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Genetic relationships between growth characters in *Salix viminalis* grown in Sweden

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Abstract From an eight by eight factorial crossing with *Salix viminalis*, 40 of the 64 families obtained were selected for further analysis. Fourteen seedplants from each of these 40 families were planted in two pairs of contrasting environments: sand and clay soil, and low and high nutrient supply. The material in the soil contrast was harvested after 1, 4 and 6 years of growth. The material in the nutrient contrast was harvested each year for 3 years and analysed after the first and the third harvests. The correlation between number of shoots and weight in the clay environment changed from being negative in the first harvests to positive at the last harvest, compared with the sand environment where this correlation was positive in all years. In the nutrient contrast this correlation was positive at the last harvest in the high nutrient environment, but no correlation could be detected in the low nutrient environment. The differences in correlations between environments may be due to a different allocation of nutrients in the plants, depending on whether the plant is under stress or not. The data suggests that the genetic relationship between growth components is the same over age and environments when the plants are grown without stress.

Key words Genetic and phenotypic correlations · Path analyses · *Salix viminalis*

Introduction

In the early phase of domestication of a crop it is important to discover the possibilities and constraints for selection. Knowledge of the relationship between different characters is necessary for understanding the indirect effect of selection. *Salix vininalis* is a fast-growing woody species, and while phenotypic and genetic correlations have already been studied in other such fast-growing woody species and breeding objectives attained that may also be applicable to *Salix* for example *Populus* sp (Wilcox and Farmer 1967; Nelson and Tauer 1987; Pichot and du Cros 1989) and *Eucalyptus* sp (Volker et al. 1990; Whiteman et al. 1992), studies in *Salix* sp are sparse (see Lin and Zsuffa 1993).

Information on changes in the structure of correlations between environments is important for breeding purposes (Cuartero and Cubero 1982; Ariyo et al. 1987; Falkenhagen 1989; Hébert et al. 1994; Lascoux et al. 1994) since a different correlation pattern from one environment to another complicates the selection. Changes in correlation pattern have also been analysed in ecological and evolutionary studies for predicting and understanding evolutionary change (Schlichting 1989 a, b; Scheiner et al. 1991; Via 1984). Hebert et al. (1994) showed that the number of significant genetic correlations increased with increasing environmental stress in his study on the correlation between 36 different characters in *Medicago lupulina*. When studying nine different characters in tomatoes (*Lycopersicon esculenta*) in four environments in order to find the best correlations to use in indirect selection for yield, Cuartero and Cubero (1982) detected large differences in correlations between the environments. In a study with okra (*Abelmoschus esculentus*), where genetic as well as phenotypic and environmental correlations were estimated during two seasons, the correlation estimates varied with age as well as between seasons (Ariyo et al. 1987). In *Pinus elliotti* the genetic correlations between growth traits and stem form changed considerably between sites (Falkenhagen 1989). Lascoux et al. (1994) showed that the correlation in *Pinus sylvestris* between two growth components changed from one nutrient level to another. Path coefficient analyses indicated that these changes were accompanied by a reversal in the contribution of the components, to the resulting traits, i.e. shoot growth.

The examples above, show that genetic correlations are influenced by environment as well as by age. This

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seems to apply to most species and could also be expected in *Salix* species.

In order to better understand how the correlations between growth characters change between environments and years, material of *Salix viminalis* planted in contrasting environments was studied. One contrast was between soil types, i.e. heavy clay and sand, and the other was between different nutrient availability, i.e. a stressed environment and one with optimum fertilization. The questions addressed in this study were:
- can phenotypic correlations be used instead of genetic correlations?
- do genetic correlations between growth characters change from one environment to another or with age?
- do growth conditions (i.e. optimal or stressed) influence the genetic correlations between characters?

