Three generations under low versus high neighborhood density affect the life history of a clonal plant through differential selection and genetic drift

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We tested whether neighborhood density affects the clonal life history of the stoloniferous plant *Ranunculus reptans* through selection and genetic drift. After three generations of sexual reproduction of 16 low and 16 high density lines, we studied traits related to growth form and reproduction in a common competition free environment. A 7.7% lower branching frequency and slightly longer internodes indicated an evolutionary shift towards a less compact growth form under high neighborhood density, but because stolons grew also more vertically, horizontal spread per ramet was slightly decreased. Neighborhood density had no directional effects on the evolution of allocation to sexual and vegetative reproduction in *R. reptans*. Variation among replicated high density lines was significantly lower than among replicated low density lines in both growth form and reproductive characteristics, indicating less pronounced genetic drift under high neighborhood density. This study demonstrates that a clonal plant can respond to selection imposed by neighborhood density. Moreover, it shows that the effect of random genetic drift increases with decreasing neighborhood density. In a declining species, such as *R. reptans* in central Europe, this may lower the potential for adaptive evolutionary change and increase extinction risk.

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Plant density is likely to affect both the ecological and genetic interactions within the neighborhood area of a plant (Antonovics and Levin 1980), and is thus likely to be an important factor in plant evolution. Theory predicts that density dependent selection affects life histories of unitary organisms in such a way that they show r selected characteristics at low and K selected characteristics at high density (MacArthur and Wilson 1967, Pianka 1970). However, it is still unclear how and to which extent the theoretical predictions may also apply for modular organisms with vegetative reproduction (Salisbury 1942, Klimeš et al. 1997), which have

as a consequence populations and life histories of both ramets and genets (Sackville Hamilton et al. 1987, Schmid 1990, Fischer and van Kleunen 2001).

Here we report results of the first selection experiment under two levels of neighborhood density using genets of a clonal plant species, *Ranunculus reptans* L. We tested whether density dependent selection imposed by high versus low ramet and correlated genet density in defined neighborhood areas leads to evolutionary changes in life history characters, particularly those of genets. We define genets as sets of ramets derived from single seeds (Harper 1977). We created replicates of an experimental

population which were allowed to evolve for three genet generations at low and high densities. This could result in genetic differentiation between low and high density lines if the genets that contributed large numbers of seedlings to the following genet generations differed between the density environments.

As a consequence of increased intraspecific competition, high neighborhood density may affect the fitness of clonal plants through a reduction in growth and changes in reproductive allocation (Abrahamson 1975, Schmid and Harper 1985, Humphrey and Pyke 1998, van Kleunen et al. 2001). Under high density, a high allocation to sexual reproduction may allow escape from locally adverse conditions (Ogden 1974, Abrahamson 1975, Loehle 1987), while on the other hand a high allocation to vegetative reproduction may increase competitive strength and result in a higher seed production in the long term (Loehle 1987). In *R. reptans* density causes a plastic increase in the relative allocation to sexual over vegetative reproduction (van Kleunen et al. 2001), but reproductive allocation does not respond to density dependent selection in populations without seed recruitment (van Kleunen and Fischer 2003). It is not known whether the allocation to sexual and vegetative reproduction can show an evolutionary response to selection imposed by density over several sexually reproducing genet generations.

As a consequence of variation in spacer length and branching frequency, clonal growth form may range from compact with highly aggregated ramets to spreading with more dispersed ramets (so called phalanx and guerilla strategies, respectively; Lovett Doust 1981). Growth form is likely to have important consequences for survival and reproductive output of clonal plants, and thus to be under selection. Therefore, growth form characteristics of clonal plants can be considered as life history traits (Fischer and van Kleunen 2001). The highly spreading guerilla plants may rapidly occupy the space available under low density, whereas the competitively stronger phalanx plants are more likely to persist under high density (Winkler and Schmid 1995, Winkler et al. 1999). On the other hand, guerilla plants such as *R. reptans* may escape from unfavourable high density patches, whereas a phalanx plant does not have this possibility (Schmid 1986, 1991).

