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Clonal structure and genet-level sex ratios suggest different roles of vegetative and sexual reproduction in the clonal moss *Hylocomium splendens*

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The allozyme haplotype was determined for 157 ramets of the unisexual, perennial, clonal moss *Hylocomium splendens* within five 10×10 cm plots, which had been the subject of demographic studies over a 5-yr period. In addition, 25 shoots were analyzed from outside the plots and from four neighbouring patches. Only four haplotypes were encountered within the plots; one female type occurred in all plots and one male type in four plots, whereas two male haplotypes occurred in only one plot. Genets grew intermingled in all but one plot. The sex ratio within the five plots was female-biased at the ramet level (male:female = 1:2.6), but male-biased at the genet level (3:1). Sporophytes were produced abundantly during the study period, but no signs of recruitment from spores were observed in the plots. Nine additional genets were encountered in neighbouring patches but from only one patch each. Four (44%) of these could potentially have been derived from spores generated within the plots. Our results suggest that each patch of *H. splendens* is colonized by a small number of genets, whereas different patches have different sets of genets, i.e. clonal diversity is determined by vegetative reproduction at within-patch scales and structured by sexual processes at among-patch scales.

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Clonal growth is a phylogenetically ancient trait among land plants, which potentially provides a long life span by avoiding senescence and limitations related to lack of sexual recruitment under unfavourable conditions (van Groenendael et al. 1996). If successful sexual reproduction is rare and involves few genets (i.e. the effective population size is small) genetic drift is likely to cause loss of genetic variation and lead to genetic differentiation among populations. Identification of individual genets by molecular markers has frequently revealed surprisingly high levels of genetic variation and clonal diversity in populations, despite lack of apparent sexual recruitment (e.g. Jonsson et al. 1996). This paradox remains unsolved since demographic parameters like recruitment and mortality rates of genets as well as total genet numbers are almost unknown.

Bryophytes have high capacity for clonal propagation, through horizontal growth, detached fragments and specialized propagules (Anderson 1980, During 1990). All propagules (spores, vegetative diaspores or fragments) are haploid, suggesting that founder events could easily result in local populations with low levels of genetic variation. Sexual reproduction is often constrained by lack of sexually mature shoots, by imbal-

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anced sex ratios, by spatial separation of sexes and by unfavourable climatic conditions (Longton 1976). In species with unisexual shoots, the distribution of male and female genets within moss patches is critical, since fertilization is mediated by freely swimming spermatozoids with action radii usually limited to centimetres (Longton and Schuster 1983, Bisang et al. 2004, Rydgren et al. unpubl.). Fertilization and subsequent production of sporophytes therefore seems to be rare in many unisexual bryophytes (Longton and Miles 1982), but the actual rate of recruitment from spores may be low even in species with high sporophyte abundance. Studies of the soil propagule bank (Jonsson 1993, Rydgren and Hestmark 1997) and tests involving experimental sowing of spores in natural habitats (Longton and Miles 1982, Kimmerer 1991) suggest that establishment of sporelings is rare in many perennial species. The relative importance of sexual and vegetative reproduction is therefore a matter of debate (cf. Mishler 1988), and the view that vegetative reproduction prevails and populations are mostly dominated by few clones has often been advanced (e.g. Anderson 1963).

The microevolutionary processes of bryophyte populations are difficult to assess through direct studies of reproduction, recruitment and dispersal, but their outcome can be evaluated by detailed mapping of male and female genets identified by molecular methods (e.g. Innes 1990, Hofman 1991, van der Velde et al. 2001). Sex ratios calculated at the ramet level can be obtained without access to molecular markers, and such studies have revealed that populations of unisexual bryophytes typically have skewed sex ratios, often with an overrepresentation of females (Bowker et al. 2000). Genet-level sex ratios have rarely been obtained and therefore estimates of effective population sizes in bryophytes are almost lacking (Cronberg et al. 2003). Recent studies of genetic population structure, primarily using allozymes, have shown that perennial unisexual bryophytes, like *Hylocomium splendens* (Cronberg et al. 1997) and *Pleurozium schreberi* (Zielinski and Wachowiak-Zielinska 1995), are genetically more variable and clonally more diverse than expected if vegetative reproduction predominates.

