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Comparison of quantitative genetic parameters between two natural populations of a selfing plant species, *Medicago truncatula* Gaertn.

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Abstract In this paper we compare mean values, heritability estimates, coefficient of genetic variation, and genetic correlations among several fitness components of two natural populations of a selfing plant species, *Medicago truncatula* L. It is shown that the population that had been found most polymorphic for molecular markers in a previous study was also the most variable for quantitative characters. Depending on the traits, the larger heritabilities in this population were due to either larger coefficients of genetic variances or smaller coefficients of environmental variances. Whereas genetic and phenotypic correlation matrices were very similar within each population, they were quite different between populations. In particular, although a positive correlation between age and size at maturity was found in both populations, the correlation between age at maturity and reproductive success was negative in the more variable population (late flowering plant, with a larger size at flowering, produced fewer pods), whereas no correlation was observed in the less variable population. We suggest that while in the less variable population all individuals have a high reproductive effort, several strategies coexist in the more variable population, with some early-flowering genotypes showing a high reproductive effort and other late-flowering genotypes showing a larger competitive ability through increased vegetative growth.

Key words Comparative quantitative genetics · Population differentiation · Natural selection · *Medicago truncatula*

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Introduction

The amount of genetic variation for life-history traits is the main determinant of a species potential response to selection. Fitness components are generally less heritable than morphological or physiological characters (Roff and Mousseau 1987; Mousseau and Roff 1987; Roff 1995). Their lower heritability could be explained by a larger environmental variance rather than by a smaller genetic variance (Barton and Turelli 1989; Houle 1992). Some fitness components, however, seem to retain more genetic variance then expected. Negative genetic correlations among fitness components may limit response to selection (Antonovics 1976; Lande 1982; Rose 1982) so that the polymorphism observed for a particular fitness component may actually be neutral and subject to genetic drift (flat fitness profile, Stearns 1992). Thus, some fitness components may retain genetic variation even though fitness itself may not be variable. Comparisons of quantitative genetics parameters (variances and covariances) among populations may provide insights into differences and similarities in their histories of selection and drift and enable the breeder to predict the degree of similarity of their response to similar selection pressures. Moreover, in order to determine the extent to which trade-offs may explain genetic variance for fitness components, it is necessary to study the extent to which these trade-offs are common in natural populations. For instance, Mitchell-Olds (1986), studying two populations of an outcrossing annual plant species, found them to be very different for phenotypic means, narrow-sense heritabilities, and genetic correlations. Such comparative studies are still quite rare.

In a previous study of the selfing annual species *Medicago truncatula* Gaertn., we showed (Bonnin et al. 1996b) that two populations from Southern France were more differentiated for quantitative characters than for molecular, supposedly neutral, markers. This was taken as evidence that selection was acting

differently in the two populations. In the present paper, we extend the comparison between these two populations to mean phenotypic values, broad-sense heritability estimates, coefficients of genetic and environmental variation, and genetic and phenotypic correlations among 24 quantitative traits.

Materials and methods

Experimental design (described in Bonnin et al. 1996b)

Two populations of *Medicago truncatula* Gaertn. (Aude and Var) were studied. The Aude population is bordering a vineyard, whereas the Var population is bordering a road. Each sampled population was subdivided into three subpopulations (10 m–50 m apart), in which 1 plant was collected every meter alongside a 30-m transect. Thirty-three genotypes (original plants) from the Var population and 44 from the Aude population were studied.

To avoid maternal effects, we obtained several seeds per original genotype by two or three generations of spontaneous selfing in the greenhouse. After germination, in order to take into account environmental variance within a genotype (Falconer 1989), 5 seeds per genotype were planted in individual pots, giving a total of 385 plants (with a randomized block design of five blocks with 77 genotypes each). Twenty-four quantitative variables (Table 1) were obtained from measurements, as described in Bonnin et al. (1996b). All phenological traits are given in days after planting in the greenhouse.

Assumptions

Given the high selfing rate of *M. truncatula* (about 98%: Chaulet and Prosperi 1994; Bonnin et al. 1996a), complete inbreeding was assumed in the calculation of heritability estimates. With complete inbreeding, individuals are homozygous, so that selfed offspring should be genetically identical. As a result, the within-family variance can be considered as environmental, while the among-family variance component is assumed to be solely genetic (Falconer 1989; Gallais 1990). However, as for clonal studies or studies not using paternal half-sibs or father/offspring regressions, our design does not distinguish any possible maternal effects from the among-family variance. Consequently, genetic variance may be overestimated. With predominant but not complete selfing, the within-family variance includes some genetic variance as well, and this effect could lead to an overestimation of the environmental variance. Since these two phenomena act upon the broad-sense heritability in opposite directions and are both expected to have small effects on heritability estimates, the final estimates should not be highly affected.

Data analysis

In Bonnin et al. (1996a,b), we described the population structure in terms of the hierarchical sampling design. As the subpopulation effect was not significant for most characters in both populations (data not shown), it was not taken into account in the present study, which allowed us to work with more degrees of freedom.

Mean phenotypic values of each population were compared for every quantitative character using a nested analysis of variance type III SS of SAS Proc GLM (1992) for unequal sample sizes. The model was the following: population, genotype within population, block, interaction population * block. Population and block were fixed effects, whereas genotype was a random variable.

