# Benefits and costs of adaptation to constant and alternating polluted environments

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## Abstract

Some populations are able to adapt to anthropogenic stressors such as pollutants, when the selection pressures entailed are not too high. However, this evolution may be accompanied with some costs related to adaptation in the novel environment. Evaluating these costs is important for our understanding of the evolution of local adaptation and specialization to certain environments. Here, we used an experimental evolution approach where we exposed *C. elegans* to uranium (U), salt (NaCl) and alternating U and NaCl treatment during 22 generations. In parallel, at generation 6, 9, 12, 15, 18 and 23, we ran experiments to compare fertility, growth, and locomotion of the populations, having evolved in the different environments: in the environment of origin (i.e. common garden) at the control temperature or at a increase of 5°C of temperature, and in the other environments of their environment of evolution (i.e. reciprocal transplant). Our results showed rapid evolutionary changes and different life history strategy for selected individuals depending on the pollution regimes but none of these populations reached the fitness of the controlled populations. Populations that had evolved in each of the three stressful environments showed a lower fitness than the control populations, when they lived at both original and the increased temperature. Furthermore, populations specialize to salt were more susceptible to uranium but the reverse was not true. Finally, populations adapted to the alternating U and NaCl environment possessed individuals with the best cumulative fitness for both of these pollutants. Assessing costs of rapid adaptation may be crucial regarding the Ecological Risk Assessment of pollutants. It permits to evaluate the impact of evolutionary response to pollutants on the susceptibility changes of populations to environmental conditions.

## Keywords

experimental evolution; costs of adaptation; life history strategy; resistance; local adaptation; evolution of generalism; *Caenorhabditis elegans*; pollution; uranium

## 1. Introduction

Environmental changes are assumed to have increased in frequency and intensity throughout the world, as a result of anthropogenic activities (Millennium Ecosystem Assessment, 2005). These sudden and important changes may be extremely critical for the future of natural populations (Tilman & Lehman, 2001 ; Bell & Collins, 2008). Genetic variation is supposed to allow populations to adapt quickly to severe and novel stressors, and thus to limit their risks of extinction (Hoffmann & Parsons, 1991 ; Charlesworth & Hughes, 2000 ; Reed *et al.*, 2003 ; Bell & Collins, 2008). Experimental and field studies have shown microevolution for populations in response of different pollutants such as xenobiotics or heavy metals (Xie & Klerks, 2003 ; Ward & Robinson, 2005 ; Morgan *et al.*, 2007 ; Lopes *et al.*, 2008 ; Brausch & Smith, 2009 ; Salice *et al.*, 2010 ; Jansen *et al.*, 2011b). Here we consider as pollutants substances that exceed a threshold concentration causing adverse effects on all or part of the ecosystem (AFNOR, 1994).

Rapid adaptation goes through the selection of individuals with characteristics providing a better fitness in the given environment, and a disappearance of less fitted genotypes in the short time frame of a few generations (Hoffmann & Parsons, 1991 ; Posthuma & Van Straalen, 1993 ; Morgan *et al.*, 2007). This adaptive response is assumed to come with a cost: the genetic impoverishment leads to a reduction of the evolutionary potential of the population, which may restrict it from dealing with novel selection pressures associated with subsequent stressors (Bergelson & Purrington, 1996 ; Coustau *et al.*, 2000 ; Xie & Klerks, 2003 ; Salice *et al.*, 2010). Understanding under which conditions adaptation to an environment is costly is a critical issue in evolutionary and in conservation biology. Firstly, rapid adaptation can be associated with a reduction of genetic diversity (Ward & Robinson, 2005 ; Athrey *et al.*, 2007) generally correlated to a decrease in individuals' fitness (Reed & Frankham, 2003). Secondly, associated to the reduction of genetic diversity, costs of adaptation can be due to selection of individuals with specific strategies conferring a selective advantage in the novel environment. The selection can be done indirectly on the life history traits. For example, the selection of genotypes allocating more resources to detoxification mechanisms at the expense of other fitness related functions (e.g. growth or reproduction). In the absence of the pollutant these highly detoxifying genotypes may show a reduced fitness compared to the lowly detoxifying genotypes (Kraaijeveld & Godfrey, 1997 ; Burdon & Thrall, 2003). There are also cases where the selection can act directly on traits, such as growth and reproduction. For example, a rapid growth and early reproduction can reduce

the internal concentration of pollutant by dilution and allow some reproduction before the damages of the pollutants are too high (Sibly & Calow, 1989). However, in the absence of the pollutant these strategies may not be the most optimal. Evidences are increasing of costs of adaptation to pollutants in multigenerational experiments (Shirley & Sibly, 1999 ; Ward & Robinson, 2005 ; Wang *et al.*, 2010 ; Jansen *et al.*, 2011b).

The occurrence of adaptation costs and their detection will depend on (i) the similarity of selection pressures between the previous and the novel environment (Travisano & Lenski, 1996 ; Jasmin & Kassen, 2007), (ii) the intensity of selection (Anderson *et al.*, 2003). We also assume that (iii) trade-offs between the measured traits may constrain their independent evolution and thus lead to potential adaptation costs. For example, the evolution of one trait could entail a cost of adaptation on other, genetically correlated, traits (Falconer & Mackay, 1996 ; Roff, 2002b ; Roff & Fairbairn, 2007). Furthermore, fundamental trade-offs have more chance to be perceptible than trade-offs between traits less related to fitness. This is the case in the trade-off between growth rate and yield of *Escherichia coli* populations (Novak *et al.*, 2006). For all these reasons, costs are not systematically found (see Coustau *et al.*, 2000 ; Reznick *et al.*, 2000 ; McCart *et al.*, 2005 ; Lopes *et al.*, 2008). Consequently, it becomes necessary to compare the effects of different (i) previous polluted environment of evolution and (ii) novel stressful environments on the existence of adaptation costs to define a comprehensive and predictive framework.

Experimental studies on the costs of adaptation generally focus on costs induced by the evolution in response to a single stressor (e.g. Ward & Robinson, 2005 ; Lopes *et al.*, 2008) or to a combination of stressors (e.g. Jasmin & Kassen, 2007 ; Jansen *et al.*, 2011a). Comparatively, few studies on the evolution in a heterogeneous environment have been proposed yet (see Turner & Elena, 2000 ; Reed *et al.*, 2003 for temporal heterogeneity), despite the fact that they probably reflect more natural conditions (Levins, 1968 ; Hedrick, 1974, 1976, 1986). Furthermore, environmental heterogeneity is assumed to help maintaining a higher level of genetic variation (Hedrick, 1986 ; Roff, 2002b). In such a heterogeneous environment, populations may not reach the same level of adaptation (but see Turner & Elena, 2000), but they may also suffer lower costs of adaptation than if they were evolving in a homogeneous environment (Reed *et al.*, 2003). It is thus necessary to conduct more studies comparing the evolutionary responses of populations to constant and fluctuating selection pressures.

Using an experimental evolution approach on *Caenorhabditis elegans*, we test in this study the hypothesis that adaptation to different polluted environments entails differential fitness costs in original (i.e. control) or novel stressful environments. We first ran a multigenerational experiment in which populations evolved for 22 generations in response to either a constant presence of uranium (U), high sodium chloride (NaCl), or U and NaCl alternating every generation (alternating environment). Then, we compared the performance (i.e. value of several phenotypic traits such as survival, fertility or growth) of populations from each pollution (i.e. uranium, salt or alternating treatments) with the control populations by putting these populations back into the original environmental conditions (common-garden experiment: Conover & Schultz, 1995). In principle every reduction of performance in the treatment populations compared to the control ones would reveal a fitness costs related to the genetic differentiation that took place through time (e.g. Shirley & Sibly, 1999 ; Ward & Robinson, 2005 ; Schulte *et al.*, 2010). We also submitted populations to a fast temperature increase (i.e. 5°C) to evaluate the adaptive costs of populations submitted to another type of stress than pollution. Fast temperature increase is a stress that many natural populations will cope with at an increasing rate in the near future (Root *et al.*, 2003 ; IPCC, 2007). Furthermore, we ran a reciprocal-transplant experiment (e.g. Hassel *et al.*, 2005 ; Iraeta *et al.*, 2006) in which we compared the performances of individuals from the four different treatments when transferred in uranium or salt environments (Figure 20A). In principle, we should expect that the fitness performance of each population should be higher in its evolved environment. Similar fitness performances in another novel environment (e.g. salt) than in the evolved environment (e.g. uranium) may indicate that adaptation to a given environment can have positive fitness effects in presence of another stressor (Figure 20B). In contrast, a fitness performance that is lower in the alternative novel environment compared to the evolved and the control environment may indicate that adaptation to a novel environment is associated to a cost in the ability of the population to cope with another stressor. Finally, to see the dynamics of appearance of costs through evolution, we measured the costs in original environmental conditions at several times of the multigenerational experiment. These experiments allowed us to identify whether costs of adaptations to a given stressor (pollutant) exist, but more importantly whether they appear on the same traits, in the same novel environments and at the same time depending on the pollutant (uranium or salt) and the type of pollution (constant or alternating).