Hylocomium splendens is a large, perennial, clonal, pleurocarpous moss with modular growth due to annual periodicity in the emergence of new modules that remain connected as segment chains until they become separated by decomposition from below (after 2–20 yr) or are damaged (Tamm 1953, Økland 1995). It is unisexual and sporophytes occur on 0.05–4.4% of segments (Økland 1995, Rydgren and Økland 2001, Cronberg 2002). Numerous wind-dispersed spores, 14–16 μm in diameter (Nyholm 1954–69), are produced in each capsule, but a soil propagule bank appears to be lacking (Jonsson 1993, Rydgren and Hestmark 1997). The level of variation in populations of *H. splendens* (gene diversity $H_S \approx 0.2–0.3$; mean no. of alleles at 10–13 polymorphic loci, $A \approx 2.0–3.0$; Cronberg et al. 1997, Cronberg 2002, 2004) is sufficient for accurate identification of individual clones. The relative differentiation among populations is low ($G_{ST} = 0.05–0.075$), suggesting a fairly high level of gene flow.

The main aim of this study is to determine the relative importance of sexual and vegetative reproduction in a sexually reproducing population of *Hylocomium splendens*. A low number of widespread genets would imply that vegetative reproduction is predominant, whereas a high number of more or less related genets with limited distribution would instead imply that sexual reproduction determinates within-patch clonal structure. In this study we therefore attempted 1) to analyse the number of genets and their spatial distribution in a patch of *H. splendens* which had earlier been the subject of a 5-yr demographic study (Rydgren and Økland 2002a); 2) to calculate the sex ratios at ramet and genet levels; and 3) to compare the genetic identity of genets from near-neighbour patches. We initially expected the investigated patch to have a high number of genetically related genets with limited distribution, since sexual reproduction had taken place regularly within the patch for a number of years, suggesting opportunities for recruitment from locally deposited spores.

Material and methods

Study site and data collection

The present study utilizes part of the population data of Rydgren and Økland (2002a, b, 2003). Demographic studies were performed during a five-year period (1995–1999) on a boulder (area: ca 0.8 m^2; average slope: 38°) almost completely covered by *H. splendens*, situated in a small *Picea abies* dominated forest valley north of Stampetjern, Skedsmo municipality, Akershus County, SE Norway (11°09′E, 59°59′N), 170 m a.s.l. A rectangular sampling site of 0.4×0.9 m was placed on the top surface of the boulder and divided into 36 grid plots, 10×10 cm each. For the entire five-year study period all *H. splendens* shoots were censused annually in the nine plots that contained two or more sporophyte-carrying segments at the start of the study in 1995. The segments were inspected for presence of sexual structures, archegonia and antheridia, in July 2000, and sporophytes were recorded at every annual census (Rydgren and Økland 2002a). A female-biased sex ratio (male:female = 1:4) has been reported for this population, but males may have been slightly underestimated as they were only searched for at one census, whereas sporophyte-carrying females were recorded at every census (Rydgren and Økland 2002a).

From five of these nine plots, all *H. splendens* segments were harvested for genetic analysis in May

2001 (see below). These five plots formed two continuous sampling areas (Fig. 1). In total, 207 shoots were sampled; 181 from the five demographic plots, 15 from the boulder outside the demographic plots and an additional 11 from four neighbouring patches. Sex ratios were calculated at three different levels (Cronberg et al. 2003): 1) at the "observed ramet level", including all ramets that expressed sex at the time of the census; 2) at the "inferred ramet level", including sterile individuals that can be inferred to be a certain sex by molecular markers; and, 3) at the genet level. At the inferred ramet level, sterile shoots were supposed to have the same sex as adjacent fertile ramets with identical haplotypes.

Germinability of spores was tested by sowing spores from 20 sporophytes on nutrient agar in Petri dishes. The nutrient solution of Rudolph et al. (1988) was used at double concentration. The cultures were kept at room temperature (ca 20°C) in daylight, but sheltered from direct sunshine, for two months and then checked for germination. Since some sporophytes were open and spores already partially released at the time of sampling, no quantitative tests were performed.

