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Heritability under benign and stressful conditions in the plant pathogenic fungus *Mycosphaerella graminicola*

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ABSTRACT

Hypothesis: Stressful environmental conditions directionally change the heritability of quantitative traits and the short-term adaptive potential of populations.

Organism: *Mycosphaerella graminicola*, a haploid pathogenic fungus causing Septoria leaf blotch in wheat.

Methods: Isolates from a population of *M. graminicola* in Switzerland were used to estimate quantitative genetic variance components and broad-sense heritability of *in vitro* mycelial colony size under six environmental conditions.

Results: Environments varied in their effect on colony size, reflecting different levels of stress for the pathogen. Three standardized variance components – genotypic, environmental, and phenotypic – increased under stress. The increase in standardized genotypic variance under stress pointed towards increased relative evolvability of the colony size. In contrast, broad-sense heritability did not vary with stress, indicating no change in absolute evolvability of the trait.

Conclusions: Environmental stress is predicted to affect heritability, but empirical research has not produced convincing generalities. One reason may be that the focus has been on heritability instead of variance components. If both genetic and environmental variances change under stress, heritability can go up or down. We found that stress positively impacted standardized genetic variance, an alternative measure of relative evolvability.

Keywords: adaptability, benign vs. stressful, environment-dependence, environmental change, evolutionary potential, favourable vs. unfavourable, genetic variance, heritability.

INTRODUCTION

Genetic variation is fundamental for evolutionary change. Genetic variation for traits with a polygenic basis is commonly expressed in terms of heritability, the ratio of additive genetic variance (V_A) to total phenotypic variance (V_P) (Falconer and Mackay, 1996). Heritability is

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relevant as a measure of genetic variation because it is proportional to the response to selection. Hence, estimating heritability for ecologically relevant traits has been an important focus in evolutionary biology. Much research has found that heritability is environment-dependent, which invokes several hypotheses for the behaviour of heritability under different environmental conditions. It has been hypothesized that heritability is affected by the amount of stress the environment imposes on organisms (Hoffmann and Parsons, 1991; Bennington and McGraw, 1996; Møller and Swaddle, 1998).

Hoffmann and Merilä (1999) described a collection of hypotheses to explain both increases and decreases in heritability under stressful conditions. Three of those hypotheses predict an immediate increase in additive genetic variance and therefore in heritability when the environment is stressful. First, when unfavourable conditions are novel, selection has not yet had the opportunity to remove genetic variants that affect the trait but also reduce fitness under the novel conditions. Second, under unfavourable conditions, alleles whose phenotypic expression would otherwise be buffered are de-canalized. De-canalization has been linked to limitations in the activity of chaperones that moderate phenotypic expression of some genetic variants under less stressful conditions (reviewed in Rutherford, 2003; Schlichting, 2008). Third, phenotypic differences among genotypes may be enhanced if stress acts to limit resource acquisition. This hypothesis goes back to enzyme kinetics and the fact that enzyme activity over a gradient of substrate concentration follows a saturation curve (Hartl *et al.*, 1985). Hartl and colleagues hypothesized that stress implied a change in the relation between enzyme activity and substrate concentration or a shift along the substrate axis, with the consequence that differences among genetic variants in enzyme activity may be more pronounced. Two further hypotheses listed by Hoffmann and Merilä (1999) predicted a decrease in heritability under stress. The first states that stressful conditions increase environmental variation. The second proposes that limited resource acquisition equalizes phenotypic differences among genotypes and reduces genetic variance (Gebhardt-Henrich and van Noordwijk, 1991). The two sets of hypotheses, either for an increase or a decrease in heritability, make specific testable predictions.

Previous experiments have produced mixed evidence for a systematic change in heritabilities under stressful conditions (Hoffmann and Merilä, 1999). A trend towards increased heritability has been observed in laboratory experiments with *Drosophila* species, whereas a trend towards reduced heritability under stress has been found in studies on birds (Hoffmann and Merilä, 1999). Charmantier and Garant (2005) performed a meta-analysis of heritability data from natural populations of animals, and concluded that heritability was somewhat higher under favourable conditions for body size but not for traits more closely related to fitness. Improved understanding of the environment-dependence of heritability may come from focusing on the environment-dependence of its components, which include (additive) genetic variance and environmental variance. It may also be useful to compare alternative measures of evolvability other than heritability, particularly for fitness-related traits (Houle, 1992). Furthermore, generality may be gained by extending the research focus to more diverse organisms and a broader set of stressors.