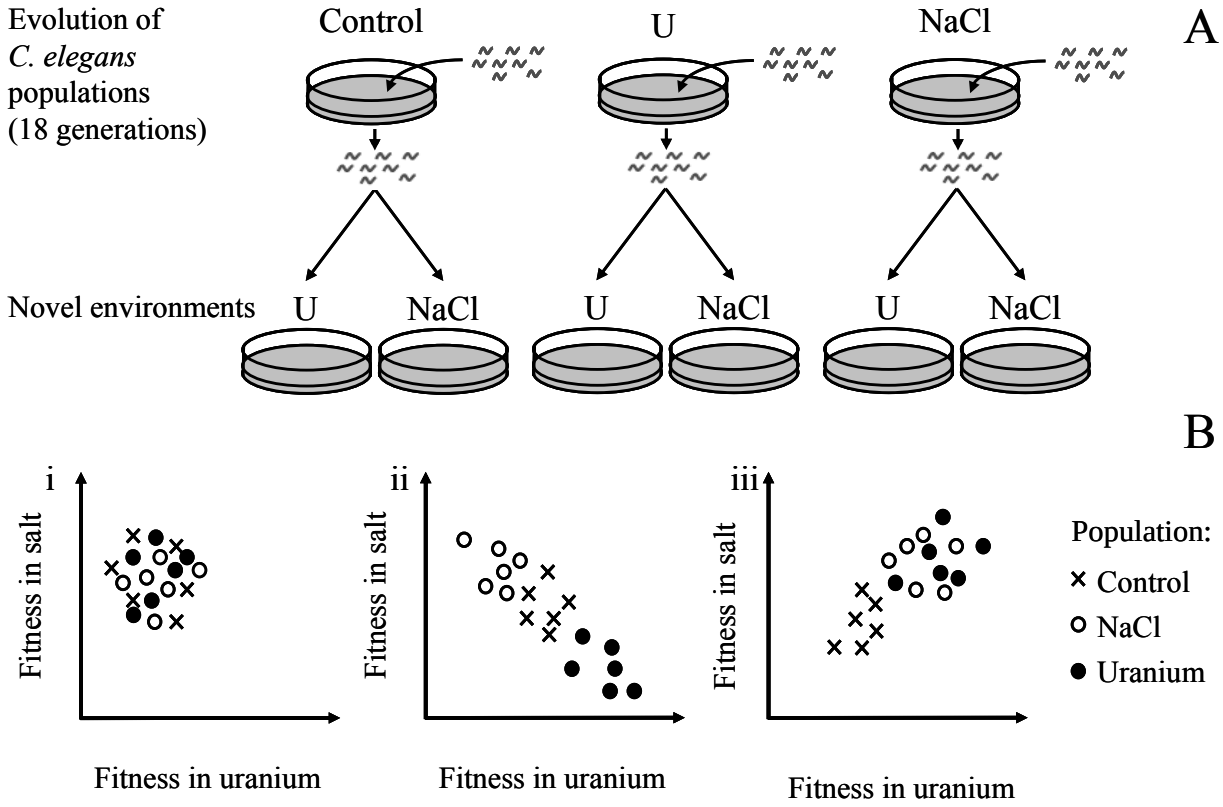


Figure 20. Reciprocal-transplant experiment design and potential responses.
In (A): Schematic overview of the reciprocal-transplant experiment design (A). First, populations lived 18 generations in a given environment (here we illustrate the experiment using control, uranium (U), and salt (NaCl) environments only). Second, each population was placed in their own environment and the other environments (e.g. here uranium, salt). In (B): Potential relationships for each population between the trait value (or fitness) measured in one environment (i.e. NaCl) as a function of the trait value measured in the other environment (i.e. U). Populations that have evolved in the control, in the uranium and in the salt environments are represented by crosses, black dots, and empty dots, respectively (each mark is the measure for an individual). (i) no evidence of adaptation; (ii) costs of adaptation to one pollutant in presence of the other pollutant so control population have a intermediate fitness in presence of each pollutant; (iii) benefit of adaptation to one pollutant in the other and vice versa, so control population have a lower fitness than other populations.

## 2. Material and methods

### 2.1. Population maintenance and prior environment of evolution

Because of its short life cycle, small body length, and ease of handling, *C. elegans* represents a good metazoan model to perform microevolution experiments (Braendle *et al.*, 2008). To obtain a study population with a large genetic diversity, we used in this study a stock population of *C. elegans* composed of a mixture of 16 wild isolates (Teotónio *et al.*, 2012). The population was kept in the experimental conditions described in Teotónio *et al.* (2012) for over 140 generations prior to our study. During the experiment we changed the conditions (see article III). Briefly, we placed 500 individuals in a 9 cm diameter Petri plate filled with an agar medium seeded with 1 ml UV-killed *Escherichia coli* OP50 strain as food source. After three days of population development we transferred 500 individuals from all developmental stages into a new Petri plate. We produced six replicated experimental populations. Generation time in *C. elegans* (i.e. time to complete a life cycle) is lower than three days (Byerly *et al.*, 1976). The population was composed of males for an androdioecious breeding system (i.e. self-fertilization of hermaphrodites and facultative outcross with males). Nematodes were cultured throughout the experiment at 20°C and 80% of relative humidity.

### 2.2. Selection experiment

For this experiment we chose to use uranium and salt environment as stressors. Uranium is a natural radioactive heavy metal which concentrations in sediments or surface soils have increased recently as a result of human activities such as mining (UNSCEAR, 2000 ; Lottermoser *et al.*, 2005). Exposure to natural uranium may induce both chemical and radiological effects, although uranium is assumed to have relatively higher chemotoxic than radiotoxic effects (Thomas & Liber, 2001 ; Kuhne *et al.*, 2002 ; Miller *et al.*, 2002 ; Zeman *et al.*, 2008 ; Mathews *et al.*, 2009). Salt concentration has increased recently in ecosystems, for many reasons, the most important one being the intensive irrigation of some cultivated lands (Rengasamy, 2006 ; Verwey & Vermeulen, 2011). High salt exposure is an extreme hypertonic stress that provokes a rapid water and solute content loss in cells of *C. elegans* (Lamitina *et al.*, 2004).

After repeating this protocol for about 40 generations, the individuals from the six replicates were mixed before being transferred into four different environmental conditions: a control environment (as described above) and three polluted environments, identical in all aspects

to the control, except for the addition in the agar medium of (i) 1.1 mM of uranium (uranyl nitrate: $UO_2$ $(NO_3)_2$, $6H_2O$; Sigma-Aldrich, France), (ii) 308 mM NaCl or (iii) alternating uranium and salt at each generation (salt for odd generations). For each type of environment we created six replicate populations of 500 individuals each. Thereafter we will refer to different population evolving in these environments as control, uranium, salt and alternating populations. Uranium and salt concentrations entailed a reduction of fertility about 60% at the first generation of exposition, which corresponds to potentially strong selection pressures. We previously described how we added pollutant in the agar medium (article III). Generation time, however, varied between the treatments. In particular, it was delayed in the NaCl-treatment compared to the other treatments, and therefore depending on the treatment, each experimental iteration (i.e. three days) may correspond to either a generation or a bit less than a generation. However, to simplify we kept using the term generation throughout the text. Here, we present measured of phenotypic traits at three different generations to observe the effects of treatments at the beginning (generation 1) and at the end (generation 22) of the experiment. Moreover, we decided to focus on genetic differentiations among populations and not to changes caused by within-individual (Scheiner, 1993) or to cross-generation phenotypic (i.e. maternal effects; Räsänen & Kruuk, 2007) plastic response to the novel environment. We thus show the results starting at the fourth generation.

### 2.3. Common-garden and reciprocal-transplant experiments

Our objective was to estimate the costs of adaptation to uranium, salt, and the alternating treatment. Thus starting from generation 6 of the multigenerational experiment, and then every three generations, we isolated 500 individuals from each replicate to run a common-garden experiment by putting the individuals from each replicate and for each treatment back into the control environment. Differences observed in the control environment between the populations that have evolved in different prior environments can be attributed to genetic changes caused by the evolution during the multigenerational experiment (Levins, 1968 ; Conover & Schultz, 1995).

At generation 18, we ran a reciprocal-transplant experiment in which samples of each replicate population from each treatment were transferred into uranium and salt environments. A negative interaction between the prior environment and the transplant environment on the trait

measured should reveal some costs of adaptation to the prior environment (see below statistical analyses).

Finally, at the end of the multigenerational experiment (i.e. generation 23), we realized one last common-garden experiment similar to the previous ones in all the conditions, with the exception that we placed the populations in incubators at two different temperatures: 20°C (i.e. similar to the control one in previous experiments) and 25°C. Such an increase provokes a reduction of development time and fertility and an increase of speed growth in *C. elegans* (Byerly *et al.*, 1976). Additional reduction of fitness at 25°C for populations that lived previously in polluted environment compared to control populations should affect the ability to deal with an environmental stress.

The costs had to be measured at least after two generations to overcome effects caused by different parental environments (Mousseau *et al.*, 2009 ; Kawecki *et al.*, 2012). Thus as mentioned above for generation 4 of selection experiment, prior to all measures of phenotypic traits each population spent three generations in the control or novel environment (same transfer at each generation than in the selection experiment) to ensure that the responses observed were due to the genetic differentiations.