Materials and methods

Clones of *Salix viminalis* L. collected in Sweden and one clone originating from Holland were used as parents in the crossing experiments. The Swedish material most probably originates from old cultivations that were established after clonal material had been brought to Sweden during the last couple of centuries.

An eight by eight factorial crossing was made in 1987, and 40 of the 64 families were used in the final analyses (Rönnberg-Wästljung et al. 1994). Fourteen seedplants per family were propagated to be planted in the two pairs of contrasting environments. The first pair of environments were field sites where the soil types differed, one site consisted of sandy soil and the other of heavy clay soil. The other pair of environments were two contrasting nutrient levels created in a sand box with inert sand where the nutrient was applied according to the method of Ingestad and Lund (1986) so as to obtain one high and one low growth rate of plants. The nutrient solution was adjusted for *Salix* (Eriksson 1981a). All environments were at or close to the Swedish University of Agricultural Sciences in Uppsala (59° 48′, 17° 39′, 25 m). The experiments were planted in an incomplete block design (Rönnberg-Wästljung et al. 1994). Twelve and eight clones per family were planted in the sand environment and in the clay environment, respectively (totals = 480 and 320 clones); of these, 278 clones were in common. In the nutrient environments 6 clones per family, making a total of 240 clones, were planted in both environments. A more detailed description of the material and environments is given in Rönnberg-Wästljung et al. (1994).

The material in the sand-clay contrast was planted in 1988 and harvested after the first growing season (= 1:1, which indicates shoot age: root age) and then after 3 (3:4) and 5 years (2:6). The number of shoots on each plant was counted at each harvest, and height of the highest shoot was measured at the first and second harvests. Diameter of the highest shoot was measured at the second harvest.

The plants in the nutrient contrast experiment were harvested each year for 3 years, and results from the first (1:1) and the third (1:3) harvests are presented in this study. The number of shoots on the plant was counted at the harvests. At the first harvest, the diameter and height of the highest shoot were also measured.

Statistics

Variance components were estimated using the REML method in the SAS VARCOMP procedure (SAS Institute 1989). The linear model used was:

$$y_{jklnp} = \mu + r_j + b_{k(j)} + m_l + f_n + mf_{ln} + c_{p(ln)} + e_{jklnp} \quad (1)$$

where y_{jklnp} = the observed value of a given character for an individual plant, μ = the overall mean, r_j = the effect of replicate $j, j = 1,2,\ldots,r; r = 5$ or 8, $b_{k(j)}$ = the effect of block k within replicate j, $k = 1,2,\ldots,b; b = 6, 8$ or 12. $k = 1,2,\ldots,b; b = 6, 8$ or 12, m_l = the effect of male $l; l = 1,2,\ldots,8$, f_n = the effect of female $n; n = 1,2,\ldots,8$, mf_{ln} = the interaction effect between male l and female n, (i.e. family), $c_{p(ln)}$ = the effect of clone p within family, $p = 1,2,\ldots,c; c = 6, 8$ or 12, e_{jklnp} = random error.

All effects except μ were considered to be random and normally distributed.

The genetic and phenotypic correlations have been estimated with the formula:

$$r = \frac{s_{xy}}{\sqrt{s_x^2 s_y^2}} \quad (2)$$

where, s_{xy} = estimated covariance between x and y, s_x^2, s_y^2 = estimated variance of x and y respectively.

The covariance between x and y is estimated from the expression: $s_{(x+y)}^2 = s_x^2 + s_y^2 + 2s_{xy}$; some rearrangements give, $s_{xy} = 0.5(s_{(x+y)}^2 - s_x^2 - s_y^2)$, where, $s_{(x+y)}^2$ = estimated variance of $x + y$.

To estimate the additive genetic correlation in the material we have used male plus female variances for the variables x, y and $x + y$, (estimated from Eq. 1) to put into Eq. 2. For phenotypic correlations, all effects in model 1 were used except for replicate and block (replicate).

The standard errors of the correlations were estimated according to Falconer (1981, p 285).

