Pollution breaks down the expression of genetic structure of life history traits in *Caenorhabditis elegans*

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Abstract

When pollution occurs in an environment, the populations present suffer numerous negative and immediate effects on their life history traits. Their evolutionary potential to live in a highly stressful environment will depend on the strength of selection pressures but also on the genetic structure: the heritability of the traits and genetic correlations between them. If expression of this structure can change in a stressful environment it becomes necessary to quantify these changes in order to estimate the evolutionary potential of the population in this novel environment. We studied the genetic structure for survival, fertility, early, and late growth in isogenic lines of a *Caenorhabditis elegans* population subject to three different environments: a control environment and two polluted environments, one with uranium and the other with a high salt concentration. We found a decrease of heritability in polluted environments for fertility and early growth, two traits that were the most heritable in the control environment. The expression of genetic structure of traits was particularly altered in uranium, probably due to extremely low heritabilities in this environment. Hence this could prevent selection from acting on traits despite the strong selection pressures exerted on them. Moreover the decrease in heritability was not linearly correlated to the fitness reduction in polluted environments since the effects on phenotypic traits in the salt environment were stronger than in uranium. Our results suggest that pollution can alter the expression of the genetic structure of a *C. elegans* population, and thus modify its evolutionary potential.

Keywords

Caenorhabditis elegans; isogenic lines; heritability; genetic correlations; uranium; pollution

1. Introduction

Anthropogenic activities can disturb biological functions. In particular, environmental pollutions entail stress on the population with negative consequences on individuals' life history and fitness, and on the demography of the population. However, individuals may not be affected in the same way according to their genotypes and phenotypes (Hoffmann & Parsons, 1991 ; Posthuma & Van Straalen, 1993). Pollution can thus create differential survival and reproductive success, which in turn can lead to microevolution in populations (Medina *et al.*, 2007 ; Morgan *et al.*, 2007). The evolutionary potential of life history traits depends on the strength of selection pressures acting on each of these traits in the population, their level of additive genetic variation, as well as the magnitude and sign of their genetic associations (Lande & Arnold, 1983 ; Falconer & Mackay, 1996 ; Roff, 2002b). The two latter are included in the matrix of additive genetic variance and covariance (**G**) (Lande, 1979). The G matrix reflects the evolutionary constraints on independent evolution of traits imposed by pleiotropy or linkage disequilibrium. An estimation of the G matrix is thus a necessary step towards the estimation of a population's evolutionary potential (Cheverud, 1984 ; Roff, 2002b).

Evolutionary change in quantitative traits can be described by the multivariate extension of the breeder's equation (Lande, 1979 ; Lande & Arnold, 1983). This equation is applicable only under the assumption that the **G** matrix is stable over time and across environmental conditions. Theoretical and empirical investigations revealed a strong **G** matrix stability through time (Arnold *et al.*, 2008), even despite climate change for example (Garant *et al.*, 2008), but others demonstrated that the stability of the **G** matrix can be altered (Steppan *et al.*, 2002 ; McGuigan, 2006 ; Doroszuk *et al.*, 2008), even following short-term environmental changes (Bégin *et al.*, 2004 ; Sgrò & Blows, 2004). Moreover, changes in environmental conditions may not only affect the additive genetic and environmental variance of traits (Hoffmann & Merilä, 1999 ; Charmantier & Garant, 2005) but also their covariance (reviewed by Sgrò & Hoffmann, 2004). These studies contradict the hypothesis of a stable **G** matrix even over short time periods. Furthermore, under harsh environmental conditions, a decrease in the genetic variance of traits may reduce the adaptive potential of populations despite strong selection pressures occurring in this type of environment, and may thus prevent microevolutionary changes (Blows & Hoffmann, 2005 ; Wilson *et al.*, 2006). Therefore, using information on the **G** matrix in one particular set of environmental conditions to predict the evolutionary potential of a population under rapid

environmental changes may be questionable. Indeed estimating the **G** matrix in the novel environmental conditions may be the only way to estimate a population's evolutionary potential. This is particularly important for studies that estimate the evolutionary consequences of novel and fast environmental changes caused by anthropogenic effects, such as the effect of pollutants (Coutellec & Barata, 2011). In this paper, we use a quantitative genetic approach to test the hypothesis that the **G** matrix for life history traits remains stable in a population of *Caenorhabditis elegans*, subject to different pollutants.

