

LIFE HISTORY VARIATION IN GAMETOPHYTE POPULATIONS OF THE MOSS *CERATODON PURPUREUS* (DITRICHACEAE)¹

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The life cycles of mosses and other bryophytes are unique among land plants in that the haploid gametophyte stage is free-living and the diploid sporophyte stage is ephemeral and completes its development attached to the maternal gametophyte. Despite predictions that populations of haploids might contain low levels of genetic variation, moss populations are characterized by substantial variation at isozyme loci. The extent to which this is indicative of ecologically important life history variation is, however, largely unknown. Gametophyte plants from two populations of the moss *Ceratodon purpureus* were grown from single-spore isolates in order to assess variation in growth rates, biomass accumulation, and reproductive output. The data were analyzed using a nested analysis of variance, with haploid sib families (gametophytes derived from the same sporophyte) nested within populations. High levels of life history variation were observed within both populations, and the populations differed significantly in both growth and reproductive characteristics. Overall gametophytic sex ratios did not depart significantly from 1:1 within either population, but there was significant variation among families in both populations for progeny sex ratio. Some families produced predominantly male gametophytes, while others yielded predominantly females. Because *C. purpureus* has a chromosomal mechanism of sex determination, these observations suggest differential (but unpredictable) germination of male and female spores. Life history observations showed that male and female gametophytes are dimorphic in size, maturation rates, and reproductive output.

Key words: *Ceratodon purpureus*; haploids; haploid sib analysis; gametophytes; life history variation; mosses; quantitative genetics.

An alternation between diploid and haploid generations is fundamental to living organisms. All land plants (bryophytes and tracheophytes) have haploid gametophytes and diploid sporophytes that are both multicellular. The relative dominance of the gametophyte and sporophyte generations in terms of temporal longevity and morphological complexity varies, but with the exception of the bryophytes, the sporophyte generation is considerably larger, more complex, and longer lived than the gametophyte generation. The bryophytes are unique among land plants in having relatively large, perennial, photosynthetic, and free-living, haploid gametophytes, and annual, relatively simple, unbranched diploid sporophytes that remain attached to the maternal gametophyte throughout their lifespan.

It has been suggested (Crum, 1976) that moss populations contain relatively low levels of genetic variation because even mildly deleterious alleles would be rapidly eliminated by selection and also because virtually all mosses have the ability to reproduce clonally. However, isozyme data that have accumulated over the last 15 yr have shown that many if not most mosses are characterized by moderate to high levels of variation at enzyme-encoding genes (Wyatt, Stoneburner, and Odrzykoski, 1989; Stoneburner, Wyatt, and Odrzykoski, 1991). Moreover, there is now ample evidence of genetic adaptation to heterogeneous environments in the form of ecotypic differentiation in mosses (Shaw, 1991, 1992), demon-

strating that ecologically relevant genetic variation occurs among populations within species. Nevertheless, no studies have elucidated patterns of variation in multiple life history traits within populations of any moss.

The goal of this research was to describe life history variation within and between two populations of the moss *Ceratodon purpureus*. The experimental protocol used a nested sib design (Shaw, Weir, and Shaw, 1997), which allows variation among families of gametophytes produced by individual sporophytes to be estimated. Because dominance variation does not occur in populations of haploids, the among-family component of variation is attributable to additive and epistatic variance only. We also partitioned variation into components attributable to differences between male and female gametophytes and between the two populations included in our study. This is the first study to dissect components of life history variation within natural bryophyte populations. The hypothesis we wished to test was that moss populations, because of their haploid genetic systems, harbor little or no variation in ecologically important life history traits.

In seed plants, fitness is generally defined as the number of viable diploid offspring to which a diploid individual contributes genetically. Fitness is thus defined as a within-ploidy-level trait (sporophyte production of next-generation sporophytes). The number of gametophytes (pollen and embryo sacs) a sporophyte produces gives an estimate of the reproductive output for that sporophyte. Reproductive output (and success) is a between-ploidy-level characteristic and is a component of fitness since it limits the number of sporophytes a particular sporophyte can produce. For consistency, the fitness and reproductive output of a moss should be defined as within-

¹ Manuscript received 12 November 1997; revision accepted 6 October 1998.

This research was supported by NSF grant DEB-9407937.

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and between-ploidy parameters, respectively (Shaw and Beer, 1997). The fitness of a moss gametophyte is the number of next-generation gametophytes it produces, estimated as the number of spores or viable gametophytes formed by the sporophytes it parents. (For present purposes, we ignore asexual contributions to fitness, although such contributions could be substantial in many mosses.) The reproductive output of a moss gametophyte is estimated as the number of sporophytes it produces by mating with other gametophytes, and this is limited by the number of gametangia (archegonia [with eggs] and antheridia [with sperm]) it produces. The number of gametangia therefore provides a measure of reproductive output in moss gametophytes. Whereas the reproductive success of a seed plant gametophyte is either zero or one (a particular gametophyte forms either one sporophyte or none), variation in the reproductive success of a moss gametophyte is potentially unlimited. In this feature, bryophytes are also unique among land plants. This paper describes variation in growth and reproductive phenology, size and vigor, and reproductive output within and between two populations of gametophytes grown experimentally.

MATERIALS AND METHODS

The study organism and experimental populations—*Ceratodon purpureus* is a widespread and common moss that typically grows on mineral soils in habitats that have been disturbed either naturally or anthropogenically. Its ecological tolerances appear to be quite broad, and the species flourishes in a variety of severe habitats, including concrete surfaces such as parking lots, on the roofs of buildings, polluted soils (Jules and Shaw, 1994), old fields, arctic and alpine tundra, and on recently formed glacial deposits. It also has a very broad geographic range that includes all the major continents (Crum and Anderson, 1981). Gametophytes are unisexual but, unlike some dioecious mosses, *C. purpureus* forms sporophytes commonly. Male and female gametophytes are dimorphic with females larger than males (Shaw and Gaughan, 1993). Sex chromosomes have been reported for *C. purpureus* (Heitz, 1932; Jachimsky, 1935), but biased sex ratios have been observed at the time of germination (Shaw and Gaughan, 1993).

