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Changes in life-history traits in an expanding moss species: phenotypic plasticity or genetic differentiation? A reciprocal transplantation experiment with *Pogonatum dentatum*

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A transplant experiment was performed to investigate whether differences in life-history traits of the bryophyte *Pogonatum dentatum* that recently expanded its distribution range, were genetically or environmentally determined, or a combination of both. Plants were transplanted reciprocally between the original mountain area and a recently colonised lowland area. Vegetative biomass of annual segments and branches tended to be higher in the mountain area than in the lowland area. Reproductive investment was higher for plants transplanted to the lowland area, and lowland shoots tended to produce larger sporophytes than mountain shoots when placed in the same environment. Age of reproducing shoots showed no consistent pattern.
Native shoots were often outperformed by non-native shoots transplanted into the same site. Non-native shoots grew larger and produced larger sporophytes than native shoots. Much of the observed variation was at the site level instead of between mountain and lowland areas, with both genetic origin and environmental effects contributing together. Range expansion of *P. dentatum* may have taken place by dispersal from populations with shoots whose growth is plastic. Such shoots grow larger and potentially produce more spores for dispersal.

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Observed differences in life-history strategies between populations can be explained by genetic differentiation or by phenotypic plasticity. Genetic differentiation can occur when there is genetic variation in the traits and the strength or direction of selection differs due to variation in factors such as climate, competition or difference in habitat parameters (Ronce and Olivieri 1997). Another force, which can be especially important for species expanding their range, is genetic drift during founding events (Hartl and Clark 1989). But observed phenotypic differences in life-history traits may also be the result of phenotypic plasticity (Via and Lande 1985). Phenotypic plasticity enables individuals to alter traits in response to environmental conditions and is expected to be an important way for sessile organisms to deal with variable environments (Schlichting 1986).

Bryophytes have a dominant haploid stage in their life cycle and both sexually and vegetatively produced diaspores are haploid. Thus natural selection is expected to act in a very direct fashion. This would lead to low genetic variation within specific habitats. However, most studies of molecular genetic variation in bryophyte populations have shown the same level of variation as in populations of vascular plants (Wyatt et al. 1989 and references therein).

Pogonatum dentatum is a bryophyte that, during the 20th century, expanded its range. In Fennoscandinavia its distribution was mainly restricted to arctic-alpine

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areas (Söderström 1992) whereas it now occurs commonly along forest roads in major parts of Fennoscandia (Fagerstén 1977, Hedenäs 1983, Hedenäs et al. 2002). Changes in habitat availability and disturbance regimes have been suggested as potential factors leading to the expansion of *P. dentatum* in the lowlands (Hassel and Söderström 2003).

Phenotypic changes in life history characters can also promote expansion. It is notable that *P. dentatum* seems to do better in the lowlands, where it reproduces freely and matures at a younger age than in the mountains (Hassel et al. unpubl.). Female shoots in the lowland area also grow faster. No overall difference in sexual reproductive investment (RI) was detected between the lowland and mountain areas with a mean RI of ca 35% (Hassel et al. unpubl.). Nevertheless, there were significant differences among populations. This was also true for other life-history traits such as gametophore size and spore size, in which phenotypic differences were found among populations rather than between areas (Hassel et al. unpubl.).

Is the observed phenotypic variation in life-history traits of *P. dentatum* related to genetic differentiation between the lowland and mountain areas or among populations, as earlier results seem to indicate. A study of the same populations using neutral molecular markers (inter-simple sequence repeats, ISSR) failed to reveal any genetic differentiation between the lowland and mountain areas, again with most of the variation among populations (Hassel et al. unpubl.). That study also showed that mountain populations were more strongly differentiated from each other than were lowland populations.

In this study we make an assessment of whether genetic or environmental variation can account for observed differences in life history traits of *P. dentatum* shoots between sites in the original mountain area and recently colonised lowland sites with a transplant experiment. Here we focus on 1) age of reproducing shoots, 2) size of annual growth segments and branches and, 3) sexual reproductive investment.

Method

Study species

Pogonatum dentatum (Bryophyta: Polytrichaceae) is a dioecious moss, in which individual shoots rarely become older than four years (Hassel and Söderström 2003) and gametophore size varies from just a few millimetres in juvenile plants to ca 2 cm in adults. The haploid gametophyte life cycle starts either with a spore (sexual reproduction) or an asexually produced fragment (e.g. leaf, detached branch, part of stem), both forming a filamentous protonema from which one or several gametophores develop. Shoots of the Polytrichaceae have a segmented appearance due to regular variation in leaf length, and these segments have been shown to represent annual growth (Longton and Greene 1967, Watson 1975). The diploid sporophyte is initiated after a successful fertilisation of a female archegonium by an antherozoid. The zygote develops into a sporophyte that is apically attached to the female gametophore, and consists of a short foot, an elongated seta (7–24 mm), and the capsule, in which haploid spores are produced by meiosis. Production of an archegonium terminates the growth of the gametophore, unless a branch (subapical innovation) is produced.

