Interplay of robustness and plasticity of life history traits drives ecotypic differentiation in thermally distinct habitats.

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Running title: robustness and plasticity of life history traits

Abstract

Phenotypic plasticity describes the ability of an individual to alter its phenotype in response to the environment and is potentially adaptive when dealing with environmental variation. However, robustness in the face of a changing environment may often be beneficial for traits that are tightly linked to fitness. We hypothesized that robustness of some traits may depend on specific patterns of plasticity within and among other traits. We used a reaction norm approach to study robustness and phenotypic plasticity of three life history traits of the collembolan *Orchesella cincta* in environments with different thermal regimes. We measured adult mass, age at maturity and growth rate of males and females from heath and forest habitats at two temperatures (12 and 22 °C). We found evidence for ecotype-specific robustness of female adult mass to temperature, with a higher level of robustness in the heath ecotype. This robustness is facilitated by plastic adjustments of growth rate and age at maturity. Furthermore, female fecundity is strongly influenced by female adult mass, explaining the importance of realizing a high mass across temperatures for females. These findings indicate that different predicted outcomes of life history theory can be combined within one species' ontogeny and that models describing life history strategies should not assume that traits like growth rate are maximized under all conditions. On a methodological note, we report a systematic inflation of variation when standard deviations and correlation coefficients are calculated from family means as opposed to individual data within a family structure.

Keywords; temperature, ecotype-specific, reaction norm, life history theory, phenotypic plasticity, random regression mixed model, *Orchesella cincta*, inflation of variance

Introduction

A key element of the ability of organisms to cope with environmental variation is phenotypic plasticity, i.e. the capacity of a genotype to produce distinct phenotypes under different environmental conditions (reviewed in Scheiner, 1993, Pigliucci, 2005, DeWitt & Scheiner, 2004). Phenotypic plasticity should be selected for if environmental variation is predictable and the different environmental conditions favour distinct optimal phenotypes (Thompson, 1991). However, individuals also require a certain level of robustness in functioning despite environmental variation (Masel & Siegal, 2009). Indeed such robustness of life history traits is observed in natural systems (Forcada *et al.*, 2008, Liefting & Ellers, 2008), and depending on the context has been referred to as buffering, environmental canalization or developmental stability (Debat & David, 2001, Masel & Siegal, 2009). For instance, traits relevant to life history such as growth rate and development time, which are directly dependent on changes in temperature (Angilletta *et al.*, 2004) have been shown to be more robust in habitats with fluctuating temperature compared to those with more stable temperatures (Liefting & Ellers, 2008).

These trait-specific levels of plasticity suggest that plasticity in one trait may facilitate robustness in other traits (Schlichting & Smith, 2002, Liefting *et al.*, 2009). For example, in some species of hoverfly, body pigmentation is plastic in response to temperature and it is assumed that this color plasticity maintains body temperature at optimal thermal conditions for activity despite seasonal variation in temperature (Holloway, 1993). Natural selection acts on the integrated phenotype, hence the fitness consequences of plasticity depend on correlated responses in other traits. There is evidence that the plasticity levels of traits are correlated through shared underlying genetic mechanisms (Gutteling *et al.*, 2007, Ellers & Driessen, 2011). We should therefore consider the plastic response of one trait in conjunction with the level of plasticity in other traits if we want to gain a better understanding of how organisms cope with environmental fluctuations (Callahan *et al.*, 2008).

The range of phenotypes produced in different environments is referred to as the reaction norm of the genotype to these environments. The level of plasticity or robustness can be represented by the slope of a reaction norm of a trait in response to an environmental variable like temperature (de Jong, 1990). A steep reaction norm reflects high thermal sensitivity (i.e. plasticity) and a relatively flat reaction norm robustness (i.e. the same phenotype regardless of temperature). Genetic variation in steepness and elevation of thermal reaction norms has been shown to exist in the laboratory (Driessen et al., 2007, Ketola et al., 2013) and in natural populations (Brommer et al., 2005, Charmantier et al., 2008). Genetic differences in temperature-induced plasticity are found across both small and large geographical ranges (Trotta et al., 2006, Liefting et al., 2009, Richter-Boix et al., 2010). Therefore differences in thermal conditions at a small spatial scale (e.g. patches with and without canopy cover) could potentially lead to different strategies in robustness and plasticity of traits, if gene flow is low relative to the strength of selection.

To examine local variation in thermal adaptation, we investigated differences in reaction norms and genetic differentiation of springtail populations (*Orchesella cincta*, Collembola, Linnaeus 1758) from two adjacent, but thermally distinct, habitats. *Orchesella cincta* is found in the litter layer of a wide variety of habitats throughout the Holarctic. The species can reach high local densities and is found in abundance in forests and woodlands (van Straalen *et al.*, 1985), as well as open habitats such as heath land and sandy dunes. Like all springtail species, *O. cincta* grows and moults indeterminately, with alternating reproductive and non-reproductive instars (Ernsting & Isaaks, 2002). Males deposit small sperm droplets on a stalk (spermatophores), even when females are absent. Receptive females locate and take up spermatophores without necessarily meeting their partner (Zizzari *et al.*, 2009). As in other small litter-dwelling ectotherms, body temperature in *O. cincta* is identical to ambient temperature. Behavioral thermoregulation in *O. cincta* is limited to moving up and down in the litter layer and vegetation. Because most life history and developmental traits are sensitive to temperature changes in *O. cincta* (van Straalen, 1994, Joosse *et al.*, 1973), differential selection on plasticity and robustness of these traits is expected. Studies have shown

that juvenile growth rate in *O. cincta* is relatively robust to temperature changes in populations from habitats with high amplitude of temperature change (heath ecotype) compared to those with low amplitude of temperature changes (forest ecotype) (Liefting & Ellers, 2008). We expect such environmental robustness to extend to other life history traits beyond the juvenile stage.

Here we study environmental robustness and phenotypic plasticity using a reaction norm approach and focus on three key life-history traits: adult mass, age at maturity and growth rate in response to thermal variation and the possible interplay between these responses. Changes in size at maturity have more severe fitness consequences for females than for males due to the relation between female size and fecundity (Fischer & Fiedler, 2000, De Block & Stoks, 2003), and we therefore have to include effects of sex in this study. Variation in growth rates in insects and other organisms may often be adaptive (Arendt, 1997), and a trade-off between juvenile development time and size at maturity is a typical component of life-history models. Yet an organism that manages to grow at a high rate can achieve both simultaneously. Therefore we also assessed correlation of slope and elevation of the reaction norms of age at maturity and growth rate to describe possible trade-offs and positive relations. By taking into account growth rate, adult mass and age at maturity simultaneously, and including possible effects on female fecundity, we aim to unravel how environmental robustness in springtails from fluctuating environments is achieved and how sex-specific strategies are entwined.

Materials & methods

The springtail populations studied here were sampled from the litter layer in two nature reserves in The Netherlands; the Kampina (51°34' N, 5°15' E) and the Hilversumse Heide (52°15' N, 5°10' E). The two reserves contain large areas of heath land surrounded by mixed forest on a sandy soil. In both reserves three heath and three forest locations were sampled (maximally 500 m apart within each reserve), resulting in 12 locations. Approx. 50 individuals were collected per location to

establish mass-bred populations. An additional 100 individuals from five heath populations and five forest populations were collected at the Kampina location to estimate genetic differentiation between the ecotypes using seven microsatellite markers (for details on methods and the genetic markers used see van der Wurff *et al.* (2005) and supplementary data S3).

