Effect of metal stress on life history divergence and quantitative genetic architecture in a wolf spider

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Introduction

Environmental stress can be defined as any environmental factor that impairs fitness when first applied (Hoffmann & Parsons, 1991; Bijlsma & Loeschcke, 2005). Despite this negative effect on fitness, natural as well as laboratory populations have been shown to persist under stressful conditions, which suggests that environmental stress may cause divergence in traits related to stress resistance among differentially exposed populations (Bijlsma & Loeschcke, 1997, 2005; Parsons, 2005). There is increasing evidence for rapid micro-evolution in populations subjected to stress, particularly for stresses of anthropogenic origin (Parsons, 1994; Hoffmann & Hercus, 2000; Reznick & Ghalambor, 2001; Räsanen et al., 2003; Badyaev, 2005). Increased resistance against ecotoxic substances such as heavy metals comprises one of the best-documented examples of rapid micro-evolutionary change in plants (Antonovics et al., 1971; Macnair, 1993; Shaw, 1999; Pauwels et al., 2006), aquatic invertebrates (Klerks & Weiss, 1987; Martinez & Levinson, 1996) and terrestrial invertebrates (Posthuma & van Straalen, 1993; Shirley & Sibly, 1999; Morgan et al., 2007). The often very intense selection pressure caused by metal exposure, its persistence and the fact that it is relatively easy to measure render metal pollution to be one of the best demonstrated classic examples of natural selection in action. Despite the growing interest in heavy metal contamination as driving force for physiological

Keywords:
animal model;
Bayesian statistics;
ecological divergence;
egg size;
heavy metal;
stress resistance;
trade-off.

Abstract

Effects and consequences of stress exposure on life history strategies and quantitative genetic variation in wild populations remain poorly understood. We here study whether long-term exposure to heavy metal pollution may result in alternative life history strategies and alter quantitative genetic properties in natural populations of the wolf spider *Pirata piraticus*. Offspring originating from a reference and a metal contaminated population and their reciprocal hybrid cross were bred in a half-sib mating scheme and subsequently reared in cadmium contaminated vs. clean environment. Results from this experiment provided evidence for a genetically based reduced growth rate and increased egg size in the contaminated population. Growth rate reduction in response to cadmium contamination was only observed for the reference population. Animal model analysis revealed that heritability for growth rate was large for the reference population under reference conditions, but much lower under metal stressed conditions, caused by a strong decrease in additive genetic variance. Heritability for growth of the metal contaminated population was very low, even under reference conditions. Initial size of the offspring was primarily determined by maternal effects, whereas egg size produced by the offspring was determined by both sire and dam effects, indicating that egg size determination is under control of the female genotype. In conclusion, these results show that metal stress can not only affect life history variation in natural populations, but also decreases the expression as well as the of the amount of genetic variation for particular life history traits.
and/or life history divergence, the genetic architecture of the traits involved and adaptive consequences remain largely unknown (Van Straalen & Hoffmann, 2000).

A thorough understanding of the genetic (co)variance of traits related to stress resistance is important for at least three reasons. First, adaptation to stress not only implies the presence of standing genetic variation for stress resistance, but the latter can also be expected to be related with fitness-related traits, hence resulting in trade-offs between environments (Shirley & Sibly, 1999; Bubliy & Loeschcke, 2005). The evolution of these ‘tolerance costs’ can best be understood through resource partitioning theory. As energy available for life history processes such as survival, growth and reproduction is limited, resource allocation to one trait generally means deprivation of another (van Noordwijk & de Jong, 1986; limited, resource allocation to one trait generally means deprivation of another (van Noordwijk & de Jong, 1986; Stearns, 1992; Reznick et al., 2000; Roff, 2002). Physiological defence mechanisms such as increased metal excretion or the production of detoxifying enzymes (Wilczek & Migula, 1996) of tolerant genotypes (Maroni et al., 1987; Van Straalen et al., 1987) can therefore be expected to reduce resource availability for growth and reproduction (Calow, 1991). In line with this, Drosophila melanogaster forced to evolve in a cadmium-contaminated medium showed a significant decrease in female weight and fecundity compared with those reared in unpolluted environments (Shirley & Sibly, 1999). Because of the limited number of studies addressing quantitative genetics of tolerance to chemical contaminants in natural populations (Posthuma et al., 1993; Forbes et al., 1999; Klerks & Moreau, 2001), basic assumptions of life history divergence because of stress tolerance remain largely untested (Van Straalen & Hoffmann, 2000). Second, exposure to stress may influence the expression of heritable variation in a trait by altering the balance between different sources of phenotypic variation, i.e. additive genetic, dominance genetic and environmental variation (e.g. Hoffmann & Merilä, 1999; Blanckenhorn & Heyland, 2004; Laugen et al., 2005). As a reduction in expression of quantitative genetic variation might hamper the evolution of stress resistance, a better insight into the sources of phenotypic variability in wild populations is required to understand changes in expression of genetic variation under ecologically relevant ecological circumstances (Hoffmann & Merilä, 1999; Pakkasmaa et al., 2003; Charmantier & Garant, 2005; Wilson et al., 2006). Third, adaptation to anthropogenic stresses often results from strong directional selection for tolerance traits. Such process might lead to alterations in the genetic architecture of populations and, eventually to genetic erosion, not only in traits directly related to stress resistance, but also in fitness traits that are genetically correlated with stress resistance (Pérez & García, 2002; Van Straalen & Timmermans, 2002; Merilä et al., 2004).