Plants for this research were collected from two populations in Tompkins County, New York. One (hereafter, the CU population) occupies 8 m² on asphalt in a driveway on the campus of Cornell University in Ithaca. The population consists of a more or less continuous, luxurious cushion of gametophytes that each year bear sporophytes in abundance. The population has existed at least since 1992. The other population (hereafter, DB) occupies a much larger area on soil in an abandoned gravel pit near the town of Danby, 35 km from Ithaca. The plants occur at this site in numerous small (4 cm²) to large (2 m²) patches on sterile gravelly soil. Sporophytes are formed abundantly, but not at such high densities as in the CU population.

Experimental protocol—Small samples (e.g., 4 cm²) of gametophytes bearing mature sporophytes were collected at both sites in August 1994. Samples were separated by 1–3 m to avoid multiple collections from the same clone. Spore progeny were obtained from 29 CU and 39 DB sporophytes using the following protocol. Each sporophyte was surface sterilized for 20 sec in undiluted bleach and the spore contents were emptied into 2 mL of sterilized distilled water. A 0.5-mL aliquot of the spore–water suspension was pipetted onto inorganic nutrient medium solidified with 1.2% agar (recipe given in Shaw, Beer, and Lutz, 1989). When the spores began to germinate (after 5 d), individual sporelings were transferred to sterile liquid medium (same as above, without the agar) by removing a small piece of the agar containing the sporeling

with a pair of forceps and placing it into the liquid culture. Individual sporelings were only a few micrometres long at this stage but were examined under a dissecting microscope to ensure that each transfer included only one individual. Seventeen individual sporelings (= haploid sibs) were isolated from each haploid sib family (= the gametophytic offspring of a single sporophyte; Shaw and Gaughan, 1994).

Individuals grew in liquid culture until 3 January 1995. At that time, each sib consisted of a small but visible mass of filamentous protonemata, the juvenile stage of the gametophyte generation. The contents of each tube were emptied onto kitty litter (clay) in a 6-cm² growing compartment in plastic trays that fit 72 such compartments. Each tray held 68 individually growing sibs, one from each of the 68 sporophytes harvested from the field populations (remaining compartments were left empty so that water could be added to the trays without disturbing the plants). The 68 sibs were randomly arranged within each tray, and the trays were placed in growth chambers that maintained a 12-h photoperiod at 16°C. Plants were kept moist by maintaining 3–5 cm of water in the bottom of each plastic tray. In order to eliminate position effects because of microenvironmental differences among trays, the 17 were repositioned every 2 wk.

Once on soil, the plants formed a larger mat of protonemata on which stem buds were produced. Numerous stems are produced by the protonemata formed from one germinated spore. Each plant (the clone growing in each pot, derived from one spore) was examined with a magnifying lens every Monday, Wednesday, and Friday and the first date at which stems were visible was recorded. In addition, the appearance of buds (perigonia) containing antheridia (male reproductive structures containing sperm) were recorded as they became visible. The dates at which the female buds (perichaetia), appeared could not be recorded in this way because they were not visible without dissecting the plants. We therefore obtained data on “days to stem formation” (from the date on which sibs were transferred from liquid to soil) and “days to antheridial formation” (from the date at which stems were first observed in that sib). Days to stem formation can be interpreted as duration of the juvenile stage and days to antheridial formation as the duration of the mature vegetative stage.

During the last week of June 1995, ten stems were harvested from each sib (unless fewer or no stems had formed) and the plants were harvested, washed to remove soil attached to the rhizoids, air dried, and weighed. The following traits were measured on stems that had been harvested before washing and drying. The lengths of three randomly selected leaves, each from a different stem, were measured using a projection microscope. The mean of the three measurements was used as raw data representing each sib. The frequency of stems bearing reproductive structures (antheridia or archegonia) was based on dissecting each of the ten stems sampled from each sib. In addition, the number of reproductive buds (perichaetia, perigonia) per stem were counted; raw data for each sib represent a mean of the ten observations. Some sibs did not form any reproductive buds during the experimental period; these were scored as sterile. The proportions of male, female, and sterile sibs were calculated for each haploid sib family. Individuals that had contained perigonia when dissected, but on which perigonia had not been observed during the experiment, were assigned a “days to antheridia” of [176 minus days to stem formation]. (The experiment was terminated after 176 d.)

Statistical analyses—The process of initiating over 1000 single spore isolates and transferring them to liquid culture took several weeks, which meant that different plants spent variable amounts of time in liquid culture prior to their transfer onto soil. Therefore, before data were analyzed to test for differences between populations, gametophyte sexes, and among families, linear regressions of each trait on the number of days in liquid culture were undertaken to determine whether this constituted a confounding factor in subsequent analyses. Days to stems formation, days to antheridial formation, frequency of sexual stems per individual, frequency of sterile sibs per family, total sib mass, and leaf

length were all significantly affected by the amount of time in liquid culture (data not shown). Therefore, subsequent analyses of variation patterns were done on the residual values from these regressions. Family sex ratios (male sibs/total sibs that expressed sex) were not affected by the amount of time in liquid culture.

Residuals from the regressions were subjected to univariate ANOVAs with populations and gametophyte gender specified as main effects, with haploid sib families nested within populations. Population \times gender and family within population \times gender interactions were also specified in the model. Significance of population effects was assessed using the family within-population mean squares as the error terms; the remaining terms in the model were tested against the residuals. Correlations among life history traits were computed from haploid sib family means, adjusted (by regression analyses as discussed above) for differences in amounts of time plants spent in liquid culture. Correlation matrices for the two populations did not differ significantly based on Mantel's Z statistic (Rohlf, 1993) so data were combined for analyses of trait correlations.