The stock populations that were established with the field collected springtails were maintained in a climate room (20°C, 70% RH, LD 12:12 h) at a population size of 500-1000 springtails for at least 3 generations to exclude possible maternal effects. All populations were fed algae (*Desmococcus* spec) on bark *ad libitum*. Juveniles from these stock populations were reared individually and sexed. To prevent differences in reproductive synchronization of the sexes that could lead to delayed egg production, each female was offered two males to produce offspring. The males and female were kept in Ø 2.5 cm pots with a bottom of plaster of Paris and were offered a continuous supply of fresh food. Each pot was checked daily for eggs, resulting in 90 egg batches of sufficient size. Egg batches (the sibs from these egg batches are referred to as a family) were divided over two temperatures with 5 eggs at 22°C and 5 eggs at 12°C, and placed individually in Ø 2.5 cm pots at 70% RH, LD 12:12 h. If the egg batch was large enough, 5 additional eggs were used to estimate egg size by measuring their diameter, which is a good proxy for total egg volume. Eggs were photographed using a Leica DC200 digital camera connected to a stereomicroscope. The diameter of the eggs was measured with Cell^D software (analySIS 5.0).

Presence of newly hatched offspring was monitored twice a day at 22°C and once a day at 12°C. Eggs hatch on average after 6 days at 22°C and after 16 days at 12°C (this experiment). From 23 days onwards (counting from the day the egg was laid), a spermatophore from a stock population of males was transferred to each individual. The pots were monitored daily for the presence of new spermatophores or eggs to determine age at maturity and the sex of the individual. Spermatophores were replaced every other day, unless they were taken or damaged, in which case it was replaced the same day. After the first eggs or spermatophores were produced, the animals were freeze-dried for dry mass measurements. This set-up allowed us to measure adult mass and age at maturity (and

growth rate as the mathematical relation between these two traits) for males and females at two temperatures and the size of the first egg batch produced.

Statistical analyses

Life history traits

Adult mass, age at maturity and growth rate were LN transformed and analyzed using a full factorial design with ecotype, temperature and sex and all possible interactions. Nature reserve, from here on referred to as location, was included as a single main effect. We fitted the models using random regression generalized linear mixed models from the MCMCglmm package (Hadfield, 2010) in R version 3.0.3 (R Core Team, 2014) to account for within-family variation in the reaction norms by including families as a random variable (for R code see S1).

Correlation of reaction norm parameters

Correlations of two reaction norm parameters (the trait mean or elevation, and trait difference over the temperature range or slope) of age at maturity and growth rate were estimated with a bivariate linear random regression model from the MCMCglmm package (for R code see S1). We focused on growth rate and age at maturity in this analysis as mass at maturity is the outcome of the interplay between growth rate and age at maturity. Correlations of reaction norm parameters both within and across traits were estimated. We do not assume that the reaction norms are linear, but calculating a slope as the difference between values at both temperatures does allows for evaluation of the temperature sensitivity of the trait.

The bivariate response random regression mixed model was defined as:

$$\begin{bmatrix} z_1 \\ z_2 \end{bmatrix}_{ijk} = \mathbf{X}_i\beta + \begin{bmatrix} a_1 \\ a_2 \end{bmatrix}_j + \begin{bmatrix} b_1 \\ b_2 \end{bmatrix}_j E_i + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}_{ik}$$

where i indexes individuals, j references the families to which individuals belong, and k represents the environment in which a given individual was reared. $\mathbf{X}_i$ is the design matrix for fixed effects associated with individual i, and β is the is the vector of (estimated) fixed coefficients. z_1 and z_2 are the traits, a_1 and a_2 are family-specific intercepts for the two traits. E_i is the environmental condition experienced by individual i. e_1 and e_2 are residuals. The trait and family-specific slopes and variances are treated as random parameters with an estimated covariance matrix according to

$$\begin{bmatrix} a_1 \\ b_1 \\ a_2 \\ b_2 \end{bmatrix}_j \sim N\left(\mathbf{0}, \begin{bmatrix} \sigma^2(a_1) & \sigma(a_1,b_1) & \sigma(a_1,a_2) & \sigma(a_1,b_2) \\ \sigma(a_1,b_1) & \sigma^2(b_1) & \sigma(b_1,a_2) & \sigma(b_1,b_2) \\ \sigma(a_1,a_2) & \sigma(b_1,a_2) & \sigma^2(a_2) & \sigma(a_2,b_2) \\ \sigma(a_1,b_2) & \sigma(b_1,b_2) & \sigma(a_2,b_2) & \sigma^2(b_2) \end{bmatrix}\right)$$

where $N(\mu,\sigma)$ represents a multivariate normal distribution with mean vector μ, and covariance matrix σ. Since no individual can be reared simultaneously in two environments, no residual covariance between environments is estimated. The residuals are estimated according to

$$\begin{bmatrix} e_1 \\ e_2 \end{bmatrix}_j \sim N\left(\mathbf{0}, \begin{bmatrix} \sigma^2(e_1) & \sigma(e_1,e_2) \\ \sigma(e_1,e_2) & \sigma^2(e_2) \end{bmatrix}_k\right)$$

A similar model structure applies to the single response random regression models used for each of the three traits independently, but with a single trait (only variances and covariances of a_1) structure.

Adult mass and female fecundity

Effects on female fecundity (number of eggs in the first batch) were analyzed using a full factorial linear mixed model with temperature, ecotype, and mass of the mother and all possible interactions and family as a random variable. Location was included as a single main effect. The model was fitted using the MCMCglmm package in R.

Egg volume

Volume of the eggs at the start of the experiment was analyzed using a full factorial linear mixed model with ecotype and location as factors and all possible interactions and family as a random variable. The model was fitted using the MCMCglmm package in R.

Comparing two approaches for estimating variance

Although it is quite common to calculate replicate means and using these mean values as input for statistical models, this can potentially inflate statistical noise. To formally test this, the slopes and elevation and their standard deviations, as well as the correlation coefficient of slope and elevation were estimated in two ways for this each of the three traits; with a univariate random regression model on individual data within a family structure (taking within-family variation into account) and on estimated family mean values. By using family means each family contributes the same to the model irrespective of the number of siblings on which the reaction norm response was established. By comparing the estimates of variation of both approaches we can flag structural differences.

Results

All three traits - adult mass, age at maturity and growth rate - responded plastically to temperature. Temperature, sex and their interaction had significant effects on all three traits (Table 1). Adult mass and age at maturity both declined with increasing temperature, growth rate increased with increasing temperature and the reaction norms were sex-specific (Fig. 1). Males matured at a younger age than females and had a lower growth rate overall (Fig. 1). The three-way interaction between ecotype x temperature x sex was significant for adult mass and growth rate, indicating that for these traits not only the reaction norms differed between males and females but they also depended on the ecotype. Adult mass of females from heath was less sensitive to temperature than adult mass of females from the forest ecotype. Males of either ecotype responded plastically to temperature in all three traits, but for males no ecotype-specific pattern was observed. Location had

no effect in the model; in fact, the observed patterns in Fig. 1 were also apparent when the reaction norms were given per location (supplementary data S1).

Reaction norms for all traits vary between families, especially for adult mass (Fig. 2, Table 2), and variation in reaction norms was larger among females than males. Elevation correlated positively with slope of the reaction norm for growth rate for heath ecotype females and males, but not in the forest ecotype (Table 3). For males of the heath ecotype the slopes of the reaction norms for age at maturity and growth rate correlated negatively. Similarly, in forest males the elevation of the reaction norm for age at maturity negatively correlated with growth rate.