Electrophoretic procedures

All harvested shoots were kept alive in a growth chamber until extraction. Some shoots were in poor shape at the time of harvesting due to a prolonged drought period. Most of these shoots regenerated after a growth period

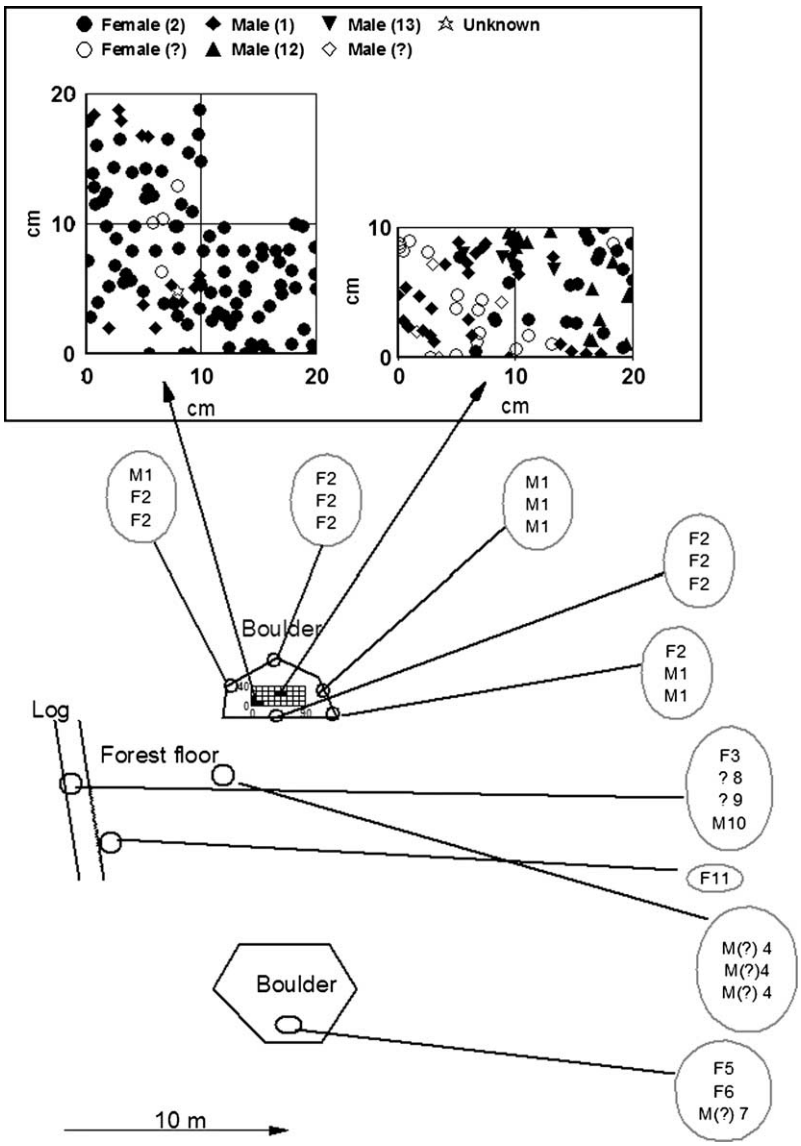

Fig. 1. Map showing haplotypes from five 10 × 10 cm grid plots in which shoots of *Hylocomium splendens* were subjected to genetic analysis. Also shown are the haplotypes of 15 shoots sampled from the same boulder outside the plots and an additional 11 shoots from neighbouring patches. Each identified haplotype is numbered (cf. Table 1) and coded according to gender (M = male, F = female, ? = unknown or uncertain gender, cf. Table 1). The boulder slopes 38° with the highest point to the right.

