

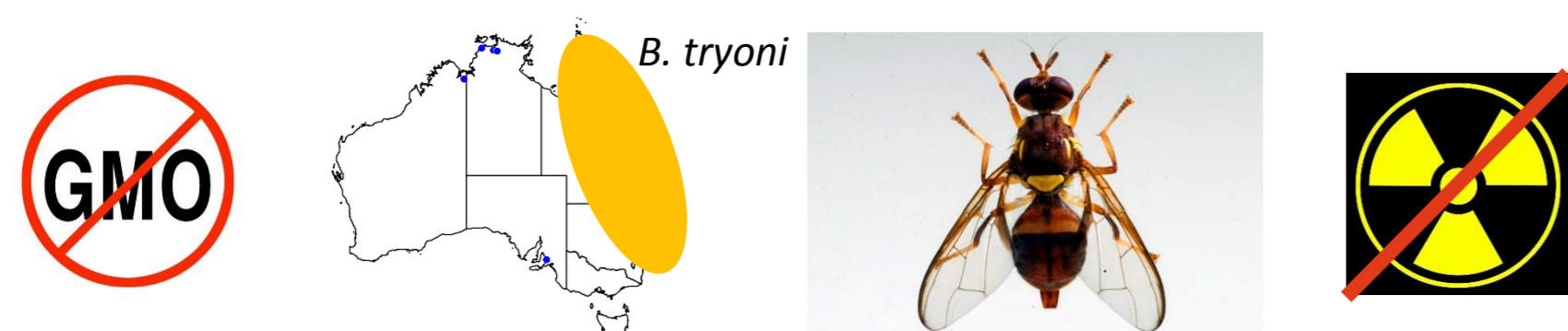
Inducing female lethality and male sterility in Queensland Fruit Fly

Christopher Hardy, Leon Court, Stephen Pearce, Owain Edwards & Steve Whyard



RNAi-mediated Sterile Insect Technology

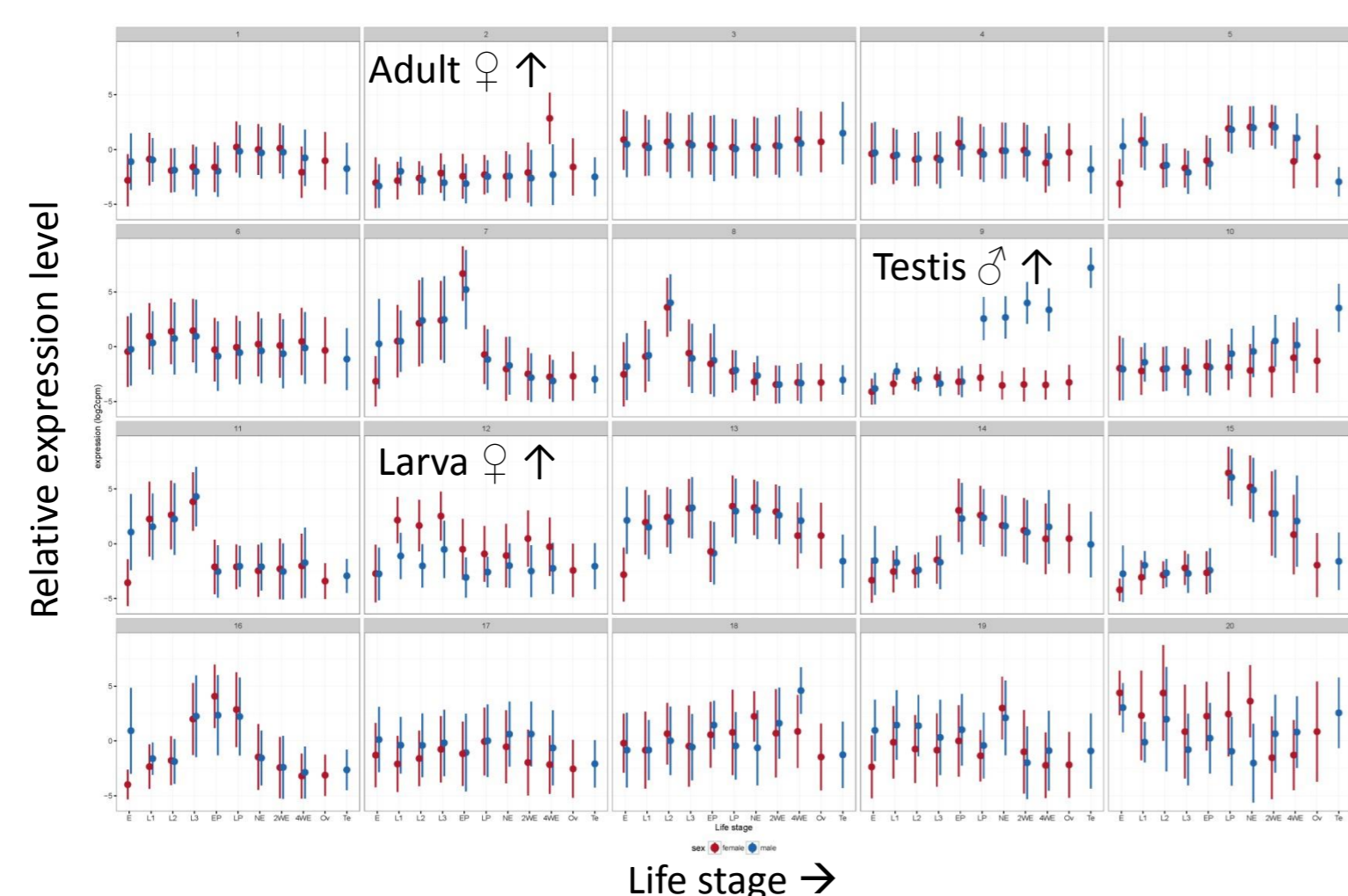
- A non-radiation method of producing sterile male only flies
- A simpler method of sex-sorting than mechanical methods
- A non-GM approach to the new SIT methods
- Adaptable to many species
- Can be used with field-caught strains – minimize assortative mating issues