This paper aims to quantify genetic variation underlying life history divergence in natural populations of the wolf spider Pirata piraticus and to assess its relationship with metal tolerance. An earlier study demonstrated altered life history patterns indicative for low growth and/or low reproductive environments in populations contaminated with Cd, Zn and Cu (Hendrickx et al., 2003b). Although clutch mass and hence, female fecundity, decreased along an increasing pollution gradient consisting of six populations, egg size significantly increased. In contaminated populations, females producing larger offspring tended to have lower levels of developmental stability, as measured by fluctuating asymmetry. This relationship was not observed for reference populations, suggesting a fitness advantage of producing larger offspring under environmental stress (Hendrickx et al., 2003a). Such life history divergence can be interpreted as adaptive under optimality models developed by Sibly et al. (1988) and Tamate & Maekawa (2000).

We here set up a half-sib breeding experiment where-by F1 offspring derived from pure as well as reciprocal hybrid crosses between individuals from the most contaminated and a reference population along this pollution gradient were reared under both contaminated and reference conditions. By estimating the levels of genetic (co)variation of life history traits size at birth, growth and egg size, we predict that (i) reduced growth and increased egg size have evolved as a result of tolerance cost and adaptive life history strategies in the metal stressed population; (ii) metal exposure decreases the additive genetic variance proportionally more than the other sources of phenotypic variance, resulting in decreased realized heritabilities and (iii) long-term metal exposure decreases the genetic variability of the investigated traits.

Materials and methods

Origin of the populations and rearing conditions

Selection of study populations was based on a previous study where adaptive life history changes were shown to co-vary with internal metal body burden along a pollution gradient (see Hendrickx et al., 2003b). The populations used in this study represented the two most extreme cases along this gradient and were characterized by profound differences in life history traits and internal metal concentration. Reference population ‘Damvallei’ (50°58'N, 3°50'E; further referred to as ‘R’) originates from a pristine lowland mire with a low degree of metal contamination. Under field conditions, females of this population had a large size, a relatively high reproductive output and produced small eggs. A severely contaminated population ‘Galgenschoor’ (51°18'N, 4°16'E, referred to as ‘C’), originates from a tidal marsh along the Schelde, a river that suffered from severe metal pollution during the last four decades (Zwolsman et al., 1996). Besides the significantly higher Cd-, Cu- and Zn-content in these spiders, adult size and reproductive output of females were significantly lower, but egg mass was significantly
higher, than for females of population R (Hendrickx et al., 2003b). The distance between both populations is 45 km.

Around 100 hibernating juveniles from each population were collected during October and reared till adulthood in a climate chamber at a constant temperature of 26 °C and a 16 : 8 light : dark photoperiod. They were kept individually in Petri dishes with at the bottom a layer of plaster of Paris. As natural populations of this species are bound to very wet and inundating habitats (Hänggi et al., 1995), Petri dishes were kept at 100% relative humidity. Spiders were fed ad libitum with fruit flies three times a week. To obtain optimal growth and minimal mortality, 40% wet weight crushed dog food was added to a banana and oatmeal based fruit fly medium (Mayntz & Toft, 2001).