Although experimental studies on evolutionary changes in response to selection are quite frequent, experimental studies on the importance of other evolutionary forces such as genetic drift on life history characters are surprisingly rare (Rich 1984, Newman and Pilon 1997). Especially in declining species, such as *R. reptans* in central Europe, genetic drift may be detrimental because it lowers the potential for adaptive evolutionary change and increases extinction risk. Therefore, we additionally tested whether neighborhood density may affect evolution of life history characters

through effects on random genetic drift. The magnitude of genetic drift is negatively related to the effective population size (Hartl and Clark 1994). Plant density is likely to affect genetic drift due to its influence on effective sizes of both the population and the genetic neighborhood of individual plants within the population (Thomas and Bazzaz 1993, van Kleunen et al. 2001). Although population size is determined by both the genet density of a population and the area covered by the population, for habitat specialists such as *R. reptans* with a restricted available amount of growing area, density is likely to be the main factor. Moreover, if within a population the neighborhood of plants is considered, in which most ecological and genetic interactions occur (Antonovics and Levin 1980), there are most likely fewer genets in a low density neighborhood than in a high density neighborhood, especially for a guerilla plant with intermingling genets such as *R. reptans*. This leads to the prediction that the magnitude of random genetic drift should be smaller under high than under low neighborhood density.

We studied evolutionary responses of size, reproductive allocation, and growth form of genets after three generations of sexual reproduction at low and high ramet density in defined neighborhood areas. Because each generation was started by planting arrays of ramets derived from several genets, genet density at the scale of local neighborhoods varied simultaneously with ramet density between the two treatments. To test our prediction about genetic drift, we replicated our selection experiment in 16 low and 16 high density lines and analysed the variation in selection response along with the mean selection response.

Material and methods

Study species

Ranunculus reptans L. (Ranunculaceae) is a stoloniferous, rosette forming herb with a circumpolar distribution, mainly in the temperate to boreal-subarctic zones of Europe, Asia, and North America (Hess et al. 1980). In central Europe, the species only grows on periodically inundated river and lakeshores with low vegetation cover (Prati 1998). Because of the regulation of water levels of most rivers and lakes, *R. reptans* has become a rare species in central Europe where it is now mainly found around Lake Constance, which borders Austria, Germany and Switzerland. There, it frequently grows in small patches of <1 m² area. Such patches of different densities consist of intermingling genets (Fischer et al. 2000b).

Rosettes (i.e. ramets) of *R. reptans* consist of up to 20 leaves. Its leaf blades are 10–50 mm long and 1–5 mm wide and gradually narrow into the petioles. Stolon branches are produced from meristems in the axils of

leaves, and consist of thin (0.5–2.0 mm diameter) internodes with a length of 3–5 cm. New ramets are produced at the nodes. Stolon branches can grow 10–20 cm within one growing season, which may result in high ramet densities. Each ramet has the potential to produce roots and a single flower, and as a consequence vegetative and sexual reproduction are not mutually exclusive in *R. reptans*. The slightly proterandrous flowers of the partially self incompatible *R. reptans* can produce 10–20 seeds (Prati and Peintinger 2000).

Collection and precultivation of plants

Collection and precultivation of plants was the same as described in van Kleunen et al. (2001) and van Kleunen and Fischer (2003). To comprehensively sample potential genetic variation in *R. reptans*, we collected a total of 40 ramets from 10 natural populations around Lake Constance from 1995–1997. RAPD analysis revealed that all 40 ramets represented different genets (Fischer et al. 2000b). Moreover, we found significant variation among these genets in the proportion of flowering and of rooted ramets, leaf length, stolon–internode length and spatial spread per ramet when they were grown in a competition free environment (all $P < 0.05$; M. van Kleunen, unpubl.). The collected genets were propagated repeatedly, and on 8 August 1997, we used ramets of the 40 genets to found one large mixed population divided over thirty two 31×44 cm trays filled with a 4:1 mixture of sand and compost. This population was allowed to reproduce only vegetatively until we had a sufficient number of ramets to start the experiment described below. Both the pre cultivation of plants and the experiment took place in a plant room at 25°C with 16 hours of artificial light (ca $300 \mu\text{E m}^{-2} \text{s}^{-2}$).