The number of eggs in the first batch depended on female adult mass at maturation (post. mean = 0.106, 95% CI 0.037 – 0.179, P = 0.010), but not on ecotype (post. mean = 8.898, 95% CI -11.155 – -27.861, P = 0.376), temperature (post. mean = 2.363, 95% CI -14.424 – 19.137, P = 0.768), location (post. mean = 0.297, 95% CI -3.350 – 4.210, P = 0.882), nor on any of the interactions. The effects contrast to 'forest, 12 °C, Hilversum' to enable interpreting the sign of posterior means. The number of eggs in the batch increased with the mass of the mother at maturity, which indicates that female adult mass is directly linked to fitness. Additionally, it is known that egg size positively correlates with juvenile size at emergence (Liefting *et al.*, 2010). Therefore differences in egg size at the beginning of the experiment could have confounding effects on life history traits measured after emergence, like adult mass. However, egg volume did not differ between ecotype (post. mean = $1.044 \cdot 10^{-4}$, 95% CI $-1.516 \cdot 10^{-4} – 3.472 \cdot 10^{-4}$, P = 0.410) or between location (Kampina or Hilversumse Heide) (post. mean = $1.979 \cdot 10^{-4}$, 95% CI $-5.173 \cdot 10^{-5} – 4.165 \cdot 10^{-4}$, P = 0.094), nor was there an interaction between these parameters (post. mean = $1.333 \cdot 10^{-4}$, 95% CI $-2.625 \cdot 10^{-4} – 4.439 \cdot 10^{-4}$, P = 0.454). The effects contrast to 'forest, Hilversum' to enable interpreting the sign of posterior means.

Microsatellite analyses revealed all seven loci to be polymorphic in the sampled Kampina populations. Numbers of alleles per locus, which are in the range described by van der Wurff *et al.* (2005) are given in Table S3a. The data were used to calculated metrics for population genetic

differentiation (D_{est} and F_{st}). Population pairwise F_{st} values were all below 0.07, indicating low to moderate genetic differentiation (see S3 for D_{est} and F_{st} values and analyses).

Variances of reaction norm parameters, i.e. of slopes and intercepts were larger when estimated as variances of family means, than when estimated with the random regression mixed models (Table 2). This occurs because variance in family means arises both from biological variation among families and from statistical noise (sampling error) in estimates of family mean values. The mixed model approach effectively integrates over uncertainty in family mean values, isolating the biologically-relevant component of the variation in the data. Subsequently, lower correlation coefficients are reported when calculated over family means.

Discussion

For most ectotherms higher temperatures lead to a decrease in adult mass and age at maturity, and an increase in growth rate (Angilletta *et al.*, 2004) and *O. cincta* forms no exception to this general pattern. What has been less appreciated is the variation in the strength of this thermal response, both among and within species (Berg *et al.*, 2010). Here we show that the strength of the thermal response differs between sexes, depends on thermal regime experienced by the ecotype, and is integrated over various life history traits.

The elevation and slope of the reaction norms differed between sexes for all the investigated traits. Across temperatures, females reached maturity with a higher mass than males, but they took longer to do so, as they could only partly compensate by a higher growth rate (Fig. 1). For two traits, adult body mass and growth rate, the slope of the reaction norm was not only sex-specific but also ecotype-specific (Table 1). Adult mass of females of the heath ecotype was more robust in response to temperature than the mass of forest females (Fig. 1a and 2a). As age at maturity is similar for both ecotypes, this means that the relative robustness of adult mass of the heath ecotype is realized by a higher plasticity in growth rate. The observed differences in adult mass did not arise because of initial differences in egg and juvenile size, as egg size did not differ between the ecotypes. Rather the

ecotype-specific reaction norms for females are the joint result of differences in the thermal response of other traits, i.e. older age at maturity for forest females at low temperature and a higher growth rate of heath females at high temperatures. Our results show that environmental robustness in one trait (adult mass) is the result of plastic responses in other traits (growth rate). This result reinforces the notion that plasticity and robustness are trait-specific terms and that it is meaningless to use these terms to refer to ecotypes, populations or even individuals. Plasticity and robustness are interrelated and both plastic and robust traits can be found within one individual.

Overall, females displayed a reduced sensitivity of adult mass to temperature compared to males, reflecting a higher degree of environmental robustness in females for this fitness related trait (Fig. 1a and 2a). We expect environmental robustness to occur when a particular phenotype is favored over a range of environmental conditions (Stearns *et al.*, 1995, Debat & David, 2001). The observed robustness of the thermal reaction norms for female adult mass can therefore be understood as a direct effect of selection on large female size across all temperatures. Because heath habitats are more exposed and experience more temperature variation than forest habitats (Liefting & Ellers, 2008), there is stronger selection on robustness of a trait like adult mass in the face of temperature change for females of the heath ecotype. The reproductive success of females depends more strongly on body size than that of males, because of the strong correlation between fecundity and female size (see also Ernsting *et al.*, 1993).

The most likely explanation as to why *O. cincta* males become reproductive at a younger age and at a smaller size than females is a lack of fitness advantage associated with large male size. The reproductive system of *O. cincta* involves indirect sperm transfer with males depositing spermatophores on the soil substrate and females taking spermatophores without the males present or interfering (Zizzari *et al.*, 2009). Because females grow indeterminately and are receptive throughout their lives (Ernsting & Isaaks, 2002) earlier maturation of males is not driven by the necessity of males to be ready before females mature, in contrast to butterfly species where female emergence is synchronized and females mate only once (Fagerström & Wiklund, 1982). Also, male

size does not correlate with number of spermatophores deposited in the first adult instar nor the total number of spermatophores deposited during seven instars (Ernsting & Isaaks, 2002). Therefore, faster male development at both high and low temperatures may enable males to reduce the time spent in the vulnerable juvenile stage while maintaining spermatophore production and hence increasing the possibility of successful fertilization.

One of the requirements for population differentiation at small geographic scale is a low level of gene flow between populations. Within Western Europe significant differentiation is observed between *O. cincta* populations from NW Europe, Central Europe and Italy, indicating limited gene flow between these areas (Timmermans *et al.*, 2005). However, studies that focused on populations within NW Europe observed low population genetic differentiation and concluded that gene flow must be high among populations (Costa *et al.*, 2013, van der Wurff *et al.*, 2005). Our genetic analyses agree with this observation and indicate low genetic differentiation between the sampled heath and forest populations (S3). Apparently, the various sites are interconnected by significant gene flows. If true, the observed differences in reaction norms must have been driven by strong local selection, overruling the homogenizing effects of gene flow at selected loci. Similar results of local adaptation have been described before in e.g. aquatic systems (Luttikhuizen *et al.*, 2003) and amphibians (Orizaola & Laurila, 2008, Richter-Boix *et al.*, 2010).

Recent studies have compared fitness consequences of elevation and slope of reaction norms in an attempt to elucidate possible mechanisms involved in performance of populations. In a study that compared different *Drosophila melanogaster* populations for thermal performance, absolute trait value changes were found to be more important than other aspects of thermal reaction norms (Klepsatel *et al.*, 2013). In order to detect possible relations between slope and elevation of reaction norms within and between traits we also considered correlation of reaction norm parameters. Correlation of reaction norm parameters like elevation and slope can reveal interdependence of traits, e.g. trade-offs. Such trade-offs can lead to different strategies, e.g. a

young age at maturity at the expense of fewer eggs in the first batch or extending the age at maturity to lay a larger batch but at the risk of being predated before reaching maturity.

The observed correlations in the current study indicate that there are indeed different strategies employed (Table 3), but there is no strong overall pattern. When considering correlations of slopes and elevation *across* traits we find that for age at maturity and growth rate a steep slope of one trait correlates with a flat slope of the other trait, indicative of a trade-off (see Fig. 2 for the reaction norms per family). Similarly, for age at maturity and growth a high elevation (mean value) of one trait correlates with low elevation of the other trait. These relations make sense in the light of life history theory; maintaining weight requires adjustments in either time to grow or growth rate. What is surprising is the apparent lack of these relations in the other subsets, especially in the females since weight is such an important trait to female fecundity. The fact that we did not find more trade-offs between age at maturity and growth rate is likely explained by the large variation in all measured traits. *Within* trait correlations of elevation and slope can give insight in whether changes in plasticity affect the mean performance. Elevation and slope of the growth rate reaction norm are positively correlated, i.e. a high mean growth rate correlates with a steep slope, but only for females and males from heath ecotypes and only for the growth rate reaction norm (Table 3). No such patterns are found in the other traits and subsets as the credible intervals are large, so there are no strong overall conclusions to be drawn from the within trait reaction norm correlations.