of up to six months, allowing their inclusion in the analyses. Sex determinations were cross-checked, again by examination of sexual structures, if present. Electrophoretic procedures for analysis of nine enzyme systems followed Cronberg et al. (1997) and Cronberg (2002). The gel-electrode buffer systems used were standard LiOH-buffer (Soltis et al. 1983; no. 7), histidine buffer (Soltis et al. 1983; no. 1, with 4% sucrose added) and morpholine-citrate buffer (Wendel and Weeden 1989; no. 2). The LiOH-buffer resolved aspartate aminotransferase (AAT, EC 2.6.1.1), phosphoglucoisomerase (PGI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 5.4.2.2) and triosephosphate isomerase (TPI, EC 5.3.1.1); the morpholine-citrate buffer resolved aconitase (ACO, EC 4.2.1.3), isocitratedehydrogenase (IDH, EC 1.1.1.42), malate dehydrogenase (MDH, EC 1.1.1.37), 6-phosphogluconate dehydrogenase (PGD, 1.1.1.43), and UDP pyrophosphorylase (UGP, EC 2.7.7.9). Homogenate from a single clone of *Pleurozium schreberi* was placed at five positions on every gel to provide a control against which relative bands in the other individuals could be scored.

Results

Thirty shoots, which had suffered severe drought before sampling, failed to regenerate and could not be analysed. Demographic data (branching patterns) made inference of the haplotype possible for six of these. Most of the remaining 24 unidentified shoots came from plots 5,3 (12 shoots) and 6,3 (8 shoots); males and females were represented by seven and 14 shoots, respectively, whereas three were sterile.

Of 12 allozyme loci, 10 were polymorphic with a mean of 2.2 alleles per locus (altogether 22 alleles) in the successfully analysed part of the sample, consisting of 177 shoots. A total of 13 different haplotypes was identified, of which most differed at several loci resulting in low expected frequencies of haplotypes in the population (Table 1). The haplotype data matched perfectly with sex determinations, indicating that the number of identified haplotypes was a close approximation of the actual number of clones.

Four haplotypes (three males and one female) were found among 157 shoots analysed from the experimental plots. Thus, males dominated at the genet level (Table 2). In contrast, the three male haplotypes (with 30, 10 and two ramets, respectively) were outnumbered more than twofold by the single female haplotype (115 ramets) at the inferred ramet level. The single female genet occurred in all plots, in one plot as the only haplotype, in three plots together with the most common male haplotype and in one plot together with all three male haplotypes (Table 3). The four haplotypes were not directly related and none could have arisen by spores produced in a cross between any combinations of the other pairs of haplotypes.

Analyses of 15 samples taken from the boulder just outside the demographic plots all belonged to the two most common haplotypes (Fig. 1); males tended to be more common towards the upper part of the boulder.

Analyses of samples taken from four neighbouring patches close to the boulder with experimental plots (Fig. 1) revealed nine additional haplotypes, each from only one patch, suggesting that different patches had originated by separate recruitment events and that patches usually were occupied by more than one clone. Four (44%) of these haplotypes (4, 7, 9, and 10) could potentially have been derived from spores that had originated by recombination of the parental haplotypes in the demographic plots, whereas the remaining five haplotypes carried additional alleles (haplotypes 3, 5, 6, 8) or a gene combination that could not be

Table 1. Haplotypes of *Hylocomium splendens* derived through allozyme analysis. M = male; F = female; ? = unknown gender (male or female structures absent); M (?) = sex structures not fully developed, propably male. Codes for alleles follow Cronberg (2004). Haplotypes 1, 2, 12 and 13 were observed in the analysed plots, haplotypes 2–11 in the surrounding patches, cf. Fig. 1. Expected frequencies of haplotypes estimated by multiplication of observed frequencies of all alleles for a given haplotype; observed frequencies of alleles being calculated at haplotype level.

Haplo-type	Gender	Aco-1	Mdh-2	Idh-1	Pgd-1	Ugp-1	Pgi-2	Tpi-1	Pgm-1	Pgm-2	Pgm-3	Expected frequency %
1	M	C	C	C	C	C	E	B	B	B	A	1.68
2	F	B	A	C	C	C	E	B	D	B	B	0.34
3	F	D	C	C	E	C	E	B	B	C	B	0.01
4	M (?)	B	A	C	C	C	E	B	B	B	B	4.14
5	F	B	C	C	C	C	E	B	B	E	B	1.32
6	F	C	A	C	C	C	E	B	B	E	B	1.16
7	M (?)	C	C	C	C	C	E	B	B	B	B	9.27
8	?	C	C	B	C	B	E	B	B	B	B	0.23
9	?	C	A	C	C	C	E	B	B	B	B	5.79
10	M	B	A	C	C	C	D	B	B	B	B	1.24
11	F	B	C	B	C	C	D	B	B	B	B	0.60
12	M	C	C	C	C	C	D	B	B	B	A	0.51
13	M	C	C	B	C	C	E	C	B	B	B	2.32

Table 2. Sex ratio estimations within five demographic plots of *Hylocomium splendens*.