Experimental setup

On 5 March 1998, we excavated all rooted ramets from the mixed population, and randomly assigned them to evenly spaced, hexagonally arranged planting positions in each of thirty two 31×44 cm trays, simulating neighborhood areas, filled with a soil mixture of the same composition as described above. To create 16 replicates of the mixed population at low density (low density lines) and 16 replicates at high density (high density lines), we planted into 16 trays 40 ramets ($293 \text{ ramets m}^{-2}$) and into the other 16 trays 200 ramets ($1466 \text{ ramets m}^{-2}$). Both densities are within the range of ramet densities observed in the field (M. Fischer, pers. obs.). Because the base population contained 40 genets and ramets were drawn randomly from it, genet density was lower in the low ramet density neighbourhoods than in the high ramet density neighborhoods. As a consequence of vegetative reproduction, ramet densities

increased during the experiment but always remained higher for the high density lines than for the low density lines (van Kleunen et al. 2001, M. van Kleunen, unpubl.). Plants were watered at two day intervals, and trays were randomly assigned to new positions in the plant room at two week intervals.

In order to get seeds to start the next genet generation, we hand pollinated all open flowers within each tray (i.e. within each replicate line) every second or third day. With a pair of tweezers, we took 4–5 anthers from each flower and moved them over the stigmata of 2–3 randomly chosen other flowers. Each flower was thus used both as pollen donor and receptor. Due to this pollination procedure, selection on genets was imposed by the density environment, i.e. at each density, genets with the highest sexual reproductive effort were likely to contribute most offspring to the next genet generation of each line. Between pollinations within different trays, we sterilized the pair of tweezers in a flame. About one month after pollinating a flower, we harvested its seeds and stored them in closed plastic boxes in a refrigerator.

On 14 September 1998, we sowed all seeds on a 1:3 mixture of sand and compost after they had been incubated in a solution of 2 mg ml^{-1} gibberellic acid for five days to break dormancy. Thirty days later, we planted the seedlings into experimental trays similar to the ones used in the first generation. Because there were fewer than 40 and 200 seedlings for most low and high density lines, respectively, we planted on average 30.8 seedlings for the low density lines (median = 32.5, range = 9–40) and 126.8 seedlings for the high density lines (median = 132.0, range = 64–165) in the second generation, and repeated the pollination and germination procedures. Because of again low seedling numbers, we planted the third generation in trays of half the size of the ones used in the previous generations. To maintain the same densities as in the previous generations, we planted into these smaller trays 20 and 100 seedlings of the low density and high density lines, respectively. Again we repeated the pollination procedure. During the experiment, two high density lines dried out and were lost. Because each selection generation started from seedlings, ramet density always varied simultaneously with genet density.

To test whether clonal life history traits had genetically diverged between low and high density lines after three generations of selection, we planted the offspring of the third generation into a common environment. First, to reduce maternal environmental carry over effects in this fourth generation, we precultivated 15 genets (derived from 15 seeds) of each of the remaining 30 lines for 82 days in $9.5 \times 9.5 \times 11$ cm pots filled with the same soil mixture as used in the previous generations. On 26 April 2001, we randomly assigned one rooted ramet of each of the 15 genets of each line to the eight planting positions in each of 56 experimental trays

similar to the ones used in the base generation with the limitation that ramets planted to the same tray belonged to different selection lines. Because some lines had fewer than 15 genets, the total number of genets in the fourth and final generation was 439 instead of 450.

Measurements

After eight weeks of growth of the fourth generation in the common environment, we counted the number of ramets, rooted ramets and flowers on each genet as estimates of size, vegetative reproduction, and sexual reproduction, respectively. We calculated the proportions of flowering and rooted ramets by dividing the number of flowers and rooted ramets, respectively, by the total number of ramets. We additionally counted the number of carpels of the oldest flower on the longest stolon branch of each plant because this may trade off with the proportion of flowering ramets (van Kleunen et al. 2002). To quantify growth form, we counted the number of branches per genet, and measured the spatial spread of the genet (i.e. the linear distance between the two most distant ramets of a genet), the length of the longest leaf and the combined length of the four oldest internodes on the longest stolon branch on each genet.