In this paper the term "population" means a group of patches of *P. dentatum* shoots occurring at a restricted site. A "patch" is a cluster of shoots that can consist of one or more individuals/genets. A "shoot" or ramet is a potentially independent gametophore, but it may be connected with nearby shoots by underground rhizoids. A "branch" is an aboveground innovation on a shoot.

Study area

Populations were sampled from two areas in northern Sweden. The mountain area is in the alpine region at Stekenjokk (65°05′N, 14°30′E, altitude 800 m), on a mountain heath above the tree limit. The lowland area is in the boreal region at Junsele (63°45′N, 17°15′E, altitude 300 m), in spruce forest. The mountain and lowland areas are part of the same valley and river system, Ångermanälven (Fig. 1). In the study area *P. dentatum* typically occurs on disturbed mineral soil. In the mountains the main disturbance factors are frost heaving and wind, whereas in the lowlands road construction and maintenance are the most common causes of disturbances that lead to creation of new habitats. Annual precipitation, mean July temperature and length of growing season are 1000–1100 mm, 8°C and 110–120 d in the mountain area compared to 600–700 mm, 12°C and 150–160 d in the lowland area (Anon. 1995). Temperature logger data for September 2001–August 2002 showed that the snow-free period was ca 183 d at site M2 (mountain) and 177 d at site L5 (lowland). The mean mid-day temperatures during September 2001 and the period from 1 May 2002 to 25 August 2002 were 16.4°C at the mountain site and 17.4°C at the lowland site. In the mountains there were several periods without or with only a thin snow cover during winter, exposing the shoots to temperatures below −20°C. In the lowland snow covered the ground during all winter.

Sampling

The experiment was initiated in early September 1999 and transplant units (patches) were collected in late

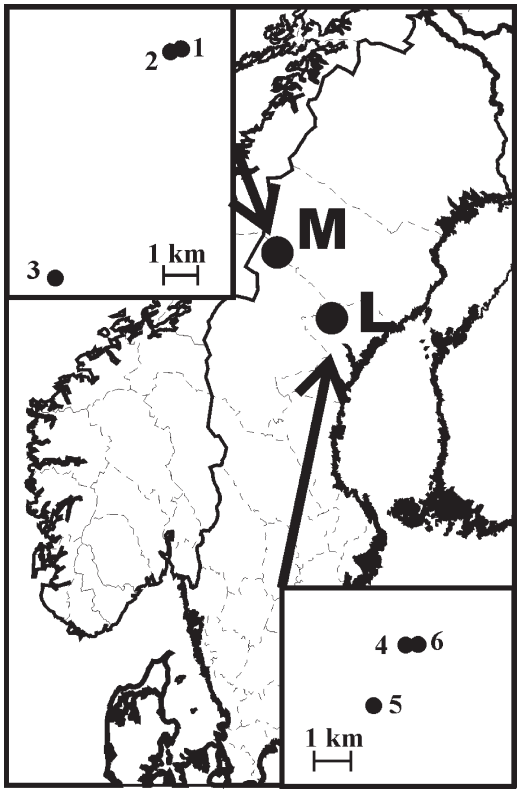

Fig. 1. Map of Scandinavia indicating the study areas, M and L, which represent lowland and mountain areas respectively. The small maps indicate the distance between the studied populations within each area numbers refer to the respective sites.

August 2002. In the mountains three populations (M1-3) were sampled from disturbed mineral soil in mountain heath and in the lowlands three populations (L4-6) were sampled from mineral soil at forest roads. Within each site seven patches (transplant units) were delimited by plastic cylinders of 103 mm diameter and 30 mm height. The plastic cylinders were pressed into the soil and marked. At each site, patches were marked and randomly assigned to one control (not moved), one internal transplant (taken up and put back at the same site), and five external transplants. The transplants were cut out of the soil at the level of the lower edge of the plastic cylinder. External transplants were transported in boxes to their new site within 48 h. Two of the external transplants were moved to the sites of the other populations within the same area, and three were moved to the three sites in the other area. The transplant units were separated from each other by at least 1 m to minimise the probability of transport of fragments between transplant/control units. It was not possible, however, to completely eliminate the possibility of spore dispersal between transplants.

Measurements

After three years, the transplants and control units were harvested and transported to the laboratory where they were stored at 4°C until examined. All reproducing female shoots (617 shoots) were collected and classified according to their stage in sporophyte development (sensu Greene 1960) and age. Shoots with sporophytes bearing swollen capsules and intact opercula (corresponding to stage 5 and 6 of Stark's (2002) classification) were dried and weighted (340 shoots). Before weighing, the shoots were dried at 70°C for 24 h, and sporophytes were detached from the gametophores by cutting the seta just above the foot. Gametophores were cut into segments representing annual growth. The G0 segment holds the current sporophyte; the G1 segment was formed the previous year (Fig. 2). Branches connected to the annual segments (G0 and G1) were detached and named B0 and B1 (Fig. 2). Sporophytes were consequently weighed without their calyptra, as some had lost the calyptra before harvest. The weight of the calyptra is so small that it should not influence the results.