Observational components of variation estimated by the ANOVAs can be related to underlying genetic and biological effects (Cockerham, 1954; Shaw, Weir, and Shaw, 1997). In addition to genetically based differences between the populations in growth and reproductive traits (specified by population main effects), significant gender effects demonstrate gametophytic sexual dimorphism. Previous work on *C. purpureus* demonstrated sexual dimorphism for plant size in an experimental population from Michigan (quantified by leaf size and biomass accumulation; Shaw and Gaughan, 1993). Differences among populations in sexual dimorphism (population \times gender interaction) or haploid sib families within populations (family within population \times gender interaction) demonstrate differences in the degree of dimorphism at the population and family levels.

Because moss gametophytes are haploid and dominance does not contribute to patterns of phenotypic variation, analyses of variation within and among haploid sib families permits separation of additive and epistatic components of variation given an appropriate experimental design (Shaw, Weir, and Shaw, 1997). However, the experimental protocol used in the present research did not involve replicating haploid sibs because the size of the experiment would have been prohibitive. Nevertheless, significant variation among haploid sib families does provide evidence of additive or epistatic genetic variation, or both. If we assume that the epistatic component of variation is minimal, then the among-family variance component gives an estimate of the narrow-sense heritability. Although we can offer no evidence that the epistatic component is, in fact, minimal, virtually all genetic studies of life history variation in natural plant populations have been forced to make this assumption (Mitchell-Olds and Rutledge, 1986; Montalvo and Shaw, 1994). Previous work on *C. purpureus* failed to reveal evidence of epistatic variance for plant size (Shaw et al., 1993).

RESULTS

There was no difference in mean seta length between the populations (data not shown). Field-collected sporophytes from the DB population were, however, substantially more variable in seta length than were those from the CU population (Figs. 1–2). The length of the seta (which is the unbranched axis of the sporophyte) is tightly correlated with capsule (sporangium) size (data not shown), so seta length is a good index of overall sporophyte size, and probably spore number. The degree of habitat heterogeneity appeared to be substantially greater at the DB site, but we could not distinguish environmental and genetic causes of increased sporophyte variation at that site.

Sex ratios were highly variable among families (Figs.

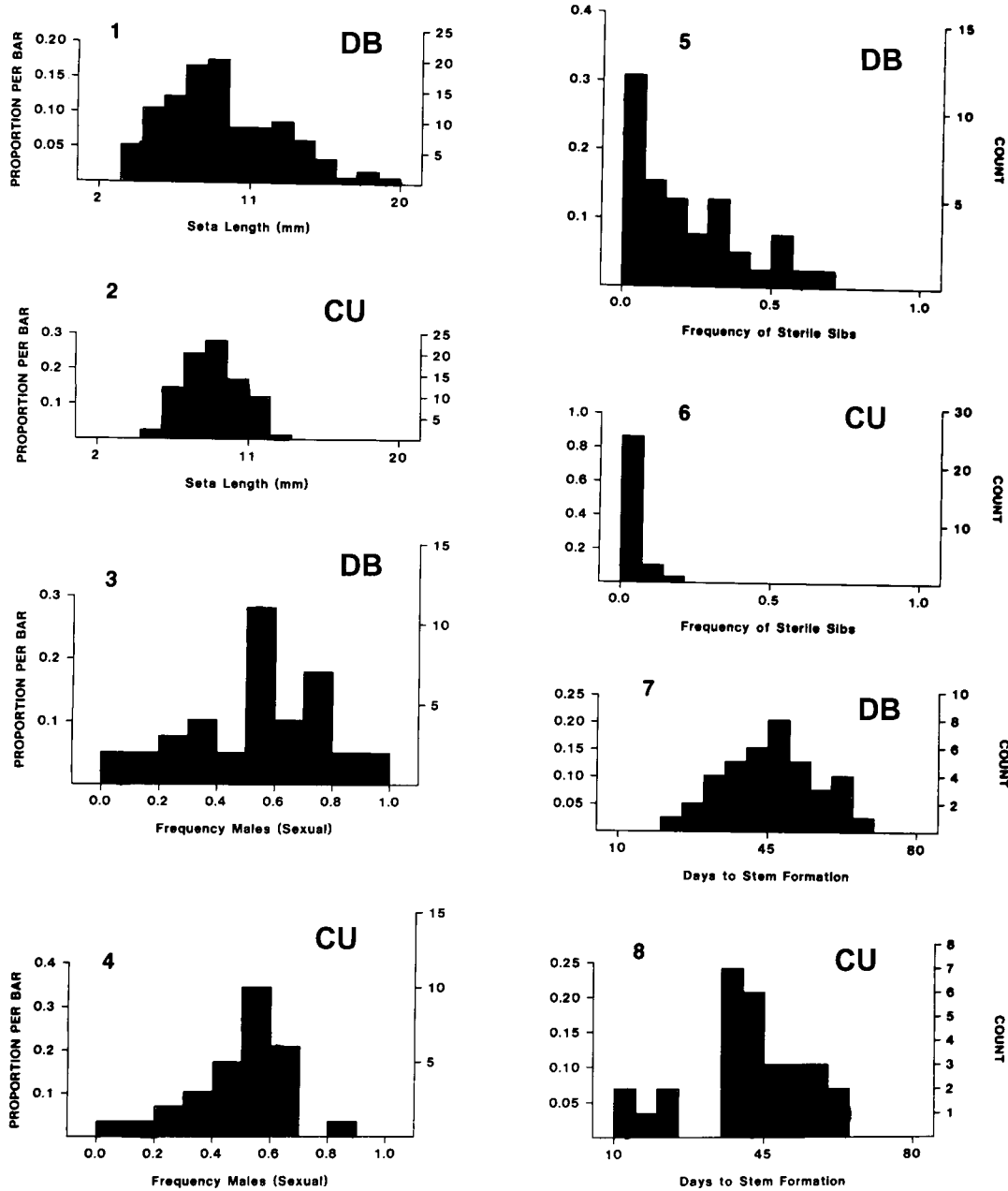
3–4), and the variation was significant within both populations (CU: $\chi^2 = 48.7$, $df = 28$, $P < 0.01$; DB: $\chi^2 = 120.12$, $df = 38$, $P \leq 0.001$). DB families included the whole range of variation from strongly female to strongly male biased (Fig. 3). Male-biased families were less common in the CU population (Fig. 4). Despite the remarkable levels of variation in sex ratios among families, both populations had mean sex ratios that did not differ significantly from 0.5, nor did the populations differ from one another in mean sex ratio (Table 1). Families that produced roughly equal numbers of male and female gametophytes were numerically most abundant in both populations (Figs. 3–4). Sibs that did not form gametangia of either sex during the experiment occurred in only three families from the CU population, but almost 70% of the DB families included at least one sterile sib, and in four families >50% of the sibs did not form gametangia (Figs. 5–6). Family sex ratio was not correlated with the frequency of sterile sibs.