We chose to work on the effects of two pollutants, uranium and a high salt concentration, known to affect differently *C. elegans*. High salt exposure is an extreme hypertonic stress that provokes a rapid loss of water and solute content in cells. *C. elegans* attempts to regulate this loss by the biosynthesis of glycerol through transcriptional upregulation of an enzyme (*gpdh-1*) in the intestine and hypodermis, which is in direct contact with the external medium (Lamitina *et al.*, 2004 ; Lamitina *et al.*, 2006). However several other genes, such as heat shock, cytoskeletal and trehalose biosynthesis also regulate hypertonic stress (Lamitina & Strange, 2005). Uranium is a heavy metal which has higher chemotoxic than radiotoxic effects because of its low specific activity (Kuhne *et al.*, 2002). Uranium affects severely the intestinal epithelium in earthworm *Eisenia fetida* (Giovanetti *et al.*, 2010). The presence of uranium in the environment of *C. elegans* increases *mtl-1* expression, which interferes with U-accumulation in cells; probably by sequestration and transport of uranium outside the cells (Jiang *et al.*, 2009).

Because of its short life cycle and ease of handling, *C. elegans* is a model of growing interest to study quantitative genetic parameters (Braendle *et al.*, 2008). For example, the genetic correlation structure of life history traits estimated from recombinant inbred lines differ between high and low food density (Harvey *et al.*, 2008) and low and high temperature (Gutteling *et al.*, 2007), at levels that are known to stress *C. elegans*.

In this paper we use the isofemale line method (David *et al.*, 2005), to analyse the changes in the expression of genetic structure of life history traits a *C. elegans* population in a control environment and in two environments polluted with uranium or a high salt concentration. Life history traits are directly involved in the demographic response of a population to any rapid environmental change and they generally encompass many other traits that can affect both survival and reproduction. Working on life history traits gives us a broad picture of the evolutionary response of an organism to changing environmental conditions. Furthermore, in the absence of

detailed mechanisms of resistance of *C. elegans* to both salt and uranium, life history traits were good candidates to examine what type of response this organism could show in these novel environmental conditions. Our hypotheses were: (i) that a genetic structure (i.e. heritability and genetic correlations) exists between traits in the three different environments; and (ii) that the expression of this structure can be altered in the polluted environments compared to the control environment, because of drastic changes in environmental conditions.

2. Material and methods

2.1. Isofemale line technique

In this study we applied the isofemale line technique (ILT), commonly used on wild populations *of Drosophila sp*. (review by David *et al.*, 2005). The relationship between narrow-sense heritability (h^2) and the coefficient of intraclass correlation (t, provided by ILT), is not so obvious but t does give a better heritability estimate than the half-sib design or the parent-offspring regression method. For example, the technique provides measures of about 95 $\pm$ 15% of the heritability for morphometric traits in natural population of *D. melanogaster* (David *et al.*, 2005), thus yielding a close estimation of additive genetic variance. It is a useful tool to rapidly estimate genetic variability and genetic correlation of traits and to compare these genetic parameters between different traits and different environments (or even different populations). Furthermore, ILT can be easily applied to *C. elegans* because of its androdioecious breeding system (i.e. self-fertilization of hermaphrodites and facultative outcross with males). Indeed after mating, each isogenic line can be conserved as an inbred line. To facilitate reading, the term heritability was used throughout the text instead of the coefficient of intraclass correlation.

2.2. Nematodes culturing

Here, we used a stock population of *C. elegans* composed of a mixture of 16 wild isolates (Teotónio *et al.*, 2012) in order to obtain a large genetic diversity. The population, composed of more than 30% of males, was maintained in laboratory conditions during 140 generations, where recombination-selection equilibrium was mostly achieved without significant loss of genetic diversity (Teotónio *et al.*, 2012). However, we used different laboratory conditions for our needs. We used 500 individuals in a 9 cm diameter Petri dish with NGM-modified agar (use of HEPES

buffer, article I). We produced six replicated experimental populations. Plates were seeded with 1 ml of a 20:1 mixture of *Escherichia coli* strain OP50 (OD_{600nm} of 3) as the food source. Every 3 days we washed the nematodes (about 20,000 individuals) off the plates with an M9-modified solution (use of HEPES buffer) for each replicate and kept each replicate in 15 ml falcon tubes. The number of individuals in a tube was estimated with five sample drops of 5 µl, following Teotónio *et al.* (2012), and then the volume corresponding to 500 individuals, from all developmental stages, was placed in a fresh Petri dish. Nematodes were cultured throughout the experiment at 20°C and 80% RH.