Analyses

We analysed all variables with analyses of variance using the statistical software SPSS (SPSS Inc., Chicago, Illinois, USA). We considered selection density as a fixed factor and the lines within selection densities as a random factor. Because genetic drift is likely to be smaller under high density than under low density, we expected the variance to be lower among high density lines than among low density lines (Falconer and Mackay 1996). Therefore, the assumption of homoscedasticity was likely to be violated. Therefore, we calculated mean squares separately for the high and low density lines, and tested the density effect conservatively against the group of lines (i.e. high or low density lines) with the highest mean squares rather than against all lines. To improve normality of residuals we log₁₀ transformed number of ramets, proportion of rooted ramets, spatial spread per ramet, and number of branches per ramet prior to analyses.

To test whether variation among high density lines was indeed lower than among low density lines, we calculated the variance components among high density and low density lines for each trait and the ratio between them from analyses of variance (Sokal and Rohlf 1995). We tested these ratios with one tailed F tests because of the specific prediction of lower variance among high density lines.

Results

Evolutionary responses of genet size and reproduction to neighborhood density

After three sexual generations, genets of the high density lines had 7.2% more ramets, a 2.9% lower proportion of rooted ramets, 0.7% higher proportion of flowering ramets, and 3.1% more carpels per flower than plants of the low density lines (Fig. 1a, Table 1). However, these differences were not significant (Table 2a), suggesting that there was no evolutionary response to neighborhood density for total size and reproductive allocation of genets in *R. reptans*.

Evolutionary responses of genet growth form to neighborhood density

After three sexual generations, plants of the high density lines had 3.5% longer leaves, 7.7% fewer branches per ramet, 2.4% longer stolon internodes, but a 10% lower horizontal spread per ramet than plants of the low density lines (Fig. 1b, Table 1). These differences were marginally significant in a multivariate analysis of variance ($F_{4,112}=2.01$, $P=0.098$) and individually significant for the number of branches per ramet ($P=0.013$; Table 2b). Lower branching frequency and longer internodes are consistent with increased guerilla like growth form, although this shift in growth form resulted in a lower rather than higher horizontal spread per ramet. This was due to stolons, which, like the leaves, escaped from neighborhood density by elongating vertically as well as horizontally (M. van Kleunen, pers. obs.).

Effects of neighborhood density on variation among selection lines

Compared to the directional effects of neighborhood density on average selection responses, density effects on the variation among lines were more pronounced. There was significant variation among lines in the proportion of flowering ramets, the number of carpels per flower, leaf and internode lengths, and marginally significant variation in the spatial spread per ramet (Table 2). The variation was lower among high density than among low density lines for the number of ramets, the proportions of flowering ($P<0.001$, Fig. 1, Table 3) and of rooted ramets ($P=0.033$), the number of carpels per flower (not significant, $P=0.185$), and the spatial spread per ramet ($P=0.007$). Only for internode length the variation was higher among high than among low density lines (Table 3; $F_{13,15}=9.43$, two tailed $P<0.001$). For the numbers of ramets and of branches per ramet, F ratios could not be calculated based on variance components because they were zero (negative)

Fig. 1. Means (± 1 SE) of (a) the proportion of flowering ramets, and (b) the number of branches per ramet for 16 low density (open symbols) and 14 high density lines (filled symbols) of *Ranunculus reptans* at the end of a three generation selection experiment. In both graphs, lines were ranked according to their mean values. Variation in the proportion of flowering ramets was significantly higher among low density lines than among high density lines ($P < 0.001$; Table 3), and the number of branches per ramet was significantly higher for low density lines than for high density lines ($P = 0.013$; Table 2b).

among high density lines (Table 3). The overall lower variation among high density lines than among low density lines is consistent with our prediction that

random genetic drift becomes a stronger evolutionary force with decreasing neighborhood density in *R. reptans*.

