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Evolution of *Lymnaea stagnalis* inbreeding depression under pesticide chronic exposure

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1. Introduction

Ecological and genetic factors may modulate the toxicity of chemicals to natural populations. In particular, inbred populations may be more sensitive to pollutants than their outbred conspecific counterparts, due to high homozygosity, loss of genetic diversity, or increased genetic load. On the other hand, strong inbreeding, such as expected in highly selfing hermaphrodites, may have a positive effect on population response to chronic (multigeneration) exposure to toxicants. First, selfing may facilitate the response to the selection exerted by a toxicant, through a transmission of coadapted gene complexes more efficient than under random mating. Furthermore, evolutionary theory predicts that spontaneous deleterious mutations are preferentially purged under selfing, whereas they are maintained for longer time in outcrossing populations, as masked in heterozygotes [1]. Thus, high and longlasting inbreeding may have positive effects on fitness, and possibly on the ability to face chronic environmental stress. We tested these hypotheses in the freshwater snail *Lymnaea stagnalis*, using a three-generation exposure to a pro-oxidant herbicide.

2. Materials and methods

A total of 16 lineages were derived from a highly diverse genetic pool stemming from five different original populations [2]. Half of these lines were maintained in control conditions, and the other half exposed to 200 µg/L diquat during 5 weeks (five successive treatments, from the age of one week). After this period, exposed lines were further reared as their control counterparts in standard conditions (charcoal-filtered dechlorinated water, 14L/10D photoperiod, 20±1°C temperature). Snail density and food supply (organic lettuce) were adjusted during the life cycle. Short before maturity, snails from the selfing lineages were isolated in order to prevent outbreeding, whereas snails from outcrossing lineages were maintained in groups and temporarily isolated for egg-laying. This protocol was implemented for three successive generations (Fig. 1).

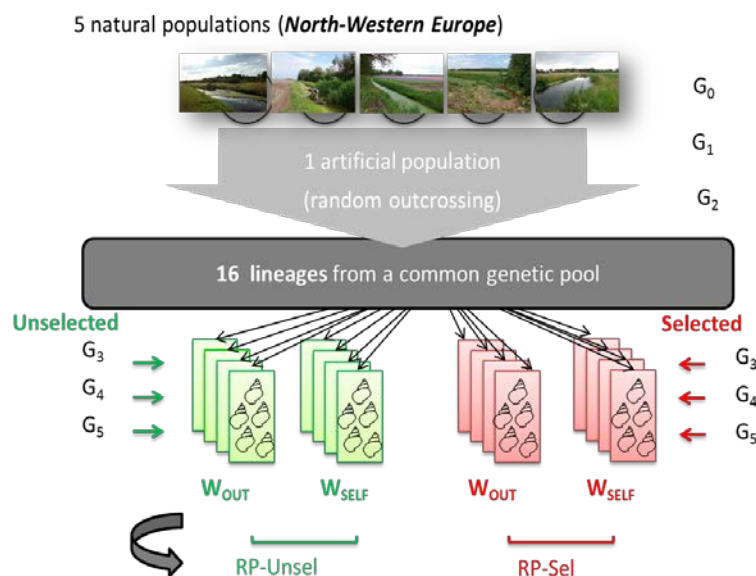


Fig.1. Experimental design. G₀ = wild-caught adults. G₁-G₅: laboratory-born generations. Mating system (SELF= selfing, OUT=outcrossing) crossed with exposure to control conditions (Unselected, in green), or to a pesticide (Selected, in red). Fitness (W) and relative performances (RP-Unsel, RP-Sel).

Chemical exposure was crossed with the mating system, i.e., in each treatment (control, diquat), four lineages were forced to self-fertilize and four other lineages were allowed to outcross. After three generations, inbreeding depression (ID), the fitness decrease of selfed progeny relative to the outcrossed one, was estimated using fitness-related traits (life history traits) and the following formula:

$$ID = 1 - \prod_{i=1}^n \frac{W_{Self_i}}{W_{Out_i}}$$

where, W_{Self_i} and W_{Out_i} are the performances at trait i under selfing and outcrossing, respectively. ID variance was estimated by bootstrapping individual values within each condition [3], and compared between diquat exposure and control conditions.

3. Results and discussion

Life history traits evolution was globally similar among control and diquat exposed lines. Inbreeding depression was mostly due to parental fecundity, hatching rate and juvenile size in the next generation (Fig.2). Inbreeding depression was slightly higher under diquat exposure (61%) than under benign conditions (53%), suggesting that selfing did not improve the response to selection by diquat. However, the difference was not significant (bootstrap test). This result, added to the fact that mortality during direct exposure to diquat was significantly higher in selfing lines (not shown), suggests an aggravation of the toxic effects under inbreeding. However, selfed lineages also showed an increase in trait variance, especially under diquat exposure. Some inbred lines performed better than some outcrossed lines, suggesting that the response to selection might in fact depend on lineage genetic composition.

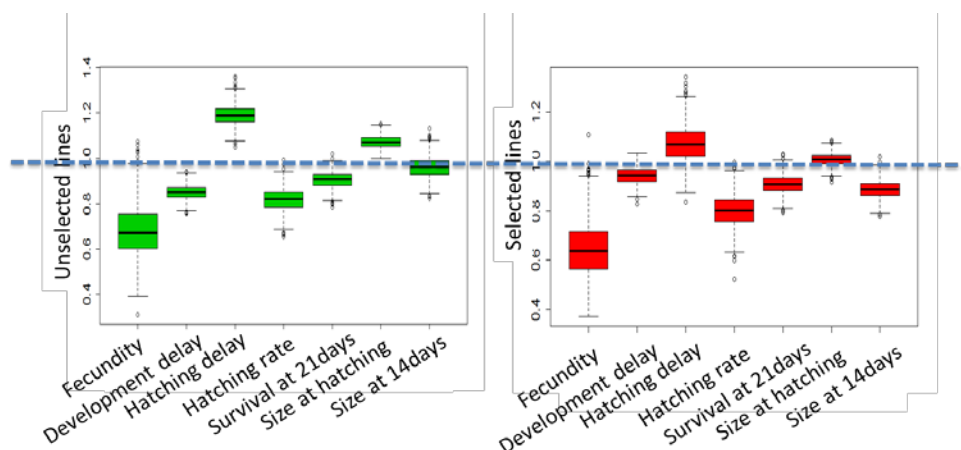


Fig 2. Relative performances (W_{SELF}/W_{OUT}) at various life history traits measured on G4 adults and G5 juveniles of *L. stagnalis*. The blue dashed line indicates equivalent trait value under selfing and outcrossing.

4. Conclusions

This study showed that the evolutionary response of *L. stagnalis* to selection by diquat was not improved by self-fertilization. Highly inbred lines were more sensitive than their outcrossed counterparts to diquat chronic exposure. In terms of toxicity testing, the use of strains maintained for generations in the laboratory may be questionable, due to high inbreeding and depleted genetic variability. To circumvent this drawback and improve ecological risk assessment, we recommend the use of at least two distinct original populations.

5. References

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