The populations, and families within populations, varied significantly in days to stem formation (Table 2). CU plants formed stems faster than DB plants (Table 1), but the difference was largely attributable to five exceptionally fast families in the CU population (Figs. 7–8). There was no detectable difference in rates of stem formation between males and females (Tables 2–3), but gametophytes that did not form any gametangia during the experiment took 25% more days to form stems compared to sexual plants (Table 3).

Male plants from the CU population also formed perigonia in fewer days than males from DB, but the difference was not significant (Tables 1–2). There was, however, significant variation among families within both populations (Figs. 9–10; Table 2; separate analyses by population not shown). Differences in times to gametangial formation between the slowest and fastest families was more than threefold in both populations (Figs. 9–10).

Moss spores give rise to an extensive mat of filamentous protonemata that form hundreds or even thousands of stems. In nature, the protonematal stage of most species is ephemeral, and the clonal stems formed from a single spore become physically separate. Not all the stems form gametangia. Plants from the CU population had a significantly higher proportion of stems that carried gametangia than DB plants (Tables 1–2; Figs. 11–12). In addition, male gametophytes consisted of a higher proportion of sexual stems than were females (Tables 2–3). Families within both populations varied in the mean proportion of sexual stems formed by individual sibs, and the degree of difference in sexual expression between males and females also varied among families, as indicated by a significant gender \times family within-population interaction (Table 2).

Stems of a clone that carried gametangia formed a variable number of gametangial buds. On average, plants from the CU population had more buds than DB plants, females had more than males, and haploid sib families within both populations were variable in bud formation (Figs. 13–14; Tables 1–3). Most of the DB families consisted of individuals that averaged fewer than 1.75 buds per sexual stem; only two families were more prolific bud formers (Fig. 13). In the CU population, in contrast, al-



Figs. 1–8. Histograms of seta length in field-collected sporophytes from the DB and CU populations of *C. purpureus* (Figs. 1–2) and of haploid-sib family means for life history traits (Figs. 3–8).

TABLE 1. Means \pm 1 SE for life history traits in *Ceratodon purpureus* summarized by population (data from male, female, and sterile gametophytes combined).

Trait	Cornell (CU)	Danby (DB)
Days to stems	42.9 \pm 1.1	45.6 \pm 1.0
Days to antheridia	53.4 \pm 1.8	68.7 \pm 2.1
Percentage sexual stems	45.1 \pm 1.3	28.4 \pm 1.2
Gametangia/plant	1.67 \pm 0.1	1.44 \pm 0.1
Leaf length (micrometer units)	14.65 \pm 0.17	16.41 \pm 0.17
Dry mass (mg)	69.8 \pm 1.9	48.1 \pm 1.3
Sex ratio (males/total sexual stems)	0.48 \pm 0.03	0.53 \pm 0.02

most a third of the families consisted of plants that formed >1.75 gametangial buds per stem (Fig. 14).