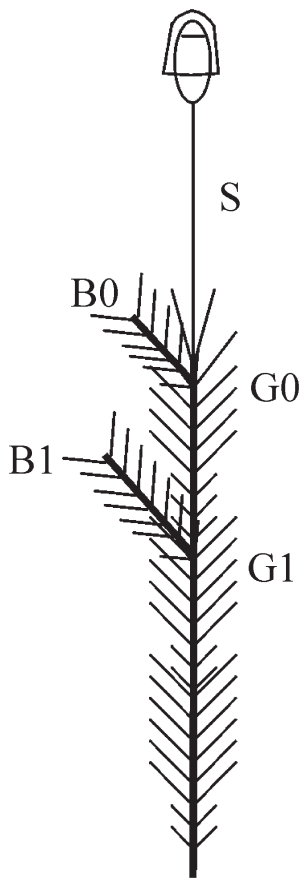

Fig. 2. Schematic illustration of a reproducing female shoot of *Pogonatum dentatum*, showing the annual growth segments (G0 and G1) with their connected branches (B0 and B1) and the sporophyte (S) attached to the G0 segment.

Perichaetal leafs were included in the weight of the G0 segment, as they besides protection of the embryo are similar to and have same function as ordinary leafs. Total biomass includes all present growth segments of the gametophore and the sporophyte. All segments were weighed separately. Reproductive investment (RI), which is the ratio between biomass invested in sexual reproduction compared to the total biomass, were used as a practical approximation to the theoretically based concept of reproductive effort (Harper and Ogden 1970, Hassel et al. unpubl.), was calculated in three different ways as:

$$RI_1 = \frac{\text{sporophyte}}{\text{sporophyte} + \text{G0}} \quad (1)$$

$$RI_2 = \frac{\text{sporophyte}}{\text{sporophyte} + \text{G0} + \text{G1} + \text{B1}} \quad (2)$$

and

$$RI_3 = \frac{\text{sporophyte}}{\text{total biomass}} \quad (3)$$

respectively. The three definitions of RI differ in which parts of the gametophore that are included in the denominator. Ideally one should only include those parts that were photosynthetically active and thereby potentially could support the development of the sporophyte (Bisang and Ehrlén 2002). However, due to the obvious difficulties of determining which parts these were, we chose to compare different measures of RI to see whether conclusions regarding reproductive investment were sensitive to details in how it was defined.

Statistical analysis

The effect of transplantation among sites of origin, new sites, and the lowland and mountain area on age at sexual reproduction, reproductive investment ($RI_1 - RI_3$) and weight of annual segments, branches, and sporophytes were examined using analysis of variance (ANOVA), Anon. For descriptions of the explanatory variables, see Table 1. The transplant design corresponds to the following linear model:

$$\begin{aligned} X_{ijklmn} = {} & \mu + A_i + S(A)_{j(i)} + nA_k + nS(nA)_{l(k)} + AnA_{ik} \\ & + AnS(nA)_{il(k)} + nAS(A)_{jk(i)} + S(A)nS(nA)_{jl(ik)} \\ & + T(S(A)nS(nA))_{m(ijkl)} \\ & + \varepsilon(T(SA)nS(nA)))_{n(ijklm)}, \end{aligned} \quad (4)$$

where μ is the overall mean shoot age or size of growth segments, branches, or sporophytes. The subscripts i, j, k, l, m and n denote area of origin, site of origin, new area, new site, transplant unit, and shoot, respectively. There is no test for the presence of interaction effects between site of origin and new site (SnS) due to lack of replication. Thus SnS and transplant unit (T) are confounded. Instead we tested the confounded factor TSS = SnS + T(SnS) against the residual variance. Thus, we analysed the response variables according to the following model:

$$\begin{aligned} X_{ijklmn} = {} & \mu + A_i + S(A)_{j(i)} + nA_k + nS(nA)_{l(k)} + AnA_{ik} \\ & + AnS(nA)_{il(k)} + nAS(A)_{jk(i)} + TSS_{m(ijkl)} \\ & + \varepsilon(TSS)_{n(ijklm)}, \end{aligned} \quad (5)$$

where μ and subscripts are as for model 4. If no effects of TSS were found, we assumed that both the variance in transplant units (σ^2_T) and the site interaction component (σ^2_{SnS}) were zero. In principle, as S(A) and nS(nA) both are random factors and orthogonal to each other, there are no ordinary F-ratios that can be used as test statistics when testing for main effects of original area (A), new area (nA) and their interaction (AnA), but such test statistics can be obtained after post hoc pooling with respect to the following terms in the linear model: S(A), nS(nA), AnS(nA) and nAS(A). To obtain test statistics for all three tests, at least two of these sources of variation should be pooled. If this was not possible, we used quasi F-ratios (Underwood 1997) as test statistics. Procedures for post hoc pooling followed Underwood (1997).