Discussion

Adaptive evolutionary responses to selection imposed by neighborhood density

Clonal growth and vegetative reproduction are important for the maintenance of populations of many plants. However, for these characters to evolve rapidly there must be genetic variation among genets and recombination via sexual reproduction (Sackville Hamilton et al. 1987). Although density dependent selection using only vegetatively produced offspring may result in evolutionary changes as shown in a previous study (van Kleunen and Fischer 2003), it has to rely exclusively on changes in the frequency of the original genotypes or on somatic mutations and perhaps epigenetic changes. In the current selection experiments, we therefore started subsequent generations of the selection lines from sexually produced seeds. Between the time from seed germination to seed production genets could show differential clonal growth, ramet mortality, ovule and pollen production, and fertilization success, which can all contribute to life history evolution.

After three generations, low and high density lines had not diverged for the number of ramets, proportions of rooted and of flowering ramets, and number of carpels per flower, even though there was significant heritable variation in the base population for these measures of genet size and reproduction (Prati and Schmid 2000, van Kleunen et al. 2000a, b, van Kleunen and Fischer 2001). Moreover, in another experiment in which we directly selected on the proportion of flowering ramets, the selected trait responded significantly within two generations (van Kleunen et al. 2002). Therefore, the present results indicate that the different components of reproduction are either selectively neutral to density or

Table 1. The mean evolutionary effect of low and high density on reproduction and growth form of *Ranunculus reptans* at the end of a three generation selection experiment. Plants of the fourth generation were grown in a common environment. Means are given ± 1 SE for non transformed data, and $\pm$ upper SE/lower SE for log10 – transformed data after back transformation. The difference between the low and high density lines was significant for the number of branches per ramet ($P = 0.013$; Table 2b).

Trait	Low density lines	High density lines
Reproduction		
number of ramets	45.7 $\pm$ 3.0/2.8	49.0 $\pm$ 2.4/2.3
proportion of rooted ramets	0.198 $\pm$ 0.008/0.008	0.192 $\pm$ 0.008/0.008
proportion of flowering ramets	0.271 $\pm$ 0.010	0.273 $\pm$ 0.007
number of carpels per flower	16.1 $\pm$ 0.4	16.6 $\pm$ 0.4
Growth form		
spatial spread per ramet (mm)	6.13 $\pm$ 0.37/0.35	5.52 $\pm$ 0.30/0.29
number of branches per ramet	0.251 $\pm$ 0.005/0.005	0.232 $\pm$ 0.003/0.003
length of longest leaf (mm)	56.2 $\pm$ 1.2	58.1 $\pm$ 1.4
length of oldest four stolon internodes (mm)	178.7 $\pm$ 2.3	183.1 $\pm$ 4.0

Table 2. Summary of analyses of variance of evolutionary effects of low and high density and of variation among lines at the end of a three generation selection experiment on (A) reproduction and (B) growth form of *Ranunculus reptans*. The number of ramets, the proportion of rooted ramets, the spatial spread per ramet, and the number of branches per ramet were log transformed prior to analyses. Because we expected inequality in variance between the low and high density lines, we tested the selection density effect conservatively against the group of lines (i.e. low or high density lines) with the largest mean squares (MS).

Effect	df	Number of ramets			Proportion of rooted ramets			Proportion of flowering ramets			Number of carpels per flower*		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P
Density	1	0.08478	0.51	0.486	0.000661	0.34	0.568	0.00022	0.01	0.922	32.205	1.17	0.296
Line(density)	28	0.12900	1.02	0.440	0.001843	1.33	0.125	0.01818	1.71	0.015	26.272	1.92	0.004
low density lines	15	0.16600	1.36	0.169	0.001956	1.50	0.107	0.02578	2.16	0.009	27.506	2.28	0.005
high density lines	13	0.08555	0.64	0.818	0.001713	1.15	0.320	0.00940	1.03	0.424	24.848	1.60	0.089
Residual	400	0.12700			0.001386			0.01062			13.697		
low density lines residual	215	0.12200			0.001300			0.01194			12.074		
high density lines residual	185	0.13300			0.001487			0.00909			15.570		