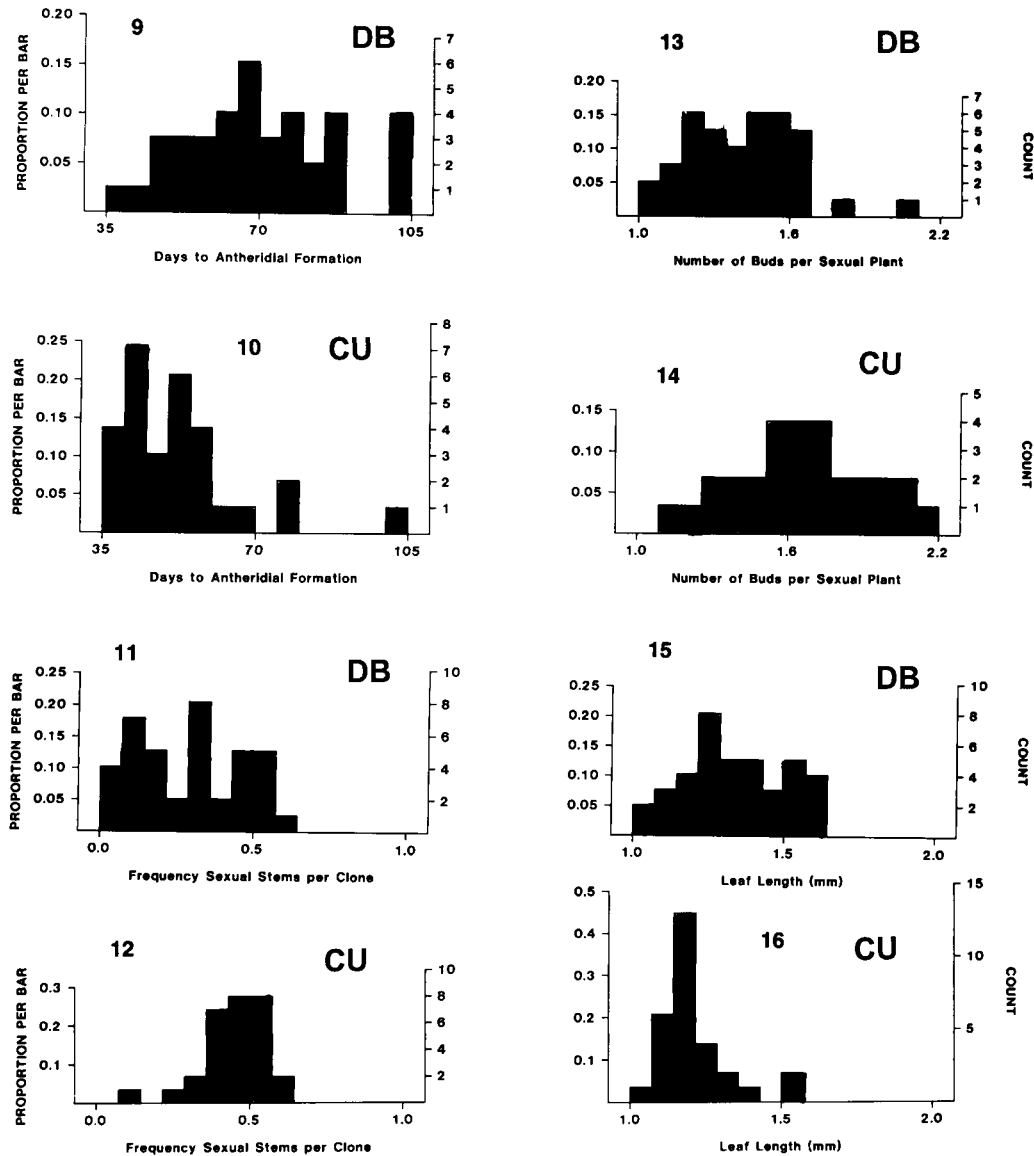
DB plants had longer leaves than CU plants, and females had longer leaves than males (Tables 1–3). In addition, leaf length was variable among families in the DB population; variation among families approached significance ($P \leq 0.06$) in the CU population (Table 2; Figs. 15–16). Significant gender \times families within-population interactions for leaf length indicate that there is genetic variability for the degree of sexual dimorphism within populations, and the significant gender \times population interaction shows that these two populations indeed differ in levels of dimorphism.

TABLE 2. Analyses of variance for growth and reproductive traits in *Ceratodon purpureus*. Data used in these analyses were residuals from linear regressions of each trait on the number of days each plant was maintained in liquid culture prior to growth on soil. See text for additional details. *** $P \leq 0.0001$; ** $P \leq 0.01$; * $P \leq 0.05$.

Trait	df	SS	MS	F
A) Days to stem formation				
Populations	1	6498.814	6498.814	6.39***
Gender	1	611.756	611.756	1.51
Families within populations	66	67 092.725	1016.556	2.51***
Population \times gender	1	251.630	251.630	0.62
Gender \times families within populations	64	25 540.105	399.064	0.99
Error	740	299 772.682	405.098	
B) Days to antheridia formation				
Populations	1	5302.752	5302.752	3.19
Families within populations	64	109 613.320	1660.808	2.19***
Error	376	284 585.311	756.876	
C) Frequency of stems with gametangia				
Populations	1	0.925	0.925	4.93*
Gender	1	2.736	2.736	48.53***
Families within populations	66	12.371	0.187	3.32***
Population \times gender	1	0.022	0.022	0.39
Gender \times families within populations	64	4.899	0.077	1.36*
Error	742	41.828	0.056	
D) Number of gametangial buds per sexual stem				
Populations	1	8.201	8.201	15.77**
Gender	1	3.015	3.015	8.44**
Families within populations	66	34.325	0.520	1.46*
Population \times gender	1	0.287	0.287	0.80
Gender \times families within populations	64	24.271	0.398	1.11
Error	646	203.749	0.357	
E) Leaf length				
Populations	1	139.265	139.265	7.31**
Gender	1	1654.750	1654.750	196.59***
Families within populations	66	2100.664	31.828	3.78***
Population \times gender	1	46.576	46.576	5.53*
Gender \times families within populations	64	1135.472	17.742	2.11***
Error	742	6245.686	8.417	
E) Dry mass				
Populations	1	0.025	0.025	8.53**
Gender	1	0.003	0.003	2.91
Families within populations	66	0.191	0.003	2.72***
Population \times gender	1	0.001	0.001	0.07
Gender \times families within populations	64	0.063	0.001	0.92
Error	742	27.634	0.037	

TABLE 3. Means \pm 1 SE for life history traits in *Ceratodon purpureus* from two populations, broken down by gametophyte gender.