Heritabilities and coefficients of variation were obtained for every quantitative character in each population using the model genotype, block. In order to estimate components of the genetic variance, we considered the genotype to be random, while block was fixed. Broad-sense heritability (H^2) was calculated for each character as the ratio of the variance arising between genotypes (σ^2_F) on the total phenotypic variance ($\sigma^2_{TP} = \sigma^2_F + \sigma_e$), where σ^2_e is the residual variance (environmental variance arising between individuals within

Table 1 List of characters measured in this investigation and their abbreviations

Seedling traits	Emergence date of cotyledons	DCOT
	Emergence date of sixth leaf	D6F
	Length of the first leaf (cm)	LGFT6
	Breadth of the first leaf (cm)	LRFT6
	Area of the first leaf (cm^2)	SFT6 = LGFT6 * LRFT6
Growth traits	Length of the main stem 62 days after planting (cm)	LG1J62
	Length of the main stem 96 days after planting (cm)	LG1J96
	Length of secondary stems 62 days after planting (cm)	LGMJ62
	Length of secondary stems 96 days after planting (cm)	LGMJ96
	Daily growth of the main stem (cm)	LG134 = (LG1J96-LG1J62)/34
	Daily growth of secondary stems (cm)	LGM34 = (LGMJ96 − LGMJ62)/34
	Length of the main stem at the flower bud stage (cm)	LG1JBF
	Length of secondary stems at the flower bud stage (cm)	LGMJBF
Reproductive traits	Date of first flower bud	DBF
	Date of first open flower	DFO
	Date of first unripe pod	DGV
	Date of first ripe pod	DGB
	Reproductive interval	GBFO = DGB − DFO
	Weight of dried stems (mg)	PTIG
	Weight of dried pods (mg)	PGOU
	Number of pods	NGOU
	Total weight (mg)	PTOT = PTIG + PGOU
	Weight of 100 pods (mg)	P100G = 100 *PGOU/NGOU
	Reproductive effort (mg)	REPRO = PGOU/PTOT

original genotype):

$$H^2 = \sigma^2_F/\sigma^2_{TP}$$

Coefficients of genetic variation (CV_G) and coefficients of environmental variation (CV_e) were obtained as:

$$CV_G = 100 * (\sigma^2_F)^{1/2}/m$$

$$CV_e = 100 * (\sigma^2_e)^{1/2}/m$$

where m is the population phenotypic mean.

Genetic correlations (r_G) between each pair of characters (X and Y) were estimated in each population from the same analysis of variance, using the MANOVA option of the GLM procedure (SAS Institute 1992):

$$r_{G^{xy}} = \sigma^2_{F^{xy}}/(\sigma^2_{F^x} * \sigma^2_{F^y})^{1/2}$$

Two main methods are used to compare quantitative genetics parameters (Shaw 1991): maximum-likelihood methods (Shaw 1987, 1991) on one hand, and randomization tests (Mitchell-Olds 1986; Cheverud 1996) or tests based on resampling methods (Mitchell-Olds and Bergelson 1990; Roff 1995) on the other hand. In this study we used resampling methods to estimate confidence intervals of heritabilities: 1000 bootstrap samples were produced by resampling over families in each population. This is the kind of test recommended by Van Dongen and Backeljau (1995) when sample size is more than 20. The estimate of variance components using bootstrap over families involves heavier computations than those involved with Jackknife, but when a sample distribution is obtained, a confidence interval can be constructed directly by ordering the estimates. Conversely, a valid confidence interval can be calculated from a Jackknife procedure only if the distribution of the parameter is normal. Because the confidence intervals of heritabilities obtained by bootstrapping or Jackknifing over families were very similar (even though the samples were not normally distributed, see Results) and because Jackknifing requires less computing, we only calculated Jackknife confidence intervals for coefficients of variation.

The significance of genetic correlations is more difficult to test (Scheinberg 1966; Becker 1984; Via 1984). Roff and Preziosi (1994) showed that phenotypic correlations calculated from family means can be used instead of genetic correlations when both matrices (phenotypic and genetic) are similar and/or when sample sizes are over 20. We thus calculated phenotypic correlations from family means (procedure CORR of SAS, SAS 1992) and compared the genetic and phenotypic correlation matrices in each populations with a Mantel test (Manly 1991) using a program written by J. Goudet (University of Lausanne). Given the large number of correlations, a sequential Bonferroni test (Holm 1979) was used to adjust the probability of type-1 error to the number of comparisons (Rice 1989).

Results

Phenotypic means

The two populations differed for most of the characters studied (Table 2). Compared to individuals from Var, individuals from Aude emerged slightly earlier (DCOT), had a larger size at the reproductive stage (LGMJBF), but grew slower (LGM34), with only secondary stems (no main axis, see LG1J62, LG1J96, LG134, and LG1JBF). They flowered about 20 days later (DBF, DFO), and produced fewer pods (NGOU) and of a smaller size (PGOU, P100G). Overall, reproductive effort (REPRO) in the Var population was more than twice that in the Aude population.