### 2.4. Phenotypic measures

To measure survival and sex ratio after 48h, we transferred approximately 100 eggs from the given generation into another Petri plate, containing the same medium that the replicate population was experiencing. From this sample we picked up three hermaphrodites per replicate randomly and measured their early (i.e. before 96h) and late (i.e. after 96h) brood size (thereafter referred to as early and late fertility, respectively). The sum of early and late brood size gave us an index of total fertility during the overall life of a hermaphrodite. We measured body bend frequency on 3 males per replicate at age 96 h, which corresponds to locomotion behaviour. Locomotion allows males and hermaphrodites to find good living conditions, but also affect male encounter rate with hermaphrodites, which is essential for males reproduction and fitness (Pannell, 2002 ; Barrière & Félix, 2005a). At age 96h these males and hermaphrodites were photographed, using a stereomicroscope (Olympus SZX12, 1.6 x 90 magnification) with a computer-connected camera (Nikon D5000). From these pictures we measured body length that we used as an index of growth from age 0 to 96h. Because of faster growth of individuals at 25°C,

we chose 72h for the separation between early and late fertility and morphological measurements, for the last common-garden experiment. Moreover, we were not able to count body bend at 25°C since we needed to change the room temperature to do it. The conditions of medium, quantity of food and how we precisely measured the traits was also previously described (article III).

### 2.5. Statistical analysis

We compared the different treatments using separate statistical models for hermaphrodites and males. We used a generalized linear mixed effects model (GLMM) approach implemented within a Bayesian Monte Carlo Markov Chain (MCMC) framework (Hadfield, 2010) in the R software (R Development Core Team, 2012).

In a first univariate mixed-effect model we tested the effects of environment and generation (i.e. Generation 1, 4, and 22) on the fitness of the population (calculated as total fertility over three hermaphrodites multiplied by survival rate of the replicate), and using replicate as a random effect. We then ran mixed models for the data on common-garden and reciprocal transplant experiments. Here again replicate within each treatment was included as a random effect in the model to control for the pseudo-replication issues. We constructed quadrivariate models for hermaphrodite traits (early, late and total fertility, and growth), bivariate models for male traits (growth and body bend), and a univariate model to analyse hermaphrodite growth in the last common-garden at two different temperatures. In the common-garden experiment analysis prior environment (i.e. the environment in which the population has evolved) and generation (i.e. the generation at which the experiment was done) were included as fixed effects. In the reciprocal-transplant models and the last common-garden at different temperatures we added novel environment as a fixed effect.

We chose a Gaussian error structure for all the other traits. For multivariate analyses, we allowed models to estimate covariances or not between the pairs of traits. To avoid any bias in the results caused by mean trait differences, we rescaled the traits prior to analysis by subtracting each value by the mean of the sample and dividing it by twice the standard deviation (Gelman, 2008). After several priors tested, we retained a slightly informative but proper prior ($nu = k - 1 + 0.002$) with a low variance parameter ($V = diag(k)*V_p*0.05$), where $V_p$ is the phenotypic variance, k the dimension of V (e.g. number of traits). After verifying for the convergence of parameters values (i.e. number of iterations, burn-in phase and thinning) and autocorrelation

issues, we retained 120 000 iterations with a burn-in phase of 20 000, for a total of 1 000 samples for each analysis (Hadfield, 2010).

We tested models including different fixed effects and selected the best-fitted model by comparing the deviance information criterion (DIC) of each model. A lower DIC signals a better fit of the model, and a different in DIC of less than 5 indicates that the two models show similar fits (Spiegelhalter *et al.*, 2007). For each series of models, when two models have DICs within a range of 5, we retained the most parsimonious (i.e. with the lowest number of parameters).

In all models we used the posterior distribution for traits that allow estimates of confidence intervals around their estimates. For common-garden experiments, we used the posterior mode of the distribution for intercept and also for slope of each treatment as a function of generation number (note that generation was used as a continuous variable). With these two parameters we were able to model the linear regression for each prior environment and each trait. We considered two parameters to show "significant different" when the 95% interval of highest posterior density (HPDI) did not overlap 0, even if with a Bayesian approach significance reflect more a difference that is considered as non negligible (differ from the significance level commonly used in a frequentist approach). To compare a trait in two conditions, we checked whether 95% HPDI of the difference between the whole posterior distributions of the trait for the two conditions overlaps 0.

In the multigenerational experiment we predicted lower trait values in each stressful environment compared to the control one in the first generations. We also predicted that values in each stressful environment will improve with time and that difference between each stressful environment and the control will decrease at the end of the experiment (indicating a potential evolutionary response in the stressful environment). A genetic differentiation caused by differential evolution each stressful environment and the control should be revealed in the common garden experiments by a difference between the intercept of the values of the trait in each stressful environment and the control. Furthermore, we expected an increase over time in the genetic differentiation between populations experiencing different treatments, revealed by differences between the slopes of the trait values as a function of the treatment. In the reciprocal-transplant experiment, we looked traits values of the populations that have evolved in the different environments (i.e. three treatments and the control) when they were placed in the two novel environments (i.e. uranium or salt). More precisely, we predicted that populations showing the

highest trait value in one environment should be the ones that had previously evolved in that environment. In contrast, these populations should show lower trait values in the other environment, indicating a cost of adaptation to the previous environment, and the control populations should have value between that of the other populations (Figure 20Bii). In the last common-garden experiment with the two different temperature treatments, we predicted a negative effect of high temperature on trait values and a stronger effect for populations that had evolved in the stressful environment compared to control.

## 3. Results

### 3.1. Multigenerational experiment

The model for fitness with the smaller DIC included the interaction between environment and generation (Table 10A). Thus, fitness changes across generations depended on the type of environment. Fitness did not change through time in the control environment (Table 11 and Figure 21). Compared to the control populations, in all the replicate populations, fitness decreased directly (i.e. generation 1) after the population was introduced in uranium, salt, or the alternating U/NaCl treatment (Table 11 and Figure 21). During the first four generations, fitness increased in the uranium and the alternating treatments but not in the salt treatment. Fitness increased between generation 4 and 22 in the salt and in the alternating treatments but not significantly in the uranium treatment. At generation 22, fitness was still weaker in the three novel environments than in the control.

### 3.2. Common-garden experiments

Changes observed between the successive common-garden experiments performed all along the multigenerational experiment represent genetic changes in each trait with time (Table 12 and Figure 22).

The model selected for hermaphrodite traits included an interaction between prior environment and generation (Table 10B). This interaction indicated that the changes in the average value of the traits across generations depended on the prior environment in which the population has evolved. The model allowing a covariance component between traits was associated with a better fit than the model without covariance. None of the traits measured in the

control populations changed with time, indicating an absence of uncontrolled evolutionary change in that environment during the experiment (Table 12A and Figure 22A). Total fertility of salt populations was similar to the control populations in the first common garden (i.e. intercept in Table 12A), but it showed a strong decrease across generations relative to control populations (Figure 22A). In contrast, total fertility of uranium and alternating populations was affected at the first generation, but then showed a steady increase across generations relative to that of control populations (the slope for total fertility of uranium populations almost overlapped 0, Table 12A). Slope for early fertility was positive for uranium populations (Table 12A and Figure 22C). Uranium and alternating populations had a lower growth compared to control populations in the first common-garden, but then they showed stronger slopes of growth over the successive common-garden experiments (Table 12A and Figure 22B).

For male traits the best-fitted model did not include the interaction between prior environment and generation, indicating an absence of evolution in male traits leading to sufficient genetic differentiation between the treatments (Table 10B). There was a reduction of male growth from the first common-garden for uranium and alternating populations and a significant positive slope for all populations, indicating a stronger growth in all treatment over the successive common-garden experiments (Table 12B and Figure 22F). However, a test with a model with interaction between prior environment and generation showed a significant slope only for uranium and alternating populations and not for control populations (data not shown). We saw any effect on body bend (Table 12B and Figure 22E).

Table 10. Comparison of models for traits measured in the different experiments.
Fitness (fertility multiplied by survival frequency) measured in the multigenerational experiment done at generation 1, 4, and 22 (A); hermaphrodite traits (total, early, and late fertility, and growth) or male traits (growth and body bend frequency) measured in common-garden experiments in control environment done at generation 6, 9, 12, 15, and 18 (B); in the reciprocal-transplant experiment done at generation 18 (C); and in the common-garden experiment at two different temperatures done at generation 23 (D). For the latter experiment we did not measured body bend frequency of males. Environment (or prior environment) corresponds to the environment in which the population has been evolving (i.e. control, uranium, salt or U/NaCl alternating treatment) and this effect was present in the models for all experiments (A to D). Generation corresponds to the generation for which we measured traits in multigenerational and common-garden experiments (A, B). Novel environment corresponds to the environment of transplant in the reciprocal-transplant experiment (C). Temperature corresponds to the temperature for which we measured traits in the common-garden experiment at two different temperatures (D). We used multivariate (or univariate in A and for male trait in D) mixed models with all the traits included as dependent variables, and compared different models using deviance information criterion (DIC). All the models contained replicates included as a random effect to control for independence of data across generations. The first DIC value corresponds to a simple model including only replicates as a random effect, and the subsequent value correspond to the associated change ($\Delta$) in DIC estimates that occurs when the effect is included within the model. Except for the models shown at the last line for each sex (and univariate models), covariance (cov) between traits was allowed in the priors. In bold, models for which $\Delta DIC > 5$, i.e. the models that had a smaller DIC, for which the replicate effect for hermaphrodite and male traits was written below table.