The relationship between the characters was studied using path coefficient analyses (Sokal and Rohlf 1995). The model was:

$$W = H \times (D/2)^2 \times \Pi \times S \times U \quad (3)$$

where W = weight of the plant, H = height of the highest shoot, D = diameter of the highest shoot, S = number of shoot of the plant, U = unknown causes, Π = constant.
To get an additive expression, a logarithmic transformation was done.

$$\log W = \log H + 2(\log D - \log 2) + \log \Pi + \log S + \log U \quad (4)$$

The path coefficients (p) were estimated as the partial regression coefficients (Sokal and Rohlf 1995). The correlation between the characters and the resultant character (weight) is the direct effect of the character (path coefficient) plus all indirect effects of the character on the resultant character via other paths (Fig. 1). For example, the correlation between height and weight is composed of:

$$r_{hw} = p_h + (r_{sh} \times p_s) + (r_{hd} \times p_d) \quad (5)$$

p_h, p_s, p_d = path coefficient for height, number of shoots and diameter, respectively (= partial regression coefficients), the direct effects on weight, r_{sh} = genetic correlations between height and number of shoot, r_{hd} = genetic correlation between height and diameter, $(r_{sh} \times p_s)$ and $(r_{hd} \times p_d)$ = the indirect effects on weight.

The correlation between U and the resultant character (weight) can be estimated from the formula for total determination of the resultant character (Sokal and Rohlf 1995, p 638).

$$\sum_i p_{wi}^2 + 2\sum_{ij} (p_{wi} p_{wj} r_{ij}) + r_{uw}^2 = 1 \quad (6)$$

where Σ_i = is the summation of all causal variables, (s, h, d) and Σ_{ij} = is the summation of all pairs of causal variables.

All variables in the model were only measured at age 3:4 in the soil environments and at age 1:1 in the nutrient environments. At age 1:1 in the soil environments, the model was reduced since the diameter of the highest shoot was not measured. This gave one path instead of two for the indirect effects of the character. At age 1:3 in the nutrient environment and at age 2:6 in the soil environments, it was not possible to estimate any path coefficients since only number of shoots and weight were measured.