All tests were model-based permutation tests (Cade and Richards 1996, Anderson and Legendre 1999). Sample sizes in these data sets were kept equal to those of the original data. Residuals were checked for homoscedasticity. Permutations were done under the full model (ter Braak and Šmilauer 1998). The dependent variables age and segment G1 were square-root transformed before analysis to fulfil the assumption of homoscedasticity.

Table 1. Explanatory variables or factors included in analyses of the transplant experiment involving *Pogonatum dentatum*.

Variable	Description	
A	Area of origin, two levels: mountain and lowland	Fixed factor
nA	New area, two levels: mountain and lowland	Fixed factor
S	Site of origin, six levels (1–6), nested within levels of A	Random factor
nS	New site, six levels (1–6), nested within levels of nA	Random factor
T	Patch (transplant unit), one unit for each combination of levels of S and nS. So T is nested within levels of both S and nS, but confounded with the SnS interaction	Random factor
ε	Shoot, number of levels varies with each transplant unit and explanatory variable	Random factor

Effects of the transplant procedure on age, weight of annual segments, and sporophytes between the internal transplants and the unmoved control were tested by a two-way ANOVA, of the following model:

$$X_{ijkl} = \mu + K_i + S_j + KS_{ij} + T(KS)_{k(ij)} + \varepsilon(T(KS))_{l(ijk)}, \quad (6)$$

where μ and subscripts are as for model 4, and the explanatory variable K is a fixed factor and has two levels represented by the internal transplant and the unmoved control.

Results

During the experiment there were no indications that shoots in the transplant unites suffered from the treatments. The transplant procedure did not have any effect on the investigated variables (shoot age $F = 0.005$, $p > 0.75$; segment G0 $F = 0.022$, $p > 0.75$; segment G1 $F = 0.723$, $p > 0.25$; sporophyte $F = 0.294$, $p > 0.50$ and RI_2 $F = 0.509$, $p > 0.50$). B1 could not be tested due to lack of degrees of freedom. Sporophyte production varied among years, at harvest in 2002 the frequency of sporophytes per transplant unit was 12 compared to 14, 26 and 16 in the three previous years. During the experiment four transplant/control units were lost for unknown reasons, and at the time of harvest 14 of the remaining 38 transplants/controls were without sporophytes. In all but two of the transplants/controls shoots with sporophytes had been found at least once the previous years. At the time of harvest the five remaining transplants at site M3 were without sporophytes. At each of the sites M1, M2 and L4, three transplants were without sporophytes, and at site L5 and L6 two and one transplants, respectively, were without sporophytes. Only transplant units and controls with sporophytes at the time of harvest are included in the analysis (Table 2). Mean age of the analysed shoots was 1.7 yr. Branch size B0 was excluded from the statistical analysis due to too few observations.

Results of the statistical analyses of the transplantation experiment are presented in Table 3. The confounded variable TSS did not contribute significantly to the total variation for any of the response variables. However, for many response variables there were significant cross level interaction effects between source area and new site and between new area and source site (i.e. AnS and nAS). Moreover, for B1 we found a significant interaction between source area and new area (AnA).

Age of shoots with sporophytes varied among sites from which they originated (Fig. 7a). The largest difference was between mountain site M2 and lowland site L5. In general, there were no large differences among mountain plants and lowland plants for this trait, except when transplanted to site L6 (Fig. 5c).

Table 2. The number of shoots with sporophytes in each transplant unit at harvest time. The other transplant units did not contain mature sporophytes at harvest. M and L denotes mountain and lowland, respectively.

New habitat	New site	Habitat of origin	Site of origin	Number of shoots
M	1	M	1	5
M	1	L	4	35
M	1	L	5	18
M	2	M	3	14
M	2	L	4	1
M	2	L	6	6
L	4	M	2	16
L	4	M	3	6
L	4	L	4	13
L	5	M	2	49
L	5	L	4	29
L	5	L	5	4
L	5	L	6	35
L	6	M	1	7
L	6	M	2	37
L	6	M	3	11
L	6	L	4	24
L	6	L	6	30

Vegetative biomass tended to be higher in the mountain area than in the lowland area (Fig. 3–5a). Shoots produced larger G1 segments when grown in the mountain area with the exception of population M3 and L5 which grew nearly equal G1 segments in both areas (Fig. 3b). G0 segments also tended to grow larger in the mountain but the pattern was less clear and shoots originating from site M3 grew better in the lowland area (Fig. 3a).