Table 2 Means and standard deviations (SD) for quantitative characters in each population. The *F*-ratio refers to a comparison of the two populations using the analysis of variance described in the text

Character	Aude ($n = 220$)		Var ($n = 165$)		*F*-ratio
	Mean	SD	Mean	SD	
DCOT	4.12	1.12	4.74	0.90	14.81***
D6F	28.90	2.96	29.75	2.62	ns
LGFT6	0.69	0.18	0.65	0.12	ns
LRFT6	0.79	0.18	0.75	0.14	ns
SFT6	0.57	0.26	0.49	0.15	4.82*
LG1J62	0.38	0.10	1.30	0.97	79.61***
LG1J96	0.67	0.31	8.29	6.39	114.93***
LGMJ62	7.06	3.39	7.91	3.72	ns
LGMJ96	45.80	11.26	69.32	15.34	117.99***
LG134	0.01	0.01	0.21	0.17	113.28***
LGM34	1.14	0.27	1.81	0.40	131.21***
LG1JBF	0.94	0.95	6.20	5.01	84.15***
LGMJBF	70.96	14.62	56.68	13.72	31.66***
DBF	110.70	7.43	91.17	6.34	269.75***
DFO	115.47	7.19	94.95	6.36	306.97***
DGV	119.18	7.17	98.99	6.11	317.29***
DGB	146.46	5.96	126.71	5.10	369.40***
GBFO	30.99	2.96	31.76	2.83	ns
PTIG	29.65	9.93	16.90	8.60	147.10***
PGOU	12.23	7.77	29.60	12.92	177.09***
NGOU	224.38	134.01	343.56	166.04	33.45***
PTOT	41.88	13.44	46.50	20.37	10.50**
P100G	5.29	0.87	8.86	1.36	386.53***
REPRO	0.28	0.14	0.64	0.08	212.26***

Significance levels: ns, not significant, ($P > 0.05$), *$P \leqslant 0.05$, **$P \leqslant 0.01$, ***$P \leqslant 0.001$

Heritabilities

Confidence intervals of heritabilities obtained in each population by bootstrapping and jackknifing are shown in Fig. 1. For most characters in both populations, the two kinds of confidence intervals were similar, although few characters (6 in Aude and 5 in Var) showed a normal distribution of the Jackknife samples (tested by the procedure UNIVARIATE of SAS, SAS 1992). The significance of heritabilities (H_0: $H^2 = 0$) was dependent, however, on the kind of resampling method used. With the Jackknife confidence intervals, 22 characters in Aude and 16 in Var were significantly heritable out of 24 (Table 3). According to the bootstrap method, 2 additional characters in Aude and 5 in Var, had significant heritability. The minima of the bootstrap intervals were, however, close to zero for the latter characters.

Average heritability over all traits was 0.46 (SD = 0.22) in Aude and 0.30 in Var (SD = 0.17). In order to compare genetic variability between populations, we will arbitrarily consider as highly heritable those characters whose heritability estimates are above 0.50. Out of 24 characters, 12 showed a high heritability in the Aude population, versus only 4 in the Var population (Table 3). In Aude, these characters were related to overall development, whereas in Var they were mainly associated to growth of the main axis. Unexpectedly for a trait likely to be under strong selective pressure, reproductive effort (REPRO) was highly heritable in both populations. Confidence intervals obtained from both bootstrap and Jackknife samples showed that heritability estimates were significantly different between the two populations for 7 characters out of 24 (LG1JBF, LGMJBF, DBF, DFO, DGV, PGOU, NGOU). The heritability of these traits, which are related to age and size at flowering and to seed production, was over 0.50 in at least one population (Table 3). Out of these 7 characters, only 1 (length of main axis LG1JBF) was more heritable in the Var population, and 4 (LGMJBF, DBF, PGOU, NGOU) showed non significant heritabilities in this population. Thus, overall, the Aude population appeared more variable than the Var population.

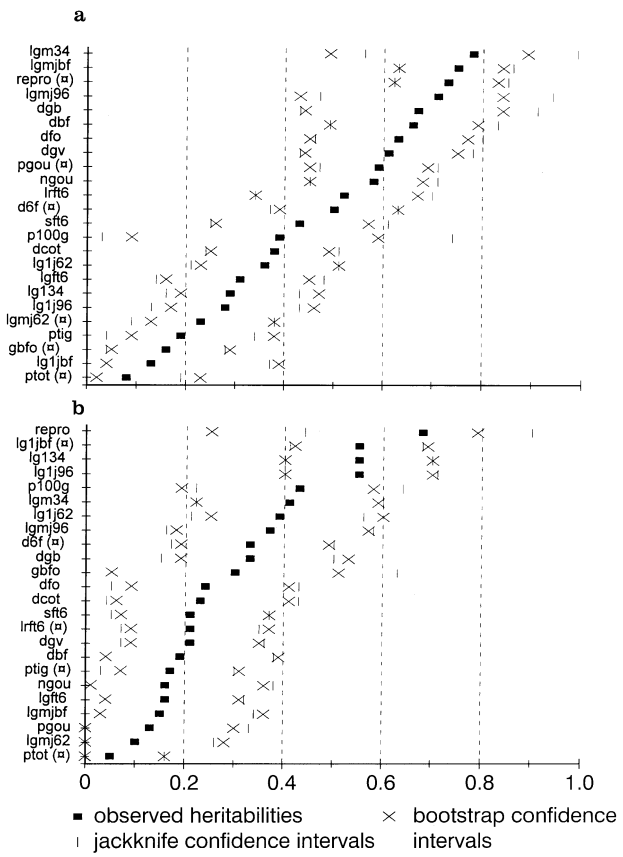

Fig. 1a, b Heritabilities estimated in each population (**a** Aude, **b** Var) and confidence intervals obtained by computing either Jackknife or bootstrap over families. ¤ indicates characters for which the distribution of Jackknife samples does not significantly depart from normality

Coefficients of genetic (CV_G) and environmental (CV_e) variation

Experiments conducted under controlled conditions are likely to over-estimate heritabilities because the environmental variance is reduced (Barton and Turelli 1989). Coefficients of genetic variation are less sensitive to this kind of biais and, as shown by Houle (1992), are likely to be better predictors of the evolvability of quantitative characters than heritabilities. We now turn to the study of such coefficients. Table 4 gives the coefficients of genetic and of environmental variation for each character and for each population. The average coefficient of genetic variation was 20% in Aude (SD = 15.1%) and 16% in Var (SD = 17.2%). The corresponding coefficients of environmental variation were 25% (SD = 23.4%) and 23% (SD = 16.6%), respectively. We will arbitrarily consider as large those coefficients above 25%.