**Experimental design**

We applied a breeding design that allowed to simultaneously test for within- and between-population genetic variation in life history traits and cadmium susceptibility. Besides pure R and C offspring (RSRD and CSD, respectively; subscript S denotes Sire population and subscript D denotes Dam population), reciprocal crosses (RSCD and CDRS) produced hybrid offspring. To extract additive genetic components of life history divergence, males and females were mated according to a half-sib mating design by allowing males of both populations to copulate with two females of each population (four females in total). However, this scheme could not be maintained in all cases due to frequent injuries or killing of males by females during copulation. Offspring originating from copulations in which a male could not copulate with at least one female from either population were excluded from the experiment. The parental population consisted of 13 R and 10 C males and 31 R and 39 C females. Parental females produced an egg cocoon from which spiderlings emerged after 3 weeks. F1 offspring was tested for within- and between-population genetic variation in response to cadmium treatment (Hendrickx et al., 2003b). Petri dishes were kept at 100% relative humidity. Spiders were fed ad libitum with fruit flies three times a week. To obtain optimal growth and minimal mortality, 40% wet weight crushed dog food was added to a banana and oatmeal based fruit fly medium (Mayntz & Toft, 2001).

**Measurement of life history traits**

Population differentiation and within-population variability was measured for the following life history traits: initial weight, growth rate and egg size. As no cadmium treatment was applied during the first 4 weeks after emergence, its effect was only assessed on the latter two traits. Initial weight was measured to the nearest 0.1 mg at the age of 3 weeks (i.e. the earliest time when spiders can be measured with high accuracy) and every third week thereafter. None of the spiders reached adulthood before the age of 12 weeks and this weight was taken as a proxy of juvenile growth. However, significant mass differences between the sexes were already present at this stage. In order to reduce statistical complexity and increase the number of observations within each full-sib group, we regressed mean juvenile female weight against juvenile male weight and transformed juvenile weights to the nearest 0.01 mm. The volume of each egg (ellipsoid) was calculated as \( \pi/6 \times \text{egg length} \times (\text{egg width})^2 \). The average volume of the eggs was calculated for each spider to obtain a single observation per female.

**Analysis of population differentiation**

We applied a generalized linear mixed model (Proc mixed in SAS v 9.1, SAS Institute, Inc., Cary, NC, USA) to test for the significance of the main fixed effects ‘sire population’ (SPOP), ‘dam population’ (DPOP), ‘cadmium treatment’ (CD) and all two- and three-way interactions. To model the most appropriate error structure for testing these fixed effects, we added the random effects ‘sire’ (nested within SPOP) and ‘dam’ (nested within Sire and DPOP) in each cadmium treatment and also estimated the degree of covariance between both treatments. Error terms were allowed to differ between treatments. Fixed effects testing was based on Type III sum of squares, with error degrees of freedom adjusted according to Satterthwaite’s approximation as multiple error terms were included in the model (Littell et al., 1996; Verbeke & Molenberghs, 2000).

**Estimation of quantitative genetic parameters**

Genetic and environmental (co)variance components were estimated by means of the animal model (Lynch & Walsh, 1998). Given that \( \mu_{s,d,t} \) is the mean value of offspring originating from the sire (S) and dam (D) population combination and cadmium treatment \( t \), the phenotype of offspring \( i \) of the \( s \)'th sire and the \( d \)'th dam receiving the \( t \)'th cadmium treatment \( Y_{s,d,t,i} \) was modelled as:

\[
Y_{s,d,t,i} = \mu_{s,d,t} + (a_{s,t} + a_{d,t})/2 + \delta_{s,d,t} + e_{s,d,t,i}
\]

where the \( a \)'s are the breeding values, \( \delta \) are the maternal and dominance effects and \( e \) are the residual errors.
Separating dam and sire additive genetic effects allowed us to estimate these breeding values for each sire and dam population separately. The vector of $x$’s are modelled as random effects as follows:

$$
\begin{bmatrix}
  z_{x-cd} \\
  z_{x+cd}
\end{bmatrix} \sim N\left(\begin{bmatrix} 0 \\
  0\end{bmatrix}, \begin{bmatrix} \sigma^2_{x-cd} & \sigma_{x-cd,+cd} \\
  \sigma_{x-cd,+cd} & \sigma^2_{x+cd}\end{bmatrix}\right)
$$

where $\sigma^2_{x-cd}$ is the additive genetic variance in the cadmium free environment, $\sigma^2_{x+cd}$ is the additive genetic variance in the cadmium contaminated environment and $\sigma_{x-cd,+cd}$ is the additive genetic covariance between breeding values in the reference and contaminated environment. Again, the variances of the $x$’s were estimated for each population separately. The latter is expressed as the genetic correlation across environment ($r_x$) rather than as a covariance and was obtained by dividing this covariance by the square root of the product of the variances within each environment. Estimates of these (co)variance components were designated as $V_{x-cd}$, $V_{x+cd}$ and $r_x$ respectively and were obtained for both reference and contaminated populations separately. The applied breeding design does not allow distinguishing between maternal effects and dominance deviations, hence, both were modelled as a single random effect represented as $\delta$ and further referred to as maternal-dominance deviations. As such, the variances in $\delta$ actually represented a quarter of the variance in dominance deviations and the variance in maternal effects (Falconer & Mackay, 1996; Lynch & Walsh, 1998). They were modelled as random vectors in an identical way as for the additive genetic effects and estimates are referred to as $V_{s,d,i}$. However, as maternal-dominance deviations can be expected to differ between cross type rather than between populations only, estimates were initially obtained per cross type.