Plants growing in the mountain area had the largest branches (Fig. 4). In addition, lowland plants moved to the mountains (L to M) had larger branches in mountain areas than plants with their origin there (M to M), suggesting genetic differentiation in addition to the environmental effects (Fig. 4). However, much of the observed variation in the measured traits is found at the site (population) level, both effects due to site of origin and effects due to the new site. Size of segment G0 showed differences in phenotypic response among populations moved to different areas (Fig. 3a). In addition there was a significant trend that shoots from sites performing well in one area also performed well in the other area (significant main effect of S), e.g. shoots originating from site L6 grew better than shoots from most other sites regardless of area.

Sporophyte biomass depended on which site they originated from (Table 3, Fig. 7b). Further, differences in sporophyte biomass depended on the site to which they were transplanted. For the most part, plants with origins in the lowlands produced larger sporophytes than plants with mountain origin except at site L6, in which sporophytes with mountain origin were largest (Fig. 5b).

The results for reproductive investment (RI) depended on which measure was used. RI_2 and RI_3 were sig-

Table 3. Analysis of variance of size of G0, G1, B1 and sporophyte and RI_2 and shoot age in *Pogonatum dentatum* according to eq. (5). Symbols for response variables are explained in Fig. 2, and sources of variation in Table 1. Significance is evaluated by permutation tests, with 999 permutations for each test. G0, G1, and Sporophyte were square-root transformed before analysis. *Test statistic calculated after eliminating *TSS* from the linear model. ** Test statistic calculated after eliminating one or more of S, nS, AnS and/or AnS from linear model following procedures in Underwood (1997). † Mean square could not be calculated due to few transplants with branching shoots.

Response variable/source of variation	DF	Mean square	Test statistic	F	p
G0					
A	1	8.903×10^{-4}	$(MS_A + MS_{res})/(MS_{AnS} + MS_S)^*$	1.854	0.197
S	4	4.512×10^{-4}	MS_S/MS_{res}^*	15.899	0.001
nA	1	4.578×10^{-4}	$(MS_{nA} + MS_{res})/(MS_{nS} + MS_{nAS})^*$	2.022	0.203
nS	3	0.770×10^{-4}	MS_{nS}/MS_{res}^*	2.713	0.033
AnA	1	0.421×10^{-4}	$(MS_{AnA} + MS_{res})/(MS_{AnS} + MS_{nAS})^*$	0.339	0.929
AnS	3	0.444×10^{-4}	MS_{AnS}/MS_{res}^*	1.564	0.243
nAS	3	1.634×10^{-4}	MS_{nAS}/MS_{res}	5.759	0.002
TSS	3	0.010×10^{-4}	MS_{TSS}/MS_{res}	0.350	0.780
Residual	320	0.285×10^{-4}			
G1					
A	1	3.105×10^{-4}	MS_A/MS_{AnS}^{**}	1.163	0.369
S	4	0.141×10^{-4}	MS_S/MS_{res}	0.635	0.626
nA	1	16.638×10^{-4}	$(MS_{nA} + MS_{res})/(MS_{nS} + MS_{nAS})^{**}$	12.435	0.002
nS	3	0.430×10^{-4}	MS_{nS}/MS_{res}^*	1.940	0.124
AnA	1	0.791×10^{-4}	$(MS_{AnA} + MS_{res})/(MS_{AnS} + MS_{nAS})^{**}$	0.281	0.967
AnS	3	2.670×10^{-4}	MS_{AnS}/MS_{res}^*	12.042	0.001
nAS	3	0.925×10^{-4}	MS_{nAS}/MS_{res}	4.174	0.008
TSS	3	0.247×10^{-4}	MS_{TSS}/MS_{res}	1.116	0.340
Residual	163	0.221×10^{-4}			
B1					
A	1	3.108×10^{-7}	MS_A/MS_{res}^{**}	6.190	0.011
S	4	0.300×10^{-7}	MS_S/MS_{res}^{**}	0.528	0.627
nA	1	7.252×10^{-7}	MS_{nA}/MS_{res}^{**}	14.444	0.001
nS	3	0.596×10^{-7}	MS_{nS}/MS_{res}	1.050	0.348
AnA	1	2.593×10^{-7}	MS_{AnA}/MS_{res}^{**}	5.164	0.033
AnS	3	0.115×10^{-7}	MS_{AnS}/MS_{res}^{**}	0.202	0.854
nAS	2	0.070×10^{-7}	MS_{nAS}/MS_{res}	0.123	0.824
TSS†					
Residual	39	0.568×10^{-7}			
Sporophyte biomass					
A	1	1.260×10^{-4}	$(MS_A + MS_{res})/(MS_{AnS} + MS_S)^*$	0.313	0.949
S	4	0.582×10^{-4}	MS_S/MS_{res}^*	2.837	0.025
nA	1	0.022×10^{-4}	$(MS_{nA} + MS_{res})/(MS_{nS} + MS_{nAS})^*$	0.201	0.993
NS	3	0.681×10^{-4}	MS_{nS}/MS_{res}^*	3.314	0.020
AnA	1	0.038×10^{-4}	$(MS_{AnA} + MS_{res})/(MS_{AnS} + MS_{nAS})^*$	0.053	0.999
AnS	3	4.103×10^{-4}	MS_{AnS}/MS_{res}^*	19.973	0.001
nAS	3	0.455×10^{-4}	MS_{nAS}/MS_{res}	2.213	0.092
TSS	3	0.104×10^{-4}	MS_{TSS}/MS_{res}	0.506	0.669
Residual	320	0.206×10^{-4}			
RI_2					
A	1	0.0211	MS_A/MS_{TSS}^{**}	1.781	0.186
S	4	0.0102	MS_S/MS_{TSS}^{**}	0.798	0.596
nA	1	0.7408	MS_{nA}/MS_{nS}	16.705	0.030
nS	3	0.0443	MS_{nS}/MS_{TSS}^{**}	3.480	0.170
AnA	1	0.0299	MS_{AnA}/MS_{TSS}	2.523	0.144
AnS	3	0.0178	MS_{AnS}/MS_{TSS}	1.397	0.407
nAS	3	0.0073	MS_{nAS}/MS_{TSS}	0.573	0.659
TSS	3	0.0127	MS_{TSS}/MS_{res}	1.866	0.128
Residual	320	0.0068			
Age					
A	1	10.824	$(MS_A + MS_{res})/(MS_{AnS} + MS_S)^*$	1.362	0.311
S	4	2.901	MS_S/MS_{res}^*	7.037	0.001
nA	1	0.445	$(MS_{nA} + MS_{res})/(MS_{nS} + MS_{nAS})^*$	0.311	0.936
nS	3	2.163	MS_{nS}/MS_{res}^*	5.248	0.002
AnA	1	0.743	$(MS_{AnA} + MS_{res})/(MS_{AnS} + MS_{nAS})^*$	0.195	0.991
AnS	3	5.352	MS_{AnS}/MS_{res}^*	12.984	0.001
nAS	3	0.586	MS_{nAS}/MS_{res}	1.422	0.248
TSS	3	0.207	MS_{TSS}/MS_{res}	0.500	0.657
Residual	320	0.414			