A - Multigenerationnal experiment

| Effect included within the model | DIC | Δ DIC |
|---|---|---|
| for hermaphrodite traits | | |
| - | 24.538 | - |
| environment | -10.864 | -35.402 |
| environment + generation | -36.198 | -25.334 |
| environment x generation | **-65.714** | **-29.516** |

Replicates effect: 0.0%

B - Common-garden experiments in control environment

| Effect included within the model | DIC | Δ DIC |
|---|---|---|
| for hermaphrodite traits | | |
| - | -704.829 | - |
| prior environment | -721.353 | -16.524 |
| prior environment + generation | -739.623 | -18.270 |
| prior environment x generation | **-744.419** | **-4.796** |
| prior environment x generation (no cov) | 1585.925 | 2330.344 |
| for male traits | | |
| - | 1046.823 | - |
| prior environment | 1041.722 | -5.101 |
| prior environment + generation | **1023.955** | **-17.767** |
| prior environment x generation | 1025.881 | 1.926 |
| prior environment x generation (no cov) | 1024.779 | -1.102 |

Replicates effect: 2.3% (hermaphrodites) and 2.0 % (males)

C - Reciprocal-transplant experiment

| Effect included within the model | DIC | Δ DIC |
|---|---|---|
| for hermaphrodite traits | | |
| - | -654.464 | - |
| novel environment | -1101.912 | -447.448 |
| novel environment + prior environment | -1117.402 | -15.490 |
| novel environment x prior environment | **-1172.517** | **-55.115** |
| novel environment x prior environment (no cov) | -2.699 | 1169.818 |
| for male traits | | |
| - | 600.821 | - |
| novel environment | 422.846 | -177.975 |
| novel environment + prior environment | 401.901 | -20.945 |
| novel environment x prior environment | 378.241 | -23.660 |
| novel environment x prior environment (no cov) | **376.365** | **-1.876** |

Replicates effect: 5.2% (hermaphrodites) and 3.0 % (males)

D - Common garden at two different temperatures

| Effect included within the model | DIC | Δ DIC |
|---|---|---|
| for hermaphrodite traits | | |
| - | -102.965 | - |
| temperature | -316.462 | -213.497 |
| temperature + prior environment | **-327.095** | **-10.633** |
| temperature x prior environment | -313.615 | 13.48 |
| temperature x prior environment (no cov) | 362.631 | 676.246 |
| for male traits | | |
| - | 191.799 | - |
| **temperature** | **179.448** | **-12.351** |
| temperature + prior environment | 176.924 | -2.524 |
| temperature x prior environment | 180.872 | 3.948 |

Replicates effect: 8.3% (hermaphrodites) and 0.6 % (males)

Table 11. Analyses of differences of fitness (i.e. total fertility multiplied by survival frequency) in multigenerational experiment of selection between generation 1 and 4 and then between generation 4 and 22.
Values correspond to the estimation of fitness, using the posterior mode of the distribution of rescaled fitness, and the limits of the 95% highest posterior density interval (HPDI: between brackets), in control and in the prior stressful (uranium, salt and alternating uranium and salt) environments relative to the control. Values in bold are those for which the 95% HPDI did not overlap 0.

| Comparison of fitness | Control | | | Uranium | | | Salt | | | Alternating U/NaCl | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Generation 1 - 4 | -0.025 | [ -0.173 ; | 0.141 ] | **-0.255** | **[ -0.440 ;** | **-0.119 ]** | 0.042 | [ -0.109 ; | 0.200 ] | **-0.382** | **[ -0.553 ;** | **-0.249 ]** |
| Generation 4 - 22 | 0.079 | [ -0.089 ; | 0.220 ] | -0.137 | [ -0.321 ; | 0.014 ] | **-0.237** | **[ -0.396 ;** | **-0.082 ]** | **-0.262** | **[ -0.404 ;** | **-0.092 ]** |

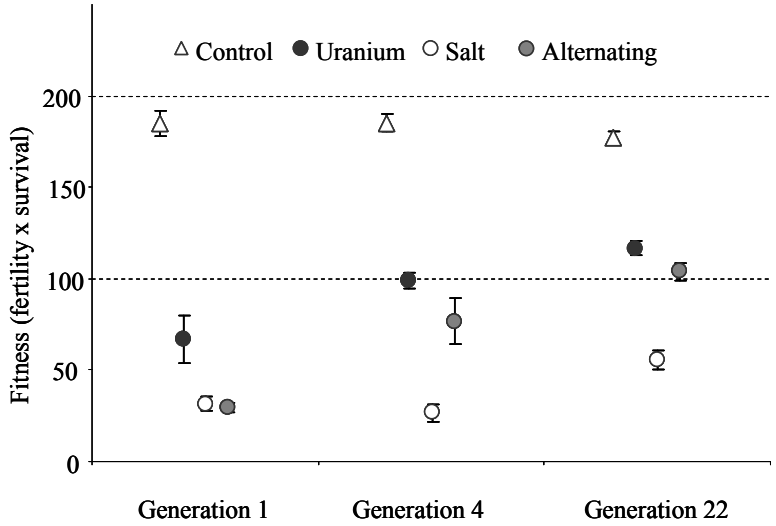


Figure 21. Fitness (i.e. total fertility multiplied by survival frequency) at generation 1, 4 and 22 in the multigenerational experiment.
Symbols represent the mean and its associated standard errors over the 6 replicated populations in each treatment: control = empty triangle; uranium = filled black dots; salt = empty dots; alternating U/NaCl treatment = filled grey dots.

Table 12. Analyses of difference of traits values for hermaphrodites (A) and males (B) in five successive common-garden experiments in control environment conducted at generation 6, 9, 12, 15 and 18 of the multi-generation experiment.

Intercept corresponds to the rescaled traits value at the first common-garden (generation 6) and slope corresponds to the slope of linear regressions across generations. Values correspond to the estimation given by the posterior mode of the distribution for each parameter (i.e. intercept and slope) in control or for each parameter relative to the control in the prior stressful (uranium, salt and alternating uranium and salt) environments, except slope for male traits (one estimation for all prior environments). Values between brackets correspond to the limit of the 95% highest posterior density interval (HPDI). Values in bold are those for which the 95% HPDI did not overlap 0.

A

| Parameter analysed | Control | Uranium | Salt | Alternating U/NaCl |
|---|---|---|---|---|
| Intercept | | | | |
| Total fertility | **0.596 [ 0.255 ; 0.911 ]** | **-1.121 [ -1.615 ; -0.690 ]** | -0.145 [ -0.655 ; 0.260 ] | **-1.130 [ -1.558 ; -0.690 ]** |
| Early fertility | **0.451 [ 0.140 ; 0.822 ]** | **-1.206 [ -1.738 ; -0.775 ]** | -0.214 [ -0.674 ; 0.267 ] | **-0.961 [ -1.445 ; -0.507 ]** |
| Late fertility | 0.365 [ -0.063 ; 0.763 ] | -0.331 [ -0.917 ; 0.152 ] | 0.014 [ -0.537 ; 0.547 ] | **-0.584 [ -1.118 ; -0.060 ]** |
| Growth | -0.071 [ -0.438 ; 0.289 ] | **-0.947 [ -1.450 ; -0.464 ]** | 0.070 [ -0.472 ; 0.566 ] | **-0.697 [ -1.196 ; -0.156 ]** |
| Slope | | | | |
| Total fertility | -0.005 [ -0.026 ; 0.016 ] | 0.024 [ -0.005 ; 0.052 ] | **-0.028 [ -0.057 ; -0.002 ]** | **0.026 [ 0.001 ; 0.053 ]** |
| Early fertility | 0.001 [ -0.021 ; 0.021 ] | **0.031 [ 0.002 ; 0.061 ]** | -0.019 [ -0.049 ; 0.009 ] | 0.019 [ -0.011 ; 0.049 ] |
| Late fertility | -0.008 [ -0.032 ; 0.019 ] | 0.003 [ -0.030 ; 0.039 ] | -0.019 [ -0.054 ; 0.015 ] | 0.016 [ -0.019 ; 0.048 ] |
| Growth | 0.004 [ -0.017 ; 0.029 ] | **0.056 [ 0.026 ; 0.088 ]** | 0.006 [ -0.026 ; 0.039 ] | **0.045 [ 0.013 ; 0.080 ]** |

B

| Parameter analysed | Control | Uranium | Salt | Alternating U/NaCl |
|---|---|---|---|---|
| Intercept | | | | |
| Growth | -0.226 [ -0.431 ; -0.015 ] | **-0.359 [ -0.548 ; -0.209 ]** | -0.127 [ -0.318 ; 0.027 ] | **-0.227 [ -0.393 ; -0.071 ]** |
| Body Bend | -0.044 [ -0.269 ; 0.175 ] | -0.051 [ -0.223 ; 0.130 ] | -0.072 [ -0.243 ; 0.102 ] | -0.047 [ -0.215 ; 0.135 ] |
| Slope (for all prior environments) | | | | |
| Growth | **0.027 [ 0.016 ; 0.038 ]** | - | - | - |
| Body Bend | 0.006 [ -0.007 ; 0.018 ] | - | - | - |