In Aude, 6 characters out of 24 showed a large CV_G; 10 traits, including the 6 just mentioned, had a large CV_e. Among the 6 traits showing a large genetic variability and a large environmental variance, 3 were highly heritable: these were traits related to reproduction (PGOU, NGOU, REPRO). The low heritability of the other 3 traits (SFT6, LG134, LG1JBF) was thus not due to a lack of genetic variability, but to an excess of environmental variance (confidence intervals obtained for LG134 and LG1JBF, which showed the highest CV_e, included 100%). For the other 9 traits that were highly heritable (D6F, LRFT6, LGMJ96, LGM34, LGMJBF, DBF, DFO, DGV, DGB), most of which describe growth and phenology, both coefficients of variation were lower than 25%. The most canalized

Table 3 Broad-sense heritabilities (H^2) calculated in each population. Confidence intervals (CI) of each heritability were obtained computing bootstrap and jackknife over families. Variance components at genotype (σ^2_F) level are also presented in absolute values. σ^2_e is the environmental variance within family (error term). Significance levels refer to *F*-tests of the genotype mean squares in the analysis of variance

Character	Aude					Var				
	H^2	Bootstrap CI	Jackknife CI	σ^2_F	σ^2_e	H^2	Bootstrap CI	Jackknife CI	σ^2_F	σ^2_e
DCOT	0.38	0.25–0.49	0.24–0.51	0.47***	0.78	0.23	0.06–0.41	0.04–0.43	0.19***	0.61
D6F	0.50	0.39–0.63	0.37–0.63	4.12***	4.11	0.33	0.19–0.49	0.17–0.50	2.16***	4.35
LGFT6	0.31	0.16–0.45	0.14–0.48	0.01***	0.02	0.16	0.04–0.31	0.00–0.32	$2\ 10^{-3}$**	0.01
LRFT6	0.52	0.34–0.67	0.34–0.70	0.02***	0.02	0.21	0.09–0.37	0.07–0.35	$4\ 10^{-3}$***	0.01
SFT6	0.43	0.26–0.57	0.25–0.61	0.03***	0.04	0.21	0.07–0.37	0.05–0.37	$5\ 10^{-3}$***	0.02
LG1J62	0.36	0.23–0.51	0.21–0.51	$3\ 10^{-3}$***	0.01	0.39	0.25–0.60	0.21–0.56	0.35***	0.56
LG1J96	0.28	0.17–0.46	0.13–0.43	0.03***	0.06	0.55	0.40–0.70	0.40–0.71	19.20***	15.45
LGMJ62	0.23	0.13–0.38	0.09–0.38	2.18***	7.23	0.10	0.00–0.28	0.00–0.26	1.09*	10.17
LGMJ96	0.71	0.43–0.84	0.47–0.94	78.09***	32.18	0.37	0.18–0.57	0.16–0.58	69.90***	118.36
LG134	0.29	0.19–0.47	0.16–0.43	$3\ 10^{-5}$***	$6\ 10^{-5}$	0.55	0.40–0.70	0.40–0.70	0.01***	0.01
LGM34	0.78	0.49–0.89	0.56–0.99	0.05***	0.02	0.41	0.22–0.59	0.22–0.60	0.06***	0.08
LG1JBF	0.13	0.04–0.39	0.00–0.37	0.11**	0.74	0.55	0.42–0.69	0.41–0.68	12.16***	10.10
LGMJBF	0.75	0.63–0.84	0.63–0.86	160.96***	54.84	0.15	0.03–0.36	0.00–0.34	25.39**	141.81
DBF	0.66	0.49–0.79	0.49–0.83	32.56***	16.63	0.19	0.04–0.39	0.00–0.38	7.78**	32.46
DFO	0.63	0.45–0.77	0.46–0.80	29.76***	17.54	0.24	0.09–0.41	0.05–0.43	9.78***	30.75
DGV	0.61	0.44–0.75	0.43–0.78	28.24***	18.14	0.21	0.09–0.35	0.07–0.36	8.03***	29.44
DGB	0.67	0.44–0.84	0.43–0.91	23.38***	11.71	0.33	0.19–0.53	0.15–0.50	8.57***	17.71
GBFO	0.16	0.05–0.29	0.04–0.28	1.11**	5.83	0.30	0.05–0.51	0.00–0.63	2.35***	5.37
PTIG	0.19	0.09–0.38	0.04–0.34	14.38***	61.33	0.17	0.07–0.31	0.03–0.30	6.53**	32.32
PGOU	0.59	0.45–0.69	0.47–0.71	31.28***	21.57	0.13	0.00–0.30	0.00–0.33	11.66*	78.84
NGOU	0.58	0.45–0.68	0.45–0.71	8887.03***	6431.15	0.16	0.01–0.36	0.00–0.38	2509.44**	12935.53
PTOT	0.08	0.02–0.23	0.00–0.19	9.30 ns	111.90	0.05	0.00–0.16	0.00–0.16	9.52 ns	188.93
P100G	0.39	0.09–0.59	0.03–0.74	0.29***	0.46	0.43	0.19–0.58	0.22–0.64	0.75***	1.00
REPRO	0.73	0.62–0.83	0.62–0.85	0.02***	0.01	0.68	0.25–0.79	0.44–0.90	$5\ 10^{-3}$***	$2\ 10^{-3}$

Significance levels: ns, not significant ($P > 0.05$), *$P \leqslant 0.05$, **$P \leqslant 0.01$, ***$P \leqslant 0.001$

traits (with lower CV_G and CV_e) were those of flowering phenology (DBF, DFO, DGV, DGB).