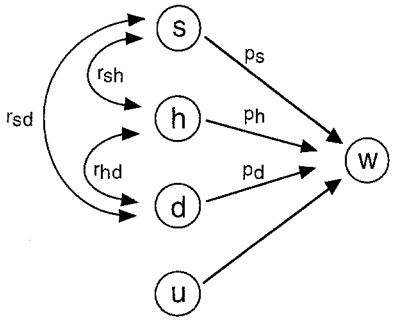


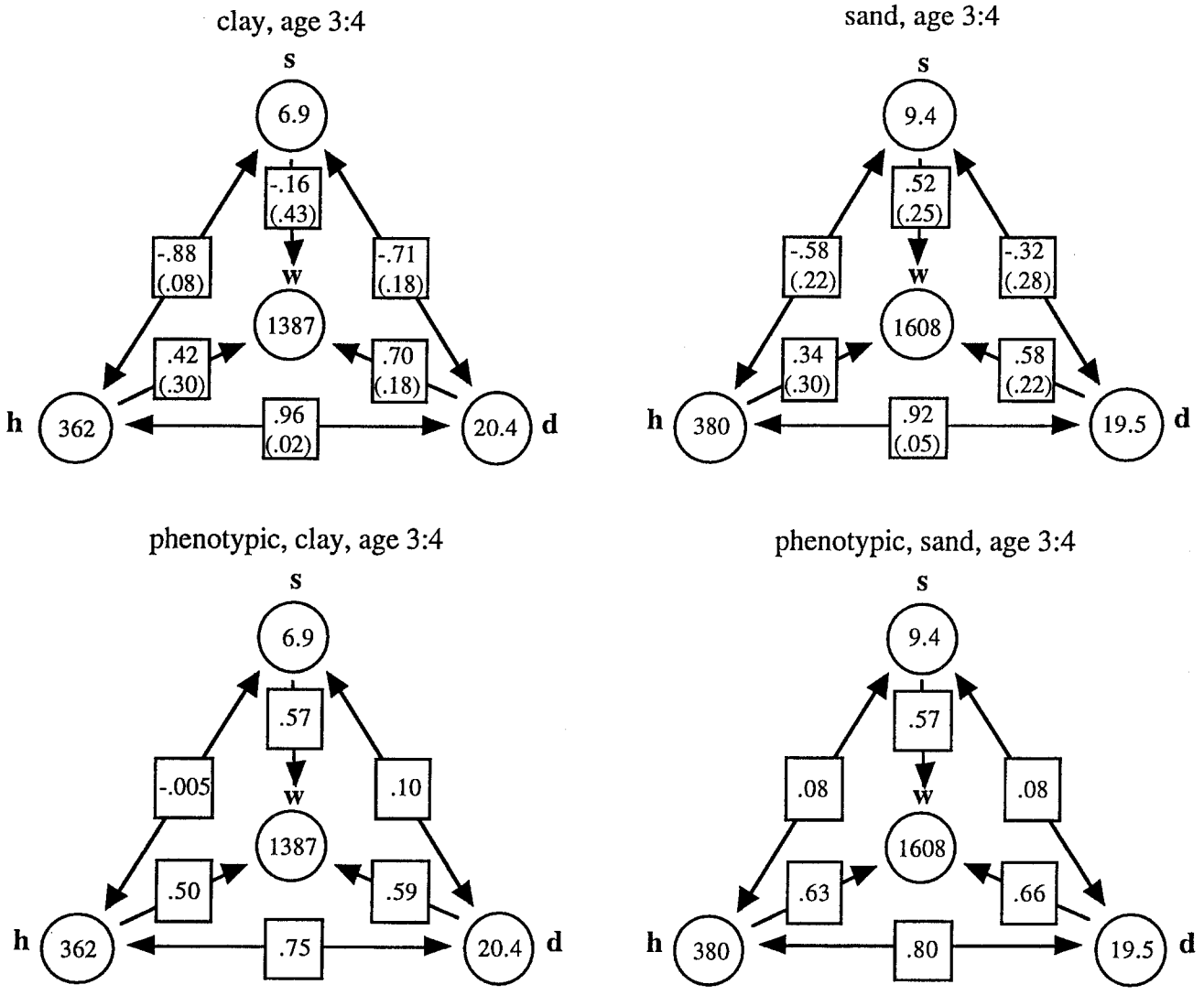
Fig. 1 Path analysis model. Path coefficients, the direct effect of the characters on weight, are indicated by *single-headed arrows*, and indirect effects on weight are indicated by *double-headed arrows*. s number of shoots, *h* height of the highest shoot, *d* diameter of the highest shoot, *w* weight of the plant, *u* unknown causes. For other abbreviations see text

Results

Genetic versus phenotypic correlations

Large differences between the genetic and the phenotypic correlations were detected, and these are illustrated for the clay and sand environments at age 3:4 (Fig. 2). In particular, correlations involving the number of shoots change sign from being positive or around zero in the phenotypic correlation to being negative in the genetic correlation. An exception is the correlation between number of shoots and weight in the sand environment where phenotypic and genetic estimates were of the same magnitude.

Fig. 2 Genetic and phenotypic correlations between growth characters in the clay and sand environments. Correlations with standard deviations (*within brackets*) are shown *within rectangles*. Mean values for the characters are *within the circles*. *Arrows* indicate the causal relationships between characters. Abbreviations: see Fig. 1



Genetic correlations and path coefficient correlations

Clay-sand

When the genetic correlations in the sand environment are compared with correlations in the clay environment, a difference can be seen at ages 1:1 and 3:4, especially between weight and number of shoots where there is no correlation, or a slightly negative correlation, in the clay environment, and a positive one in the sand (Figs. 2, 3). The two other correlations with number of shoots at age 3:4 are also more negative in the clay environment. At age 2:6, the correlation between number of shoots and weight show similar, and positive, values in the different soil environments (Fig. 3).