nificantly higher for plants transplanted to the lowland area (only results for RI_2 shown, Fig. 6), whereas RI_1 did not vary among treatments (results not shown).

The significant interaction effects for G1 segment size, sporophyte size and shoot age between area of origin and new site all seem mainly to be due to site L6, where

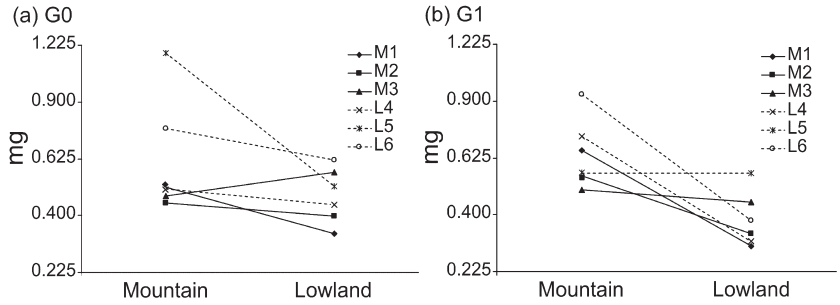

Fig. 3. Mean size (mg) of (a) segment G0 and (b) segment G1 in *Pogonatum dentatum* shoots grown in their area of origin and in a new area. M and L denote mountain and lowland source sites.

shoots responded differently compared to all other sites (Fig. 5).

Discussion

Transplant experiments are useful in genecological research when the aim is to differentiate between environmental and genetic effects on the phenotype (Shaw 1991, Briggs and Walters 1997), but caution is necessary when interpreting differences due to origin as genetic effects. One recurrent problem with transplant experiments is the possibility of carry-over effects (i.e. effects due to the original environment from which the transplant was taken). When present, such effects cannot be separated from genetic effects. Carry-over effects can occur when some element of the original environment is transplanted with the bryophytes, when epigenetic effects are present (Jablonka and Lamb 1995), or when developmental and physiological processes in the original habitat continue to determine the phenotype after transplanting (Bakken 1995). In this experiment, nearly all harvested shoots originated from diaspores after transplanting. However soil was transplanted together with the shoots and soil most likely differed between mountain areas and the forest road localities sampled in the lowland. Effects due to origin are mostly observed at the site level while effects of area of origin are dependent on which site (and not area) the shoots were transplanted to. Even though such patterns are hard to reconcile with carry over effects due to soil properties, such effects cannot be ruled out.

Another factor that can obscure the results is establishment from spores or fragments from the neighbourhood area or from other transplant unites. This would have a homogenising effect among transplants within sites, and thereby obscure any genetic differentiation. As most of the shoots included in the experiment are two- to three-years-old, this is a possibility. Nevertheless effects of original site were found for nearly all traits examined.