Residual errors ($e$) are composed of additive genetic error ($e_a$) and dominance error and special environmental effects. The latter two errors are modelled as one component ($e_{s,d}$) as dominance effects cannot be estimated with high precision in the current breeding design. Hence, the error of the $i$’th offspring of the $s$’th sire and $d$’th dam is modelled as:

$$
e_{i,s,d,i} = \left(\frac{e_{a,s,i} + e_{s,d,i}}{2}\right)/\sqrt{2} + e_{w,s,d,i,1}
$$

Additive genetic errors ($e_a$) were assumed to follow the same distribution as the breeding values. Error variances were assumed to follow a normal distribution with mean zero and variance $\sigma^2_{e,a}$ and were allowed to differ between the four crossing types and both cadmium treatments. Estimates of this variance are further referred to as $V_e$.

Estimating these additive, maternal-dominance and error variances allowed us to obtain separate heritabilities for each population and cadmium treatment and were calculated as:

$$h^2(t) = \frac{V_A}{V_A + V_{M,D} + V_E}$$

where $t$ refers to the cadmium treatment.

The model was fitted using a Bayesian, MCMC approach with Gibbs sampling in winbugs 1.4 (Spiegelhalter et al., 2003). This permitted to estimate the full posterior distribution of the different (co)variance components and heritabilities directly (Merila et al., 2004). All estimated parameters were given non-informative prior distributions. For the fixed effects these were normally distributed with mean 0 and a variance of 1000. For the precision of the variances, these were chosen to follow a uniform [0,1000] distribution. For the multivariate random effects the prior distribution of the precision of the variance–covariance matrix was chosen to follow a Wishart distribution with $R = [1 0; 0 1]$ and 2 d.f. It should be noted that setting a prior distribution that is bound to zero always results in positive variance component estimates and lower credibility intervals are consequently always larger than zero. Hence, only variance components with credibility intervals that are substantially larger than zero were interpreted as being substantial. Selection of the most parsimonious model was based on Deviance Information Criterium (Spiegelhalter et al., 2002). Three MCMC chains, each with different starting values, were run simultaneously for 12 000 iterations. Convergence of the three chains was checked by visual inspection of plots depicting the Gibbs chains. Posterior summary statistics of the parameters were also highly comparable for the three chains. The first 2000 iterations were treated as burn-in and discarded for the estimation of the parameters.

**Results**

**Differentiation between populations**

**Week 3 weight**

As the cadmium treatment was only applied from the age of 4 weeks onwards, cadmium effects could not be observed. Initial weight of the spiderlings strongly depended on female origin (DPOP: $F_{1,771} = 10.57; P = 0.001$) whereby offspring of population R were significantly smaller (mean = 1.87 mg, SE = 0.13) than of population C (mean = 2.36 mg, SE = 0.10). At this age there was no significant effect of male origin (SPOP: $F_{1,771} = 0.76; P = 0.38$) nor was there a significant interaction between male and female origin ($F_{1,771} = 0.8; P = 0.37$). This demonstrates that differences in offspring size in early developmental stages are predominantly determined by female population origin.

**Week 12 weight**

Weight at the age of 12 weeks was strongly reduced under the cadmium treatment, although its effect differed between the crossing types (Table 1; Fig. 1). Differences between crossing types were more
pronounced when no cadmium treatment was applied (Fig. 1; SPOP and DPOP effect in reference environment: $F_{1,19.4} = 6.45; P = 0.02$ and $F_{1,42.5} = 13.78; P = 0.0006$ respectively). The fact that sire origin significantly affected growth confirms the (partly) involvement of genetic effects in population differentiation under reference conditions. Female origin affected weight to a similar magnitude across cadmium treatments. Absence of significant interactions between SPOP and DPOP indicated that differences were largely due to additive genetic effects. Differences in offspring weight were no longer significant when they were exposed to cadmium (Fig. 2; SPOP and DPOP effect in cadmium environment: $F_{1,21.4} = 1.98; P = 0.17$ and $F_{1,46.6} = 0.06; P = 0.81$ respectively). The response to cadmium was significantly dependent on female as well as male origin (Table 1). Although evidence for influences of the female origin appeared to be stronger, we found no evidence that influences of male and female origin on cadmium response included some dominance effects, as revealed from a non-significant three-way interaction.