It is quite common to calculate replicate means and using these mean values as input for statistical models (e.g. Berger *et al.*, 2014), but depending on what type of analysis is used this is not always without consequence. Here, we estimated standard deviation of slope and elevation of the reaction norms and the correlation coefficient between slope and elevation for each trait based on individual data within a family structure and based on family mean values (as represented in the reaction norms at the family level in Fig. 2). We report systematically higher variation when the analysis is based on family means than when within-family variation is accounted for. Subsequently, lower correlation coefficients are reported when calculated over family means. The fact that all

families in this dataset consisted of few siblings with moderate levels of within-family variation explains why the family means model still behaves relatively well. However when within-family variance is larger, which is not unlikely in many ecological studies, this effect will increase drastically. We therefore warrant caution for readily using replicate mean values.

What do these results mean from an ecological point of view? Dealing with environmental fluctuations is often dependent on life history traits being either flexible or buffered in response to these fluctuations (Richards *et al.*, 2006). We hypothesized that robustness of life history traits would be ecotype-specific and that this robustness might require a higher level of plasticity of supporting traits. We found evidence for ecotype-specific robustness of female adult mass to temperature, facilitated by plastic adjustments in growth rate and partially age at maturity. However, for males this pattern is lacking. The robustness in female adult mass is the result of different outcomes of the trade-off between growth rate and age at maturity at the two temperatures. We see different outcomes of life history theory combined within one species' ontogeny, with different outcomes being dependent on the local ecological conditions. This is a complex scenario that is nevertheless quite realistic and likely to be found in other species and at a local spatial scale. Although it is widely accepted that development and growth rates can be plastic and can even be life stage dependent (Fischer *et al.*, 2014), maximization of growth rates under different conditions is still too often wrongly assumed (Monro & Marshall, 2014). Given the fact that selection pressures change during life stages and with ecological conditions varying in time and space, it is meaningful to monitor life history strategies throughout an organism's life. These requirements have rarely been met in experimental set-ups and the results of this study demonstrate that such knowledge is essential for further understanding of life history theory.

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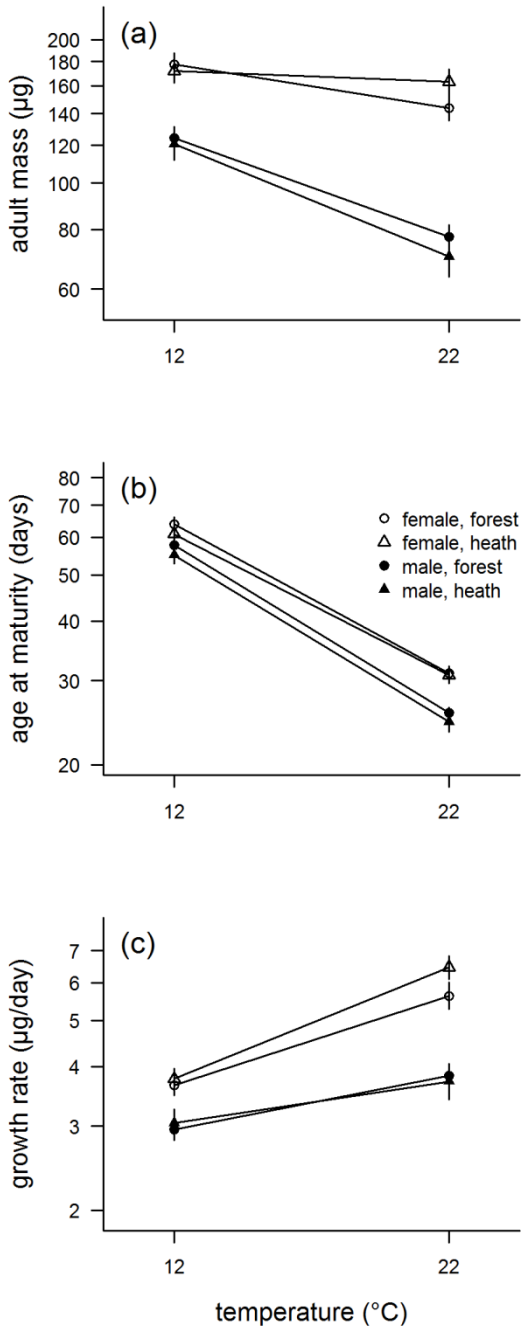

Fig. 1 Adult mass (a), age at maturity (b) and growth rate (c) of *O. cincta* in relation to temperature, habitat and sex. Means of re-transformed data on an LN-scale are given with 95% CI. Patterns for the nature reserves are highly similar and therefore data of both locations was lumped (see Fig. S2 for reaction norms per location). Throughout, adult mass of males is significantly lower than that of females, corresponding with younger age at maturity and a lower growth rate.

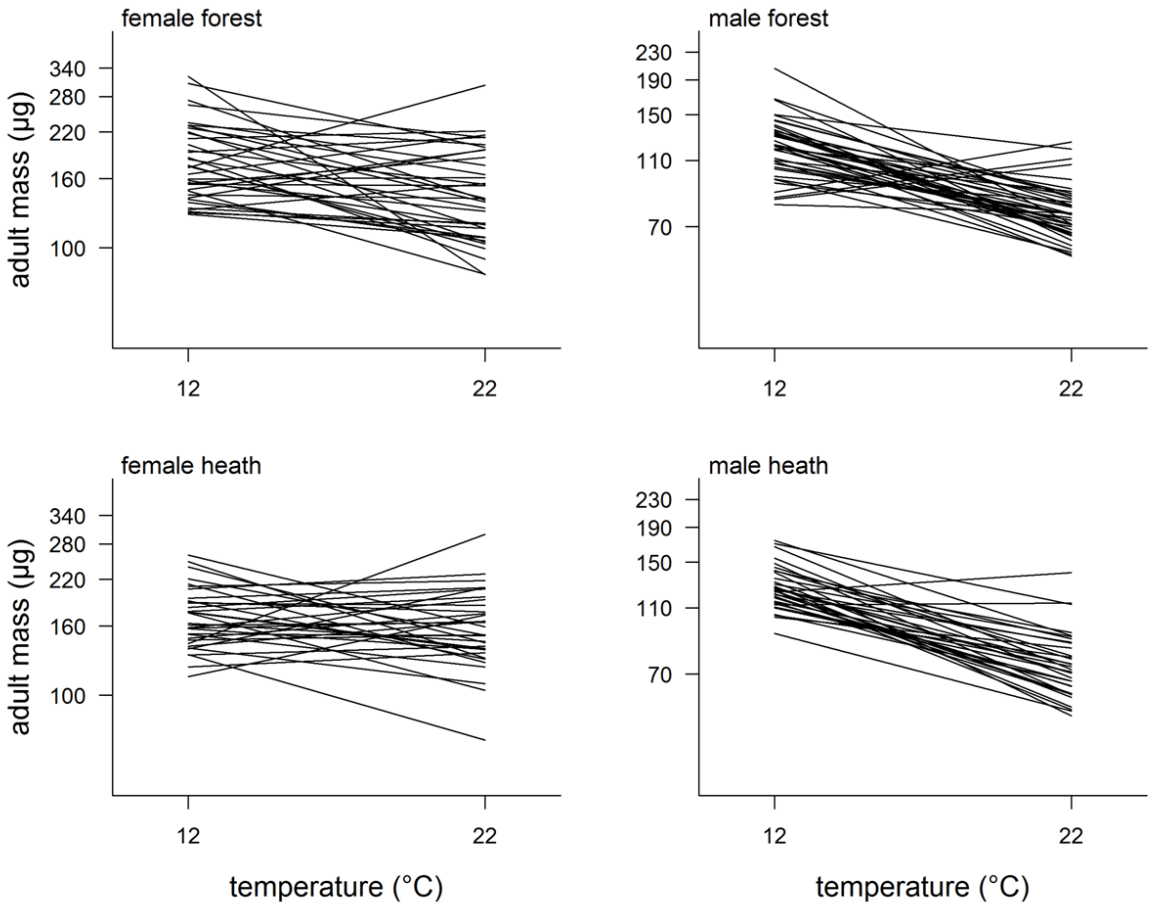

Fig. 2a Reaction norms per family for adult mass of *O. cincta* in response to temperature in relation to ecotype and sex. On average, adult mass declines with higher temperatures but typically less so for females than for males.