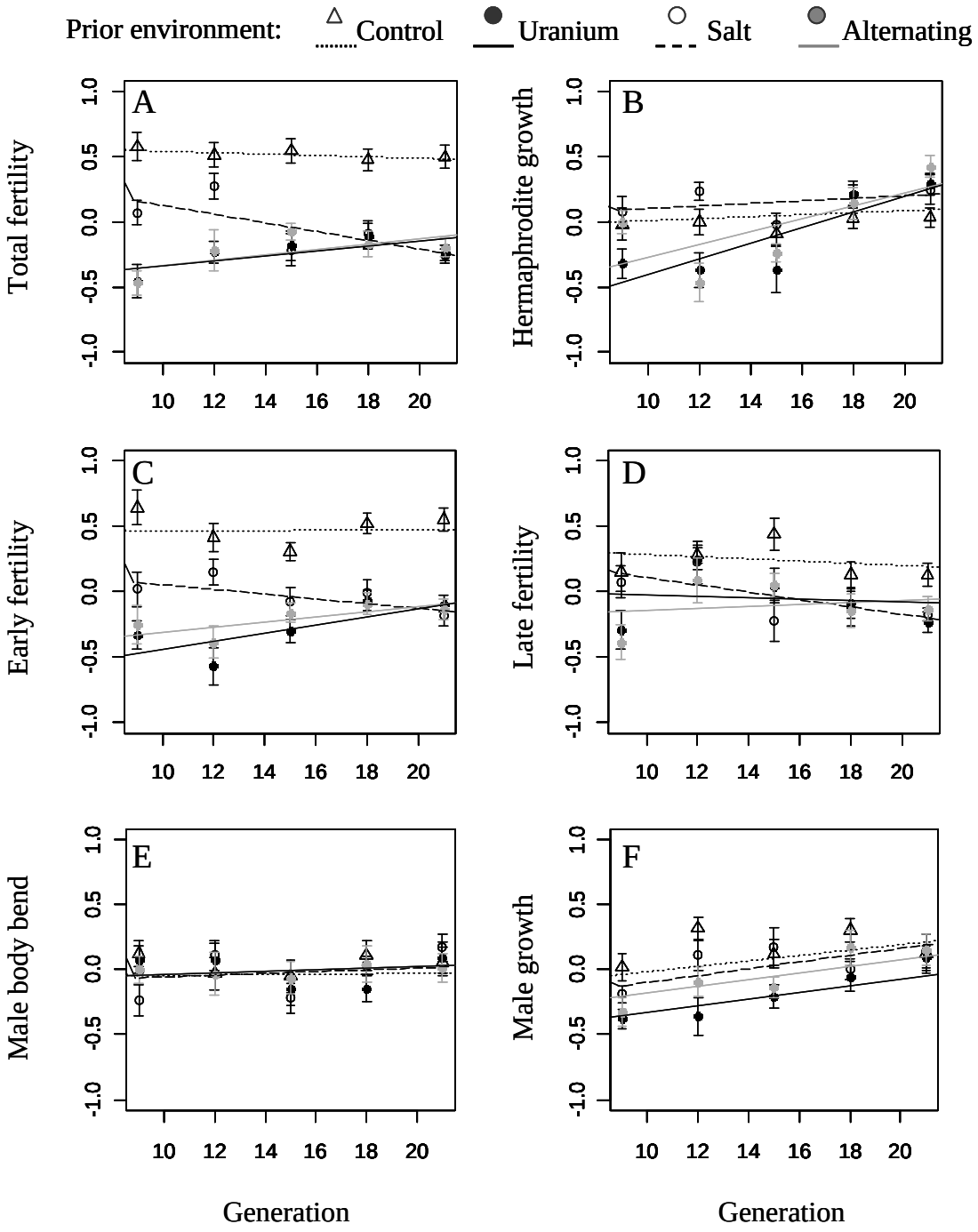


Figure 22. Total fertility (A), hermaphrodite growth (B), early fertility (C), late fertility (D), male body bend (E) and male growth (F) during five successive common-garden experiments conducted at generation 6, 9, 12, 15 and 18 of the multi-generation experiment.

Traits (rescaled) were measured after individuals have spent three generations in the control environment. Symbols represent the mean and its associated standard error for 18 randomly sampled individuals in each treatment. Control = empty triangle; uranium = filled black dots; salt = empty dots; alternating U/NaCl treatment = filled grey dots. Regression lines correspond to posterior mode of the distribution for intercept and slope for each treatment: control = small dashed line; uranium = black line; salt = large dashed line; alternating U/NaCl treatment = grey line.

### 3.3. Reciprocal-transplant experiment

For traits in hermaphrodites, the model with the smaller DIC was the one that included covariance between traits and an interaction between prior and novel environment (Table 10C). Therefore, trait expression in the novel environment depended on the environment populations had previously evolved in.

Total fertility in uranium was similar between populations from prior control, prior uranium, and prior alternating environment (Table 13 and Figure 23A), whereas prior salt populations showed a reduced total fertility compared to the populations that had evolved in these three other prior environments. In contrast, in the novel salt environment, prior alternating and salt populations showed higher values of fertility than prior uranium or control populations (Table 13 and Figure 23A). The same patterns were found for late fertility (Table 13 and Figure 23D). However, for early fertility, control populations showed intermediate values between salt and uranium environments in the novel uranium environment, and control populations showed lower values of early fertility than uranium populations and equivalent value than salt populations in the novel salt environment (Table 13 and Figure 23C). Overall, populations that had evolved in the alternating environment showed the highest total fertility in both of the two stressful environments: hermaphrodites from these populations produced on average more than 95 larvae against 80-86 larvae for the prior control, uranium, and salt populations.

Prior uranium and alternating populations produced bigger individuals at 96h than prior control populations in both the uranium and the salt environments, and prior salt populations did not differ from the prior control populations in both novel environments (Table 13 and Figure 23B).

The model with the smaller DIC for the male traits was the one including an interaction between prior and novel environment and with no covariance between traits (Table 10C). Prior uranium populations produced bigger males than the three other types of populations in both salt and uranium environment (Table 13 and Figure 23F). Prior control populations showed the smallest males in the salt environment.

Individuals of prior salt populations showed a smaller frequency of body bends in the uranium environment, whereas the three other types of population did not differ with each other (Table 13 and Figure 23E). No differences in body bends were observed between the different types of populations in the salt environment.

### 3.4. Common-garden experiment at different temperatures

In hermaphrodites no interaction was detected between temperature (20 and 25°C) and prior environment (Table 10D). In other words, the effects of prior environments on traits were the same at both temperatures. Compared to 20°C, at 25°C all the populations lowered their total fertility and shortened their reproductive longevity; almost 90% of eggs were produced before 72h (Table 14 and Figure 24A, C and D). Hermaphrodites also grew up faster, whatever their population of origin (Table 14 and Figure 24B). Independent of the temperature, control and salt population showed the highest early fertility values, followed by both uranium and alternating populations. Patterns were less clear for late fertility, and total fertility showed a marked difference between the control and the other populations.

Male growth was not affected by prior environments, and increasing temperature reduced growth in males (Table 10D, Table 14 and Figure 24E).

### 3.5. Replicate effects

Differences between replicate populations represented between 0 and 8.3% of the overall trait variation (Table 10), indicating that the random sampling of the individuals that founded the different populations was appropriate.

Table 13. Analyses of difference of traits values between the populations that previously lived in the control or stressful (uranium, salt or alternating uranium and salt) environments for hermaphrodites and males, and in each novel environment (i.e. uranium or salt).

It corresponds to the reciprocal-transplant experiment at generation 18. Values correspond to the estimation of traits, using the posterior mode of the distribution of rescaled traits, and the limits of the 95% highest posterior density interval (HPDI: between brackets).Values in bold are those for which the 95% HPDI did not overlap 0.

| Comparison | Hermaphrodite traits | | | | | | | | | | | | Male traits | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total fertility | | | Early fertility | | | Late fertility | | | Growth | | | Body bend | | | Growth | | |
| In uranium | | | | | | | | | | | | | | | | | | |
| Control-Uranium | -0.086 | -0.193 ; | 0.107 | **-0.143** | **-0.234 ;** | **-0.047** | 0.212 | -0.100 ; | 0.419 | **-0.201** | **-0.347 ;** | **-0.050** | 0.005 | -0.246 ; | 0.261 | **-0.263** | **-0.465 ;** | **-0.077** |
| Control-Salt | **0.209** | **0.082 ;** | **0.385** | **0.113** | **0.031 ;** | **0.210** | **0.296** | **0.019 ;** | **0.549** | -0.077 | -0.196 ; | 0.108 | **0.261** | **0.073 ;** | **0.586** | 0.176 | -0.025 ; | 0.396 |
| Control-Alternating | 0.039 | -0.082 ; | 0.224 | -0.075 | -0.167 ; | 0.019 | 0.193 | -0.005 ; | 0.531 | **-0.177** | **-0.313 ;** | **-0.016** | -0.123 | -0.428 ; | 0.104 | 0.074 | -0.134 ; | 0.271 |
| Uranium-Salt | **0.309** | **0.139 ;** | **0.436** | **0.255** | **0.176 ;** | **0.354** | 0.073 | -0.148 ; | 0.369 | 0.137 | -0.018 ; | 0.281 | **0.296** | **0.042 ;** | **0.558** | **0.449** | **0.255 ;** | **0.657** |
| Uranium-Alternating | 0.112 | -0.037 ; | 0.267 | 0.071 | -0.022 ; | 0.159 | 0.082 | -0.138 ; | 0.386 | 0.018 | -0.112 ; | 0.188 | -0.159 | -0.402 ; | 0.136 | **0.320** | **0.159 ;** | **0.546** |
| Salt-Alternating | **-0.159** | **-0.318 ;** | **-0.006** | **-0.195** | **-0.279 ;** | **-0.102** | 0.113 | -0.240 ; | 0.308 | -0.127 | -0.269 ; | 0.043 | **-0.414** | **-0.753 ;** | **-0.209** | -0.158 | -0.323 ; | 0.088 |
| In salt | | | | | | | | | | | | | | | | | | |
| Control-Uranium | -0.064 | -0.205 ; | 0.088 | **-0.115** | **-0.231 ;** | **-0.040** | 0.139 | -0.126 ; | 0.385 | **-0.222** | **-0.366 ;** | **-0.055** | -0.041 | -0.396 ; | 0.140 | **-0.708** | **-0.932 ;** | **-0.514** |
| Control-Salt | **-0.244** | **-0.371 ;** | **-0.067** | -0.100 | -0.173 ; | 0.012 | **-0.321** | **-0.588 ;** | **-0.062** | -0.038 | -0.178 ; | 0.123 | -0.015 | -0.367 ; | 0.189 | **-0.352** | **-0.554 ;** | **-0.172** |
| Control-Alternating | **-0.403** | **-0.533 ;** | **-0.249** | **-0.214** | **-0.295 ;** | **-0.113** | **-0.393** | **-0.641 ;** | **-0.134** | **-0.272** | **-0.415 ;** | **-0.103** | -0.181 | -0.459 ; | 0.078 | **-0.427** | **-0.630 ;** | **-0.235** |
| Uranium-Salt | **-0.140** | **-0.315 ;** | **-0.010** | 0.046 | -0.034 ; | 0.161 | **-0.443** | **-0.667 ;** | **-0.120** | **0.189** | **0.030 ;** | **0.338** | 0.050 | -0.229 ; | 0.299 | **0.420** | **0.186 ;** | **0.587** |
| Uranium-Alternating | **-0.324** | **-0.478 ;** | **-0.177** | -0.096 | -0.165 ; | 0.016 | **-0.515** | **-0.744 ;** | **-0.220** | -0.045 | -0.204 ; | 0.107 | -0.098 | -0.356 ; | 0.173 | **0.287** | **0.091 ;** | **0.507** |
| Salt-Alternating | **-0.139** | **-0.324 ;** | **-0.012** | **-0.141** | **-0.222 ;** | **-0.034** | -0.057 | -0.350 ; | 0.194 | **-0.215** | **-0.383 ;** | **-0.072** | -0.201 | -0.394 ; | 0.158 | -0.065 | -0.260 ; | 0.131 |