The path coefficient analyses revealed that the direct effect of the number of shoots on weight is much higher in the sand environment than in the clay environment at age 1:1 (Table 1). The negative correlation in the clay between these characters is due to a strong negative indirect effect of height on number of shoots (Table 1). In the clay environment, height influences the total weight more than the number of shoots, which can be seen in the path analyses studying the direct effects of these characters on weight (Table 1).

In the sand environment at age 3:4, the path coefficients are all positive. The negative correlations between number of shoots and height, and between number of shoots and diameter, cause negative indirect effects (Table 1).

Nutrient level

The genetic correlations at age 1:1 in the two nutrient levels are similar (Fig. 4), but there are some differences between the nutrient levels in the direct and indirect effects of the characters on weight (Table 2). Height of the highest shoot is more important for total weight at the low nutrient level than at the high nutrient level, and diameter is the most important character for the total weight at the high nutrient level.

Fig. 3 Genetic correlations in the clay and sand environments. *Empty circles* show that the character has not been measured. Abbreviations: see Fig. 1. For explanation of symbols see Fig. 2.

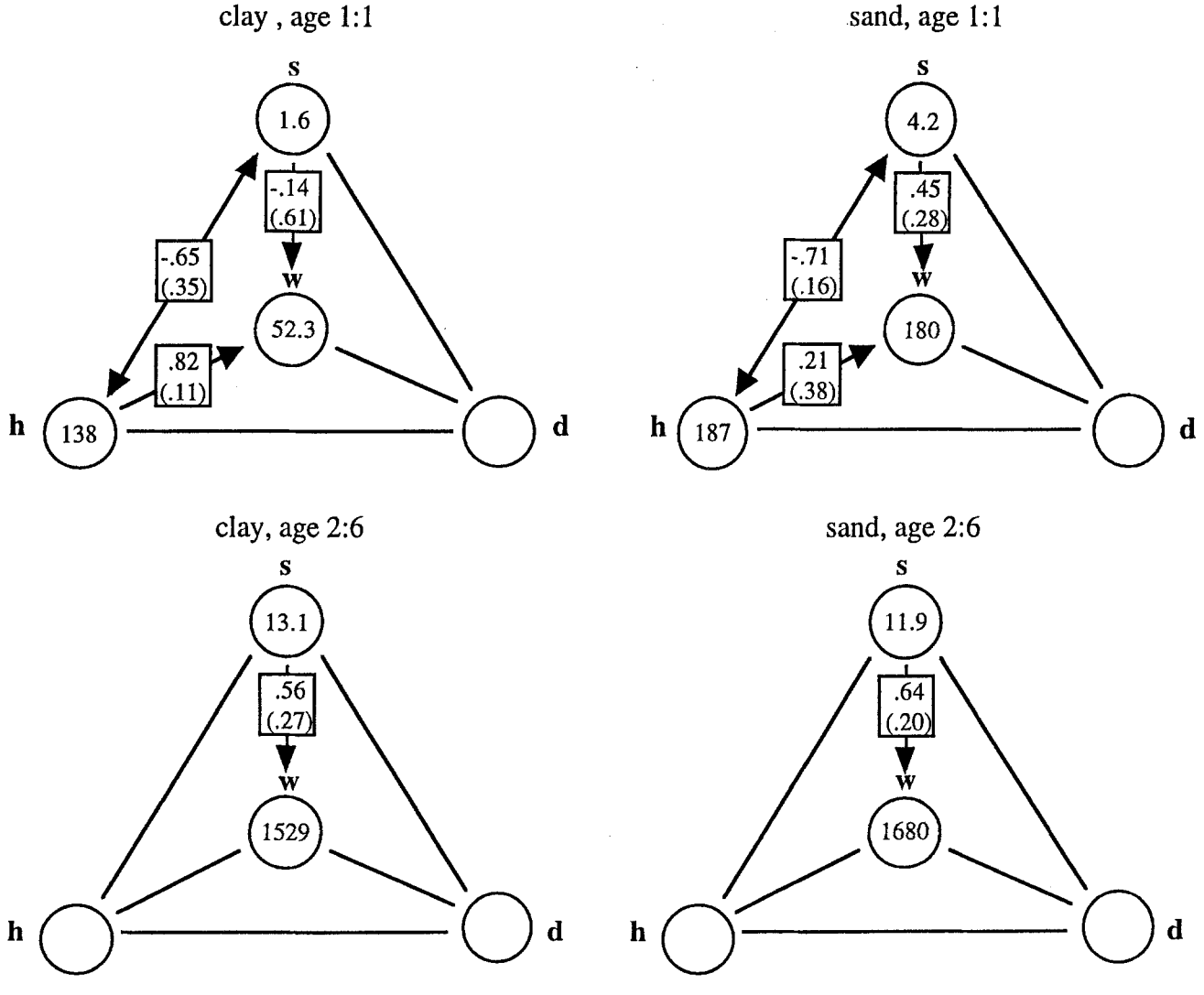


Table 1 Path analyses in the soil contrast

	Plant age[a] 1:1				Plant age 3:4				
	Corr[b] clay/sand	Direct effect[c] clay/sand	Indirect effect[d] clay/sand		Corr sand	Direct effect sand	Indirect effect sand		
Character[e]			S	H			S	H	D
S	−0.14/0.45	0.68/1.22		−0.82/−0.77	0.52	0.89		−0.20	−0.17
H	0.82/0.21	1.26/1.08	−0.44/−0.87		0.34	0.34	−0.51		0.50
D	–	–	–	–	0.58	0.55	−0.28	0.31	
U	0.27/0.48				0.33				

[a] Plant age is given as age of shoot : age of root