2.3. Creation of lines

After repeating this protocol about forty times (~ 40 generations despite the discrete overlapping of developmental stages), we selected 100 gravid hermaphrodites, and placed them on a new Petri dish, for each of the six replicates. Potential evolutionary changes caused by such changing conditions, should be similar in both the treatment and control environments. We allowed the hermaphrodites to lay eggs for a 2-hour period, and then transferred approximately 200 embryos on a new Petri dish (200 embryos per replicate). After 40 hours, when sexual differentiation is visually possible but individuals are not yet mature (i.e. L3 larval stage; Sulston *et al.*, 1980), we randomly picked a single hermaphrodite and a single male, from different Petri dishes, and placed them separately (by couples) in 6 well tissue-plates. We created 14 couples. NGM in wells were seeded with 150 µl of a 5:1 mixture of OP50. After leaving plates in a laminar flow hood one hour to allow them to dry, plates were then top-exposed to UV doses for 90 s to stop bacterial growth (Bio-Link Crosslinker; λ=254 nm; intensity = 200 µwatt.cm^{-2}). The main aim of this UV treatment was to avoid different bacterial growth in control and polluted plates. Reproduction by mating produces a high frequency of male in the progeny, whereas hermaphrodite self-fertilization produces only 0.1% of males (Ward & Carrel, 1979 ; Prahlad *et al.*, 2003 ; Teotónio *et al.*, 2006). Therefore, 72 hours after the separation of the couple, we checked if the ratio of males and hermaphrodites in the progeny was approximately 1:1, to guaranty that mating between the hermaphrodite and the male was successful. We then picked one L3 stage hermaphrodite in each of the progeny of 14 couples to initiate 14 isogenic lines. This was done 72 hours after couple creation to avoid the risk of self-fertilization that could occur within the few hours after mating

(Hart, 2006). Each line reproduced by self-fertilization for two generations in a 6 cm Petri dish, composed of the same environment as presented above.

2.4. Contamination design and measurement of traits

At the second generation of self-fertilization, we transferred 20 gravid hermaphrodites of each line in a new Petri dish. We considered this moment as t = 0. After 2 hours of egg-laying, eggs were individually placed in 12-well of a tissue culture plate. Twelve eggs per line and per environment were placed in three different NGM media: control, uranium (addition of 1.1 mM U) and high salt concentration (addition of 308 mM NaCl). Wells were seeded with 75 µl of a 5:1 mixture of OP50 (same UV treatment as for 6-well plates). In our experiment, the term "isogenic" does not correspond exactly to our lines, as there is heterozygosity in this population (Teotónio *et al.*, 2012). However, individuals have reproduced twice by self-fertilization before the experiment; we thus expected a high reduction of heterozygosity (Hedrick, 1980 ; Reed & Frankham, 2003 ; Honnay *et al.*, 2007).

Individuals growing in salt showed a slower development than in the control and in uranium. Therefore, individuals were transferred twice into a new well at 96h in the control and uranium environment or 168h in the salt environment and then again 36h later. Hatched progeny were counted the day following each transfer to measure brood size, an index of fertility. We photographed individuals using a stereomicroscope (Olympus SZX12, 1.6 x 90 magnification) with a computer-connected camera (Nikon D5000). Using a micrometer scale measure and ImageJ software (Rasband, 2012), body length was measured at 72 and 144h. These two hours correspond approximately to the population's onset and to the end of the egg-laying period in a control environment, respectively (*pers. obs.*). These two measures will be called early growth and late growth thereafter. Survival was estimated by counting the number of parental individuals still alive at 144h.

2.5. Genetic parameters estimation

We estimated the genetic parameters by using a Bayesian model approach in the MCMCglmm package for generalized linear mixed-effects model (Hadfield, 2010) in the R software (R Development Core Team, 2012). We fitted multivariate generalized linear mixed-effects models for different traits within the same environment (a quadrivariate model per environment) and for

the same trait across different environments (a trivariate model per trait). So, models did not contain fixed effects but we included lines as random effects in order to estimate between- and within-line variance (respectively *Vb* and *Vw*) for each trait. We modelled the survival with a binary error structure and a Gaussian one for the other traits. Expanding the binomial data into binary data allows us to fit a model in which we can estimate the correlation between survival and the others traits. Estimations were generated by a Bayesian approach to obtain the entire posterior distributions of (co)variance matrices of traits rather than only point estimates. To avoid any biased results caused by the fact that traits differ in their means, we rescaled the traits prior to analysis by dividing the mean by twice its standard deviation (Gelman, 2008). After several priors tested, we retained proper prior ($nu = k - 1 + 0.002$) with a very low variance parameter ($V = \text{diag}(k) * V_p * 0.05$), where V_p is the phenotypic variance, k the dimension of V (i.e. number of traits). There is one exception for survival for which we fixed the environmental (also called residual) variance to 1. For multivariate analyses with four different traits we allowed models to estimate different random and environmental variances, and covariances between the pairs of traits. In the models analysing the same trait across the three different environments, traits were measured on different individuals, and we thus fixed the environmental covariance to zero (see appendix C Table C1 for comparison of models with or without genetic covariance between traits allowed in prior). Despite the use of slightly informative priors used in the models, starting with low variance and any kind of covariance allowed us to say that the generated (co)variances were tangible. After verifying the convergence of parameters values (i.e. number of iterations, burn-in phase and thinning) and autocorrelation issues, we retained 2 500 000 iterations with a burn-in phase of 500 000, for a total of 1 000 samples for each analysis (Hadfield, 2010).