Table 14. Analyses of difference of traits values between the populations that previously lived in the control or stressful (uranium, salt or alternating uranium and salt) environments for hermaphrodites and males, and at 25°C.

It corresponds to the common-garden experiment at two different temperatures. Values correspond to the estimation given by the posterior mode of the distribution of rescaled traits at 25°C between two prior environments (hermaphrodites) or for all prior environments (males). Slope corresponds to the slope of linear regressions between traits values at each temperature (from 25 to 20°C) for all prior environments. Values between brackets correspond to the limit of the 95% highest posterior density interval (HPDI). Values in bold are those for which the 95% HPDI did not overlap 0.

| Comparison | Hermaphrodite traits | | | | | | | | | | | | Male trait | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total fertility | | | Early fertility | | | Late fertility | | | Growth | | | Growth | | |
| At 25°C | | | | | | | | | | | | | | | |
| Control-Uranium | **0.296** | **0.150 ;** | **0.433** | **0.392** | **0.201 ;** | **0.580** | 0.096 | -0.023 ; | 0.174 | 0.026 | -0.199 ; | 0.235 | **-0.394** | **-0.510 ;** | **-0.270** |
| Control-Salt | **0.193** | **0.097 ;** | **0.393** | 0.108 | -0.109 ; | 0.298 | **0.188** | **0.080 ;** | **0.273** | -0.222 | -0.451 ; | 0.011 | for all prior environments | | |
| Control-Alternating | **0.231** | **0.077 ;** | **0.365** | **0.394** | **0.171 ;** | **0.562** | 0.042 | -0.056 ; | 0.140 | -0.007 | -0.263 ; | 0.178 | | | |
| Uranium-Salt | -0.033 | -0.169 ; | 0.124 | **-0.290** | **-0.475 ;** | **-0.091** | 0.083 | -0.018 ; | 0.187 | -0.240 | -0.460 ; | 0.001 | | | |
| Uranium-Alternating | -0.011 | -0.216 ; | 0.069 | -0.064 | -0.245 ; | 0.148 | -0.059 | -0.150 ; | 0.060 | -0.075 | -0.297 ; | 0.151 | | | |
| Salt-Alternating | -0.043 | -0.176 ; | 0.109 | **0.315** | **0.040 ;** | **0.456** | **-0.129** | **-0.249 ;** | **-0.043** | 0.190 | -0.045 ; | 0.417 | | | |
| Slope between 25 and 20°C | **0.742** | **0.633 ;** | **0.837** | **-0.645** | **-0.781 ;** | **-0.505** | **0.927** | **0.853 ;** | **0.999** | **-0.479** | **-0.644 ;** | **-0.339** | **0.286** | **0.130 ;** | **0.436** |

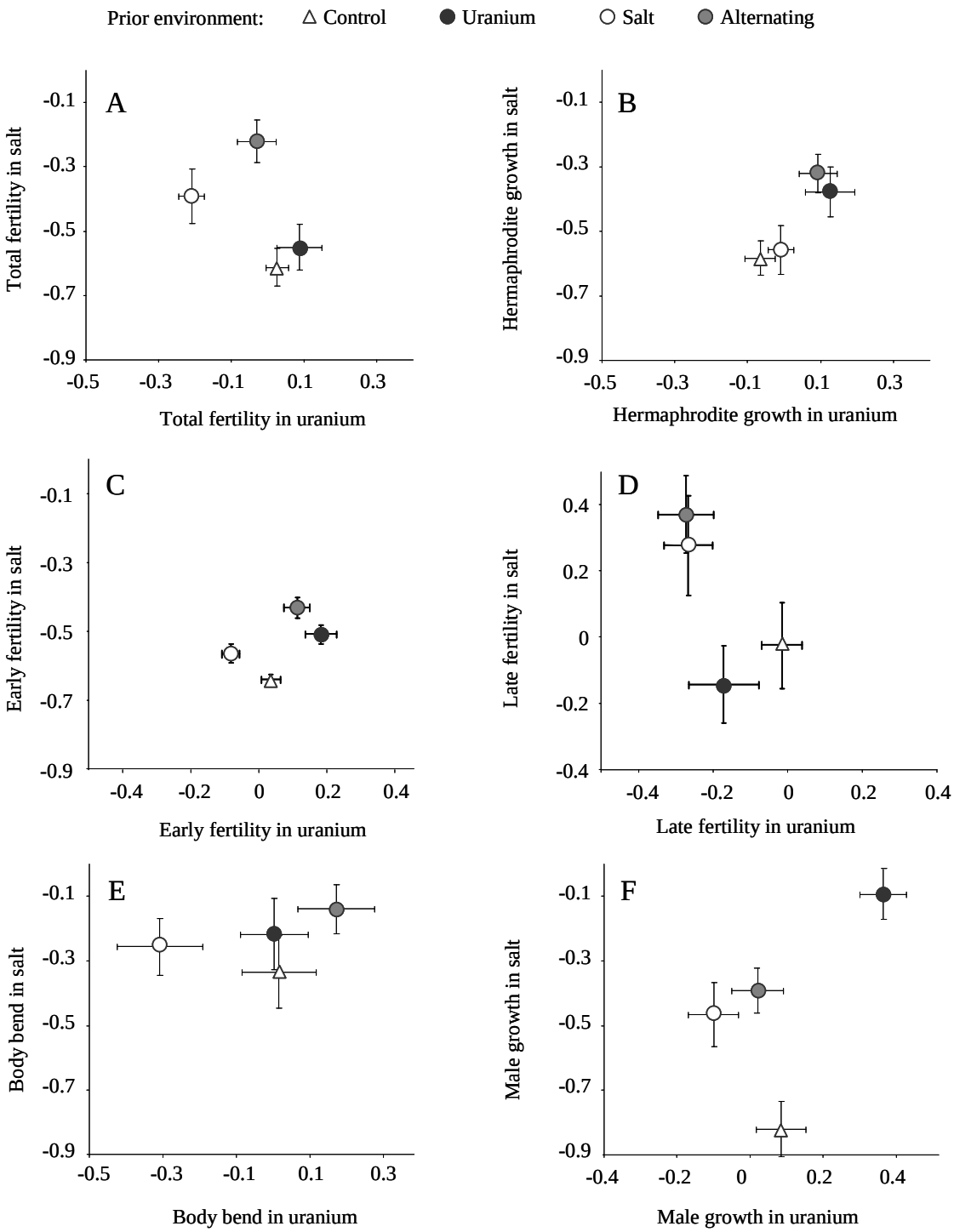


Figure 23. Average traits values in the reciprocal-transplant experiment.
Average values and their standard errors (n = 18 individuals) for populations that have evolved in the four different treatments (i.e. control, uranium, NaCl and alternating uranium and NaCl) and then been put in the uranium (x axis) or NaCl (y axis) treatment during the reciprocal-transplant experiment at generation 18 of the multi-generation experiment. Traits (rescaled) were measured after individuals have spent three generations in the novel environment. Traits: total fertility (A); hermaphrodite growth (B); early fertility (C); late fertility (D); male body bend (E); male growth (F). Prior environment: control = empty triangle; uranium = filled black dots; salt = empty dots; alternating U/NaCl treatment = filled grey dots.