Trait	Males	Females	Steriles
A) Cornell population (CU)			
Days to stems	44.5 \pm 1.5	40.5 \pm 1.5	72.4 \pm 12.6
Days to antheridia	53.4 \pm 1.8	—	—
Percentage sexual plants	55.1 \pm 1.9	37.4 \pm 0.1	—
Gametangia/plant	1.56 \pm 0.04	1.77 \pm 0.05	—
Leaf length	12.70 \pm 0.21	16.56 \pm 0.19	12.40 \pm 0.80
Dry mass (mg)	68.7 \pm 2.7	73.8 \pm 2.7	14.9 \pm 5.1
B) Danby population (DB)			
Days to stems	43.9 \pm 1.4	42.7 \pm 1.6	54.1 \pm 2.8
Days to antheridia	68.7 \pm 2.1	—	—
Percentage sexual stems	42.8 \pm 2.0	27.8 \pm 1.5	—
Gametangia/plant	0.64 \pm 0.04	0.45 \pm 0.03	—
Leaf length (units)	15.00 \pm 0.25	18.06 \pm 0.23	16.26 \pm 0.37
Dry mass (mg)	52.2 \pm 1.9	55.2 \pm 2.3	27.6 \pm 2.0



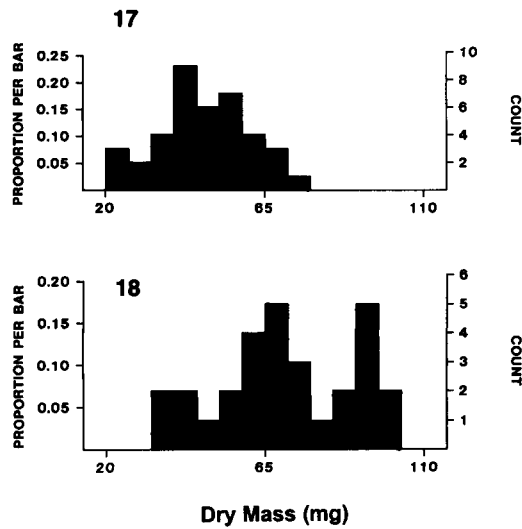
Figs. 9–16. Histograms of haploid-sib family means for life history traits in *Ceratodon purpureus*.

CU plants formed substantially more biomass during the experiment than did DB plants (Tables 1–2). Variation among families within both populations was significant (Figs. 17–18; ANOVA results by population not shown). Although the range of variation in family means for the two populations was broadly overlapping, lightweight CU families were virtually absent, as were heavyweight DB families (Figs. 17–18).

A principal components analysis of family means suggests that gametophyte developmental rate is a major axis of variation (Fig. 19). Days to stem and antheridial formation, proportion of sexual stems per individual, the frequency of sterile individuals, the number of buds per sexual stem, and total biomass have high loadings in relation to the first principal component, which accounts for 45% of the total variation (Table 4). In general, CU families appear to mature more quickly than DB families (Fig. 19). Thus, CU plants tend to have shorter juvenile and vegetative stages, each individual formed a higher

proportion of sexual stems during the experiment, fewer individuals remained completely vegetative, they formed more buds per sexual stem, and they accumulated more biomass (Fig. 19, Table 4). DB plants tended to have longer leaves, but this feature is probably independent of developmental rate, since leaf growth is determinate and the leaves that were measured were comparably mature.

The second and third principal components reflect variation related to sexual dimorphism (Table 4). Combined, the second and third components account for 34% of the total variation, highlighting the contribution of sexual dimorphism to morphological and life history variation in both populations. Loadings of life history traits on the second component show that families with predominantly female offspring (low scores for the component) tend to form stems more quickly, form more biomass, and have larger leaves. The third component, although it summarizes only 14% of the total variation, suggests a different pattern of sexual dimorphism. Some families with pre-



Figs. 17–18. Histograms of haploid-sib family means for life history traits in *Ceratodon purpureus*.

dominantly female offspring have a longer juvenile stage (days to stem formation) and form less total biomass.

DISCUSSION

This study shows that there are high levels of variation in presumed fitness components within the two experimental populations of *C. purpureus*. This complements accumulating data from isozyme studies of mosses that have shown that despite the haploid nature of moss gametophyte populations, high levels of genetic variation are the rule rather than the exception (Wyatt, Odrzykoski, and Stoneburner, 1989; Wyatt, Stoneburner, and Odrzykoski, 1989; Stoneburner, Wyatt, and Odrzykoski, 1991). Ironically, the CU and DB populations of *C. purpureus* are depauperate in terms of isozyme variation, as are a number of other populations of this species from New York State and other parts of the eastern United States (unpublished data).

Phenotypic variation among haploid sib families can include additive and/or epistatic genetic components and although our experimental design does not allow us to separate these causal effects, it is likely that at least some of the variation is due to the additive effects of genes. Clear differences in life history traits between the two populations confirm the potential for divergent evolution implied by among-family variation within the popula-

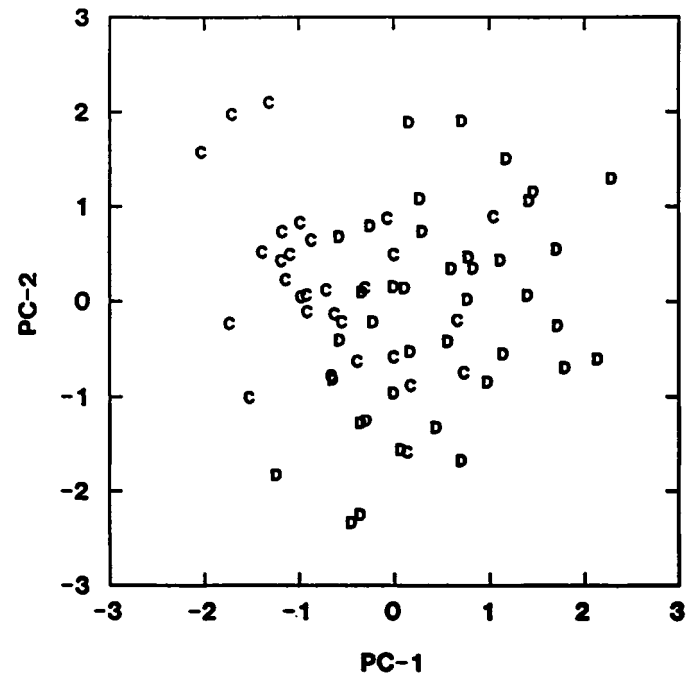


Fig. 19. CU (C) and DB (D) families plotted in relation to the first two components from a principal components analysis of family means for eight life history traits (see Table 4).

tions. Plants from the CU population tend to have a shorter juvenile stage (days to stem formation), a shorter mature vegetative stage at least in males (days to antheridial formation), a higher reproductive output (frequency of stems with gametangia per clone, and number of gametangial buds per sexual stem), and greater biomass accumulation. DB plants had longer leaves, but this trait does not appear to be correlated with other components of fitness. These characteristic differences between plants in the CU and DB populations conform to the patterns predicted by r- and k-selection, respectively (MacArthur and Wilson, 1967). The habitat of the CU population, a driveway surface, may be on the ephemeral end of the spectrum of *Ceratodon* habitats and may be related to differences between the populations in life history characteristics.

