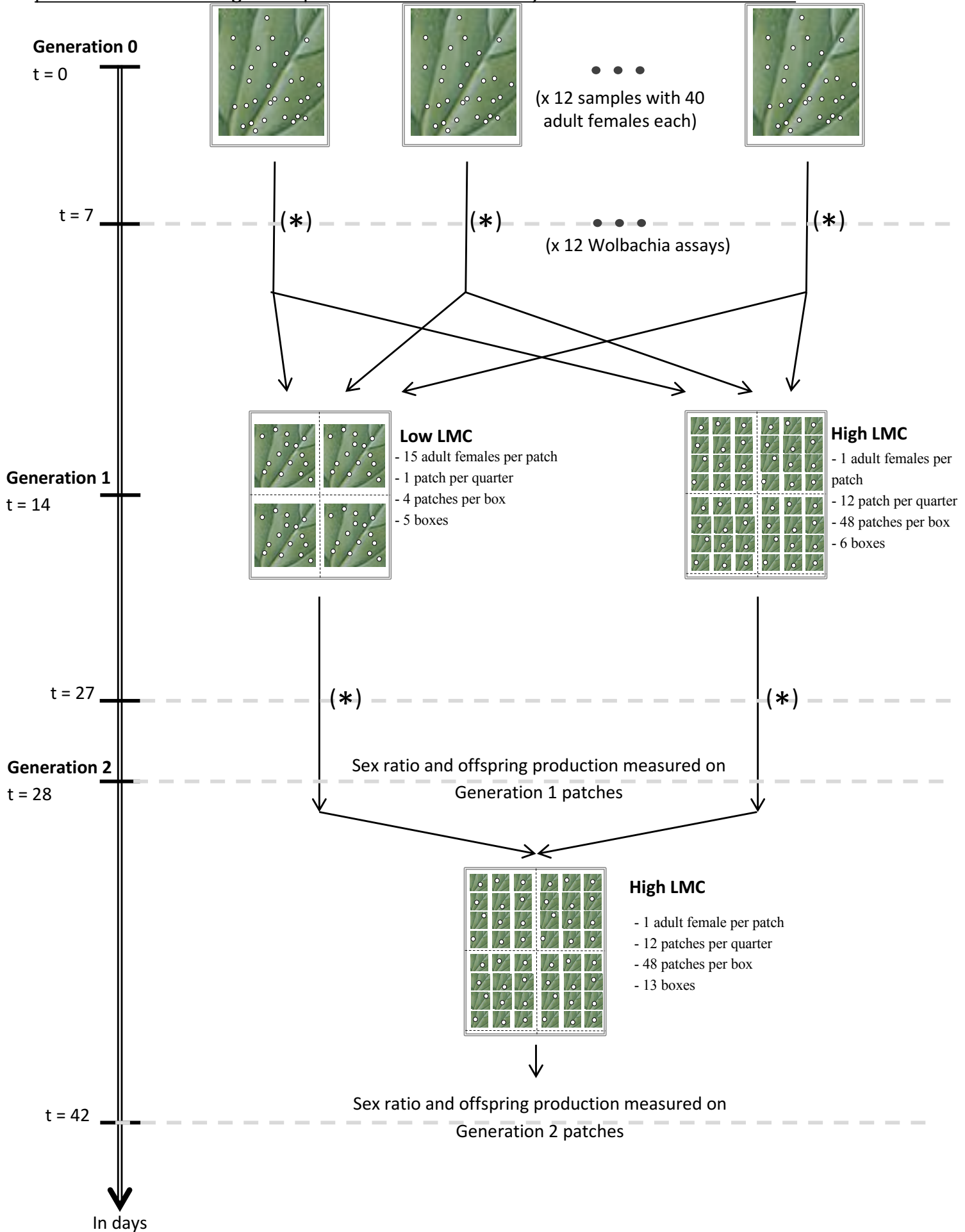


Figure S1: design for Experiment 1: sex allocation in response to LMC across generations. Leaf patches are shown as green squares and females *Tetranychus urticae* as white circles.



## Supplementary Materials

### Appendix 1: Testing for the presence of *Wolbachia* in our *Tetranychus urticae* populations

We tested for the presence of *Wolbachia* on four occasions during our experiments. Prior to starting the experiment, we tested that the base population was not infected with *Wolbachia*. This first test was on a pool of 50 adult females haphazardly chosen from the population. Subsequently, we tested that each of the 12 independent samples (generation 0) were *Wolbachia* free. To do this we pooled between 21 and 39 adult female mites (mean  $27.92 \pm 1.35$  SE) from each sample, after they had laid eggs. We also tested that pools of 12 adult, female offspring from each 'quarter' of each box from the 'maternal generation' remained uninfected with *Wolbachia*. Similarly, we tested pools of 5 adult, female offspring from each patch from the 'sib-mating' experiment. All tests confirmed that *T. urticae* used in experiments were not infected with *Wolbachia*.