We estimated heritability in the multivariate models for traits within environments using $t = nVb / (nVb + (n - 1)Vw)$, where n was the number of lines (David *et al.*, 2005). Following David *et al.* (2005) we also estimated genetic correlations without correcting for variance and covariance within-line, although n was less than 20. Using this correction would generate, posterior distribution for correlations containing values greater than 1. We used the posterior mode of the distribution of heritability, genetic and phenotypic correlations as quantitative genetic parameter estimates. For each trait we also estimated phenotypic ($V_P = Vw + Vb$), environmental ($V_E = V_P - V_G$) and genetic variances ($V_G = tV_P$) and genetic covariance (Cov) between traits was calculated as $\text{Cov}_{1,2} = r_G \sqrt{V_{G1} V_{G2}}$, where r_G corresponds to genetic correlation between traits 1

and 2 and V_{G1} and V_{G2} to the genetic variance for trait 1 and 2, respectively (see appendix C Figure C1). We considered that each estimate was significantly different from zero when its 95% Bayesian posterior intervals of highest density (HPDIs) did not include zero. We also tested the differences of an individual's traits and genetic parameters in different environments. We considered differences to be significant between two environments when 95% HPDIs of subtraction between the posterior distributions of the trait in these two environments did not overlap 0.

3. Results

Phenotypic values for all traits were lower in the salt environment than in the uranium and the control environment (95% HPDIs of the subtraction did not include 0; Table 3). When compared to the control environment, fertility decreased by 55.0% in uranium and by 86.4% in the salt environment (95% HPDIs did not include 0). The same pattern was found for both early and late growths. Survival in the uranium environment was similar to that of the control environment (95% HPDI included 0), but it nearly halved in the salt environment.

Table 3. Average trait values of 14 isogenic lines of *C. elegans* in the control, the uranium and in the salt environment. Fertility is measured by the number of eggs of a hermaphrodite. Early and late growth is measured as the increase in total length (in µm) between 0 to 72h and 72 to 144h respectively. Significant differences corresponded to 95% intervals of highest posterior density of subtraction between the posterior distributions of a trait in two environments that do not overlap 0, and are represented by different superscript letters.

	Mean ± sd			Percentage
Environnement	Fertility	Early growth	Late growth	Survival at 144h
Control	173.8 ± 43.5 [a]	953.1 ± 131.3 [a]	345.7 ± 106.9 [a]	80.9 [a]
Uranium	78.2 ± 26.4 [b]	721.5 ± 108.8 [b]	184.2 ± 100.1 [b]	88.8 [a]
Salt	23.6 ± 34.1 [c]	223.2 ± 79.6 [c]	127.2 ± 121.0 [c]	46.5 [b]

Heritability estimates varied between 0.07 and 0.31 in the control environment and were significantly higher than zero for fertility and early growth only (Table 4A). In the uranium environment we found lower (i.e. 0.04 to 0.08) and non-significant heritabilities (Table 4B). In the salt environment heritability values varied between 0.07 and 0.27, but were only significant for early growth (Table 4C). For fertility and early growth, heritability did not differ significantly between the control and the salt environments, but heritability in the control environment was

higher than in the uranium environment (95% HPDIs did not include 0). Phenotypic variance for these two traits was reduced in both polluted environments compared to the control. However, in uranium, compared to the control, there was a stronger reduction of genetic variance than environmental variance (95% HPDIs did not include 0, Figure 15) which was directly related to lower heritabilities in this polluted environment (Table 4A, B). Phenotypic variance for fertility was twice as high in the control environment ($V_P = 0.114$ [0.083; 0.180]) than in salt ($V_P = 0.059$ [0.042; 0.086]) and three times higher than in uranium ($V_P = 0.040$ [0.034; 0.054], Figure 15A and see also appendix C Figure C3 for representation of phenotypic correlation structures). The difference was less pronounced for early growth but also significant (Figure 15B).

Table 4. Matrices of heritabilities (diagonal), phenotypic (below the diagonal) and genetic correlations (above the diagonal).
Parameters with their 95% intervals of Bayesian credibility, for traits measured on C. elegans in the control, the uranium, and the salt environment. Values in bold are significant estimates.