Age

The results indicate that there may be genetic differentiation among populations of *P. dentatum* in age of female gametophores bearing sporophytes. An earlier study (Hassel et al. unpubl.) concluded that lowland shoots reached maturity at an earlier age than mountain shoots. The present study does not reveal any consistent differences between lowland and mountain phenotypes with respect to either genetic or environmental effects. It is noteworthy that the largest difference is between populations at site M2 and L5, the most intensively sampled in the study by Hassel et al. (unpubl.). However, here we found a significant interaction between area of origin and site. Thus, it seems that mountain and lowland genotypes may have differentiated under certain environmental conditions as illustrated by the observations from site L6.

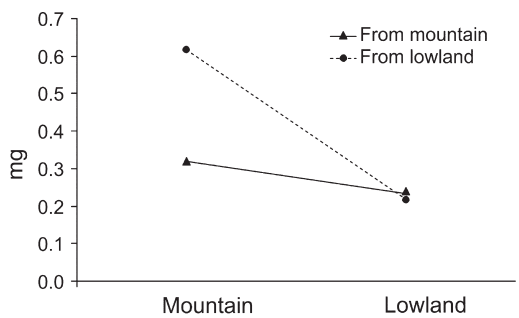

Fig. 4. Mean size (mg) of branch B0 in *Pogonatum dentatum* shoots grown in their area of origin and in a new area.

Annual segment and branch size

We found genotype-environment interactions may be present for traits related to gametophore performance. Such interactions appear when different environmental conditions locally select for different traits or trait combinations, resulting in local adaptations both within and among populations (Turkington and Harper 1979, Antonovics et al. 1987, Wyatt et al. 1989, Stratton 1994, 1995, Såstad et al. 1999). Genetic differentiation among populations is maintained when gene flow among populations and habitat types are outweighed by strong

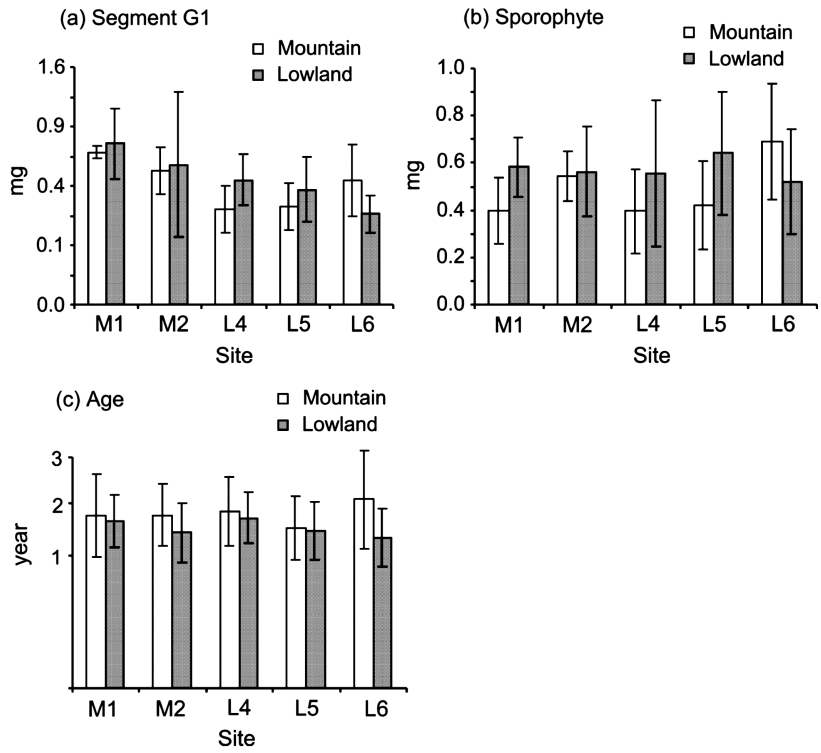

Fig. 5. Mean size (mg) of segment G1 (a), size (mg) of sporophyte (b) and age of reproducing shoots (c) and the standard deviation in shoots of *Pogonatum dentatum* growing in mountain (M1 and M2) and lowland (L4-L6) sites and originating from mountain and lowland areas.

local selection and when adaptation to one environment occurs at the cost of being less adapted to another (van Tienderen and van der Toorn 1991). We found, however, that shoots transplanted from other sites or areas grew larger than native shoots, indicating that local adaptation has not occurred. Instead, we believe that the genetic differentiation among populations of *P. dentatum* is maintained through metapopulation dynamics including founder events (cf. Taggart et al. 1990). Local founder effects can appear in metapopulations, in which extinction and recolonisation events are frequent. In such systems a small number of diaspores may establish a new population that consists of only a subset of the genotypes in the whole metapopulation. This is the situation in the lowland region, where local populations disappear regularly and new populations appear on previously uncolonised sites (Hassel and Söderström 1998, 1999, 2003). In the mountain area, however, the situation may be different because local populations are more long-lived and extinctions probably are much less frequent. Here, the occasional spores that are dispersed between populations act to increase genetic variation, as it adds to already existing genotypes. The amount of genetic variation therefore represents more of a balance between gene flow and local processes, such as selection, and is not so much determined by founder effects. These differences in gene flow effects are consistent with the genetic patterns found by Hassel et al. (unpubl.) in this species.