It is not possible to directly compare levels of variation in the two populations because the sample sizes and error variances are different, but it appears from the histograms of family means that the CU population is more variable

TABLE 4. Loadings of life history traits on the first three components from a principal components analysis of *Ceratodon purpureus*.

Trait	Principal component		
	1	2	3
Days to stem formation	0.543	-0.557	0.456
Days to antheridial formation	0.785	0.040	-0.393
Proportion sexual stems per individual	-0.852	-0.387	0.071
Frequency of sterile individuals	0.831	0.266	-0.020
Buds per sexual stem	-0.779	0.276	0.009
Sex ratio	0.129	-0.512	-0.787
Leaf length	0.427	0.749	0.056
Biomass	-0.698	0.456	-0.319
Percentage of total variance	45.23	20.49	13.66

in some traits, while DB families are more variable in others. CU plants are notably variable in days to stem formation, number of gametangial buds per sexual stem, and dry mass, while DB plants are especially variable in the frequency of sterile sibs per family, days to antheridial formation, and leaf length. DB families are also exceptionally variable in progeny sex ratios. Field-grown sporophytes from the DB site were extremely variable, but this may reflect the more heterogeneous environment (because of its far greater aerial extent) in which they matured.

Five families in the CU population were exceptionally fast in forming stems and gametangia. In fact, these families account for 58% of the variance of family means in the population for length of the juvenile stage, although they only account for 7% of the variance in length of the mature vegetative stage (days to antheridia). It is noteworthy that Shaw, Weir, and Shaw (1997) likewise found that three exceptional families of *C. purpureus*, out of 40 grown from a Michigan population, accounted for 50% of the additive variance for biomass accumulation among gametophytes in that population.

The classic question that is raised by observations such as these is how such high levels of variation are maintained in traits that are likely to be important components of gametophyte fitness. Mechanisms that rely on heterozygosity shielding deleterious alleles from selection pressures do not, of course, apply to moss gametophytes. Chromosome numbers of $n = 11$, 12, and 13 have been reported in *C. purpureus*, with $n = 13$ by far most common (V. Bryan, unpublished data). Bryan (unpublished data) found a single population of *C. purpureus* from Austria with $n = 6$ and invoked autopolyploidy followed by several episodes of aneuploidy to explain the origin of higher chromosome numbers. Patterns of isozyme expression in *C. purpureus* do not indicate diploid gene expression despite the fact that this species, like most other mosses, might have experienced at least one event of chromosome doubling in its distant phylogenetic history (Smith, 1978). Multiple niche selection has been invoked to explain the maintenance of high levels of variation in populations of other haploid organisms such as bacteria (Selander, Beltran, and Smith, 1991), and similar explanations have been put forth to explain high levels of isozyme variation in some mosses (Wyatt, Odrzykoski, and Stoneburner, 1989). Experimental data from moss populations to support the suggestion that these organisms respond to fine-scale multiple niche selection are, however, lacking.

The levels of life history variation we observed are too high to attribute to mutation pressure. Likewise, the maintenance of genetic variation in these populations via gene flow from nearby plants simply pushes the problem to a different site. It is hard to imagine how some of the variants we observed could be maintained in any population. One family from the DB population, for example, yielded gametophytes that produced few or no stems during the experiment, during which other plants formed mature stems and abundant reproductive structures. How could such gametophytes persist in the population? If only a few gametophytes scattered in different families had such seemingly maladaptive phenotypes, one or more mutations during meiosis could be invoked as a viable

explanation. However, the existence of entire haploid sib families with nearly inviable individuals must involve a mutation during early developmental stages of the sporophyte with subsequent incorporation into all spore mother cells.

Although we do not have an answer to the question of how such high levels of genetic variation can be maintained in *C. purpureus*, we suggest two testable hypotheses that we believe could provide contributing mechanisms. One is that interlocus interactions (epistasis) could maintain variation in a manner analogous to the role of dominance in diploids. Our experimental design does not permit the separation of additive and epistatic components of variance, but epistasis can be separated using either a nested haploid sib analysis (Shaw, Weir, and Shaw, 1997) or by crossing studies. In the absence of epistasis, the gametophyte progeny mean for some trait from a cross should be midway between the genotypic value of the parental gametophytes (Burnett, 1975). These approaches could be applied to the *Ceratodon* system to assess the contribution of epistatic variance to variation in life history traits. The nested haploid sib analysis does not involve crosses, but requires a very large experiment that was not feasible (or directly relevant) for present purposes of documenting variation within the two populations.

Another possible mechanism for maintaining variation in populations of moss gametophytes is through trade-offs between fitness components in the sporophyte and gametophyte generations. To the extent that there is overlap in gene expression between the generations, pleiotropic effects of individual genes could, for example, cause increases in sporophyte fitness while causing decreases in gametophyte fitness. Such constraints on evolution are analogous to well-documented trade-offs between life history components at different developmental stages in the sporophyte generation of higher plants (Roach, 1986; Montalvo and Shaw, 1994). In general, there appears to be a high level of genetic overlap (in terms of gene expression) in the sporophyte and gametophyte generations of flowering plants. Some 50–75% overlap in gene expression has been estimated for angiosperms (Ottaviano and Mulcahy, 1989), which have far more different gametophytes and sporophytes than do mosses. Many genes of mosses are probably expressed in both generations and patterns of genetic covariance in sporophytes and gametophytes could have significant effects on moss evolution (Shaw and Beer, 1997).