	Males	Females	Sterile	Sex ratio
Observed ramet level	42	109	30	1:2.60
Inferred ramet level	49	129	3	1:2.63
Genet level	3	1		3:1

derived from recombination of extant haplotypes (haplotype 11).

Germination of spores was fast and germination was recorded from all 20 sporophytes that were tested.

Discussion

The studied plots represent 6% of the area of the entire *Hylocomium splendens* patch on the boulder. Only four haplotypes were encountered within this area. We cannot exclude the possibility that additional genets occur in the plots that were not screened. The unscreened area most likely was dominated by the single female clone, however, as 1) the 15 samples systematically taken from the boulder outside the screened plots turned out to belong to either the dominant male or the single female and 2) the shoots from the four plots used for demographic studies but not screened for genetic variation consisted mostly of females (Rydgren and Økland 2002a, Rydgren et al. unpubl.).

Recruitment

The clonal population structure revealed in this study agrees with earlier studies of *H. splendens*, in which sampling was done at coarser scales. For example, Cronberg (2004) found that five samples from each of 60 patches, sized 10×10 cm, included 1, 2, 3, 4 or 5 haplotypes (relative frequencies: 28, 30, 30, 7 and 5%, respectively). Patches are apparently often occupied by more than one genet, but the number of coexisting genets seems to be limited. Similarly, demographic studies suggest that continuation of the segment chain by clonal growth is the main means of local persistence of *H. splendens*. Successful recruitment, either by vegetative fragments or by spores, seems therefore to be restricted relative to vegetative proliferation of existing genets in *H. splendens*. A similar clonal structure has been observed for the arctic rhizomatous sedge *Carex bigelowii* (Jonsson 1995, Jonsson et al. 1996). Despite apparent lack of recruitment from seeds, the *Carex* population was highly diverse genetically and clonally, with each patch occupied by few different genets, one of which was usually dominant.

It is impossible to determine whether sexual or vegetative recruitment was responsible for initial colonization of the boulder. Studies involving experimentally created small vegetation gaps have shown that regeneration by vegetative proliferation is a common strategy among forest floor bryophytes (Lloret 1994, Frego 1996, Heinken and Zippel 2004, Rydgren et al. 2004). The position of the studied population on a steeply sloping boulder is likely to slow down this form of colonization, since ground contact occurs only at one margin. On the other hand, the conditions for establishment and growth of *H. splendens* have most likely been favourable at least since the immigration of *Picea abies* to this area ca 2500 yr ago (Hafsten 1992, Økland et al. 2003).

The present-day genets of *H. splendens* on the boulder are unrelated, and spores produced by the present three pairs of potential parents have not resulted in recruitment within the patch. This agrees with earlier studies, which suggest that recruitment from spores is limited in *H. splendens* (Jonsson 1993, Rydgren and Økland 2002b) as well as is in other perennial forest-floor bryophytes (Longton and Miles 1982, Innes 1990, Cronberg et al. 2003). Evidently, spores are unable to germinate in established populations of *H. splendens* or,

Table 3. Number of ramets belonging to different haplotypes in five demographic plots of *Hylocomium splendens* at the census 2001. Also shown are the numbers of ramets that could not be identified to haplotype due to drought damage.

Haplotype no.	Gender	Plot no. 1,1	1,2	2,1	5,3	6,3	Sum
1	M	9	4		12	5	30
2	F	28	26	36	4	21	115
12	M					10	10
13	M					2	2
Unidentified	M				4	3	7
Unidentified	F	1	3		6	4	14
Unidentified	?				2	1	3
Total no. of genets		2	2	1	2	4	
Total no. of ramets		38	33	36	28	46	
Total no. of M ramets		9	4	0	16	20	49
Total no. of F ramets		29	29	36	10	25	129
Total no. of ? ramets					2	1	3

alternatively, sporelings are suppressed by the mature ramets. Recent experimental studies have revealed that specific conditions (Wiklund 2002), often in combination with disturbance (Sundberg and Rydin 2002), are essential for recruitment of bryophytes from spores. A comparison of populations of *H. splendens* on islands, dated by their height above sea level in a land uplift area, suggests that a slow, but successive recruitment from spores occurs over time, leading to an increased number of clones (Cronberg 2002). Our spore germination tests clearly show that the spores are viable, but experiments in natural environments are needed to reveal under which circumstances recruitment from spores actually takes place.