[b] Corr = genetic correlation between the character and weight
[c] Direct effect = path coefficient for the character
[d] Indirect effect = genetic correlation between the character and another character multiplied by the path coefficient for the other character
[e] S, Number of shoots of the plant; H, height of the highest shoot; D, diameter of the highest shoot; U, unknown causes

Table 2 Path analyses in the nutrient contrast

	Plant age 1:1				
	Corr high/low	Direct effect high/low	Indirect effect high/low		
Character			S	H	D
S	0.36/0.35	0.31/0.16		−0.11/0.14	0.16/0.05
H	0.74/0.95	0.50/0.57	−0.07/0.04		0.31/0.34
D	0.92/0.89	0.55/0.46	0.09/0.02	0.28/0.42	
U	0.13/0				

See Table 1 for explanations

At age 1:3, the correlation between number of shoots and weight is highly positive at the high nutrient level while there is no correlation between these characters at the low nutrient level (Fig. 4).

Discussion

Genetic versus phenotypic correlations

Phenotypic correlation consists of both additive genetic variance/covariance and environmental variance/covariance, which suggests that a genetic understanding of the relationships between characters can be difficult with only phenotypic estimations of the correlations. The formula

$$r_p = h_x h_y r_a + e_x e_y r_E \quad (7)$$

from Falconer (1981) explains the relationships between genetic, phenotypic and environmental correlations and heritabilities where, r_p = phenotypic correlation between the two characters x and y, r_a = genetic correlation between x and y, r_E = environmental correlation between x and y, h^2 = heritability, $e^2 = 1 - h^2$.

The formula shows that if the heritabilities of both characters are low, then the phenotypic correlation is mainly determined by the environmental correlation, and if the heritabilities of the characters are high, then the genetic correlation have more impact on the phenotypic correlation (Falconer 1981).