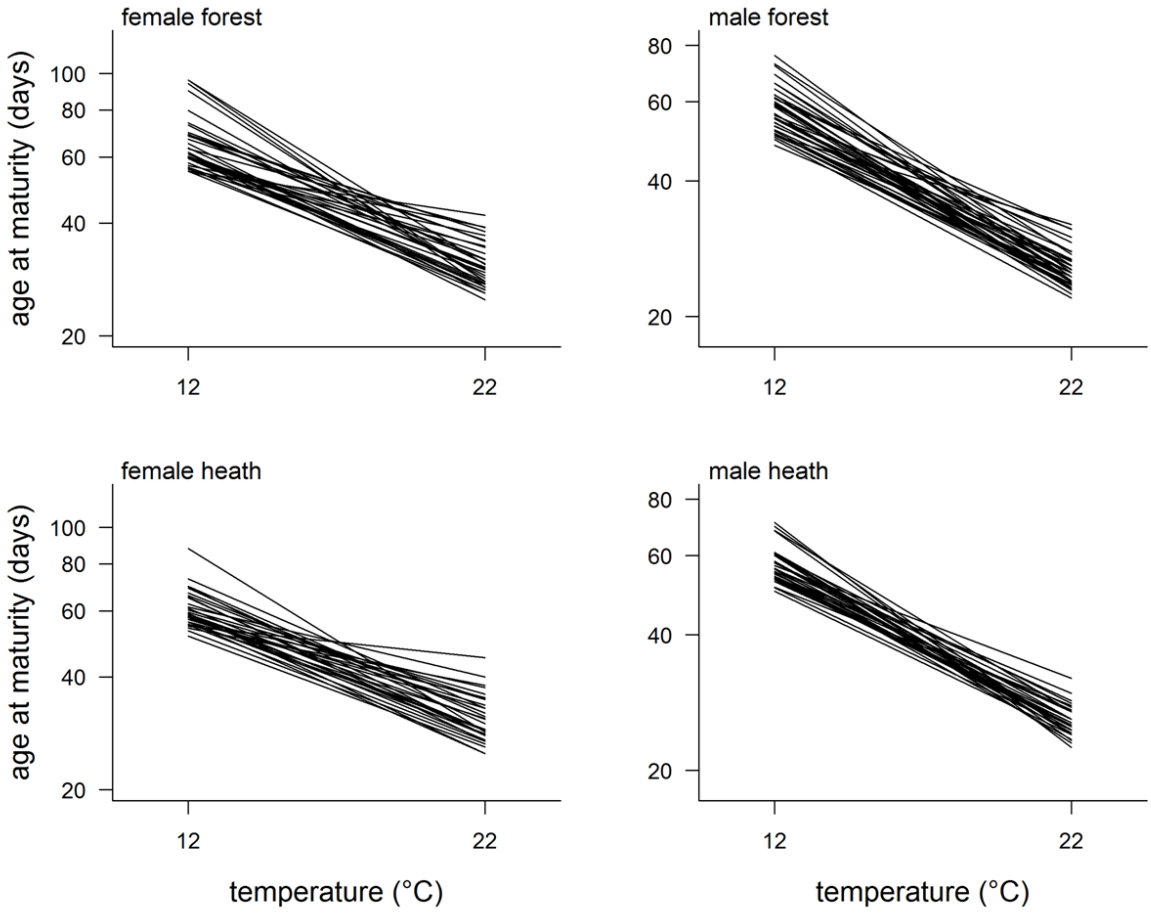

Fig. 2b Reaction norms per family for age at maturity of *O. cincta* in response to temperature in relation to habitat and sex. Animals of both sexes mature earlier at higher temperatures.

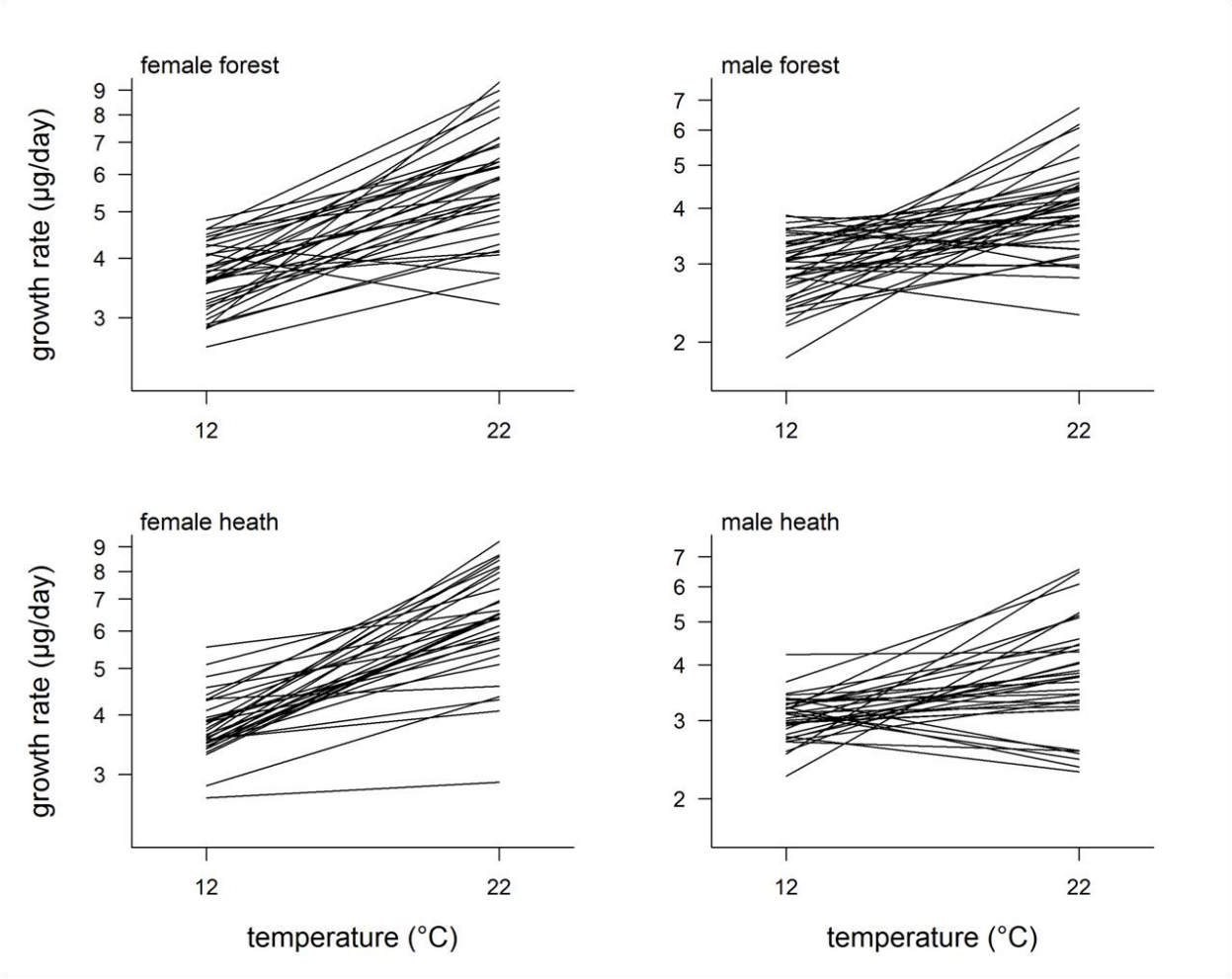

Fig. 2c Reaction norms per family for growth rate of *O. cincta* in response to temperature in relation to ecotype and sex. Growth rates typically increase with higher temperatures although there is substantial variation between families, both for males and females.