Effect	df	Spatial spread per ramet ^Δ			Number of branches per ramet			Length of longest leaf			Length of oldest four stolon internodes [†]		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P
Density	1	0.1250	1.23	0.285	0.005588	7.98	0.013	346.436	0.95	0.348	2280.08	0.77	0.396
Line(density)	28	0.0871	1.43	0.075	0.000466	0.62	0.937	344.631	2.47	<0.001	2039.68	1.90	0.004
low density lines	15	0.1020	1.66	0.061	0.000700	0.69	0.793	327.309	2.59	0.001	1246.65	1.19	0.281
high density lines	13	0.0703	1.16	0.313	0.000195	0.43	0.957	364.617	2.36	0.006	2954.72	2.68	0.002
Residual	400	0.0611			0.000754			139.339			1071.87		
low density lines residual	215	0.0615			0.001016			126.254			1045.02		
high density lines residual	185	0.0606			0.000449			154.546			1103.46		

* For the number of carpels per flower, the df of residual, low density lines residual and high density lines residual are 362, 194 and 168, respectively.

^Δ For the spatial spread per ramet, the df of residual, low density lines residual and high density lines residual are 386, 207 and 179, respectively.

[†] For the length of oldest four internodes, the df of residual, low density lines residual and high density lines residual are 394, 213 and 181, respectively.

Table 3. Summary of analyses of inequality of variance in reproduction and growth form between the 16 low density and 14 high density lines of *Ranunculus reptans* at the end of a three generation selection experiment. Plants of the fourth generation were grown in a common environment. The number of ramets, the proportion of rooted ramets, the spatial spread per ramet, and the number of branches per ramet were log10 transformed prior to analyses. Differences between variation among low density lines and among high density lines were tested with one tailed F tests of the ratio between the variance among low density lines and among high density lines. For the number of ramets and the number of branches per ramet, we found negative variance components (which were set to zero) and as a consequence we could not calculate F values for these traits.

Trait	Variance among low density lines	Variance among high density lines	F _{15,13}	P
Reproduction				
number of ramets	3.051·10 ⁻³	0	—	—
proportion of flowering ramets	9.601·10 ⁻⁴	2.218·10 ⁻⁵	43.29	<0.001
proportion of rooted ramets	4.552·10 ⁻⁵	1.591·10 ⁻⁵	2.86	0.033
number of carpels per flower	1.178	0.715	1.65	0.185
Growth form				
spatial spread per ramet	2.890·10 ⁻³	7.033·10 ⁻⁴	4.11	0.007
number of branches per ramet	0	0	—	—
length of longest leaf	13.946	14.791	0.94	0.550
length of oldest four stolon internodes	14.109	133.027	0.11	1.000

that adaptive evolution is constrained by genetic correlations among traits. We favour the first explanation because it is in accordance with a simulation study of Sackville Hamilton et al. (1987), who showed that, for non overlapping generations of guerilla species such as *R. reptans*, selection on sexual reproductive allocation does not differ between high and low density environments. In another density dependent selection experiment in which populations of *R. reptans* reproduced exclusively vegetatively for four generations, there was also no response in reproductive allocation (van Kleunen and Fischer 2003).

Genets of our high density lines showed a shift towards a more guerilla like growth form of lower branching frequency and longer internodes (Sutherland and Stillman 1988, Stoll et al. 1998) compared with genets of the low density lines. However, this shift did not lead to increased horizontal spread because the internodes were not growing purely horizontally to escape density stress but also grew more erect. Higher vertical growth may allow the genets to capture more light when grown under competition (van Kleunen and Fischer 2001), and may thus be more beneficial than horizontal growth. Genets of high density lines also showed a tendency to evolve longer leaves, which was also found in another experiment with density dependent selection in which populations of *R. reptans* reproduced exclusively vegetatively (van Kleunen and Fischer 2003). This can be seen as a further adaptive response to shading when grown under high density because the light gradient is more predictable in the vertical plane (Huber et al. 1998).