### Egg size

Egg size produced by adult F1 females was not significantly affected by the cadmium treatment but only sire and dam population origin (Table 2, Fig. 2). Female offspring from parents that both originated from the contaminated population produced significantly larger eggs (0.34 mm$^3$, SE = 0.007) than female offspring from reference parents (0.28 mm$^3$, SE = 0.007). The absence of a significant interaction between male and female origin confirmed the additive genetic origin of genetic differentiation in egg size. Egg size produced by females from the two hybrid crosses was not significantly different ($P > 0.07$).

### Table 1

<table>
<thead>
<tr>
<th>Effect</th>
<th>d.f.</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>15.6</td>
<td>27.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SPOP</td>
<td>20.3</td>
<td>5.34</td>
<td>0.03</td>
</tr>
<tr>
<td>DPOP</td>
<td>45</td>
<td>4.84</td>
<td>0.03</td>
</tr>
<tr>
<td>SPOP × DPOP</td>
<td>45</td>
<td>0.02</td>
<td>0.89</td>
</tr>
<tr>
<td>CD × SPOP</td>
<td>15.6</td>
<td>4.57</td>
<td>0.048</td>
</tr>
<tr>
<td>CD × DPOP</td>
<td>43.5</td>
<td>19.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(CD × SPOP × DPOP)</td>
<td>43.5</td>
<td>1.38</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Non-significant interaction terms (between brackets) were removed from the final model. Sire, dam and their respective interactions with the cadmium treatment were included as random effects to model an appropriate error structure for the fixed effects.

### Table 2

<table>
<thead>
<tr>
<th>Effect</th>
<th>d.f.</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>245</td>
<td>0.12</td>
<td>0.73</td>
</tr>
<tr>
<td>SPOP</td>
<td>23.5</td>
<td>5.88</td>
<td>0.02</td>
</tr>
<tr>
<td>DPOP</td>
<td>48.4</td>
<td>39.84</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(SPOP × DPOP)</td>
<td>48.4</td>
<td>0.50</td>
<td>0.48</td>
</tr>
<tr>
<td>(CD × SPOP)</td>
<td>245</td>
<td>0.24</td>
<td>0.62</td>
</tr>
<tr>
<td>(CD × DPOP)</td>
<td>245</td>
<td>0.05</td>
<td>0.82</td>
</tr>
<tr>
<td>(CD × SPOP × DPOP)</td>
<td>245</td>
<td>0.00</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Non-significant interaction terms (between brackets) were removed from the final model. Sire, dam and their respective interactions with the cadmium treatment were included as random effects to model an appropriate error structure for the fixed effects.
Variation within populations

**Week 3 weight**
Sources of phenotypic variance for initial weight were of similar magnitude for the four crossing types, and obtaining estimates for each crossing type separately did therefore not result in the most parsimonious model. A common additive genetic, maternal-dominance and environment specific variance was therefore estimated over crossing types. Maternal-dominance effects contributed to the highest amount of phenotypic variance \( (V_{M,D} = 0.277; 95\% \text{ CI: } 0.157–0.437) \), followed by environment specific residual variance \( (V_E = 0.104; 95\% \text{ CI: } 0.025–0.183) \) and additive genetic variance \( (V_A = 0.096; 95\% \text{ CI: } 0.029–0.190) \). Consequently, heritabilities for initial weight were estimated to be low \( (h^2 = 0.20) \), and not substantially higher than zero \( (95\% \text{ CI: } 0.05–0.37) \).

**Week 12 weight**
We obtained additive genetic, maternal-dominance and environmental variance estimates for each population (cross) and cadmium treatment. The relative magnitude of the variance components differed strongly depending on population origin and cadmium treatment (Table 3). For the reference population, expression of additive genetic variance for growth was considerably larger when spiders were reared under reference conditions compared to the cadmium treatment. However, the genetic correlation across environments approximated one, indicating an absence of genotype–environment interaction. Maternal-dominance and environment specific residual variance was in contrast highly comparable under both cadmium treatments and hence only one variance was estimated across environments. The stronger reduction in additive genetic variance compared to the other components consequently resulted in a significantly lower heritability for weight for the reference population under cadmium contaminated conditions (Fig. 3).