A CONTROL	Fertility	Early growth	Late growth	Survival
Fertility	**0.249 [0.036; 0.525]**	**0.904 [0.361; 0.965]**	**-0.912 [-0.987; -0.450]**	0.924 [-0.520; 0.985]
Early growth	**0.543 [0.345; 0.718]**	**0.314 [0.105; 0.608]**	**-0.780 [-0.967; -0.188]**	0.930 [-0.279; 0.991]
Late growth	-0.154 [-0.427; 0.093]	**-0.299 [-0.566; -0.101]**	0.110 [-0.009; 0.424]	-0.839 [-0.978; 0.588]
Survival	**0.695 [0.454; 0.881]**	0.346 [-0.007; 0.673]	0.132 [-0.310; 0.553]	0.073 [-0.087; 0.394]

B URANIUM	Fertility	Early growth	Late growth	Survival
Fertility	0.039 [-0.075; 0.068]	-0.023 [-0.635; 0.693]	-0.063 [-0.683; 0.784]	0.371 [-0.691; 0.863]
Early growth	**0.607 [0.485; 0.699]**	0.042 [-0.073; 0.122]	-0.521 [-0.835; 0.637]	-0.401 [-0.869; 0.717]
Late growth	0.140 [-0.031; 0.326]	**-0.347 [-0.479; -0.125]**	0.034 [-0.059; 0.241]	-0.720 [-0.948; 0.749]
Survival	**0.736 [0.411; 0.852]**	**0.512 [0.062; 0.724]**	0.208 [-0.372; 0.576]	0.083 [-0.089; 0.154]

C SALT	Fertility	Early growth	Late growth	Survival
Fertility	0.186 [-0.034; 0.442]	**0.896 [0.514; 0.987]**	**0.774 [0.045; 0.966]**	0.892 [-0.804; 0.961]
Early growth	**0.551 [0.392; 0.723]**	**0.270 [0.001; 0.512]**	**0.811 [0.188; 0.967]**	0.901 [-0.825; 0.973]
Late growth	**0.537 [0.340; 0.694]**	**0.708 [0.584; 0.813]**	0.126 [-0.039; 0.363]	0.901 [-0.722; 0.967]
Survival	**0.404 [0.037; 0.742]**	**0.515 [0.144; 0.738]**	**0.725 [0.448; 0.880]**	0.067 [-0.086; 0.217]

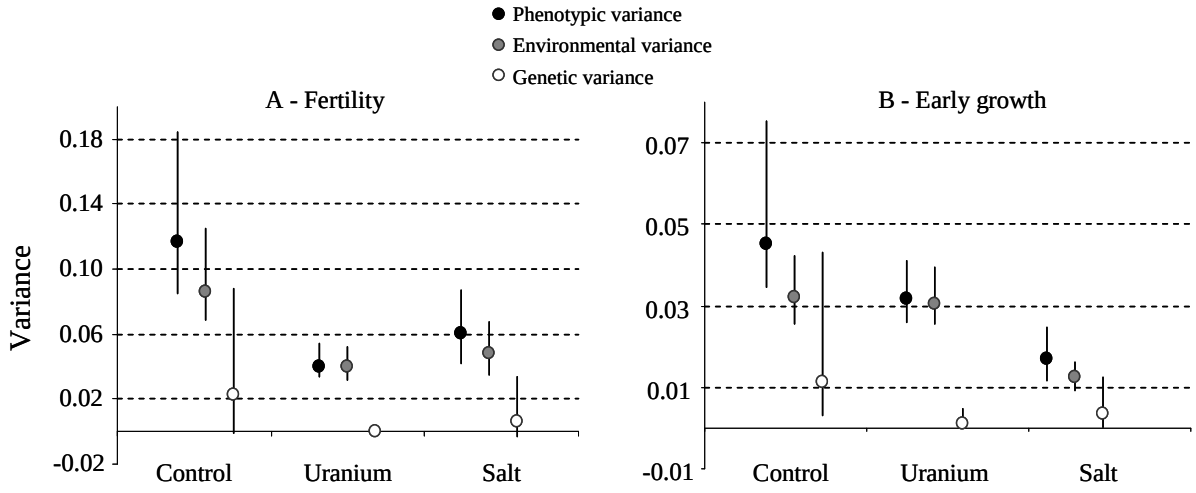

Figure 15. Variance estimates with their 95% intervals of Bayesian credibility, for fertility (A) and early growth (B) measured on *C. elegans* in the control, the uranium, and the salt environment.
Phenotypic variance (V_P) is decomposed in environmental (V_E) and genetic variances (V_G). Estimates were obtained using multivariate models for different traits within the same environment (see appendix C Figure C2 for late growth).

We found moderate phenotypic correlations between traits in the three different environments (Table 4). In all environments, individuals that had a higher fertility also had a stronger early growth and better survival. The phenotypic correlation between early and late growths was negative in the control and in uranium but positive in salt. In the control environment, fertility and early growth were positively genetically correlated, but these two traits were negatively correlated to the late growth. The range of these correlations was strong (> 0.75 in absolute value, Table 4A). Moreover, the genetic correlation between fertility and late growth was hidden since phenotypic correlation was non-significant. However the environmental correlation (r_e) was opposite in sign but non-significant (r_e = 0.100 [-0.128; 0.300], see appendix C Table C2 for environmental correlations). In turn, we did not detect any genetic correlation in uranium. In particular, posterior modes for genetic correlation between fertility and growth traits are close to 0 (-0.023 and -0.063, Table 4B). We found strong genetic correlation for the same combination of traits in salt and in the control (Table 4C) but with changes of sign for the correlation involving late growth due to the change of genetic covariance signs (see appendix C Figure C1 for genetic covariances between traits). We did not find any significant genetic correlation involving survival in all three environments (Table 4). For phenotypic correlations that were not detected at the genetic level, environmental correlations were significant (see appendix C Table C2).

