Chlamydomonas reinhardtii: a model organism for exploring the evolution and management of herbicide resistance?

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Dynamics of resistance evolution

- PROCESS OF SELECTION
- OUTCOME OF SELECTION

Chart showing the dynamics of resistance evolution over generations.
Approaches for studying ‘evolution in action’

Selection experiments

Simulation models

Biological models

*Arabidopsis thaliana*

*Chlamydomonas reinhardtii*
Chlamydomonas: key experimental attributes

- a simple unicellular plant
- haploid genome
- complete genome sequence
- 10-12 generations / week
- 1.5 million cells / mL growth medium
- facultative asexual and sexual cycles
- evolved lines can be cryopreserved

Chlamydomonas is a photosynthetic model in the thriving ‘new’ discipline of microbial experimental evolution
Chlamydomonas: the study system

Chlamydomonas cultured in 20mL Bold's medium supplemented with herbicide.

Cultures maintained at 28°C in an orbital incubator with lighting from above.

After 7 days, population growth rates are determined by measuring optical density at 750nm.

At 7 day intervals cells are transferred into fresh growth medium.

At completion of selection experiments evolved lines are transferred to tubes containing Bold's medium (A), the selective herbicide (B) and other herbicide modes of action (C, D) to assay for fitness costs, resistance and cross-resistance, respectively.
Resistance management questions

- Does mode of action rotation slow evolution of resistance?
- Are herbicide mixtures an effective resistance management strategy?
- Do rotations and mixtures increase selection for cross-resistance?
- Does selection of resistance to one mode of action accelerate evolution of resistance to subsequent MoA?
- Costs of resistance?
Herbicide rotation

A0
G0
C0
AG1
AG2
AG3
AC1
AC2
AC3
CG1
CG2
CG3

A – atrazine  G – glyphosate  C - carbetamide
Herbicide rotation: resistance dynamics

- Rotation can accelerate evolution of resistance
- Rotation can prevent evolution of resistance
- Rotation can increase selection for cross-resistance (data not shown)

Lagator et al. Evol Appl. 2013
Experimental Design:

- 3 herbicides - atrazine, glyphosate, carbetamide
- Pairwise and three-way mixtures
- 6 replicates per regime
- Evolved for 12 weeks (approx. 80 generations)
Herbicide mixtures: resistance dynamics

Lagator et al. New Phyt. 2013
Herbicide mixtures: resistance dynamics

- ‘Low’ dose mixture combinations accelerate selection for resistance
- ‘Full’ dose mixture combinations generally slow or prevent evolution of resistance
- ‘Low’ dose mixtures select for greater cross-resistance (data not shown)
Sequential evolution of resistance

Experimental Design:

• 3 herbicides - atrazine, glyphosate, carbetamide
• 6 replicates per regime
• Evolved for 20 weeks (approx. 150 generations)
Sequential evolution: resistance dynamics

- **Herbicide sequence**: A, GA, GCA
  - **Weeks to resistance**: A: n=6/6, GA: n=6/6, GCA: n=2/2

- **Herbicide sequence**: G, AG, ACG
  - **Weeks to resistance**: G: n=6/6, AG: n=6/6, ACG: n=1/4

- **Herbicide sequence**: C, AC, GC, AGC, GAC
  - **Weeks to resistance**: C: n=0/6, AC: n=4/6, GC: n=2/6, AGC: n=4/6, GAC: n=3/6
Sequential evolution: costs of resistance

- **a**
- **b**
- **c**
Conclusions

- *Chlamydomonas reinhardtii* can be a useful model for exploring resistance management strategies.

- Herbicide rotation can slow or increase the rate of resistance evolution.

- Herbicide mixtures are effective if both mixtures components applied at full doses

- Prior selection with one herbicide mode of action can accelerate selection of resistance to subsequently applied MoA.

- Responses are diverse and depend on MoA

- *Is Chlamydomonas a useful model?*
A parting thought

When we attack agricultural pests with pesticides (or any other management) we are picking a fight with natural selection.

“Going into a fight like that without Darwin is like going to the moon without Newton”

Andrew Read, evolutionary biologist, Penn State