Estimated additive genetic variance of the contaminated population was in contrast much lower under uncontaminated conditions and not statistically different when fed with cadmium flies. As maternal-dominance and residual variation were of comparable magnitude under both environmental conditions, and of comparable magnitude as for the reference populations, heritability estimates were lower, particularly under uncontaminated conditions (Fig. 3).

**Egg size**
Variation in egg size was mainly determined by additive genetic and maternal-dominance effects and only for a minor part by environment specific residual effects.

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**Table 3** Mean estimated additive, maternal and environmental variance components (2.5 and 97.5 percentiles between brackets) for weight at week 12.

<table>
<thead>
<tr>
<th>Source ( (\text{-Cd}) )</th>
<th>( R )</th>
<th>( C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_A )</td>
<td>47.96 ([23.70; , 84.6])</td>
<td>1.13 ([0.14; , 4.37])</td>
</tr>
<tr>
<td>( V_A ) ( (+ \text{ Cd}) )</td>
<td>4.07 ([0.46; , 12.40])</td>
<td>1.32 ([0.16; , 5.33])</td>
</tr>
<tr>
<td>( r_G )</td>
<td>0.80 ([0.51; , 0.99])</td>
<td>0.20 ([-0.76; , 0.92])</td>
</tr>
<tr>
<td>( r_G ) ( (\text{-Cd}) )</td>
<td>( R_S R_D )</td>
<td>( C_S R_D )</td>
</tr>
<tr>
<td>( V_{M,D} )</td>
<td>2.92 ([0.23; , 9.18])</td>
<td>5.35 ([1.86; , 11.61])</td>
</tr>
<tr>
<td>( V_E )</td>
<td>11.19 ([8.47; , 14.82])</td>
<td>9.89 ([7.31; , 13.32])</td>
</tr>
<tr>
<td>( h^2 ) ( (\text{-Cd}) )</td>
<td>0.75 ([0.58; , 0.86])</td>
<td>–</td>
</tr>
<tr>
<td>( h^2 ) ( (+ \text{ Cd}) )</td>
<td>0.27 ([0.04; , 0.60])</td>
<td>–</td>
</tr>
</tbody>
</table>

Cross type labels refer to parental origin of sires (subscript S) and dams (subscript D) with \( R = \) reference population and \( C = \) metal contaminated population.

\( V_A \), genetic variance in breeding values; \( r_G \), genetic correlation across cadmium treatments; \( V_{M,D} \), maternal-dominance variance; \( V_E \), residual variance.
resulting in high estimates of narrow sense heritability across cadmium treatments (Table 4). In parallel with population responses, egg size variation in response to the cadmium treatment was of small magnitude. Estimates of heritability and variance components did not differ substantially between both populations.

**Discussion**

**Differentiation between populations**

Results from this paper provide evidence for life history divergence in two populations of wolf spiders that differ in exposure to heavy metal contamination. The significant effect of paternal origin on growth rate and egg size suggests that the previously field observed population divergence along the pollution gradient is – at least partly – genetically based. The intermediate levels of both life history traits in hybrid crosses, and an absence of interactive effects between male and female origin, further point that additive genetic components are the main source of the observed life history variation (Lynch & Walsh, 1998).

The weight of early instars originating from parental reference (R) females was considerably smaller than that of instars originating from females of the contaminated (C) population, which is in concordance with egg size differences recorded in both populations under natural conditions (Hendrickx et al., 2003b). Instar size appeared to be only affected by the population origin of the maternal population. However, variation in egg size produced by F1 females was additionally affected by paternal origin. The intermediate size of eggs from both hybrid crosses further suggests additive paternal and maternal effects. Combining data on early instar size and egg size demonstrate that progeny size is determined by the mother’s genes and hence a maternal genetic effect as has been shown for other invertebrates (Mousseau & Dingle, 1991; Mousseau & Fox, 1998).