Table 1 Fixed effects from random regression mixed models of three traits. Posterior means are given with 95% CI for each factor, the effects contrast to 'forest, 12 °C, female, Hilversum' to enable interpreting the sign of posterior means.

	Adult mass (LN, µg)		Age at maturity (LN, days)		Growth rate (LN, µg/day)	
Factor	Post. mean (95% CI)	P	Post. mean (95% CI)	P	Post. mean (95% CI)	P
Intercept	5.180 (5.114 - 5.235)	**<0.001**	4.157 (4.120 - 4.189)	**<0.001**	1.300 (1.251 - 1.351)	**<0.001**
Ecotype	-0.032 (-0.106 - 0.050)	0.422	-0.048 (-0.091 - -0.008)	**0.030**	0.029 (-0.035 - 0.088)	0.348
Temperature	-0.210 (-0.276 - -0.134)	**<0.001**	-0.721 (-0.759 - -0.687)	**<0.001**	0.431 (0.373 - 0.483)	**<0.001**
Sex	-0.354 (-0.409 - -0.300)	**<0.001**	-0.102 (-0.134 - -0.068)	**<0.001**	-0.213 (-0.254 - -0.171)	**<0.001**
Location	-0.039 (-0.090 - 0.0178)	0.170	-0.010 (-0.040 - 0.017)	0.496	-0.010 (-0.053 - 0.039)	0.622
Ecotype x temp	0.159 (0.068 - 0.250)	**<0.001**	0.040 (-0.014 - 0.090)	0.128	0.107 (0.033 - 0.180)	**<0.001**
Ecotype x sex	0.074 (-0.007 - 0.147)	0.064	0.045 (0.002 - 0.086)	**0.042**	0.011 (-0.053 - 0.067)	0.736
Temp x sex	-0.270 (-0.361 - -0.182)	**<0.001**	-0.088 (-0.134 - -0.046)	**<0.001**	-0.171 (-0.251 - -0.097)	**<0.001**
Ecotype x temp x sex	-0.220 (-0.331 - -0.098)	**<0.001**	-0.037 (-0.108 - 0.020)	0.266	-0.166 (-0.277 - -0.060)	**0.002**

P-values <0.05 in bold

Table 2 Standard deviation and correlation coefficients of the reaction norm parameters for each traits, partitioned for each possible subset, calculated with a random regression mixed model based on individual data within a family structure or family means.

			Adult mass (LN, µg)		Age at maturity (LN, days)		Growth rate (LN, µg/day)	
			Individual data (95 %CI)	Family means	Individual data (95 %CI)	Family means	Individual data (95 %CI)	Family means
Female	Forest	SD elevation	0.205 (0.140 - 0.259)	0.220	0.090 (0.060 - 0.115)	0.098	0.152 (0.102 - 0.192)	0.168
		SD slope	0.161 (0.105 - 0.207)	0.184	0.107 (0.075 - 0.137)	0.114	0.109 (0.063 - 0.141)	0.139
		Cor. elevation, slope	0.171 (-0.511 - 0.401)	0.193	-0.127 (-1.000 - 0.178)	-0.125	0.430 (-0.071 - 0.582)	0.459
	Heath	SD elevation	0.161 (0.110 - 0.209)	0.176	0.077 (0.049 - 0.100)	0.087	0.162 (0.110 - 0.209)	0.168
		SD slope	0.135 (0.088 - 0.179)	0.159	0.081 (0.052 - 0.107)	0.092	0.109 (0.074 - 0.142)	0.120
		Cor. elevation, slope	0.329 (-0.410 - 0.516)	0.249	0.298 (-0.432 - 0.478)	0.243	0.580 (0.463 - 0.709)	0.496
Male	Forest	SD elevation	0.097 (0.064 - 0.127)	0.121	0.072 (0.050 - 0.089)	0.076	0.109 (0.068 - 0.140)	0.129
		SD slope	0.128 (0.085 - 0.163)	0.149	0.067 (0.048 - 0085)	0.074	0.129 (0.092 - 0.165)	0.150
		Cor. elevation, slope	-0.034 (-1.000 - 0.286)	-0.062	-0.199 (-1.000 - 0.118)	-0.198	0.250 (-0.350 - 0.448)	0.251
	Heath	SD elevation	0.115 (0.070 - 0.155)	0.145	0.046 (0.030 - 0.060)	0.051	0.121 (0.074 - 0.164)	0.148
		SD slope	0.089 (0.046 - 0.124)	0.120	0.062 (0.041 - 0.078)	0.066	0.131 (0.084 - 0.170)	0.153
		Cor. elevation, slope	0.507 (-0.397 - 0.618)	0.408	-0.140 (-1.000 - 0.176)	-0.139	0.721 (0.535 - 0.757)	0.690

Table 3 Correlation coefficients (r) of slopes and elevation, either *within* the same trait or *across* traits for females and males of different ecotypes, RN refers to reaction norm. The considered traits are; age at maturity (referred to as 'Age' in the table) and growth rate (referred to as 'Growth' in the table).

	Females		Males	
r RN parameters *within* trait	Heath r (95% CI)	Forest r (95% CI)	Heath r (± 95% CI)	Forest r (95% CI)
elevation Age x slope Age	0.323 (-0.449 - 0.508)	-0.119 (-1.209 - 0.206)	-0.153 (-1.467 - 0.209)	-0.222 (-1.550 - 0.111)
elevation Growth x slope Growth	**0.609** (0.370 - 0.674)	0.410 (-0.502 - 0.551)	**0.720** (0.532 - 0.786)	0.255 (-0.485 - 0.482)
r RN parameters *across* traits				
slope Age x slope Growth	-0.145 (-1.792 - 0.232)	-0.170 (-2.593 - 0.164)	**-0.731** (-3.200 - -0.106)	-0.373 (-1.975 - 0.055)
elevation Age x elevation Growth	-0.304 (-2.001 - 0.081)	0.003 (-0.003 - 0.010)	-0.306 (-2.309 - 0.099)	**-0.482** (-2.238 - -0.038)

Significant correlations based on 95% credible interval range in bold

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Additional Supporting Information:

S1 R code for univariate random regression model and bivariate response linear regression model.

Figure S2 Reaction norms for adult mass, age at maturity and growth rate for each of the two locations (Kampina and Hilversumse Heide).

S3 materials and methods for estimating genetic differentiation.

Table S3a Allele frequencies and heterozygosities.

Table S3b Average D_{est} and pairwise F_{st} values.