Height and diameter have the highest heritabilities of the measured characters in the field environments for this study (Rönnberg-Wästljung et al. 1994), and thus an agreement between the genetic and the phenotypic estimate for the correlation between height and diameter could be expected. The estimates of the phenotypic and genetic correlation between height and diameter in this study show different values, but they are both high and positive. Still, most of the phenotypic variation is non-additive, which may explain why phenotypic correlations differ from genetic correlations. The theoretical background to the relationships between phenotypic and genetic correlations (Eq. 7) and also our data show that for characters with lower heritabilities the genetic correlation differs from the phenotypic correlation. For characters with high heritability, on the other hand, the phenotypic correlation probably reflects the genetic correlation.

Genetic correlations and path analyses

The genetic causes behind a correlation between characters are pleiotropic effects or linkage between genes (or

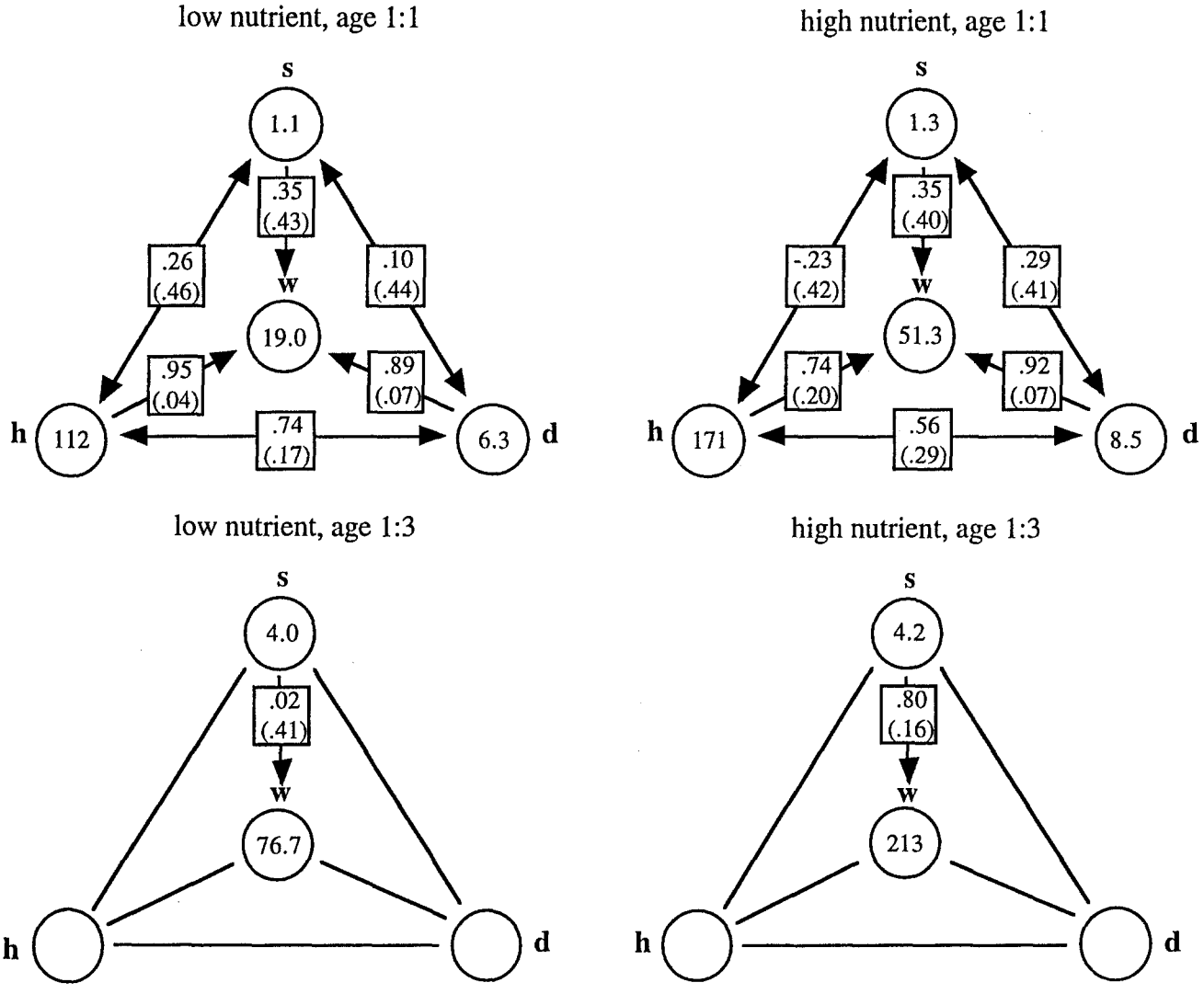


Fig. 4 Genetic correlations in the nutrient environments. *Empty circles* show that the character has not been measured. Abbreviations: see Fig. 1. For explanation of symbols see Fig. 2

both) (Falconer 1981). In this material some parents came from populations in linkage disequilibrium for a set of allozyme loci (Lascoux et al. 1995). In spite of that, the results are discussed as if the genetic causes of the correlation are due to pleiotropy.

An important result of this study is the resemblance in correlation patterns between growth characters in optimal environments. We consider optimal environments to be environments where the plants are well-established and have a high nutrient availability. Thus, apart from the low nutrient environment, we consider all environments in the sand to be optimal for the plants. Furthermore, at age 2:6 in the clay environment, we also consider the plants to be well-established with a good availability of nutrients from the clay soil.

In all of the environments mentioned above as optimal, the correlation between number of shoots and weight is positive except for the high nutrient environment at age 1:1. In the latter case, the correlation with number of shoots had a large standard deviation, an explanation of which possibly being the low mean value for number of shoots, 1.3, which makes it difficult to get a significant correlation. Path analyses show similar relations between direct and indirect effects for the correlation between number of shoots and weight in the optimal environments where it was possible to conduct path analyses.