An additional factor that cannot be discounted is that variation observed under controlled growth conditions would not be expressed in the field. We have shown that the length of time sporelings grew in liquid culture affected subsequent life history characters. Nevertheless, if such effects contributed to variation among families, our results suggest that families responded differently to growth in the liquid cultures.

The existence of high levels of variation in progeny sex ratios was perhaps the most surprising and intriguing observation to come from our experiment. Plant sex chromosomes were originally discovered in the liverwort *Sphaerocarpos* (Allen, 1917, 1919) and sex-determining, or at least sex-correlated (Anderson, 1980), chromosomes are known in a substantial number of mosses (Ramsay

and Berrie, 1982). In a chromosomal system of sex determination, moss sporophytes are always XY, and meiotic segregation in capsules should result in male and female spores in a 1:1 ratio. Typically, doubling of the chromosome number via polyploidization leads to bisexual gametophytes (because they carry both X and Y chromosomes). Sex chromosomes have not been observed in all mosses and the possibility that other sex-determining mechanisms exist in some species cannot be eliminated. Nevertheless, no other system has been demonstrated for any species, and there is no evidence of environmentally controlled determination.

Sex chromosomes were reported in *C. purpureus* by Heitz (1932) and Jachimsky (1935). Nevertheless many populations of *C. purpureus* exhibit skewed sex ratios and female gametophytes tend to be more common than males in most populations (Shaw and Gaughan, 1993). Shaw and Gaughan (1993) found that females outnumbered males (by 3:2) among gametophytes isolated at the time of germination from a Michigan population. In our experiment, the population-wide sex ratios among gametophytes from both CU and DB did not differ significantly from 1:1, but individual haploid sib families exhibited highly skewed ratios. Indeed, given the exceptional variation among families in progeny sex ratio, it is remarkable that the overall ratio in both populations was so close to 1:1 and may suggest a role for selection in maintaining equal proportions of male and female gametophytes in the population.

Assuming that gametophyte sex is, in fact, chromosomally determined in *C. purpureus*, nonrandom survival of spores could be effected by either genetic or environmental factors. Sex ratio distorter genes have been implicated in biased (sporophyte) sex ratios in the dioecious plant *Silene alba*, which also has sex chromosomes (Taylor, 1994). Both maternal and paternal effects on sex ratios in *Silene* suggest that several genes are involved in the modification of sex ratios. Mogensen (1981) proposed a system involving balanced lethals to explain his observation that 50% of the spores from all sporophytes of *C. purpureus* are abortive, but our observations do not corroborate such a consistent level of spore abortion, nor does the balanced lethal system offer a viable explanation for the variable sex ratios we observed among families. There are, however, sufficient numbers of nongerminating spores in all capsules of *C. purpureus* to make nonrandom spore mortality a viable mechanism to explain biased sex ratios among haploid sib progeny.

Theoretical considerations on the evolution of biased sex ratios require that among-family differences in progeny sex ratio are heritable, but relatively few studies have addressed the genetic basis of sex ratio variation in plants, and none have involved mosses. While our results suggest the existence of heritable differences in sex ratio among haploid sib families in *C. purpureus*, the possibility that progeny sex ratio is controlled or at least affected by environmental factors cannot be eliminated. In fact, among-family variation could result from a combination of genetic and environmental factors, and the two are not mutually exclusive. The environment in which spore mother cells undergo meiosis, for example, and in which postmeiotic spores mature, is a function of both the genotype of the parental sporophyte and the external

environment of that sporophyte. In fact, because the sporophyte completes its full development attached to the maternal gametophyte, the environment provided for that sporophyte (and consequently the spores developing within it) is affected by the maternal gametophyte to which it is attached (Shaw and Beer, 1997). Thus, developing spores that yield biased sex ratios are in direct connection with not only the parental sporophyte but also the maternal gametophyte that produced that sporophyte. Large gametophytes (resulting from their genetic makeup and/or their nutritional status) tend to form large sporophytes, and large sporophytes may nurture spores differently than do small sporophytes (Shaw and Beer, 1997). It has now been well established that the nutrient status of flowering plant sporophytes can influence the performance of pollen they produce (Young and Stanton, 1990; Lau and Stephenson, 1993, 1994). In *Zea mays*, pollen from F₁ hybrid plants perform differently than pollen from inbred individuals (Johnson and Mulcahy, 1978). Because of the physical and nutritional connection between moss sporophytes and several generations of gametophytes, very complex interactions between the generations could have substantial influences on progeny performance, including sex ratio.

In addition to potential complications introduced by the moss life cycle, the X/Y system of sex determination in mosses is quite different than X/Y systems in angiosperms because in mosses the sporophyte is always XY and is hermaphroditic. Gametophytic sex determination occurs at meiosis rather than at syngamy. Biased sex ratios in mosses, instead of involving mechanisms such as differential success of X- vs. Y-bearing pollen (Mulcahy, 1967), must involve events that occur during or subsequent to meiosis, and/or during germination. While the proximate mechanisms that effect biased sex ratios in mosses must differ from those underlying analogous phenomena in angiosperms, evolutionary mechanisms through which selection might favor such skewed ratios, including the relative cost of producing males vs. females, and X- or Y-linked meiotic drive, could occur in populations of mosses as in higher plants (Fisher, 1930; Sandler and Novitski, 1967). Crossing studies to determine the genetic basis of sex ratio variation in *C. purpureus* are needed.

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