We found a positive and significant genetic correlation between the control and the uranium environment for fertility (Table 5), indicating a genetic dependence across environments. We also found a positive correlation for late growth across these two environments and a negative one for early growth between the two polluted environments despite a slight overlap of 0 for 95% HPDIs. Because of a potential lack of power, we did not present cross-environment genetic correlations for survival.

Table 5. Cross-environment genetic correlations.
Parameters with their 95% intervals of Bayesian credibility, for traits measured on *C. elegans*.

	Fertility	Early growth	Late growth	Survival
Control-Uranium	0.718 [0.024; 0.968]	0.438 [-0.478; 0.838]	0.833 [-0.049; 0.954]	0.978 [-0.967; 0.998]
Control-Salt	0.444 [-0.585; 0.787]	0.087 [-0.473; 0.716]	0.554 [-0.488; 0.900]	0.967 [-0.940; 0.996]
Uranium-Salt	-0.070 [-0.746; 0.721]	-0.811 [-0.943; 0.076]	0.625 [-0.292; 0.942]	-0.943 [-0.999; 0.929]

4. Discussion

Previous theoretical and empirical investigations have shown differences in the expression of genetic structure of morphological and life history traits between stressful and control environments (Hoffmann & Merilä, 1999 ; Roff, 2002b ; Charmantier & Garant, 2005). Here we show that living in a polluted environment can decrease both genetic and environmental variances. Yet the stronger reductions of genetic variance rather than environmental variance explained the reductions in heritability. Heritability for fertility and early growth were lower in uranium than in salt, despite the stronger effects of the salt environment on phenotypic traits. Concerning changes of heritability in uranium, we found no genetic correlations contrarily to the salt and control environments. There were strong genetic correlations between fertility, early and late growth in control and salt but changes of sign for correlations involving late growth in salt. Cross-environment, there was a positive genetic correlation between control and uranium environment for fertility and probably also for late growth. Between uranium and salt environments, there was a negative genetic correlation for early growth.

Heritability (t) from our experiment did not only yield additive genetic variance probably because there is a large amount of epistatic interactions due to the experimental bottleneck, linked to the foundation of lines, as David *et al.* (2005) explained. This additional effect would be cryptic

in a large panmictic population. Other quantitative genetic designs may not suffer from that issue. For example, an 'animal model' (Kruuk, 2004) using complete information on a pedigree can provide very appropriate estimates of quantitative genetic parameters. However, it is almost impossible to build up a pedigree for *C. elegans*, a species for which we cannot differentiate between individuals without separating them, as in our experiment. For the same reasons and the difficulties to control for all the offspring if they come from outcrossing or self-fertilization, it is very hard to create a half-sib/full-sib design experiment in this species. Besides, ILT has been shown to provide a better estimation of heritability than the parent-offspring regression method (Bubliy *et al.*, 2001). Therefore, ILT remains a very appropriate and convenient approach to quantitative genetic analyses in this species.

4.1. Effects on heritability

Compared to the other traits, survival heritability was absent or extremely low (thus difficult to detect) in the control environment. This may be due to its genetic regulation. Survival is regulated by a few genes of large effects contrary to growth or reproductive traits which are regulated by many genes of small effects for which heritability increases (Hoffmann & Parsons, 1991 ; Barata *et al.*, 2002; in *C. elegans* Knight *et al.*, 2001). Heritabilities in the control environment were moderate for early growth and fertility and low for late growth. Gutteling *et al.* (2007) studied broad-sense heritability on recombinant inbred lines of *C. elegans* for body mass at maturation and fertility at different temperatures. Heritability was similar to that of our study for fertility but it was higher for growth. However the difference can be explained by the differences in temperature between our study (20°C) and theirs (12 and 24°C), which affects average growth and reproduction (Byerly *et al.*, 1976) as well as heritability (Gutteling *et al.*, 2007).

The decrease in heritability for early growth and fertility in both polluted environments is consistent with several laboratory or field studies, although some studies show the opposite response (reviewed by Hoffmann & Merilä, 1999 ; Charmantier & Garant, 2005). Heritability reduction could be caused by a decrease in additive genetic variance or an increase in environmental variance. Our results showed that both environmental and genetic variance were higher in the control, resulting in a decrease of phenotypic variance in both polluted environments (Figure 15). Despite the decrease in phenotypic variance, the reduction of heritabilities was mainly due to the reduction of (additive) genetic variance. Although a reduction of heritability is

commonly caused by an increase in the environmental variance component, there can be cases where it results from a reduction of the genetic variance components (Charmantier & Garant, 2005). In this latter case, the decrease in heritability could be triggered by a limitation of the genetic potential due to constraining developmental conditions (Wilson *et al.*, 2006), e.g. despite strong additive genetic effects responsible for individual differences in body size, potential size may not be attained because of a stress-induced reduction of growth rate (Gebhardt-Henrich & Van Noordwijk, 1991).