In the Var population, 4 characters out of 24 had a large CV_G; 10 traits, including the 4 just mentioned, had a large CV_e. Of the 4 characters with both a large CV_G and a large CV_e, 3 (LG1J96, LG134, LG1JBF), all related to the growth of the main axis, had a large heritability. The lower heritability of the remaining trait (LG1J62) was probably due to its larger environmental variance, although differences in CV_e were not significant among the 4 traits. Only 1 other trait was highly heritable in Var (reproductive effort, REPRO), for which both CV_G (10.7%) and CV_e (7.4%) were particularly low compared to the Aude population (respectively 43.6% and 26.3%). Like in Aude, characters related to flowering phenology showed the lowest CV_G and CV_e.

For the 5 out of 7 traits whose heritability estimate differed significantly between the two populations (LG1JBF, LGMJBF, DBF, DFO, DGV), the difference in heritability was due to both larger CV_G and smaller CV_e, although only LGMJBF showed a significant difference in CV_e between populations. It therefore appears that variance for these traits was either genetic, or environmental, but not both. This was not true, however, for traits related to seed production (NGOU and PGOU); their CV_es were similar between the two populations, so that the larger heritabilities in Aude could only be due to significantly larger CV_Gs.

Genetic and phenotypic correlations

Phenotypic correlation matrices calculated from family means were similar to the genetic correlations matrices in each population (Mantel tests: $r = 0.98$, SD = 0.06 and $r = 0.94$, SD = 0.07 in Aude and Var, respectively). Phenotypic and genetic correlations matrices were also positively correlated between populations, but to a much lesser extent ($r = 0.53$, SD = 0.06 and $r = 0.43$, SD = 0.06 for phenotypic and genetic correlations, respectively). We then used phenotypic correlations and *t*-tests calculated in each population to compare the two populations.

Tables 5 and 6 present the phenotypic correlation matrices in Aude and Var, respectively. There were slightly more significant correlations in Aude than in Var (50% versus 42% at $P = 0.05$ for 276 comparisons tested in each population), and this result was confirmed by the sequential Bonferroni test (the experiment-wise

Table 4 Coefficients of genetic variation (CV_G) and coefficients of environmental variation (CV_e) estimated in each population. Confidence intervals of each coefficient were obtained computing Jackknife over families. (¤) indicates characters for which the distribution of Jackknife samples does not significantly depart from normality

Character	Aude				Var			
	CV_G		CV_e		CV_G		CV_e	
DCOT	16.7	11.5–21.8	21.4	18.5–24.3	9.1	4.4–13.8	16.5	12.2–20.8
D6F[a]	7.0	5.6–8.4	7.0	6.2–7.8	4.9	3.4–6.5	7.0	5.9–8.1
LGFT6	14.3	9.0–19.5	21.2	18.4–24.0	7.3	3.2–11.3	16.7	14.2–19.2
LRFT6[a]	16.8	11.7–21.8	16.1	13.4–18.8	8.3	5.3–11.3	16.1	13.2–19.1
SFT6	29.2	20.0–38.5	33.5	28.7–38.3	14.2	8.2–20.2	27.7	23.8–31.7 (¤)
LG1J62	14.9	11.6–18.2	19.7	17.5–21.8	45.8	33.9–57.7	57.7	45.7–69.6
LG1J96[b]	23.9	16.7–31.1	38.0	31.3–44.7	52.9	35.3–70.4	47.4	37.3–57.5 (¤)
LGMJ62	20.9	13.3–28.5	38.1	33.1–43.1	13.2	0.9–25.4	40.3	34.5–46.1 (¤)
LGMJ96[a]	19.3	10.5–28.0	12.4	10.6–14.2	12.1	7.1–17.0	15.7	13.7–17.7
LG134[b]	57.6	41.9–73.2 (¤)	89.1	72.1–106.0	55.4	36.3–74.5	49.9	39.3–60.5 (¤)
LGM34[a]	20.2	10.9–29.5	10.8	9.2–12.4	13.3	8.2–18.4	15.9	13.9–17.9 (¤)
LG1JBF[b, c]	35.8	19.7–51.9	91.5	53.3–129.5	56.2	39.3–73.1	51.2	41.7–60.8 (¤)
LGMJBF[a, c]	17.9	13.6–22.2	10.4	9.0–11.9	8.9	3.0–14.7	21.0	15.2–26.8
DBF[a, c]	5.2	3.4–6.9	3.7	3.1–4.2	3.1	1.2–4.9	6.2	3.9–8.6
DFO[a, c]	4.7	3.2–6.3	3.6	3.0–4.3	3.3	1.5–5.1	5.8	3.9–7.8
DGV[a, c]	4.5	3.1–5.8	3.6	2.9–4.2	2.9	1.4–4.3	5.5	3.9–7.1
DGB[a]	3.3	2.0–4.6	2.3	1.7–3.0	2.3	1.4–3.2	3.3	2.2–4.4
GBFO	3.4	2.0–4.8	7.8	6.8–8.8	4.8	0.9–8.8	7.3	5.9–8.6
PTIG	12.8	8.2–17.3	26.4	21.9–30.9	15.1	9.1–21.1	33.6	28.5–38.8
PGOU[a, c]	45.7	34.0–57.5	38.0	32.2–43.8 (¤)	11.5	0.0–23.2	30.0	24.9–35.1
NGOU[a, c]	42.0	30.9–53.1	35.7	30.7–40.8 (¤)	14.6	3.1–26.0	33.1	24.5–41.7
PTOT	7.3	2.1–12.4	25.3	21.6–28.9	6.6	0.0–14.8	29.6	25.3–33.8
P100G	10.1	3.0–17.2	12.8	10.9–14.7	9.8	5.4–14.1	11.3	9.0–13.5
REPRO[a, b]	43.6	32.7–54.5	26.3	20.8–31.8 (¤)	10.7	3.0–18.4	7.4	4.6–10.2