In this study, we investigated the behaviour of three important quantitative genetic variance components – genotypic, environmental, and phenotypic variance – in the wheat fungal pathogen *Mycosphaerella graminicola* under six environmental conditions with variable effect on *in vitro* growth. *In vitro* growth, or size when assessed at one point in time, is measured on dishes filled with growth medium by placing a droplet of quantified spore solution on the medium; spores then germinate and give rise to a dense net of mycelial

hyphae that spread radially with clear edges at the mycelial forefront. *Mycosphaerella* is an ideal organism for *in vitro* experiments because of its relatively fast mycelial growth under various laboratory conditions and easy assessment of growth (Zhan *et al.*, 2005). Furthermore, the species is haploid, which means that intra-locus gene interactions are absent. Thus, dominance gene effects do not affect genotypic and phenotypic estimates.

METHODS

Isolates of *Mycosphaerella graminicola* were collected in 1999 in a wheat field in Eschikon near Zürich, Switzerland, and stored on silica gel at −80°C. To ensure that we worked with genetically distinct isolates, DNA was analysed at inter-microsatellite locus regions (ISSR) and by rep-PCR fingerprinting of ERIC and BOX elements combined (Versalovic *et al.*, 1994). The isolates differed in several electrophoresis bands under both approaches. Before the start of the experiment in the fall of 2007, isolates were removed from the freezer, propagated on petri dishes with yeast-malt agar, and transferred twice to new petri dishes ($4\ g \cdot l^{-1}$ yeast, $4\ g \cdot l^{-1}$ malt, $4\ g \cdot l^{-1}$ sucrose, $12\ g \cdot l^{-1}$ agar, $50\ mg \cdot l^{-1}$ kanamycin). Spores were collected from a 5-day-old precursor petri dish, and spore concentration was counted with a haemocytometer. Each inoculum was diluted in water to a final concentration of 200 spores per microlitre and distributed over three aliquots. Then four drops of 3 μl spore solution from an aliquot were placed equidistant from one another on a fresh petri dish with yeast-malt agar, and six such dishes were set up per aliquot. After about an hour, dishes were exposed to six treatments, representing six environments varying in stress: (a) 22°C constant, which is within the range of optimal temperatures for mycelial spread and pycnidia production (Chungu *et al.*, 2001); (b) 27°C constant; (c) 0°C after the beginning of spore germination, for 24 h and subsequent storage at 22°C; (d) alternating 8°C and 27°C for 24 h each; (e) drying of the droplets with germinating spores for 3 h at room temperature before re-humidification with purified water over the dishes and storage at 22°C; and (f) increased pH of 9 (compared with a pH of 6 in all other treatments) in the medium and storage at 22°C. Total numbers of dishes were: 7 isolates × 6 environments × 3 replicates with four drops minus 5 petri dish losses, resulting in 121 dishes. After 13 days, photographs of each petri dish were taken and the surface area of *M. graminicola* was measured with the image analysis software ASSESS v2.0 (Lamari, 2002). We measured each image twice. The area was recorded in units of pixels, with 168 pixels per square millimetre.

Data (in units of pixels) were natural logarithm-transformed, so that size was expressed on a proportional scale. We then investigated the effect of environment, genotype, and their interaction on petri dish means of mycelial colony size (PROC GLM, SAS Institute, 2008). The error term for testing the effect of environment was the environment × genotype interaction, whereas for all other effects it was the residual error. Variance components were derived for each environment separately from a mixed effects model [PROC MIXED, restricted maximum likelihood (SAS Institute, 2008)] with the random effects of isolate, dish nested within isolate, and droplet position within dish and isolate. Variance explained by isolate was interpreted as genotypic variance (V_G), while variance among dishes within genotype was considered to be environmental variance (V_E). Variance at the level of droplets within dishes estimated the error stemming from heterogeneity in spore density among drops, and the error variance was measurement error. Broad-sense heritability was calculated as the ratio of genotypic to phenotypic variance (where $V_P = V_G + V_E$). Variance components were standardized by the squared mean of colony size for the particular environment to make

data comparable across environments [now I_P, I_G, and I_E (Houle, 1992)]. Standardized genotypic variance, I_G, represents a measure of evolvability that differs from heritability in that the response is expressed relative to the trait mean before selection (Houle, 1992). Standard error of the variance components were calculated by jackknifing over isolates.

RESULTS

The six environments significantly influenced aggregate mycelial colony size ($F_{5,30} = 28.98$, $P < 0.0001$), genotypes differed in aggregate colony size ($F_{6,79} = 5.32$, $P = 0.0001$), and environmental effects on colony size varied among genotypes ($F_{30,79} = 3.88$, $P < 0.0001$). Mean environment-specific colony size was interpreted as a measure of the degree of stress; least square means for the environments are listed in Table 1. The fact that isolates differed in aggregate colony size indicated that size had a genetic basis, and the interaction between isolate and environment reflected that genotypes differed in colony size across environments. Estimates of genotype means did not correlate across environments except for the two environments of 22°C constant and cold shock ($r = 0.97$, $P = 0.0004$; all other comparisons $P \geq 0.05$; Bonferroni-adjusted $\alpha = 0.0033$). Table 1 shows unstandardized genotypic and environmental variance estimates with jackknifed standard errors.