Furthermore, heritabilities were extremely low in the uranium environment. In several studies, phenotypic variation in stressful environments seems to be rather related to the provided novelty (Holloway, 1990 ; Kristensen *et al.*, 2003). The original populations at the origin of the wild isolates used in our study may have experienced either similar stresses or different stresses affecting the same sets of genes as the uranium. Moreover evolutionary response of a trait to selection cannot occur in an environment where the trait heritability is null, no matter how strong selection pressures are. Wilson *et al.* (2006) showed a decrease of maternal genetic variance in a stressful environment for wild sheep although the strength of selection was strongly and positively correlated to the quality of the environment. Cadmium (a heavy metal like uranium) induced reduction of broad-sense heritability in *Daphnia magna* at 20°C, but apparently this was mostly due to the decrease in dominance variance (Messiaen *et al.*, 2012). Nonetheless, Hendrickx *et al.* (2008) observed a strong decrease in heritability of growth in the wolf spider *Pirata piraticus* associated to a decrease in additive genetic variance in a cadmium environment, compared to the control. The authors proposed that the expression of growth in that species involved different sets of genes in the different environments. In a stressful environment, gene expression governing the traits can be masked by other alleles involved in detoxification (Hoffmann & Parsons, 1991). Indeed, for several heavy metals including uranium, resistance is due to one or a few major genes in *C. elegans* (Klerks, 2002 ; Aschner & Martinez-Finley, 2011), which could mask the genes' expression of quantitative traits such as life history traits. Nonetheless if heritability is only hidden but existent, selection may still act on it, thus more studies are required to confirm the effects of polluted environments on heritability reduction.

4.2. Cross-environment genetic correlations

We detected cross-environment genetic correlations between control and uranium for fertility and apparently also for late growth. This indicated a partial overlap between the genes involved in the expression of these traits in both environments (Falconer & Mackay, 1996), despite different genetic variances between control and uranium. For other measures between control and the polluted environments, the 95% HPDI largely overlapped 0 and it is thus possible that the correlation is negligible or this result may be attributed to a potential lack of power related to the number of lines in our experiment. Given that our aim was only to detect the presence of negative genetic correlations rather than obtain an accurate measure of it, our method based on line mean was sufficient as it generally produces lower correlations than other methods thus resulting in a more conservative estimate (see Astles *et al.*, 2006). All the posterior modes of correlations with the control environment are either positive or close to 0, implying that there is no trade-off for the measured traits between the uranium or salt environment and the control environment. Therefore, adaptation to uranium or to salt does not seem to be associated with a strong coevolution between environments for these traits in our experiment.

However, there was a negative genetic correlation for early growth between uranium and salt, which indicates a cost to adapting to the presence of one of these pollutants after adapting to the other. In their experimental evolution study, Lopes *et al.* (2008) found that once *C. elegans* was adapted to an environment polluted with a pesticide, there was no adaptation cost for reproduction in the control environment or an environment polluted with a second pesticide. Contrarily, Jansen *et al.* (2011) showed an adaptation cost, which was conditional on the novel environment for *D. magna* populations that were exposed to a pesticide. Adapted populations of *D. magna* suffered no costs in the control environment but were more parasitized, therefore suggesting a higher susceptibility of this population to other stresses. In contrast, adaptation costs to cadmium existed in the control environment, for growth in *D. magna* (Ward & Robinson, 2005) or fertility in *D. melanogaster* (Shirley & Sibly, 1999). The advantage of cross-environment estimates of genetic correlation to measure the potential adaptation cost to pollutants is that it can be performed over a short period compared to other approaches such as experimental evolution. The absence or presence of adaptation cost to pollutants revealed by this method, however, would need to be validated by direct multigenerational experiments of adaptation to uranium and salt, before one can generalize its appropriateness.