[a] Characters showing a heritability over 0.50 in Aude
[b] Characters showing a heritability over 0.50 in Var
[c] Characters whose heritabilities are significantly different between the two populations

significance levels were $P = 0.0002$ for Aude and Var, which showed, respectively, 28% and 19% significant correlations). In Var, most of the highly significant correlations were found among growth traits; these correlations were positive. In Aude, the highly significant correlations were mostly found among characters related to either flowering phenology or seed production, both of which were more heritable in this population. Flowering traits were positively correlated to each other (DBF, DFO, DGV, DGB) and negatively correlated to seed production traits (PGOU, NGOU, P100G, REPRO): late-flowering plants produced fewer pods and of a smaller size than early-flowering plants. In contrast, in the Var population, this negative trend between age at reproduction and traits related to offspring production was only significant for the reproductive effort (REPRO). Moreover, plants from Aude produced either numerous and heavy pods or few and small pods ($r = 0.50$, $P < 0.001$, between NGOU and P100G), whereas in Var, there was a trade-off between pod production and pod size ($r = -0.58$, $P < 0.001$, between NGOU and P100G).

Both populations showed a similar trend between growth rate, size, and age at reproduction: slow-growing plants flowered later and at a larger size than fast growing plants (e.g. negative correlations between LGMJ96 and DBF, DFO, DGV, DGB, and positive correlations between LGMJBF and these phenological traits). Whereas larger (later) flowering plants in Aude produced fewer pods than smaller (earlier) plants (negative correlations between LGMJBF and PGOU, NGOU, P100G, REPRO), there was no relationship between size at reproduction and any trait related to pod production in Var.

Signs of correlations among traits related to seedling stage (DCOT, D6F, LGFT6, LRFT6, SFT6) were also similar in both populations: earlier emerging seedlings had larger first leaves. These characters were correlated to only 1 trait of growth in both populations, which was estimated at the beginning of the life cycle (LGMJ62). Unexpectedly, highly significant correlations were found between seedling characters and traits of flowering phenology in Aude (DBF, DFO, DGV, DGB) (earlier emerging plants came earlier into flower), while plants from Var did not show any such relationship.

Table 5 Significant phenotypic correlations calculated from family means in the Aude population

Traits	Traits											
	DCOT	D6F	LGFT6	LRFT6	SFT6	LG1J62	LG1J96	LGMJ62	LGMJ96	LG134	LGM34	LG1JBF
D6F	0.43b											
LGFT6	−0.56c*	−0.48b										
LRFT6	−0.56c*	−0.55c*	0.92c*									
SFT6	−0.56c*	−0.49c*	0.97c*	0.97c*								
LG1J62												
LG1J96												
LGMJ62	−0.41b	−0.32	0.69c*	0.67c*	0.67c*							
LGMJ96			0.39b	0.32a	0.37a			0.66c*				
LG134							0.95c*					
LGM34								0.52c*	0.98c*			
LG1JBF							0.63c*			0.58c*		
LGMJBF	0.35a	0.47b	−0.30a	−0.38a	−0.38a				0.43b		0.46b	
DBF	0.45b		−0.57c*	−0.59c*	−0.63c*			−0.39b	−0.48c*		−0.46b	
DFO	0.43b		−0.56c*	−0.60c*	−0.63c*			−0.39b	−0.46b		−0.44b	
DGV	0.45b	0.31a	−0.57c*	−0.61c*	−0.64c*			−0.38a	−0.42b		−0.39b	
DGB	0.37a		−0.54c*	−0.59c*	−0.62c*			−0.34a	−0.43b		−0.41b	
GBFO	−0.39b		0.34a	0.33a	0.33a			0.32a	0.31a			
PTIG							0.43b			0.32a		0.35a
PGOU				0.37a	0.38a							
NGOU							0.31a					
PTOT	−0.34a		0.31a	0.40b	0.42b							
P100G				0.36a	0.36a							
REPRO												