Data deposited at Dryad: doi:xxxx

Code S1
R code analyses

```
## one trait random regression model

d<-as.data.frame(read.table("Springtails.csv",header=TRUE,sep=','))


d$stTemp<-d$temp
d$stTemp[which(d$temp==12)]<- -0.5
d$stTemp[which(d$temp==22)]<- +0.5

d$familie<-as.factor(d$familie)
d$temp<-as.factor(d$temp)


## dummy variable for different strata of the residual
d$tempEcotypeSex<-as.factor(paste(d$temp,d$sex,d$ecotype))


p<-list(G=list(G1=list(V=0.01,nu=1)),
    R=list(V=diag(8)*0.01,ny=1))


m1.ln.dev.time<-
MCMCglmm(lntime~ecotype+temp+sex+location+ecotype:temp+ecotype:sex+temp:sex+ecotype
:temp:sex,
            random=~familie,
            rcov=~idh(tempEcotypeSex):units,
            data=d,prior=p,family=c("gaussian"))

summary(m1.ln.dev.time)

m1.ln.mass<-
MCMCglmm(lnmass~ecotype+temp+sex+location+ecotype:temp+ecotype:sex+temp:sex+ecotype
:temp:sex,
            random=~familie,
            rcov=~idh(tempEcotypeSex):units,
            data=d,prior=p,family=c("gaussian"))

summary(m1.ln.mass)

m1.ln.rate<-
MCMCglmm(lnrate~ecotype+temp+sex+location+ecotype:temp+ecotype:sex+temp:sex+ecotype
:temp:sex,
             random=~familie,
             rcov=~idh(tempEcotypeSex):units,
             data=d,prior=p,family=c("gaussian"))

summary(m1.ln.rate)
```

```r
## bivariate response linear random regression model
## with block diagonal residual covariance matrix


d<-as.data.frame(read.table("Springtails.csv",header=TRUE,sep=','))


d$stTemp<-d$temp
d$stTemp[which(d$temp==12)]<- -0.5
d$stTemp[which(d$temp==22)]<- +0.5

d$familie<-as.factor(d$familie)
d$temp<-as.factor(d$temp)

## for each subset; e.g. female forest:
d_females_forest<-subset(d,d$sex=="f"&d$ecotype=="f")

## and male heath:
d_females_forest<-subset(d,d$sex=="m"&d$ecotype=="h")


p<-
list(G=list(G1=list(V=diag(4)*0.01,nu=1)),R=list(R1=list(V=diag(2)*0.01,nu=1),R2=li
st(V=diag(2)*0.01,nu=1)))

m<-MCMCglmm(cbind(lntime,lnrate)~-1+trait+trait:temp,
            random=~us(trait+trait:stTemp):familie,
            rcov=~us(trait:at.level(temp,"12")):units
                  +us(trait:at.level(temp,"22")):units,
            data= d_females_forest,prior=p,family=c("gaussian","gaussian"))

famEffects<-matrix(apply(m$VCV[,1:16],2,mean),4,4)

## Reorganise matrix

famEffects<-famEffects[c(1,3,2,4),c(1,3,2,4)]

famEffects


## To get the upper and lower bounds of the credible interval

lower_bound<-matrix(HPDinterval(m$VCV[,1:16])[,1],4,4)
lower_bound<-lower_bound[c(1,3,2,4),c(1,3,2,4)]

upper_bound<-matrix(HPDinterval(m$VCV[,1:16])[,2],4,4)
upper_bound<-upper_bound[c(1,3,2,4),c(1,3,2,4)]


famEffects
lower_bound
upper_bound
```

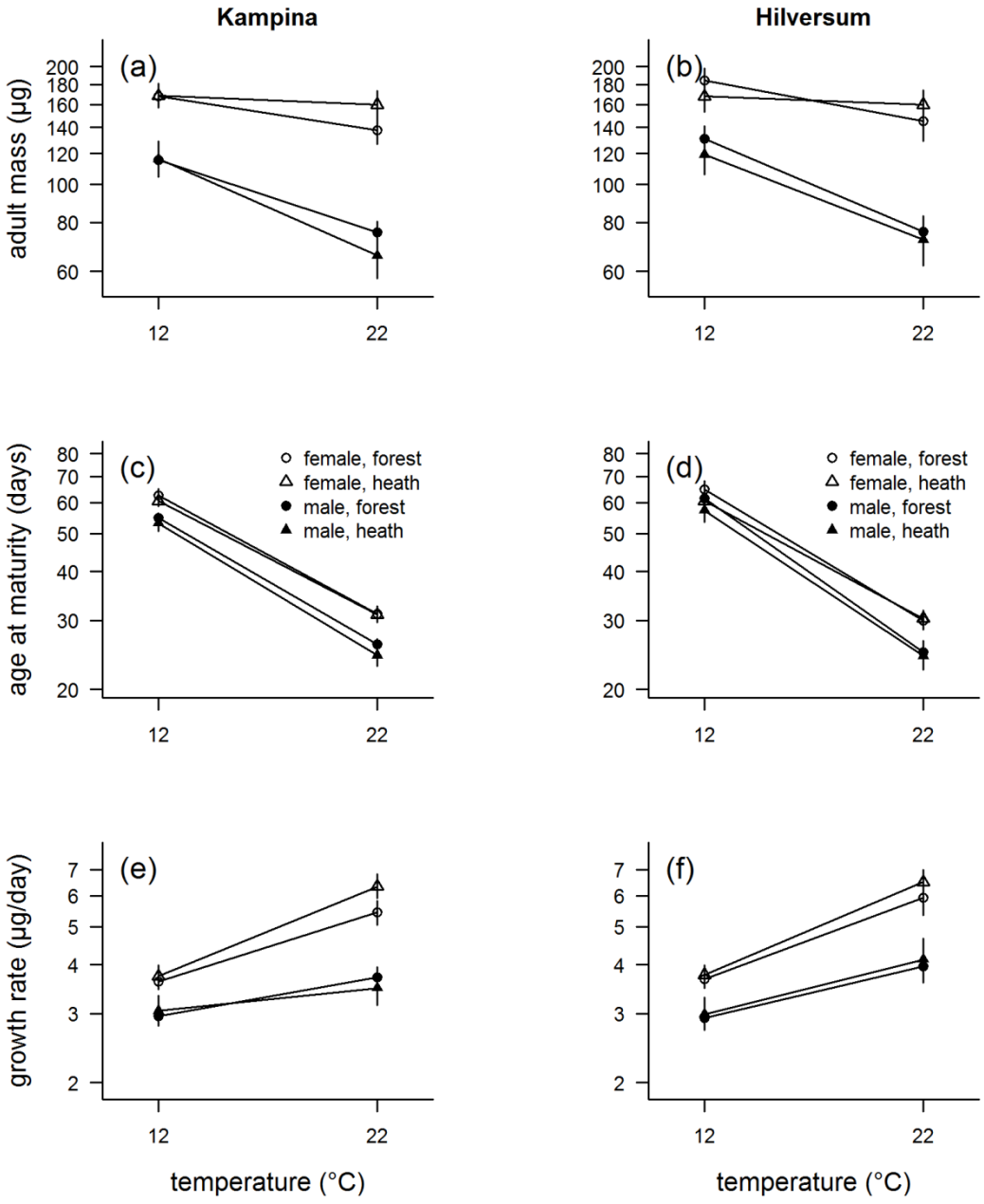

Figure S1. Adult mass (a-b), age at maturity (c-d) and growth rate (e-f) of *O. cincta* in relation to temperature, ecotype, sex for each of the two locations (nature reserves Kampina and Hilversumse Heide). Means of re-transformed data on an LN-scale are given with 95% CI.

Data S1 – genetic differentiation

Estimating genetic differentiation with neutral markers

A total of 100 individuals from five heath populations and five forest populations were collected at the Kampina location for population genetic analyses using microsatellite markers. This analysis used the six variable markers of van der Wurff (2001) and one newly developed marker (GenBank: FJ009051-FJ009054). This latter marker was retrieved from an *O. cincta* Expressed Sequence Tag dataset (Occ00334: Oc_SSHCd_18F04 / Oc_SSHCd_18B06; and PCR-amplified using the primers ORCmsGA_F (5'-ACGATGATCGTCATGATCAAC- and ORCmsGA_R (5'- TGATCCGTGACTTTTTCTGG-3'). DNA was extracted (Promega Wizard DNA Extraction Kit) from each individual and microsatellite markers were subsequently amplified using Cy5 labeled forward primers separated on an ALF express automatic DNA sequencer. Alleles were visualized using Fragment Manager V1.2 software (Pharmacia Biotech) and scored manually. The microsatellite toolkit for Windows Excel (Park *et al.*, 1999) was used to obtain allele frequencies. The software program Arlequin Ver. 2.000 was applied to test for deviations from Hardy-Weinberg equilibrium following the method of Guo & Thompson (1992). The populations were divided into two groups: those originating from heath and those originating from forest habitat. Population genetic differentiation was estimated by calculating population-pairwise F_{st} values (Weir & Cockerham, 1984) and arithmetic means of Jost's unbiased estimator of differentiation, D_{est} (Jost, 2008). Calculations were performed using FSTAT 2 (Goudet, 1995) and the R package DEMEtics (Gerlach *et al.*, 2010).