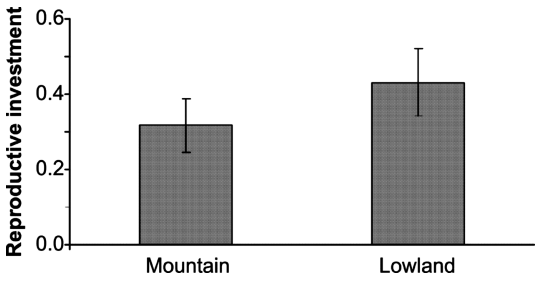

Fig. 6. Mean RI_2 and standard deviation of *Pogonatum dentatum* shoots growing in the mountains and shoots growing in the lowlands, irrespective of origin.

Annual segments and branches tend to grow larger in mountain areas. This seems to contradict Hassel et al. (unpubl.), who studied the same populations and found higher growth rates for lowland females than for mountain females. However, they studied shoots growing in their native sites only. In the present study there are no clear pattern emerging when comparing characters for mountain and lowland gametophores grown in their native areas. In particular this is true for populations at site M2 and L5, representing the sources of most of their material.

Annual segments show large variation in size among populations moved to different sites. Shoots from lowland sites seem, with few exceptions, to be more plastic than shoots from mountain sites and to grow larger in both areas. For branch size (B1) we see the same pattern. Hassel et al. (unpubl.) found a positive relation-

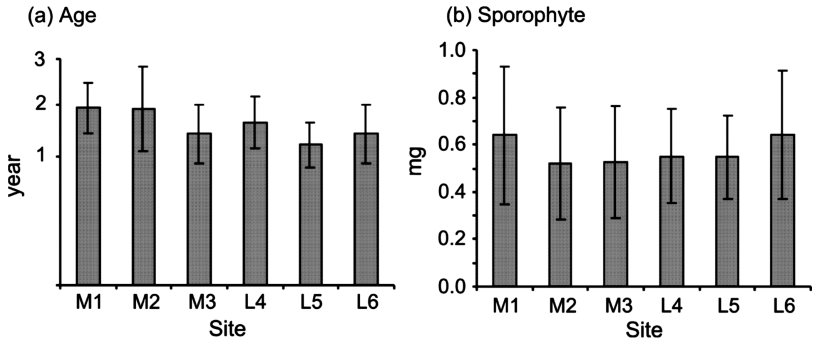

Fig. 7. Mean age (a) and sporophyte size (mg; b) and standard deviation of reproducing shoots of *Pogonatum dentatum* growing in the mountain (M1-M3) and lowland (L4-L6) sites (S).

ship between gametophore size and number of spores produced in *P. dentatum*. Large mountain gametophores produce more spores than small gametophores and have therefore had a higher probability to contribute to the origin of lowland populations, resulting in an over-representation of genes that give large gametophores. Such differences will persist as long as the plant depends on massive spore production.

The production of branches in shoots (of both lowland and mountain origin) at the lowland sites contradicts results from a study of resource allocation at first reproduction (Hassel et al. unpubl.), which showed that branches were only produced in the mountains. This may be partly explained by a one-year earlier maturation in the lowland and that growth of branches may be induced during the late sporophyte development stages. However, in the current study even some one-year-old lowland shoots were found with branches. This could be due to variation in the length of the growth season between years.

Sexual reproduction and RI

High reproductive investment and production of easily dispersed diaspors would seem to be an advantage in the lowlands where *P. dentatum* are dependent of finding available substrate at new sites, as it will disappear as succession proceeds at the current site. However, we found an indication of genetic differentiation only at the population (site) level and only for sporophyte biomass. Thus, as with the other traits included, most of the variation is found at the population level. As with age and segment G1 we found a significant interaction between area of origin and new site, due to the deviating trend at site L6 compared with all other sites. The reason for the deviations at site L6 remains unresolved. The variation in sporophyte biomass follow the same main patterns as for G0 and G1 and the discussion above would also apply to sporophyte biomass.

The different calculations of RI gave different results. RI_1 had no significant explanatory variables while RI_2 and RI_3 both were dependent on new area. As predicted RI were higher in the more dynamic lowland area. RI_1 will in most cases be an overestimate of RI as not all photosynthetically active parts are included. RI_3, on the other hand, is prone to overestimating the biomass of photosynthecially active gametophore tissue. This is especially true in older shoots where a high proportion of dead tissue would be included, which would lead to an underestimate of RI. In the current study the great majority of sampled shoots are one- or two-years old and consequently the RI_2 and RI_3 estimates gave quite similar values.

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