Dispersal of bryophyte propagules is strongly leptokurtic, and related to size (Söderström 1994), with the vast majority deposited within a radius of one or two metres (Longton 1976, 1994). In the present case, spores deposited in situ (i.e. on the boulder) seem in general to be wasted. A significant fraction of bryophyte spores are borne by air currents over longer distances (Longton 1994, Söderström 1994). Indirect data from molecular studies suggest that local gene flow mediated by wind-dispersed spores is random in *H. splendens* (Cronberg 2002). Some of the genets identified from the surrounding area could indeed have been derived from spores generated in the boulder population, but most possessed alleles that were not found among the boulder haplotypes and must have been derived from elsewhere, unless some other genet occurred within the parts of the boulder that were not screened for genetic variation.

Clonal size and distribution

Two or more genets grew intermingled in all but one of the five plots. Similarly, Cronberg (2002) found that intermingling was common and increased with increasing population age. A substantial degree of clonal intermingling has been found for some forest floor bryophytes, such as *Plagiothecium undulatum* (Hofman 1991) and *Pleurozium schreberi* (Zielinski and Wachowiak-Zielinska 1995), whereas several *Polytrichum* species appear to form uniclonal colonies (Wyatt and Derda 1997, van der Velde et al. 2001). The terms "phalanx" and "guerrilla" have been coined for clonal vascular plants characterized by different types of modular organization; those with closely aggregated ramets versus those with more widely spaced stolons or rhizomes, respectively (Doust 1981). The equivalents for bryophytes would be species growing in dense tufts or cushions, "acrocarps", like for instance *Polytrichum* species (van der Velde et al. 2001), as opposed to those forming wefts or mats, "pleurocarps", like *H. splendens* (Cronberg 2002). It has been proposed that genets of guerilla species should intermingle to a larger extent than genets of phalanx species (McLellan et al. 1997), facilitating outcrossing and increasing the effective population sizes of populations. Jonsson (1998) found from a literature survey of allozyme variation in Cyperaceae that rhizomatous species had significantly higher levels of within-population varation and less differentiated populations than caespitose species. A similar trend can be seen when the phalanx species *Polytrichum commune* is compared to the guerrilla species *H. splendens*. Both are perennial forest-floor species, but studies of allozymes (Cronberg et al. 1997, Derda and Wyatt 1999, van der Velde and Bijlsma 2000, Cronberg 2004) suggest that variation within populations (H_S) of *H. splendens* is consistently higher and relative genetic differentiation among populations (G_{ST} or F_{ST}) lower than in *P. commune*.

Two of the clones were detected at opposite ends of the boulder, at distances of ca 100 cm. This suggests that each of these clones has been growing on the boulder for a long time. It is possible to calculate a minimum time span for full colonization of the boulder by means of clonal expansion, if we ignore the possibility that vegetative fragments may have been detached and relocated. If the colonization started at the lower part of the boulder by invasion from the ground and expanded radially with a mean distance of 2 cm annually (which is a reasonable estimate for horizontal growth of *H. splendens* shoots with mean size equal to the average size observed on the boulder in the five-year period; Økland unpubl. data), the expansion would have been completed within 50 yr. The expansion may have been even slower because *H. splendens* requires an existing humus-layer, most likely already occupied by other bryophytes, prior to colonization (Tamm 1953, Økland 1995). On the other hand, establishment of detached and relocated *H. splendens* segment chains (Økland 1995, 2000) may have accelerated the colonization process considerably (a mean of 3.6% of the total stock of segments were detached annually; Rydgren unpubl.). In any case, frequent colonization from adjacent patches would have led to increased levels of clonal diversity, and it therefore seems likely that most relocation events occur within patches.