4.3. Effects on genetic correlation structure

The strong genetic correlation in the control environment indicated a coevolution between growth and fertility (Table 4A). Genotypes that grew faster before sexual maturity (early growth) were more fertile but had a reduced late growth. This evolutionary strategy is generally that of small, short-lived organisms as *C. elegans* (r-selection, MacArthur & Wilson, 1967 ; Roff, 2002b). *C. elegans* produces juveniles as soon as possible (Hodgkin & Barnes, 1991). Sperm production takes place during larval stages and then stops at the molt, which produces the adults while the remaining germ line cells generate exclusively oocytes until the end of the egg-laying period (Antebi *et al.*, 1997). Investment in growth during the costly oocyte production affected directly fertility (Goranson *et al.*, 2005). Consequently, the likely outbreeding depression caused by the hybridization between diverse wild isolates (Dolgin *et al.*, 2007), to create the population used in our experiments, may be the cause of its low fertility (about 174 larvae in control, Table 3) compared to other populations of *C. elegans* (Hodgkin & Barnes, 1991). However, outbreeding depression happening at the construction of the *C. elegans* population, when the wild isolates were crossed, and did not break down the expression of genetic structure of the study population under stabilizing selection (Teotónio *et al.*, 2012).

Genetic correlations in the salt environment existed for the same combination of traits. When compared to the control, the changes in sign for correlations involving late growth in salt could be explained by a considerable developmental delay in this environment. Unlike in other environments, most individuals started laying eggs after 72h, and even after 144h (data not shown). So, we could say that early and late growths correspond to early growth in salt, and that the expression of genetic structure between growth and fertility is close to that of the control environment. In contrast, the genetic correlations in the control became null in uranium, in particular those involving fertility. Interestingly in uranium the phenotypic correlations were similar than in the control environment, due to significant environmental correlations (see appendix C Table C2). It has even been shown that environmental correlations can have opposite signs than genetic correlations, in particular due to acquisition and allocation of resources for both traits (de Jong & Van Noordwijk, 1992 ; Roff, 2002b). Besides this could also explain, the presence of more significant environmental correlations in the stressful environment, in our study, where individuals have probably suffered from effects on resources acquisition and allocation (Lamitina & Strange, 2005 ; Jiang *et al.*, 2009 ; Giovanetti *et al.*, 2010) compared to

the control. This highlights the importance of looking at the genetic structure: despite the same phenotypic structure in different environments, the expression of genetic structure may be broken down, implying a different evolution of life history traits (Willis *et al.*, 1991 ; Gibert *et al.*, 1998 ; Roff, 2002b ; Hadfield *et al.*, 2007).

Mechanisms of resistance, governed by gene expression, enable maintenance of individuals under highly stressful conditions such as pollution, but affect expression of genetic structure (Hoffmann & Parsons, 1991). Indeed Shirley & Sibly (1999) analysed the processes by which *D. melanogaster* increased its tolerance to cadmium. Apparently the links between genes involved in fertility, survival and development period in a control environment may be disrupted due to the addition, in the previous links, of resistance genes expressed in cadmium. It is also the case for natural birch-feeding insect, *Priophorus pallipes*; the genetic correlation between body size and development time was positive when quality of foliage resources were good and negative or close to zero for low resource quality (Kause *et al.*, 2001). Evolutionary trajectories depend on the effect of environmental conditions on organisms (Sgrò & Hoffmann, 2004). Hence, even if selection experiments in polluted environment require considering the temporal heterogeneity of the **G** matrix, for example by emergence of trade-off (de Jong, 1995 ; Steppan *et al.*, 2002 ; Fuller *et al.*, 2005), we showed here that the expression of genetic structure is already changed when individuals are subject to sudden pollution. In contrast, a change in the genetic structure in the uranium environment, by selection on heterozygosity for example, is hardly possible given that mortality was similar in the uranium and the control environments. There are also different degrees of change, for uranium and salt, probably depending on their mode of action. Consequently, evolution of the **G** matrix is already affected from the moment pollutants appear in the environment. It should now be important to consider why some pollutants or specific genetic conditions promote stability or instability of the **G** matrix.

4.4. Relation between heritability and fitness

Another important aspect of our result is that, when compared to the control, the decrease of heritability was higher in uranium than in the salt environment (Table 4) although salt concentrations used in our study affected more strongly the individuals' fitness (Table 3). Consequently, changes in heritability are more complex than a simple positive relation between stress effects and fitness reduction. The decrease of heritability in polluted environments may also

depend on how interference disrupts gene expression (Medina *et al.*, 2007). However this needs to be clarified with studies that focus on heritability in different polluted environments with different concentration ranges of pollutant as well.

5. Conclusion

Isogenic lines of *C. elegans* allowed us to analyse in one generation the effects of pollutants on the expression of genetic structure of life history traits. Our findings confirm the decrease of heritability in polluted environments probably due to the expression of different sets of genes, such as resistance genes, that disrupt the expression of genetic structure, especially in sudden uranium pollution. Moreover the decrease of heritability is not linearly correlated to fitness reduction in polluted environments. The extremely low heritability in uranium could prevent selection to act despite strong selection pressures on traits. Finally, when individuals are adapted to one polluted environment, there seems to be a genetic cost to living in the other polluted environment. Pollution can break down the expression of genetic structure of life history traits of a population of *C. elegans* upon arrival in its environment, and thus affect its evolutionary potential.

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