RNAi target identification using QFF transcriptome analyses

- Males and females separately considered for all life stages (genetic sorting test developed)
- 48 genetic libraries covering 7 major life stages (tissues include ovaries and testes)
- Strand-specific library construction

Gene expression profiling



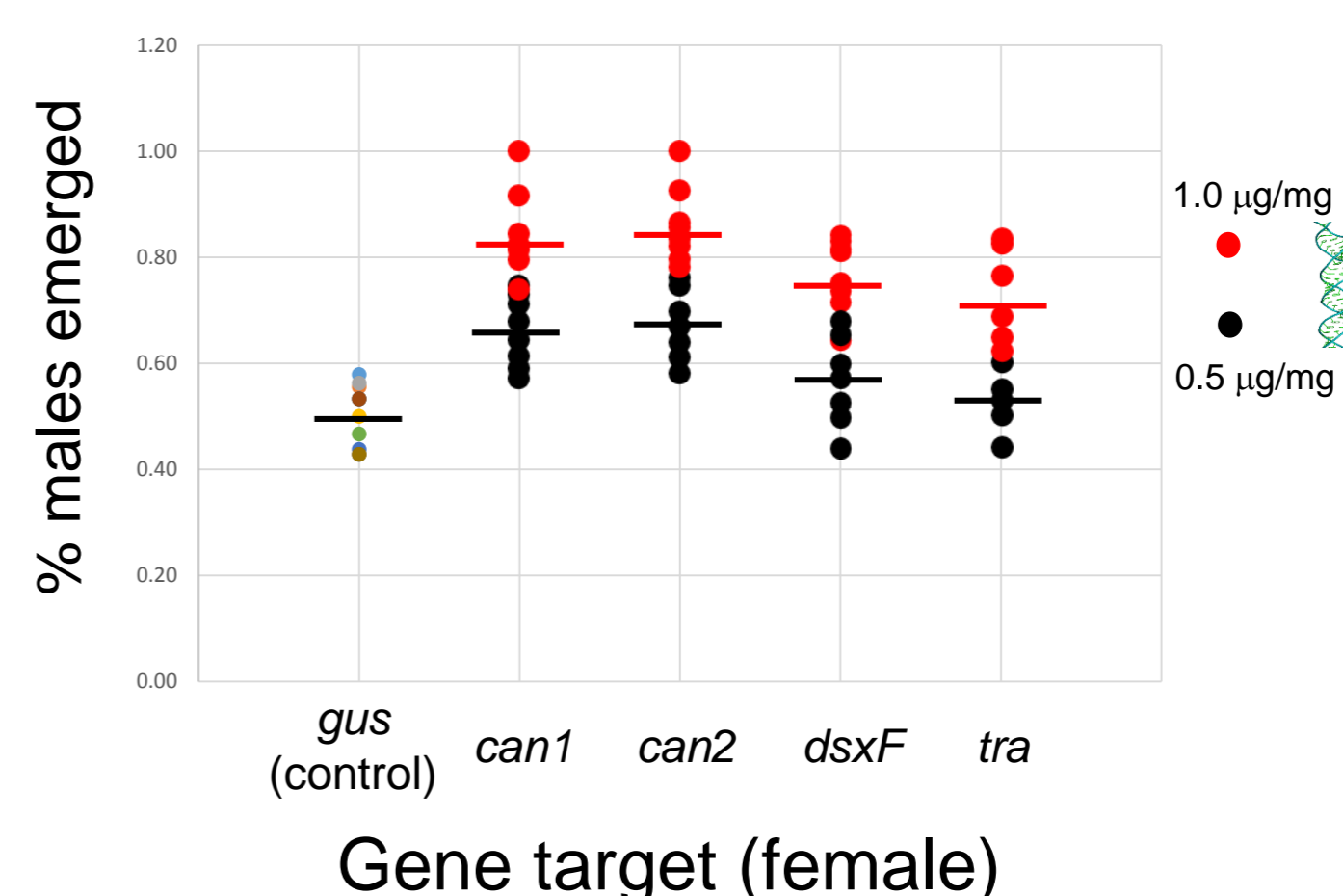
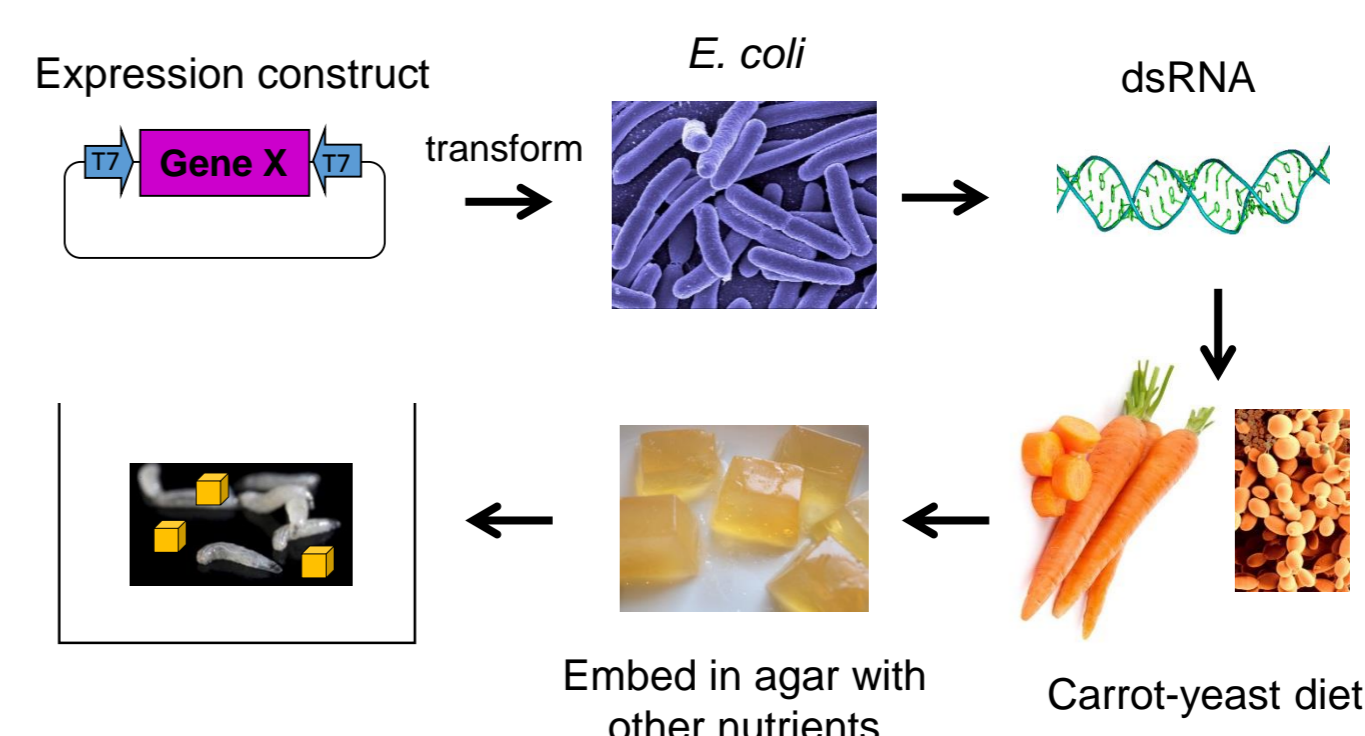
RNAi production options

- Express and purify dsRNA from bacteria
 - Clone target sequence in *E. coli*
 - Induce expression with IPTG
 - ~200 mg dsRNA/L culture (enough for 300 QFF)
- Killed bacteria/yeast expressing dsRNA
 - Can add directly to the diet without purification
- Synthetic dsRNA

Inducing QFF male sterility (larvae fed purified dsRNA)

dsRNA in diet (testis genes)	males emerged	% transcript knockdown	fully sterile males	% sterile males	viable progeny for non-sterile males	% impact on fertility on non-sterile males
<i>gus</i> (control)	21	-	2	9.5	16 ± 6	0
<i>bol</i>	19	46 ± 11	6	32	14 ± 5	12
<i>gas8</i>	22	42 ± 16	7	32	13 ± 5	19
<i>dynein light chain</i>	17	39 ± 13	6	35	13 ± 6	19

Preventing QFF female development (larvae fed purified dsRNA)



Project AI13001 Highlights

- Establishment of RNA protocols for creating and analysing QFF transcriptome data
- A genetic test to determine the sex of any QFF life stage in under 2 hrs
- Creation of the first developmental transcriptome map for QFF, covering all life stages of both sexes from egg to mated adult
- Demonstration that male sterility can be induced in QFF by including RNAi in the diet
- Demonstration that female development in QFF can be prevented by RNAi in the diet



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