Table 5 Continued

Traits	Traits										
	LGMJBF	DBF	DFO	DGV	DGB	GBFO	PTIG	PGOU	NGOU	PTOT	P100G
DBF	0.51c*										
DFO	0.53c*	0.99c*									
DGV	0.55c*	0.98c*	0.99c*								
DGB	0.54c*	0.95c*	0.97c*	0.97c*							
GBFO		−0.57c*	−0.57c*	−0.55c*	−0.35a						
PTIG	0.54c*	0.39b	0.36a	0.38a	0.39b						
PGOU	−0.66c*	−0.80c*	−0.82c*	−0.84c*	−0.83c*	0.36a	−0.49c*				
NGOU	−0.63c*	−0.76c*	−0.78c*	−0.80c*	−0.77c*	0.39b	−0.44b	0.97c*			
PTOT		−0.49c*	−0.54c*	−0.54c*	−0.52c*	0.33a	0.39b	0.60c*	0.62c*		
P100G	−0.49c*	−0.61c*	−0.60c*	−0.63c*	−0.63c*		−0.46b	0.68c*	0.50c*		
REPRO	−0.72c*	−0.78c*	−0.78c*	0.81c*	−0.79c*	0.34a	−0.69c*	0.95c*	0.92c*	0.38a	0.70c*

Significance levels: a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$
Probability for experiment-wise significances using the Bonferroni test $*P < 0.05$

Discussion

Given that directional selection is expected to reduce additive variance, life-history traits are expected to show lower heritabilities than traits presumably subject to less strong selection and thus evolve less readily (Mousseau and Roff 1987; Roff and Mousseau 1987; Falconer 1989; Platenkamp and Shaw 1992; but see Venable and Burquez 1989; Till-Bottraud et al. 1990). In a review of about 800 quantitative characters, Houle (1992) found that fitness components possessed coefficients of additive variation that were higher than those of morphological traits, and that their low heritability was best explained by their high residual variation. Two explanations can be suggested: either these characters are more affected by the large number of genetic and environmental events occurring throughout the lifetime of the organism (Price and Shulter 1991), or their genetic and environmental variances are less reduced by stabilizing selection, i.e., they are more often subject to directional selection (Houle 1992). According to other authors (Stearns et al. 1995), however, traits more closely related to fitness are expected to be more

Table 6 Significant phenotypic correlations calculated from family means in the Var population

Traits	Traits											
	DCOT	D6F	LGFT6	LRFT6	SFT6	LG1J62	LG1J96	LGMJ62	LGMJ96	LG134	LGM34	LG1JBF
D6F	0.50b											
LGFT6	−0.47b*											
LRFT6	−0.58c*	−0.48b	0.83c*									
SFT6	−0.53b*	−0.44a	0.95c*	0.95c*								
LG1J62				0.36a	0.35a							
LG1J96						0.83c*						
LGMJ62	−0.53b		0.49b	0.67c*	0.59c*							
LGMJ96						0.73c*	0.91c*					
LG134						0.77c*	1.00c*		0.92c*			
LGM34		0.37a				0.71c*	0.91c*		0.98c*	0.91c*		
LG1JBF				0.38a		0.81c*	0.98c*		0.85c*	0.97c*	0.85c*	
LGMJBF						0.35a	0.43a		0.37a	0.43a	0.38a	0.53b
DBF						−0.40a	−0.48b		−0.60c*	−0.47b	−0.56c*	−0.35a
DFO						−0.44b	−0.56c*	−0.38a	−0.66c*	−0.55b	−0.62c*	−0.43a
DGV						−0.49b	−0.59c*	−0.36a	−0.69c*	−0.58c*	−0.66c*	−0.48b
DGB						−0.59c*	−0.73c*		−0.84c*	−0.73c*	−0.82c*	−0.65c*
GBFO												
PTIG												
PGOU						0.35a	0.42a		0.39a	0.41a	0.41a	0.35a
NGOU						0.39a	0.52b		0.44b	0.52b	0.46b	0.49b
PTOT												
P100G							−0.44a		−0.36a	−0.45b	−0.35a	−0.47b
REPRO							0.44a		0.40a	0.45b	0.46b	0.38a

Table 6 Continued

Traits	Traits										
	LGMJBF	DBF	DFO	DGV	DGB	GBFO	PTIG	PGOU	NGOU	PTOT	P100G
DBF	0.50b										
DFO	0.40a	0.98c*									
DGV	0.36a	0.98c*	0.99c*								
DGB		0.87c*	0.89c*	0.90c*							
GBFO	−0.64c*	−0.50b*	−0.49b	−0.43a							
PTIG	0.39a	0.50b	0.48b	0.50b	0.57c*						
PGOU											
NGOU									0.88c*		
PTOT								0.66c*	0.86c*	0.73c*	
P100G										− 0.58c*	
REPRO		−0.50b	−0.47b	−0.52b	− 0.59c*		−0.67c*	0.58c*	−0.55b		− 0.35a

Significance levels: a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$
Probability for experiment-wise significances using the Bonferroni test $*P < 0.05$

canalized than others. Stabilizing selection should favor this process by which the phenotypic variation due to genetic or environmental disturbances is reduced by developmental mechanisms (Waddington 1957 in Falconer 1989). If we take into account both of the populations we studied here, the heritabilities of characters likely to be related to fitness, such as age and size at flowering and pod production, were not significantly different from the heritabilities of characters less obviously related to fitness, such as juvenile characters. The measure of coefficients of variation shows, however, that all characters of phenology, including age at reproduction, present simultaneously the lowest environmental variation and the lowest genetic variation in both populations, whereas the largest coefficients of variation are found for characters related to growth of the main axis. Thus, among fitness components, age at flowering seems to be very canalized, whereas no general trend appears for the other components. This could be explained by stabilizing selection acting on flowering phenology.