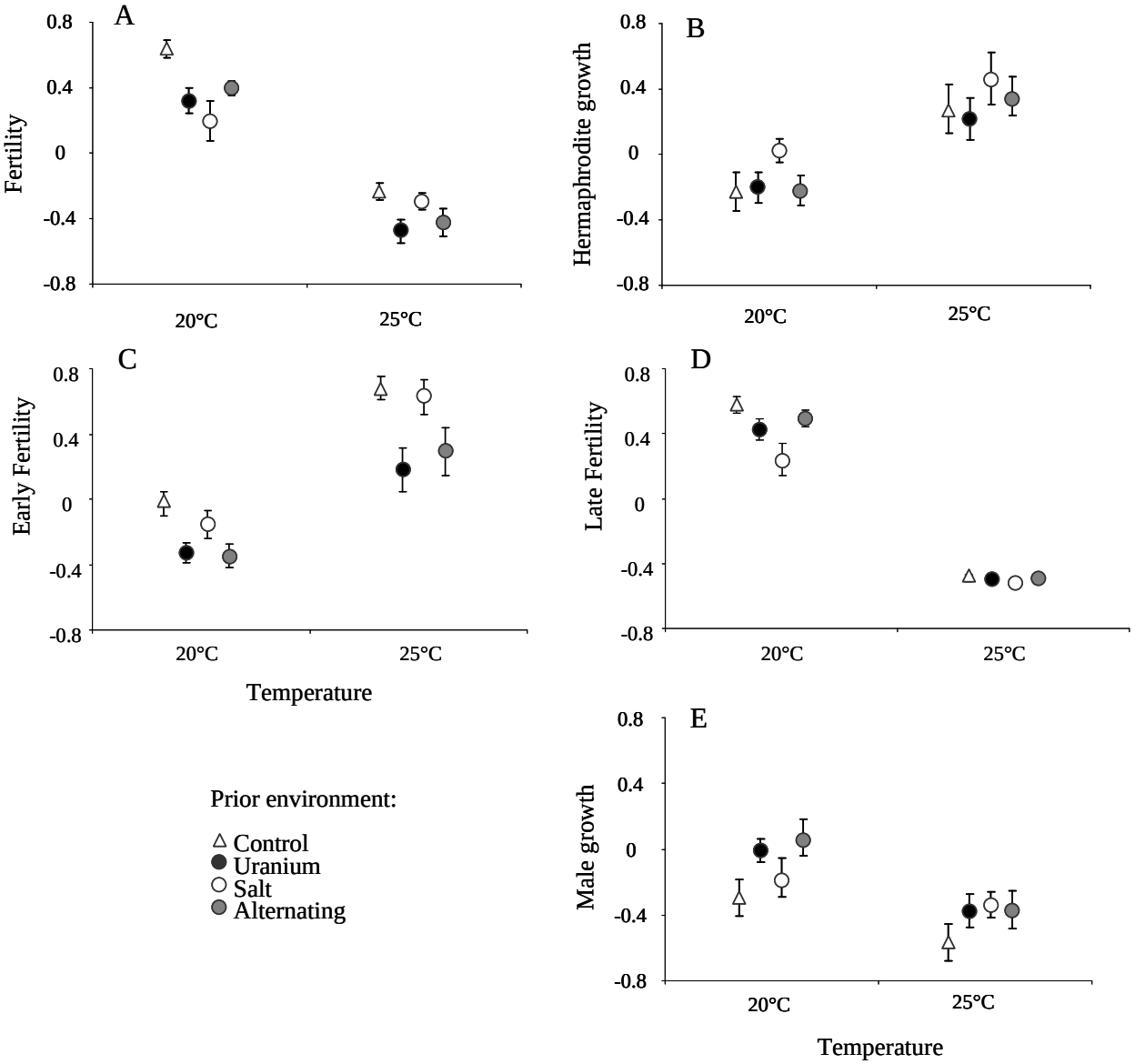


Figure 24. Common-garden experiment comparing populations that have evolved within different environments for 23 generations and put in a control environment at two different temperature treatments (20 and 25°C). Traits (rescaled) were measured after individuals have spent three generations in the novel environment. Traits measured: total fertility (A); hermaphrodite growth (B); early fertility (C); late fertility (D); male growth (E). Symbols represent the mean and its associated standard error for 18 randomly sampled individuals in each treatment. Prior environment: control = empty triangle; uranium = filled black dots; salt = empty dots; alternating U/NaCl treatment = filled grey dots.

## 4. Discussion

We found that the populations in the three different polluted environments had rapidly started to adapt to their stressful environment since their fitness increased between the fourth generation and the end of the multigenerational experiment, although they never reached the fitness level of control populations. The absence of temporal changes in the control environment, during the multigenerational or the successive common-garden experiments, indicates an absence of changes in the traits caused by uncontrolled, environmental, genetic drift, or selection effects during the experiments. Consequently it allows us to interpret any change observed in the treatment populations as genetic differentiation in response to selection pressures entailed by the pollutants. However, adaptation to the pollutants entailed a cost on fitness once populations were transferred into another stressful environment (reciprocal-transplant and common-garden at two different temperatures experiments) or back in the original environment (common-garden experiments). After having adapted to the alternating environment, populations showed higher fertility in both stressful environments (i.e. uranium and salt) and did not seem to show any supplementary cost in other environments, compared to populations adapted uniquely to uranium or salt.

Working on phenotypic measures, we may not be able set apart completely the epigenetic from the genetic basis of the phenotypic changes observed throughout the experiment. For example plants and rats exposed to some stressors can transmit epigenetic modification to unexposed progeny, down to the fourth generation (Anway *et al.*, 2005 ; Molinier *et al.*, 2006 ; Vandegehuchte & Janssen, 2011). However, in both the common-garden and the reciprocal transplant experiments we sought to overcome maternal effects by measuring traits only after the fourth generation. This precaution guarantied us to eliminate all the changes observed across generation that could be caused by phenotypic plasticity, maternal effects, grand-maternal effects, and probably also epigenetic effects. However, populations transferred back to their original conditions may experience their previous selection pressures. Then the rapid evolution of *C. elegans* in our experiments suggests that some genetic differentiation could have been induced by selection during that first four generations. Indeed, the measures were realized at the fourth generation in these experiments, and in the common-garden experiment, genetic changes could have occurred backward as a result of reverse evolution (e.g. Teotónio *et al.*, 2012), i.e. a return to the phenotypes of "ancestral" individuals as in control populations. This is possible, particularly when adaptation to the novel environmental conditions is costly (Lenski, 1998 ; Morgan *et al.*,

2007). In the reciprocal-transplant experiment we expect that novel selection pressures will drive the population towards novel phenotypic values in as fast as four generations. Such a reverse evolution in the common-garden experiment and novel evolutionary direction in the reciprocal-transplant experiments may have the disadvantage of reducing our ability to detect genetic differentiation between the populations that had evolved in different prior environments. Exposing *Daphnia magna* to organic pollutants (an insecticide and naphthalene) during twelve generations, Brausch & Smith(2009) have demonstrated that in only four generations populations were able to develop resistance to each of these pollutants. Then, after being put back into an unpolluted environment, populations lost their resistance to pollutants at only a few generations. Nevertheless, we think that the disadvantage of reverse or novel evolution in our experiment is circumvented by the possibility to eliminate almost all the epigenetic effects on the changes observed.

Furthermore, our multi common-garden experiment approach clearly illustrates the danger of doing only one common-garden experiment at a stage where populations have not been completely differentiated yet. For example, we would have miss change in fertility in salt, or changes in growth in uranium. To achieve several measures across generations of phenotypic traits permits to observe the implementation of genetic differentiation in a stressful environment and not only compare to the control population at one point, as is usually done. Furthermore, using the combination of results from common-garden and reciprocal-transplant experiments permits us to identify different adaptation costs depending on several prior and novel environments. Moreover reciprocal-transplant gave us supplementary information, if the specialization at each pollutant gave a lower performance in the other pollutant or a better one.

### 4.1. Changes during the first four generations

Populations subjected to each of the three stressful environments showed a strong reduction in fitness during the first four generations, although the increase in fitness between generation 1 and 4 was found only in the uranium and in the alternating environment. This early difference between salt and other populations was likely caused by a better capacity of *C. elegans* to response to uranium by acclimation in the first few generations of exposition (see more details in article III), and this particular capacity may be related to within-individual or to cross-generation phenotypic plastic response to the novel environment (Scheiner, 1993 ; Mousseau & Fox, 1998 ; Räsänen &

Kruuk, 2007). At this stage, it is not clear yet for what reason *C. elegans* shows this particular ability to responds to uranium but not to salt

### 4.2. Responses to differential selection pressures

Evolution in the three polluted environments was made visible by the differences in the slopes of the trait values with generations in the successive common-garden experiments. Such evolutionary (i.e. genetic differentiation) responses of populations subjected to uranium or salt confirms our predictions based on our estimation of both the intensity of selection during multigenerational experiment (article III) and the moderate but existing heritability found on the studied traits (article II). Furthermore, our results indicate that populations evolved towards different life history strategies in response to the different stressors.

*C. elegans* responded to salt by reducing its fertility, and by increasing its generation time and its survival (article III and here Figure 22). There was no effect of the prior salt environment on total fertility at the first common-garden but then fertility decreased regularly over time. Consequently the effects of salt could entail a selection of particular life history strategy: individuals with longer life cycle, lower fertility, and a higher survival. Indeed survival before reproduction (here at 48h of age) was affected by salt environment, a trait more essential for fitness than the production of a large number of embryos.

