of Drosophila melanogaster worldwide during the past 70 years. P element-bearing strains are represented by closed triangles. Strains lacking P elements are represented by open squares. (Kidwell & Ribeiro 1992)

What: System components





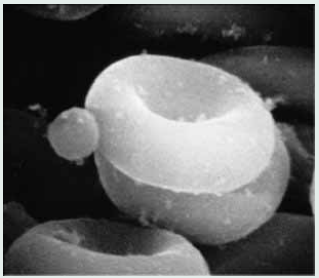


Figure 13: Main system components

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What: questions related to TE?

TE are intragenomics parasites

- fixation
  - density dependent transpositional increase in copy number
  - oposing forces: selection(germ-cell death, zygotic lethality) and rate of transposition (dependent on copy number), host fitness, excision, inactivation (formation of a number of truncated, nonautonomous elements that produce a defective transposase that inhibits transposition)
- influence on chromossome organization
- theory of speciation and “selfish gene”

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Model: TE population genetics

Static Parameters

m number of occupable sites in a haploid genome

n number of copies of a given family of elements per individual in a population

$\bar{n}$ mean number of copies of a given family of elements per individual in a population

V_n variance in copy number between individuals within a population

N number of breeding individuals in a population

x_i frequency of elements at the ith occupable site in a population

$\bar{x}$ mean of x_i over all sites ($\bar{x} = \frac{\bar{n}}{2m}$)

σ_x^2 variance of x_i between sites

D_{ij} coefficient of linkage disequilibrium in element frequency between the ith and jth occupable sites

(Charlesworth & Langley 1989)

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Model: TE population genetics

Dynamic Parameters

μ_n the germ-line probability of transposition per generation of an element belonging to a given family, in a host individual carrying n elements of that family. (The functional dependence of μ on n, denoted by the subscript n, allows for possible regulation of the rate of transposition in response to copy number.)

ν the germ-line probability of excision per generation of an element of a given family

w_n the fitness of a host individual carrying n members of a given family, relative to a value of one for an element-free individual

$\bar{w}$ the mean of w_n over all individuals in the population

(Charlesworth & Langley 1989)

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Examples of TE equations

- distribution of elements belonging to a given family between different individuals within a population

$$V_n = \bar{n}(1-\bar{x}) - 2m\sigma_x^2 + 4\sum_{i<j} D_{ij}$$

- change in copy number per generation

$$\Delta\bar{n} \approx \bar{n}(\bar{n}-\bar{x})\frac{\delta \ln \bar{w}}{\delta \bar{n}} + \bar{n}(u_{\bar{n}} - \nu)$$

TE-mosquitoes (Ribeiro & Struchiner submitted)

- host system (3-parameter density dependent)

$$N_{t+1} = r \times N_t \times (1 + \alpha \times N_t)^{-\beta}$$

  N_t number of zygotes at generation t

  r net rate of increase

  α scaling term determining population size

  β term determining the strength of density dependence (intraspecific competition)

- TE

$$c_{t+1} = c_t + c_t \times T_0 \times U(c_t)$$

  c_t TE copy number at generation t

  T_0 maximum efficiency of transposition

  U_c T_0 density dependence decreasing factor

- Host-TE interaction (mating and fitness)

$${}^{c+c'}N_{t+1} = (r - d\,(m + m')) \times {}^{c}_{m}G_{t+1} \left(\frac{{}^{c'}_{m'}G_{t+1}}{{}^{\text{all}}G_{t+1}} \right) \times (1 + \alpha \times N_t)^{-\beta}$$

  ${}^{c}_{m}G_t$ number of gametes at time cycle t harboring c copies of TE, out of which m were recently mobilized

  d decrease in fitness caused by each recently mobilized transposition

  $m + m'$ number of recent transposition events in gametes

TE-mosquitoes-parasite (Boete & Koella 2002)

- benefit of refractoriness due to avoiding malaria parasite's detrimental effects on fecundity and mortality (sex dependent fitness)
- cost of refractoriness associated with maintaining and mounting an immune response in insects
- the efficiency of the transformation system
- cost of the transformation system on fitness

$$p_{f,t+1} = \frac{p_{f,t}p_{m,t}W_{f,RR} + 0.5(1+\partial)\left[p_{f,t}(1-p_{m,t}) + (1-p_{f,t})p_{m,t}\right]W_{f,RS}}{\bar{W}_f}$$

$p_{f,t}$ frequency of refractory gene in female gametes at generation t (and similarly for males)

$W_{f,RR}$ fitness of females that homozygous for the refractory gene (and similarly for those heterozygous)

$\bar{W}_f$ $p_{f,t}p_{m,t}W_{f,RR} + \left[p_{f,t}\left(1-p_{m,t}\right) + \left(1-p_{f,t}\right)p_{m,t}\right]W_{f,RS} + \left(1-p_{f,t}\right)\left(1-p_{m,t}\right)W_{f,SS}$

∂ efficiency of the genetic drive

Disease in Humans

$$y = \frac{R_0 - 1}{R_0 + \frac{a}{\mu}}$$

$$R_{0,t} = R_0^* \left\{p_{f,t}p_{m,t}\left(1-s\right) + \left[p_{f,t}\left(1-p_{m,t}\right) + \left(1-p_{f,t}\right)p_{m,t}\right]\left(1-hs\right) + \left(1-p_{f,t}\right)\left(1-p_{m,t}\right)\right\}$$

s effectiveness of protection conferred by the refractory allele

h cost of refractoriness

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Model Predictions

- TE fixation seems plausible
- Efficacy of protection (refractoriness) must approach 100% to have any impact on transmission

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Remaining questions

- changes in population immune profile
- impact on pathogen virulence
- stabilty of refractoriness

References

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