There were significant negative relationships between the three standardized variance components (genotypic, environmental, and phenotypic) and least square mean aggregate size, which represented the level of stress (slope ± S.E.: I_G: -0.012 ± 0.001, $t = -16.81$, $P < 0.0001$; I_E: -0.013 ± 0.002, $t = -5.71$, $P = 0.0047$; I_P: -0.025 ± 0.003, $t = -9.90$, $P = 0.0006$) (Fig. 1A, B, and C). Broad-sense heritability was not related to the mean aggregate colony size (H^2: 0.034 ± 0.084, $t = 0.41$, $P > 0.7$) (Fig. 1D).

DISCUSSION

Environmental stress experienced by the wheat pathogenic fungus *Mycosphaerella graminicola* led to neither a systematic increase nor decrease in broad-sense heritability. However, inspection of the individual variance components revealed that the lack of significant directional effect on heritability was due to a simultaneous increase of genotypic and environmental variance with stress. The results imply that whereas both genotypic and environmental variance can change, heritability does not change in a predictable manner

Table 1. Least-square means (LSM) with lower and upper 95% confidence limits (LCL, UCL) and non-standardized variance components (±1 standard error) of aggregate mycelial colony size in *Mycosphaerella* for the six environmental treatments

Environment	LSM	LCL, UCL	$V_G \pm$ S.E.	$V_E \pm$ S.E.
Initial cold shock	8.741	8.428, 9.055	0.116 ± 0.006	0.028 ± 0.003
22°C constant	8.680	8.366, 8.994	0.065 ± 0.004	0.018 ± 0.001
Initial humidity drop	8.346	8.021, 8.671	0.014 ± 0.003	0.077 ± 0.007
pH 9	7.151	6.837, 7.465	0.927 ± 0.068	0.012 ± 0.002
8°/27°C fluctuating	5.682	5.346, 6.017	1.289 ± 0.146	1.220 ± 0.131
27°C constant	4.543	4.208, 4.879	1.022 ± 0.183	1.134 ± 0.185

Note: The six environments are sorted in order of increasing stressfulness.