Apart from genetic effects, growth rates were also affected by environmental stress levels. In absence of cadmium stress, pure R offspring were larger than C offspring, whereas hybrid crosses obtained intermediate sizes. This contrasts with the larger initial size of C offspring and indicates reduced growth of C offspring, even in absence of environmental stress. In spiders, exposure to heavy metals has been observed to increase the production of detoxifying enzymes such as metallothioneins (Wilczek & Migula, 1996), which is considered to act as a physiological defence mechanism (Maroni et al., 1987). Likewise, selection experiments with *D. melanogaster* indicated genetically based production of these enzymes under heavy metal contamination, eventually causing decreased fitness under favourable conditions (Shirley & Sibly, 1999). In wolf spiders, such mechanism is evident from the reduced growth rates under favourable conditions in individuals locally adapted to cadmium stress. Although in our experiment, mean growth rates of C offspring did not exceed those of R offspring (i.e. non-crossing reaction norms), this may well be the case under natural conditions where cadmium body burdens can be expected to be even higher then the levels reached in this experiment (50–150 µg Cd g⁻¹ spider, F. Hendrickx personal observation, Hendrickx et al., 2003c). Moreover, natural spider populations are generally contaminated with a mixture of heavy metals (Hendrickx et al., 2003b, 2004) that have been shown to act synergistically (Lock & Janssen, 2002).

Egg size showed no plastic response to metal contamination and differences between cross types remained fixed across cadmium treatments. This suggests that female wolf spiders are unable to adjust their egg size in response to the environmental conditions experienced during growth at population level (but see Ernsting &

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**Table 4 Mean estimated additive, maternal and environmental variance components (2.5 and 97.5 percentiles between brackets) for egg size.**

<table>
<thead>
<tr>
<th>Source</th>
<th>R</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_A)</td>
<td>0.128 [0.080; 0.241]</td>
<td>0.108 [0.064; 0.219]</td>
</tr>
<tr>
<td>(V_A) (+ Cd)</td>
<td>0.158 [0.096; 0.274]</td>
<td>0.162 [0.100; 0.366]</td>
</tr>
<tr>
<td>(r_g)</td>
<td>0.96 [0.67; 0.99]</td>
<td>0.78 [0.42; 0.97]</td>
</tr>
<tr>
<td>(V_{MD})</td>
<td>0.125 [0.05; 0.350]</td>
<td>0.117 [0.051; 0.257]</td>
</tr>
<tr>
<td>(V_E)</td>
<td>0.023 [0.01; 0.06]</td>
<td>0.051 [0.027; 0.142]</td>
</tr>
<tr>
<td>(h^2) (+ Cd)</td>
<td>0.469 [0.257; 0.674]</td>
<td>0.627 [0.329; 0.923]</td>
</tr>
<tr>
<td>(h^2) (+ Cd)</td>
<td>0.411 [0.221; 0.594]</td>
<td>0.586 [0.301; 0.813]</td>
</tr>
</tbody>
</table>

Cross type labels refer to parental origin of sires (subscript S) and dams (subscript D) with R = reference population and C = metal contaminated population.

\(V_A\), genetic variance in breeding values; \(r_g\), genetic correlation across cadmium treatments; \(V_{MD}\), maternal-dominance variance; \(V_E\), residual variance.
Several patterns can explain the observed difference in $V_A$ in this study. First, patterns of changes in additive genetic variation were in close relationship with the mean growth response of the different populations–cadmium treatment combinations, indicating that the observed differences can be attributed to scale effects (Houle, 1992; Falconer & Mackay, 1996), i.e. a change in the mean value of a trait following a change in its (additive genetic) variance. However, such pattern is unlikely to explain the change in additive genetic variance in the present study. This can be derived from calculating the coefficients of variation (i.e. square root of the additive genetic variance divided by to the mean value of the trait) which averaged 21.4% for the reference population in the cadmium-free environment, 12.7% when cadmium treatment was applied and 5.5% for the contaminated population across cadmium treatments. Moreover, in case of scale effects, all variance components would be expected to change consistently whereas heritability estimates would not change in response to changes in mean trait value.

Second, the decrease in heritable trait variation can be understood within the framework of traits plasticity in response to environmental variation (i.e. reaction norms). In case of a negative additive genetic covariance between the breeding value of growth in the reference environment and growth rate reduction in response to cadmium, levels of additive genetic variance will vary along the environmental gradient and may become close to zero at particular environmental values (Scheiner, 1993; Lynch & Walsh, 1998; Nussey et al., 2007). However a similar pattern might arise if different loci determine growth rate in both environments. For example, the expression of growth promoting alleles in the reference environment can be masked in part by the expression of alleles that enhance detoxification in the contaminated environment. If heritable variation of the latter is low, growth heritability can be strongly reduced under contaminated conditions. Results of the present study can however not distinguish which of both mechanisms prevail as the genetic correlation across environments could not be estimated with high accuracy.