Genetic differentiation

All seven microsatellite loci were polymorphic in the sampled populations. Furthermore, the numbers of alleles per locus were all in the range described earlier (van der Wurff *et al.*, 2005). Significant departures from Hardy-Weinberg equilibrium were only observed in one forest population (for loci rt10d9 and t17f2) and two heath populations (for locus rt10d9) (Table S1a). When comparing both D_{est} and F_{st} values for genetic differentiation (Table S1b) we can conclude that there is very low to none genetic differentiation between the considered populations.

Table S1a. Microsatellite allele frequencies and heterozygosities for the 10 populations. Microsatellite alleles are identified by their repeat number, except locus t2h2 where a) A(gT)3 and b) (gT)4g(gT)3. Significant departures from H-W expectation are indicated by * (P < 0.05) or ** (P < 0.01)). Ho = observed heterozygosity, He = expected heterozygosity.
Ave. He and Ave. Ho indicate mean expected and mean observed heterozygosity over all loci and Ave # indicates average number of alleles.

	Population									
	forest1	heath2	heath3	heath4	forest6	heath7	heath8	forest9	forest10	forest11
ft29b3										
5	0.25	0.20	0.10	0.10	0.35	0.15	0.33	0.00	0.06	0.05
6	0.35	0.20	0.15	0.35	0.30	0.40	0.28	0.50	0.50	0.20
7	0.20	0.35	0.45	0.25	0.30	0.35	0.28	0.35	0.28	0.55
8	0.20	0.25	0.30	0.30	0.05	0.10	0.11	0.15	0.17	0.20
He	0.77	0.77	0.71	0.75	0.73	0.72	0.76	0.64	0.68	0.65
Ho	0.80	0.80	0.80	0.60	0.60	0.50	0.67	0.80	0.78	0.60
rt10d9										
6	0.10	0.20	0.50	0.40	0.40	0.40	0.50	0.10	0.55	0.50
7	0.35	0.35	0.10	0.00	0.40	0.30	0.40	0.50	0.15	0.20
8	0.15	0.10	0.25	0.20	0.10	0.10	0.00	0.15	0.10	0.15
12	0.40	0.35	0.15	0.40	0.10	0.20	0.10	0.25	0.20	0.15
He	0.72	0.74	0.69	0.67	0.69	0.74	0.61	0.69	0.66	0.70
Ho	0.80	0.60	0.50*	0.50	0.30*	0.50	0.60*	0.80	0.70	0.70
rt23d11										
7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.90
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10
He	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20
t17f2										
7	1.00	0.65	1.00	0.95	0.70	0.85	0.70	0.95	0.85	0.95
8	0.00	0.35	0.00	0.05	0.30	0.15	0.30	0.05	0.15	0.05
He	0.00	0.48	0.00	0.10	0.44	0.27	0.44	0.10	0.27	0.10
Ho	0.00	0.70	0.00	0.10	0.00**	0.30	0.20	0.10	0.10	0.10
rt18d7										
6	0.35	0.20	0.15	0.40	0.20	0.40	0.15	0.20	0.30	0.30
7	0.65	0.80	0.85	0.60	0.80	0.60	0.85	0.80	0.70	0.70
He	0.48	0.34	0.27	0.51	0.34	0.51	0.27	0.34	0.44	0.44
Ho	0.50	0.20	0.30	0.40	0.20	0.60	0.10	0.20	0.60	0.40
t2h2										
a	0.30	0.25	0.40	0.20	0.35	0.20	0.45	0.40	0.35	0.15
b	0.70	0.75	0.60	0.80	0.65	0.80	0.55	0.60	0.65	0.85
He	0.44	0.39	0.51	0.34	0.48	0.34	0.52	0.51	0.48	0.27
Ho	0.60	0.50	0.80	0.40	0.70	0.40	0.30	0.40	0.30	0.30
GA										
5	0.60	0.50	0.45	0.45	0.75	0.40	0.55	0.40	0.44	0.35
6	0.35	0.35	0.30	0.30	0.25	0.35	0.45	0.55	0.39	0.60
7	0.00	0.00	0.20	0.10	0.00	0.15	0.00	0.05	0.11	0.00
9	0.05	0.15	0.05	0.15	0.00	0.10	0.00	0.00	0.06	0.05
He	0.54	0.64	0.70	0.71	0.39	0.72	0.52	0.56	0.67	0.54
Ho	0.40	0.90	0.70	0.80	0.50	0.40	0.70	0.30	0.78	0.80
Results over all loci										
Ave. He	0.42	0.48	0.41	0.44	0.44	0.47	0.45	0.40	0.46	0.41
Ave. Ho	0.44	0.53	0.44	0.40	0.33	0.39	0.37	0.37	0.47	0.44
Ave. #	2.43	2.57	2.57	2.57	2.43	2.71	2.29	2.43	2.71	2.71

Table S1b. Population genetic differentiation. Bottom half: Average D_{est} and p-value (between brackets, corrected for multiple testing using method of Benjamini and Hochberg. H_0 = no genetic differentiation, i.e. panmictic populations). Calculations performed using R package DEMEtics (Gerlach *et al.* 2010). Upper half: Population pairwise F_{st} calculated using FSTAT (Goudet 1995).

Population	1 forest		2 heath		3 heath		4 heath		6 forest		7 heath		8 heath		9 forest		10 forest		11 forest
1 forest	X		0.0142		0.0528		0.0026		0.0253		-0.0036		0.0452		0.0027		0.0224		0.0578
2 heath	-0.014	(0.67434)	X		0.0475		0.0274		-0.0073		-0.0025		-0.0004		0.0284		0.0212		0.042
3 heath	0.073	(0.08)	0.038	(0.18658)	X		0.0156		0.0465		0.0193		0.0367		0.053		0.0029		0.0198
4 heath	0.010	(0.42843)	0.021	(0.25669)	0.008	(0.42843)	X		0.0602		-0.0226		0.0704		0.0566		-0.0153		0.0169
6 forest	0.027	(0.22676)	0.003	(0.46532)	0.056	(0.13125)	0.085	(0.05625)	X		0.0024		-0.0431		0.0469		0.0107		0.0683
7 heath	0.002	(0.47321)	-0.007	(0.61706)	0.014	(0.40985)	-0.014	(0.70746)	0.006	(0.42843)	X		0.0097		0.0094		-0.0349		-0.0081
8 heath	0.041	(0.19475)	0.004	(0.44833)	0.042	(0.19475)	0.082	(0.05625)	-0.038	(0.969)	0.009	(0.42843)	X		0.0335		0.0001		0.054
9 forest	0.005	(0.42843)	0.024	(0.22676)	0.079	(0.05625)	0.079	(0.08)	0.060	(0.08)	0.017	(0.37584)	0.050	(0.13125)	X		0.0163		0.0471
10 forest	0.034	(0.19475)	0.030	(0.22239)	-0.001	(0.46532)	-0.018	(0.7245)	0.025	(0.22676)	-0.038	(0.969)	0.009	(0.42843)	0.031	(0.19475)	X		0.0108
11 forest	0.079	(0.072)	0.038	(0.19475)	0.005	(0.42843)	0.028	(0.22676)	0.068	(0.05625)	-0.005	(0.57173)	0.053	(0.09)	0.059	(0.08)	0.008	(0.42843)	X

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