Despite an extensive literature on density dependent selection, experimental studies on evolutionary responses to density are, surprisingly, very scarce, and the few existing ones are biased towards animals and species with short generation times such as *Drosophila melanogaster* (Mueller et al. 1991, Joshi et al. 1998).

In these studies, significant evolutionary responses to density dependent selection were generally found and depended on the particular life history of the organism (Mueller 1997). Artificial selection experiments on organisms with longer generation times, such as most plants, show that evolutionary responses can be observed already after a few generations (Mazer et al. 1999, Marek et al. 2000, van Kleunen et al. 2002). In these studies, however, selection was not exerted by different environments but directly by the scientists themselves and therefore relatively strong. In another study on evolutionary responses to selection exerted by low and high density, Law (1979) found that populations of the annual grass *Poa annua* selected for 21 months at high density had a slightly but significantly longer prereproductive period than plants selected at low density. This confirms our finding that evolutionary responses can be observed already after a few generations of selection by different densities.

Effect of neighborhood density on genetic drift

In clonal plants, it is the number of genets that is relevant for effective population size. Although genet number is related to ramet number, the latter can be considerably higher because of vegetative reproduction of genetically identical ramets by genets. Therefore, effective population size has been theoretically predicted to be generally smaller in clonal than in non clonal plants (Orive 1993). In our experiment, ramet and genet density varied simultaneously (Materials and methods). Consistent with our hypothesis of more pronounced effects of random genetic drift at low than at high neighborhood density, we found larger variation among replicate low density lines than among replicate high density lines for most traits (Table 3). Another possible explanation for

larger variation among low density lines could be that under low neighborhood density, selection has been weaker than under high density. However, there are no reasons to expect different strength of stabilizing selection between low and high density environments, and the only trait for which the outcome of directional selection differed between low and high density treatments (i.e. number of branches per ramet) did not differ in variation among replicate lines between the density treatments. Therefore, we conclude that the generally higher among line variation for low density lines is caused by more pronounced genetic drift rather than by weaker selection.

Another effect that increases the difference in effective population sizes between high and low density populations in clonal plants even more can result from a difference in the mode of competition between genets of clonal plants as compared with nonclonal plants. Whereas in nonclonal plants intraspecific competition is generally asymmetric, leading to increased size variation (Weiner and Thomas 1986, Weiner 1988), in horizontally spreading clonal plants it is likely to be symmetric (de Kroon et al. 1992, Hara 1994), which can lead to lower size variation under high density as previously observed in *R. reptans* (van Kleunen et al. 2001). Thus, because lower variation in genet sizes increases the effective population size (Hartl and Clark 1994), high density populations of *R. reptans* have even disproportionately larger effective population sizes than low density populations predicted only from the density differences. This results in an even stronger increase in the importance of random genetic drift with decreasing density.

Conclusions

We conclude, that density can impose directional selection on the life history evolution of a clonal plant such as *R. reptans*, and that density effects on random genetic drift as an evolutionary force can be even more pronounced, and certainly have been less often considered so far.

Possibly, in a declining species, such as *R. reptans* in Central Europe, more pronounced genetic drift due to low density lowers the potential for adaptive evolutionary change and increases extinction risk, at least in the smallest populations (Fischer et al. 2000a). In an invading species, on the other hand, this may possibly lead to pronounced genetic variation between neighborhoods and populations, which could then lead to more rapid evolutionary change once these groups coalesce.

Acknowledgements – We thank Rahel Schmid, Gillian Rutherford Rauh, Andrea Lienert, Claudia Pfister, Rebecca Gopfert, Kati Dräger, Mirjam Friedli, Franz Fritzsche, Philipp

Kocyan, Lukas Wotruba and Thomas Pflüger for practical assistance. We acknowledge financial support by the Swiss National Science Foundation (Grant 31 49728.96).

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