Besides this observed decrease in heritability of growth in the reference population, a strong decrease in growth heritability under reference conditions was also observed for population C. This reduction in heritability for growth, caused by a low additive genetic variance, is likely due to the decreased mean growth rate, suggesting a rather permanent expression of physiological defence mechanisms that decrease growth rate. This might hamper the expression of loci that determine growth in the reference population under reference conditions. A decrease in additive genetic variance in response to selection on the mean value of a trait has until now primarily been investigated in experimental populations and by theoretical models. These studies reveal that 'antagonistic

Isaaks, 1997; Fox et al., 1999; Fox & Czesak, 2000; Guinnee et al., 2007 for examples of increased offspring size in response to environmental variation). Given the apparent absence of egg size plasticity in our study, optimal egg size differences appeared to have evolved through local adaptation, as predicted by various theoretical models (Smith & Fretwell, 1974; Lloyd, 1987; McGingley et al., 1987) and demonstrated empirically in wild populations (Einum & Fleming, 1999; Räsänen et al., 2005).

The observed differentiation in life history traits is in line with the pattern observed under field conditions, where both populations are situated on the extremes of a pollution gradient consisting of six different populations. Average metal body burdens of field captured individuals are about 6, 5 and 1.4 times higher for Cd, Cu and Zn respectively (Hendrickx et al., 2003b). However, as only two populations could be included in this breeding design, other causal factors than metal pollution cannot be excluded unambiguously. First, results from this study provide no evidence whether the differentiation is directly caused by metal pollution or rather because of indirect effects such as reduced prey availability. Although the density of suitable prey items is hard to estimate, densities of adult individuals are highly comparable under field circumstances and average about 8–10 individuals m$^{-2}$ (F. Hendrickx personal observation). Second, contamination of the river Schelde also includes other pollutants besides heavy metals, which may cause, or at least reinforce, this life history differentiation.

**Within population variability**

Our results show that cadmium contamination strongly decreased the heritability for growth, but only for the reference population. For the contaminated populations, heritabilities for this life history trait were low, and not affected by the applied cadmium treatment.

Although earlier studies, mainly laboratory studies on *Drosophila* populations, investigating changes in heritability in response to stressed conditions report an increase in heritable variation (Hoffmann & Hercus, 2000), decreased heritabilities under unfavourable conditions are no exception in natural populations under more realistic types of environmental stress (Hoffmann & Merilä, 1999, Charmantier & Garant, 2005). Most of these studies showed that the decrease in heritability could be attributed to an increase in environmental variation due to stress, which results in a relative decrease of the additive genetic variation and, consequently, the heritability of that trait. We here provide evidence that the decrease in growth heritability in the reference populations is principally caused by a decrease in the additive genetic variation as other sources of phenotypic variation remained constant over cadmium treatments.
selection’, i.e. selection towards the overall mean, being upward in unfavourable conditions and downward in favourable conditions, decreases environmental sensitivity, which might ultimately lead in a decreased additive genetic variance (Falconer, 1990; Scheiner & Lyman, 1991; Scheiner, 2002). It remains however unclear if these results can be generalized towards wild animal populations, as the few studies that compared genetic variability of populations subjected to long term stress exposure gave contrasting results (e.g. Charmantier et al., 2004; Merilä et al., 2004). Other mechanisms that might explain the observed decrease in genetic variability is a reduction in effective population size in stressful environments (Hoffmann & Hercus, 2000; Van Straalen & Timmermans, 2002), which has been shown to decrease the amount of additive genetic variance as well as heritability (e.g. Kristensen et al., 2005). This appears however less likely in the present population as neutral genetic diversity, investigated on six allozymes, was not different between both populations (F. Hendrickx, F. Langenbick & J.-P. Maelfait unpublished data).

Egg size variation showed relatively high heritabilities in both populations in the two environments, indicating that there is evolutionary potential for egg size adjustment to local conditions. In line with the absence of a change in mean egg size in response to cadmium, genetic determination appeared to be unaffected by the cadmium treatment. The high genetic correlation demonstrated that no genetic variation is present in egg size adjustment in response to the applied cadmium treatment.

Conclusion
With this experiment we give evidence that cadmium exposure might not only reduced the growth rate of a previously unexposed population, but moreover influenced the genetic architecture of this life history trait. Decreased realized heritabilities were observed under the cadmium treatment, suggesting a profound decrease in the evolutionary potential of the tested reference population. Comparing these observations with those of a population subject to metal pollution for several decades revealed that growth and growth heritability were lower, particularly under reference conditions. This demonstrates that long-term metal pollution may cause severe fitness costs and decrease the evolutionary potential of particular life history traits, even when the pollution source ceases to exist. In accordance to life history theory, a genetically based increase in egg size is likely to have evolved for counteracting these adverse fitness effects.

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References


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