DNA was extracted from each pool of mites using the Dneasy Cell and Tissue Kit (Qiagen) following the protocol described in Boubou *et al.* (2011). DNA amplification was performed in a total volume of 10 $\mu$ l (5 $\mu$ l of Qiagen Multiplex buffer, 3 $\mu$ l of sterile water, 1 $\mu$ l of primer premix and 1 $\mu$ l of template DNA). The primer premix comprised *Wolbachia* primers for the 222bp fragment of the *wsp* gene (WSPTG forward: 5'-GTT GGT GTT GGT GCA GCG TAT GTA AGC-3'; WSPTG reverse: 5'-AGT GCT GTA AAG AAC TTT GAT TCC GCC ATC-3') at a 2 $\mu$ M concentration, as well as the positive control for *Tetranychus urticae* using primers for the 386bp fragment of the ITS1 gene (forward: 5'-AGG TGA ACC TGC GGA AGG ATC ATT AAC G-3'; reverse: 5'-CCT TCT TTA AAC CTT GCC GTC AGC ATA AGC-3') at a 0.5 $\mu$ M concentration. PCR conditions were as follows: denaturation at 95 °C for 15 minutes, followed by 30 cycles consisting of a denaturing step at 94 °C for 30 seconds, an annealing step at 61 °C for 1 minutes 30 seconds and an extension step at 72 °C for 1 minute. A final extension step at 72 °C for 30 minutes was added after 30 cycles.

#### REFERENCES

Boubou, A., Migeon, A., Roderick, G.K. & Navajas, M. 2011. Recent emergence and worldwide spread of the red tomato spider mite, *Tetranychus evansi*: genetic variation and multiple cryptic invasions. *Biol. Invasions* **13**: 81–92.

## Appendix 2: SAS script for analyses

### 1. Generation 1: mean offspring production per female

```
data play;
  infile '/folders/myfolders/sasuser.v94/ME_gen1_fec.txt';
  input Box$ Treatment$ Offspring_no;
run;

proc print data=play;

proc glimmix data=play;
  class Box Treatment;
  model Offspring_no = Treatment / dist=Poisson link=log solution;
  random Treatment(Box);
  lsmeans Treatment/DIFF;
  output out=sortie;
run;
```

### 2. Generation 2: sex ratio

```
data play;
  infile '/folders/myfolders/sasuser.v94/ME_GEN2_no2.txt';
  input Quarter$ Box$ Patch$ Treatment$ males females total_offspring;
run;

proc print data=play;

proc glimmix data=play;
  class Quarter Treatment;
  model males/total_offspring = Treatment / dist=Binomial link=logit solution;
  random Treatment(Quarter);
  lsmeans Treatment/DIFF;
  output out=sortie;
run;
```

### 3. Generation2: offspring production

```
data play;
  infile '/folders/myfolders/sasuser.v94/ME_GEN2_no2.txt';
  input Quarter$ Box$ Patch$ Treatment$ males females total_offspring;
run;

proc print data=play;

proc glimmix data=play;
  class Quarter Treatment;
  model total_offspring = Treatment / dist=Poisson link=log solution;
  random Treatment(Quarter);
  lsmeans Treatment/DIFF;
```

```
output out=sortie;  
run;
```

#### 4. Sibmating: sex ratio

```
data play;  
  infile '/folders/myfolders/sasuser.v94/ME_Sib_8_8_17.txt';  
  input Male_family$ Female_family$ Block$ Treatment$ males females  
total_offspring;  
run;
```

```
proc print data=play;
```

```
proc glimmix data=play;  
  class Female_family Block Treatment;  
  model males/total_offspring = Treatment / dist=Binomial link=logit solution;  
  random Female_family Block;  
  lsmeans Treatment/DIFF;  
  output out=sortie;  
run;
```

#### 5. Sibmating: offspring production

```
data play;  
  infile '/folders/myfolders/sasuser.v94/ME_Sib_8_8_17.txt';  
  input Male_family$ Female_family$ Block$ Treatment$ males females  
total_offspring;  
run;
```

```
proc print data=play;
```

```
proc glimmix data=play;  
  class Female_family Treatment;  
  model total_offspring = Treatment / dist=Poisson link=log solution;  
  random Female_family;  
  lsmeans Treatment/DIFF;  
  output out=sortie;  
run;
```