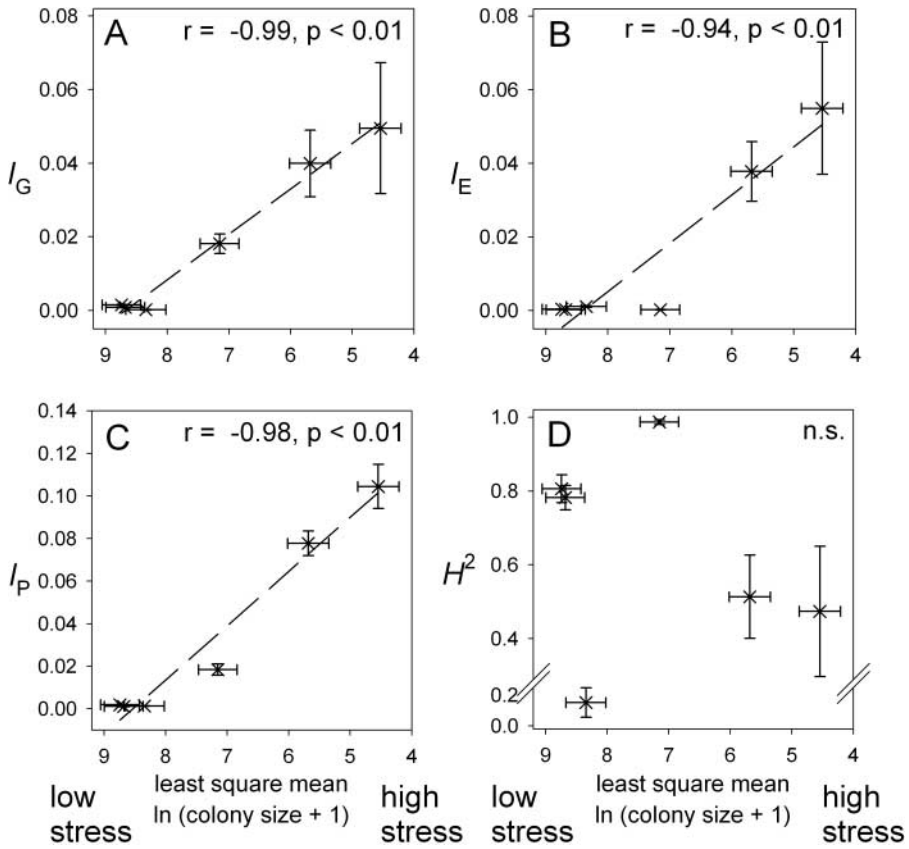

Fig. 1. Relationship between variance components (standardized by the square of trait mean) and the least square mean of aggregate colony size in *Mycosphaerella graminicola* under six treatments differing in environmental stress (higher colony size represents lower stress). The terms I_G, I_E, and I_P stand for standardized genotypic, environmental, and phenotypic variance respectively; H^2 stands for broad-sense heritability. Error bars indicate ± 2 S.E. for variance components, and the 95% upper and lower confidence limit for colony size. Relationships between all variance components and the level of stress were significant, but broad-sense heritability showed no significant association with stress.

under increasingly stressful conditions. Heritability of aggregate colony size varied among environments from very low to over 90% (Fig. 1D), which suggests that the potential for an evolutionary response to selection differs substantially among stressful environments. However, we also found that an alternative measure of evolvability, standardized genotypic variance I_G, increased with environmental stress. The two measures of evolvability, heritability and I_G, differ slightly in what they say about the predicted response to selection. While heritability is proportional to the absolute predicted response (in units of standard deviation), I_G for a fitness-related trait is proportional to the predicted response relative to the trait mean before selection (Houle, 1992).

The results for the variance components in our study support two of the three hypotheses predicting an increase in genetic variance under stress (Hoffmann and Merilä, 1999). One possibility

is that genotypic variation was enhanced under stress due to de-canalization. De-canalization via temperature stress has been associated with the reduced functioning of heat-shock proteins as chaperones. Rutherford and Lindquist (1998) found that when the heat-shock protein Hsp90 was experimentally impaired in *Drosophila*, morphological genetic variants were expressed that otherwise were not, particularly when tested under heat stress. The second possible explanation is that limited resource acquisition under stress resulted in accentuation of phenotypic differences among genotypes. However, more direct empirical tests of this hypothesis have so far produced equivocal results; some studies in which stress was reliably traced back to weak nutritional status or in which nutritional status was directly manipulated report lowered genetic variance under stress [e.g. in birds (Gebhardt-Henrich and van Noordwijk, 1991; De Neve *et al.*, 2004); in wheat and maize (reviewed in Laperche *et al.*, 2006)]. The novel-stress hypothesis is unlikely in our case, because the above-optimal temperatures that created stressful conditions (27°C) are not uncommon in most wheat-growing areas.

This study illustrates that progress in understanding how evolvability depends on the environment comes from studying changes in the underlying variance components. One reason is that Hoffmann and Merilä (1999) brought together specific hypotheses for the behaviour of heritability under stressful conditions. In the end, these hypotheses make predictions about the components of heritability – genetic and environmental variances. Our results show that while both variance components may change with stress, heritability itself may not change directionally. In line with this, several earlier studies observed no change in heritability between favourable and unfavourable conditions while finding significant shifts in genetic and/or environmental variance, at least for some traits (e.g. Blanckenhorn and Heyland, 2004). A second reason is that evolvability measures other than heritability may reveal more clear-cut trends. Houle (1992) derives several measures, each appropriate under different kinds of selection regime. For both of these reasons, it is desirable to design studies that can estimate variance components.

The highly variable relationships between heritability and stress reported by Hoffmann and Merilä (1999) and Charmantier and Garant (2005) may arise from differences in other factors between studies. For example, Charmantier and Garant (2005) argued that laboratory experiments have employed more novel and stronger agents of stress than field studies, and this may account for the trend towards increased heritability observed in the laboratory. It may be useful to follow the approach that we have taken in expressing stress in a standard way, as a proportional impact on fitness or fitness-related traits. More emphasis on different types of stressors may reveal the importance of past selection regimes for changes in variance components, as has been shown for the magnitude of inbreeding depression under stress (Bijlsma *et al.*, 1999; Willi *et al.*, 2007). The timing of stress across developmental stages may turn out to be relevant as well. A recent study found that food stress and developmental stage interacted in their effect on heritability in body size of a beetle (Dmitriew *et al.*, 2010).

The impact of environmental stress on species abundance and distribution is relevant within evolutionary biology, but also in applied fields with an interest in evolutionary change such as animal and plant breeding or epidemiology (Hoffmann and Willi, 2008). Short-term adaptation, at least, will depend on the standing level of genetic variation that exists in populations. As environmental stress impacts variance components, more research will be needed to identify the underlying patterns and ultimate causes.

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