In contrast, survival did not seem to be affected by uranium (see article III and appendix L1 for graphical representation of survival). Moreover, populations subjected to uranium or to the alternating environment showed a strong reduction in fertility and growth at the first common-garden (see also discussion of article I) possibly caused by epigenetic effects, followed by an evolutionary increase in fertility (in particular early fertility) and growth between generation 4 and 22. It should also be noted that fertility and growth were correlated (at least phenotypically) as there was covariance between them, i.e. effects on one trait affected the other. Furthermore, individuals from populations adapted to uranium had faster growth in uranium and also in salt environment compared to prior control and salt populations in the reciprocal-transplant. In uranium we have selected individuals that grew faster. Growing faster can have a selective advantage. For example, best fitted individuals in a polluted environment can detoxify their body, prevent the internalization of pollutant or to reduce the negative effects of the pollutants by being bigger can reduce their internal pollutant concentration (Sibly & Calow, 1989 ; Guedes *et al.*,

2006). However, this reallocation of energy will be done at the expense of other traits or of fitness in other environments (Hoffmann & Parsons, 1991 ; Reznick *et al.*, 2000). Consequently, we may have selected individuals with higher fertility, high growth rate, and faster generation time (and may be lower life span). In other words, uranium seems to be associated to rapid life cycle adapted to reduce the period of contact with the pollutant.

Body bend frequency was not affected in the different experiments except a reduction in uranium for prior salt populations compared to the other populations. We also showed a significant evolutionary response of body bend in the salt environment in the multigenerational experiment (article III). Nonetheless this reduction in body bend probably hardly affected the encounter rate between males and hermaphrodites as male frequency was kept similar for all populations (see appendix L1 for graphical representation of the ratio of males). In *C. elegans* the ratio of males depend on the encounter rate between males and hermaphrodites as Lopes *et al.* (2008) showed it and locomotion behaviour promotes encounter rate between males and hermaphrodites (Pannell, 2002 ; Barrière & Félix, 2005a). In uranium, the reduction of body bend frequency (article III) were more probably due to effect on neurons and muscle cells (e.g. Wang & Wang, 2008a, 2008b). The cells require to form correct connections and assemble signalling proteins into synapses but any disruption would affect the locomotion behaviours (Loria *et al.*, 2004). These kinds of effects are mostly due to environmental constraints, which entail the change; they are not selected.

### 4.3. Costs of adaptation

Populations adapted to a stressful environment show a lower fertility than the control populations when placed in another stressful environment. Populations adapted to salt were more susceptible to uranium and populations adapted to each of the three stressful environments showed a lower fertility than the control populations when they lived at both original and the increased temperature.

Similar findings were observed in several studies on adaptation to pollutants, with populations adapted to a stressor showing a reduction in fitness compared to control population when confronted to another stressor such as higher temperature, parasites or other heavy metals (Xie & Klerks, 2003 ; Salice *et al.*, 2010 ; Jansen *et al.*, 2011b). In our multigenerational experiment, mutation rates was most probably not sufficient to generate genetic diversity over 22

generations, and thus the major evolutionary force was probably selection on the standing genetic variation in this *C. elegans* population (Mackay *et al.*, 1994 ; Denver *et al.*, 2009). Consequently we can assume a reduction of genetic diversity for the populations that evolved in our polluted environments, as that was demonstrated in other studies with pollutants (Ward & Robinson, 2005 ; Athrey *et al.*, 2007 ; Nowak *et al.*, 2009).

Costs of adaptation to pollutants for population in their original environment were also previously found (Shirley & Sibly, 1999 ; Ward & Robinson, 2005 ; Mireji *et al.*, 2010). Indeed the specific strategies of individuals selected in a polluted environment may not be optimal in a favourable environment. For example, it can consist in a reduction of pollutant assimilation (Xie & Klerks, 2003), the improvement of pollutant excretion (Posthuma & Van Straalen, 1993 ; Lagauzère *et al.*, 2009), the sequestration of the pollutant (e.g. metallothionein synthesis; Shirley & Sibly, 1999 ; Gillis *et al.*, 2002 ; Jiang *et al.*, 2009), or every other biochemical, physiological or cellular modification that can limit the impact of the pollutant on the organism. In the presence of a pollutant the selected genotypes with a better capacity to use one of these mechanisms may thus be at a disadvantage if the population encounters more favourable environment. If the induction of these mechanisms is not plastic, it may not be shut down in the non-polluted conditions (i.e. the "facultative response" of Calow & Sibly, 1990). Combined with the reduction of genetic diversity, we assume that this phenomenon happened for the populations adapted to each of our three stressful environments.

However, costs of adaptation were not systematic. In the reciprocal-transplant experiment populations adapted to the prior salt environment showed some signs of specialization. Their fertility was higher than that of the control populations in salt but was lower in the novel uranium environment. In contrast, uranium populations never showed a lower performance than the control population when transferred in the salt environment. It thus seems that costs of adaptation were asymmetric between uranium and salt adapted populations (i.e. one specialist paid a cost but not the other). Several studies on microorganisms of viruses have shown asymmetric costs of adaptation (Kassen, 2002 and references therein; Jasmin & Kassen, 2007). The causes of such asymmetry must correspond to different pleiotropic effects accompanying genetic differentiation depending on environmental conditions (Travisano & Lenski, 1996 ; Rose *et al.*, 2005 ; Jasmin & Kassen, 2007). We thus need to know the biochemical, physiological and genetic mechanisms responsive of adaptive costs to start building a global framework to predict the presence of costs.

### 4.4. Changing environment and the evolution of generalism

It is recognized that fluctuating or changing environments promote generalist genotypes, and constant environments promote specialist genotypes (Reboud & Bell, 1997 ; Cooper & Lenski, 2000 ; Turner & Elena, 2000). Interestingly, when placed in both uranium and salt, populations adapted to the alternating U and NaCl environment showed a better overall fertility than the populations that have evolved in constant polluted environment (note that in each specific environment the fertility of the alternating populations was not yet higher than the one of the other populations). Then these alternating populations performed as well if not better than the control populations in the uranium or in the salt environment. Furthermore, confronted to an increase in temperature or in original environment, these alternating populations did not show lower performance than the other populations. Our results, thus confirm the possibility that alternating or changing environmental conditions generate generalists genotypes that could have an advantage when confronted to one of these stressors.

A similar result was found for populations of viruses adapted to a novel host (Turner & Elena, 2000). Populations confronted to an alternating regime between two hosts were as well adapted to each host as population adapted to only one of the two hosts. Moreover, fluctuating or changing environments have been shown to better promote the diversification of populations than environments that contain all the novel conditions simultaneously (Buckling *et al.*, 2000 ; Cooper & Lenski, 2010 ; Collins, 2011). In this study we cannot show any evidence for a strong cost of adaptation to such a changing environment. The existence of cost-free generalists has been found in long-term evolution of microorganisms (Buckling *et al.*, 2007). In the same way, Reed *et al.* (2003) have shown that *Drosophila melanogaster* populations adapted to two stressors alternating in time had enhanced fitness in a novel stressful environment, than populations adapted to only one of the stressors. These results change the vision that adaptation to a variety of environment could decrease the adaptive potential of populations.

### 4.5. Implications for natural populations in polluted environments

Understanding the mechanisms and consequences of evolution of populations that suffered pollution has recently become a major challenge in our need to improve Ecological Risk Assessment process (Medina *et al.*, 2007 ; Morgan *et al.*, 2007 ; Coutellec & Barata, 2011 ; Klerks *et al.*, 2011). Our results confirm the existence of trade-offs between the adaptation to a particular

stressor and the capacity to cope with other future stressors or to compete with other genotypes in a favourable environment (Coustau *et al.*, 2000 ; Roff & Fairbairn, 2007). Moreover, we show that the costs can appear in only six generations with pollutants (see also Xie & Klerks, 2003 ; Salice *et al.*, 2010 ; Jansen *et al.*, 2011b). The interaction of anthropogenic stressors with other selection pressures may have severe consequences on the risks of extinction of natural populations.

The significant slopes in the successive common-garden experiments have practical implications for conservation biology. Running only one common-garden experiment at any time during a potential evolutionary process should be interpreted with caution, as it generally did with natural populations. If we had limited our study to the fifth common garden, we would not have seen the differentiation for growth during evolution in uranium and the longer implementation of cost on fertility during evolution in salt.

Adaptation in a predictably changing environment may not restrict the adaptive potential of the population to novel stressors (Reed *et al.*, 2003 ; Frankham & Kingsolver, 2004). Consequently, we assume that if the strength of selection pressures entailed by pollution is well beyond that of other environmental stressors or if all the selection pressures go in the same direction, the population will respond to the pollutant. However, if the pressures entailed by other environmental stressors are opposed to those of pollution, the population will not have the ability to respond to the pollutant (Bell & Collins, 2008).

## 5. Conclusion

The circumstantial presences of adaptive costs reported in our study have an important role on the susceptibility of *C. elegans* populations. Life history strategy of selected individuals and adaptive costs depend on the type of stressor that the population is confronted to. Moreover, the costs did not appear in the same time, thus a better evaluation of the evolutionary processes and of the adaptive costs involve performing several measures of phenotypic traits across time. The increase of benefits after adaptation to the alternating environment without increase of costs in novel environments was particularly surprising. Nevertheless, we cannot be sure that supplementary costs could exist in other stressful environments that we have not studied. Future experiments on adaptive costs should focus on the causes of costs to be able to predict more largely if natural

populations are more susceptible to environmental changes after adaptation to polluted and heterogeneous environments.

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