The long establishment phase for *Salix* on clay soils (Ledin and Ahlriksson, 1992) probably results in the plants being under more stress at early ages (1:1, 3:4) compared with plants in the sand. One possible hypothesis to explain the differences we found in the correlations between growth characters, and in the path analyses, between clay and sand environments for plants at these ages might be differences in the allocation of nutrients in the two contrasting environments. We do not have any data on the allocation of nutrients in the plants, but Eriksson (1981b) has shown that a lower availability of nutrients and water in *Salix* causes a shift in the shoot/root ratio towards higher root growth. Differences in the allocation of nutrients can also give

differences in gene activites and pleiotropic effects of the plants and, thus, explain the differences in the genetic correlations.

In the clay environment, the change from a no or a slightly negative correlation between number of shoots and weight for plants at ages 1:1 and 3:4 to a positive correlation for plants at age 2:6 might also reflect differences in the allocation of nutrients. The plants are probably well established at age 2:6 and then show an allocation pattern similar to the plants in the sand environments.

In the low nutrient environment at plant age 1:3 there was no correlation between number of shoots and weight compared with the plants in the high nutrient environment that showed a high positive correlation. Since nutrient and water availability have been shown to influence the shoot/root ratio (Eriksson 1981b), differences in allocation of nutrients could also in this case be an explanation of why the correlation differed between nutrient environments.

Conclusions

Phenotypic correlations should be used with caution to describe the genetic relationships between growth characters unless there are high heritabilities of the characters involved. The genetic correlation pattern changes both between environments and between years within environment. In optimal environments, there is a high degree of resemblance in the correlation patterns. Our main suggestion for the change in correlations between optimal and suboptimal environments is differences in the way the plants allocate nutrients.

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