Sex ratio and effective genetic population size

In the total set of nine plots, the observed ramet sex ratio has remained approximately the same during the five-year period of demographic screening, with a 1:4 surplus of females (Rydgren and Økland 2002a). In the subset of five plots that have been analysed genetically, the observed-level ramet sex ratio is 1:2.6, still with an excess of females. The inferred-level ramet

sex ratio does not change this pattern; the haplotype identifications show that both males and females occurred among the ramets that were sterile. Rydgren and Økland (2002a) suggested that the biased sex ratio could have arisen as a result of random processes during colonization of *H. splendens* (e.g. because a higher number of colonists happened to be females, either vegetatively dispersed or recruited from spores). Our results show that the boulder is presently occupied by a single female and three male clones. At the genet level the sex ratio is thus opposite to the observed ramet sex ratio, and cannot explain the excess of female ramets. It is possible that the single female clone arrived on the boulder first and has remained dominant even after the arrival of the three males. One of the male clones, however, is almost equally widespread as the single female, and may therefore have been present for a long time as well. The ramet-level sex ratio differs between plots, and males are underrepresented in the lower plots and relatively more common in the upper two plots. The distribution of the two dominant clones thus appears non-random and may reflect differences in genetic capacity to respond to micro-environmental gradients. In any case, it is clear that the scale of sampling needs to be taken into consideration when sex ratios are calculated. As a comparison, Cronberg (2002) found that the inferred ramet-level sex ratios were often skewed in 10 island populations of *H. splendens*, but balanced in the composite sample including all islands.

Bryophytes, as illustrated by *H. splendens*, differ in many respects from the ideal populations assumed in the Wright-Fisher model of genetic drift (e.g. Hartl and Clark 1989). In particular, generations are overlapping and generation time is likely to be long, which makes it difficult to separate contemporary and historic processes (Petit et al. 2001). The effective genetic population size at plot level is low. Assuming that all males take an equal part in sexual reproduction, this estimate arrives at values between 2 and 3, after correction for imbalanced sex ratios. Since the studied males are of different size, they are unlikely to contribute equally to sexual reproduction in the population and this would reduce the population size even more. Such a low value would imply that genetic drift is strong at the patch level, since loss of a single genotype means loss of a large fraction of the total genetic variation. Drift could still be slow if the exclusion rate of genets is low. The effective population size at the among-patch scale is likely to be much larger, since different patches are colonized by different genets. If gene flow by spores is essentially random at the stand level, the rate of genetic drift depends on the frequency of sporophyte-producing patches, the net recruitment rate and the distance to other stands with sexually reproducing populations of *H. splendens*. Model studies of genetic variation in partially asexual diploid organisms have revealed that even a small number of sexually derived individuals per generation is sufficient to give a population the same pattern of allelic variation as found in a fully sexually reproducing organisms (Bengtsson 2003). One such example may be *Carex bigelowii* (Bengtsson 2003), which displays high clonal diversity and genetic variability despite no observed recruitment (Jonsson et al. 1996). These models do not directly apply to organisms with a dominant haploid generation, such as bryophytes (Bengtsson 2003), but it is worth noting that the genetic population structures of *Carex bigelowii* and *H. splendens* are quite similar in terms of genetic variation, clonal diversity and numbers of co-existing genets within patches.

Conclusions

In a clonal plant, the local population could consist of a single or a few widespread clones. Our results, together with earlier results (Cronberg et al. 1997, Cronberg 2002, 2004), suggest that this is not the case for *H. splendens*; different patches are occupied by different sets of genets. The local population of *H. splendens* could instead be described as a number of patches of limited size, each dominated by a small number of more or less intermingling clones. This suggests that static growth or slow expansion by lateral growth is more important in the studied population than relocation of detached fragments between patches. Recruitment by sexually produced spores is rare but appears to be more common than extinction of clones, so that a net recruitment into the total population occurs over time (cf. Cronberg 2002). In this way clonal diversity is determined by vegetative reproduction at the within-patch level and structured by sexual processes at the among-patch level. Similar population structures are probably common among clonal plants, although appropriate information is currently lacking for most species. Such a population structure can explain how high levels of genetic variation, as observed by molecular markers, can be maintained in populations despite extensive clonal growth.

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