Heritabilities of characters related to overall development were particularly high in the Aude population. Non additive effects such as epistatic or maternal

effects could explain the high values of broad-sense heritabilities found in Aude. We have assumed, however, that the populations consisted of homozygous lines or clones because of an inbred mating system; in this case, epistatic variance should be inherited and reduced by directional selection in the same way as additive variance. Moreover, it is difficult to distinguish any possible maternal effects from the among-family variance in our study. Maternal effects are usually attributed to non-genetic factors, such as mother-plant vigor, which influence the maternal provisioning of offspring (Roach and Wulff 1987). This kind of maternal effect has generally been found to be more important in early stages of the life-cycle (Mitchell-Olds and Bergelson 1990) but it can also remain important in later stages (I. Till-Bottraud, personal communication). Among the characters highly heritable in Aude, only those of flowering phenology were correlated to seedling traits. Thus, differences in initial maternal vigor could influence the variability of these characters, which showed both very low genetic and environmental variances. Furthermore, estimates of heritabilities in greenhouse and garden studies may not reflect those in the wild. Environmental sources of variation are different and probably much higher in natural conditions and, consequently, heritabilities lower (Mitchell-Olds 1986; Schwaegerle and Levin 1991; but see Wolff and Van Delden 1987; Venable and Burquez 1989). However, low-realized heritabilities *in natura* cannot explain the very large coefficients of genetic variation observed for pod production traits in the Aude population.

Spatially and temporally variable selection is expected to prevent the erosion of genetic variability, in particular with respect to quantitative characters (Via and Lande 1985; Endler 1986; Schemske and Horvitz 1989; Kelly 1992). This could be happening in the Aude population, where environmental variation seems larger, both in space and in time. The Aude population borders a vineyard which is ploughed and harvested every year; it is likely that disturbances are very frequent at this site. Moreover, being subdivided, the population from Aude could maintain a higher level of heritable variation than expected under drift acting at the population level as a whole (Lande 1991; Goldstein and Holsinger 1992; Widén and Andersson 1993). We have also suggested that some outcrossing events following rare migrations events could explain the high level of variability found within subpopulation of this population (Bonnin et al. 1996a). In this case, the magnitute of heritabilities we found in Aude would be even under-estimated.

Other mechanisms could explain the maintenance of additive variance for fitness components. Under most models of mutation-selection balance, the genetic variance of a trait is correlated with the number of loci involved (Barton and Turelli 1989; Houle 1991). If fitness is influenced by many or most genes of an organism, the increase in genetic variance due to new mutations could be larger for fitness components than for other characters. Bürger (1993) showed that, in large populations, a slow steady flow of moderately advantageous mutations under directional selection could actually increase the additive genetic variance.

Trade-offs among traits could also protect the variability of fitness components from directional selection. In this case a change in one character in a direction that increases fitness implies a change in another trait that decreases fitness, so that genetic variability for a given fitness component may appear selectively neutral (Stearns 1992). However, very few negative correlations were observed in both populations. As discussed by several authors (van Noordwijk and de Jong 1986; Charlesworth 1990; de Laguérie et al. 1991; Houle 1991), this does not preclude the existence of trade-offs: a large variance in resource acquisition will lead to positive covariation among life-history traits, whereas a negative covariance will be obtained if variance in acquisition is small. This could well explain why no trade-off was observed between pod size (P100G) and pod number (NGOU) in the Aude population, where, compared to Var, there seems to be more genetic variation for at least some growth components (LGMJBF, LGM34, LGMJ96) and for flowering date (both kinds of traits are obviously related to resource acquisition). Moreover, in the case of covariance between traits determined through successive steps of allocations of a resource, de Jong (1993) showed that the sign of the covariance will also depend on the respective position of the traits in the allocation 'tree' and on allocation magnitudes.

In annual plant species, early reproduction may be favored if adult mortality is high, as a consequence of predation or disturbances. Conversely, late reproduction may allow a higher acquisition of resources during the time spent in the vegetative stage, leading *in fine* to a better reproductive success. In both populations, correlations between age and size at flowering were indeed found to be positive (see also Farris and Lechowicz 1990; Dorn and Mitchell-Olds 1991; Aarsen and Clauss 1992). According to Dorn and Mitchell-Olds (1991), the absence of both large and early-flowering plants could be explained by physiological constraints on photosynthesis and growth, while small and late-flowering plants would be eliminated by natural selection. In Aude, although larger at flowering, late-flowering individuals produced fewer pods than smaller, early-flowering genotypes. It could be that re-allocation of resources towards reproduction was impossible under the experimental conditions, or that such re-allocation was not part of their competitive strategy. The competitive conditions experienced in the Aude population could explain the individuals growth pattern (lack of main axes) observed in this population.

The two populations of *Medicago truncatula* described in this paper showed a very different level of

variability for quantitative traits, thus confirming our previous results on molecular markers (Bonnin et al. 1996a): the population from Var is significantly less variable than the one from Aude for both genetic markers and quantitative characters. Moreover, the two populations have diverged for most quantitative traits. Because the divergence of molecular markers is less marked (Bonnin et al. 1996b), it is likely that the divergence of quantitative characters has been caused by heterogeneous selection acting upon them. The reasons why the Aude population is more polymorphic than the Var population could be related to the stronger population structure in the former, but also to historical differences, or differences in the mating system, with some rare outcrossing events in the Aude population allowing the retention of a larger polymorphism.

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