

Effects of Metals on Growth, Morphology, and Reproduction of *Ceratodon purpureus*

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Abstract. *Gametophytic plants from six populations of the moss Ceratodon purpureus (Hedw.) Brid. were grown on substrates with varied degrees of heavy-metal contamination in order to assess the effects of metal pollution on growth, leaf size, and formation of archegonia and antheridia. Sex ratios and the overall levels of sexual expression varied greatly among samples from natural populations. Sex ratios in four populations were biased in favor of females, one in favor of males, and one contained asexual gametophores only. Experimental substrates had a significant effect on the overall levels of sexual expression but not on the relative frequencies of male versus female stems. Innate differences in leaf size among populations were maintained in a common garden but leaf size was also affected by substrate. Patterns of phenotypic plasticity characterizing the six populations were indistinguishable. Plants from several populations that originated in uncontaminated habitats grew as well on mine soil as plants from a mine-site population, suggesting an absence of ecotypic differentiation.*

It is a fundamental problem in evolutionary biology to distinguish environmental from genetic sources of phenotypic variability, since predictions about future evolutionary change require that heritable components of variability be recognized. It is well known that many gametophytic traits in bryophytes are susceptible to environmentally induced modifications (reviews in Schuster 1983). Environmental factors can also have an effect on population-level processes. Success of sexual reproduction is obviously affected by moisture supply through limitations on dispersal of motile gametes, and more subtle environmental effects on such processes as gametangial formation and abundance, or gamete viability, are also likely, though less well understood. These effects, acting on individual organisms, have important consequences for the genetic structure of populations.

Bryophytes with bisexual gametophytes generally form sporophytes more abundantly than do those with unisexual gametophytes (Gemmell 1950; Wyatt 1982). Sporophyte formation in many bryophytes, whether monoecious or dioecious, is relatively uncommon in habitats subjected to environmental pollution (LeBlanc & De Sloover 1970; Longton 1985; Shaw 1989). LeBlanc and De Sloover (1970) felt that such populations are simply less vigorous and that poor health is reflected in reduced sexual expression. Little is actually known, however, about why sexual reproduction frequently fails in mosses growing in polluted environments.

Questions about why sexual reproduction occurs infrequently in polluted environments constitute a

subset of more general issues regarding the factors that limit sex in bryophytes. Some populations of dioecious species appear to consist of one sex only (Anderson 1943; Longton & Schuster 1983). Populations could be unisexual because of founder events in which gametophytes of only one sex colonized the area. It is also possible that unisexual populations occur because of differential mortality between male and female plants. On the other hand, some populations that appear to contain plants of only one sex may in fact contain both males and females with gametangium formation inhibited in one sex or the other (Reese 1984). Environmental factors might determine whether male or female gametangia occur in any particular population, even when the population is genetically bisexual.

Ceratodon purpureus (Hedw.) Brid. is widespread throughout the Northern Hemisphere and occurs in a range of habitats including both polluted and unpolluted sites. It is common in urban and industrial environments subjected to a variety of pollutants, along highways, and on the tailings and refuse associated with both coal and heavy-metal mining activities. The species is dioecious but sporophytes are formed abundantly at unpolluted sites. In many urban areas of eastern North America *C. purpureus* is abundant along roadsides but in such places sporophytes are often absent or rare. Likewise, populations growing on mine tailings or in other habitats contaminated by heavy metals often lack sporophytes in spite of vigorous gametophytic growth.

In this paper we document sex ratios in six populations of *C. purpureus*. These originated in both

polluted and unpolluted sites. We wished to determine whether natural populations exhibit 1:1 ratios of male to female gametophores as expected if sex determination is chromosomal (Ramsay & Berrie 1982). Secondly, we grew bulk population samples under uniform experimental conditions in order to test the hypothesis that phenotypic sex ratios estimated from populations in the field reflect the relative proportions of genetically male and female plants. Many populations contain a high frequency of sterile gametophytes and, except in sexually dimorphic species or when a cytological distinction can be readily made, there is no practical way to determine the sex of sterile gametophores. We reasoned that if sex ratios under natural conditions reflect primary genetic sex ratios, the proportions of male and female plants regenerated under experimental conditions from a random sample of the population should match those observed in the original population. We acknowledge that environmental influences might carry over more than one generation of clonal propagation and affect sex expression, but we consider experimental evidence of this type suggestive. Thirdly, we wished to determine whether metals, supplied as either a mist or in soil, had measurable effects on gametophytic growth, levels of sex expression, sex ratios, or morphology. Finally, we wanted to determine whether these effects differed among populations, reflecting genetically based variation in patterns of "phenotypic plasticity." In order to answer these questions, samples were collected from six populations growing in diverse environments, and these plants were then grown on four soil treatments with varying degrees of heavy-metal contamination, plus a treatment in which lead was supplied in solution at weekly intervals as a mist.

MATERIALS AND METHODS

Gametophytic plants were collected from six localities in the eastern United States in May 1989. At each locality, small (2–4 cm²) clumps of gametophytes were collected and pooled in a paper bag in order to obtain a composite sample of the population. Information on the physical position of individuals within the populations was not recorded. Locality data for the populations are: 1) Blue Wing Mine, Granville Co., North Carolina, on copper-contaminated mine tailings; 2) Blue Wing Baptist Church, Granville Co., North Carolina, on uncontaminated sandy soil in the shade of a large *Quercus phellos* L., behind the church, ca. 0.75 km from the Blue Wing Mine; 3) Durham, Durham Co., North Carolina, under a large *Quercus phellos*, ca. 2 m from one of the main roads within the city of Durham; 4) Baltimore, Baltimore Co., Maryland, on disturbed soil ca. 50 m from Highway 51, in a semirural area just NW of the city of Baltimore; 5) Scranton Co., Pennsylvania, on grassy soil of a roadbank ca. 10 m from the heavily-travelled Interstate-81; 6) Ithaca, Tompkins Co., New York, on soil in sidewalk crevices within the city of Ithaca.

Both sexual expression and sex ratios were estimated for each population. Sexual expression was defined as the frequency of stems bearing antheridia or archegonia (as opposed to stems that were completely asexual). Sex ratios were defined as the ratio of males to sexual stems (males plus females). Fifty stems from each field-collected population were dissected to determine the presence or absence of archegonia and antheridia.

Gametophytic samples from each natural population were analyzed for the concentrations of 18 macro- and micronutrients. Samples were prepared by thoroughly washing each under distilled water, and elemental concentrations were measured in the Department of Pomology, Cornell University, using inductively coupled argon plasma emission spectrometry. It is very difficult to remove all traces of soil contamination from gametophytic samples of bryophytes (e.g., Shacklette 1965), so our elemental concentrations probably include some external contamination. Although our measurements should not be interpreted as internal, physiologically active concentrations, the data do allow general comparisons of nutrient and metal abundances between the natural habitats. Details of the analytical procedures employed for the elemental analyses were provided by Shaw et al. (1989).

In order to assess the effects of environmental variables on gametangial formation, samples from each population of *C. purpureus* were grown experimentally in a greenhouse at Ithaca College. In the first experiment, plants were grown on four soil treatments; 1) sand, 2) soil from tailings of the Union Copper Mine, Rowan Co., North Carolina, 3) two parts sand mixed with one part mine soil (v/v), and 4) one part sand and two parts mine soil. The Union Copper Mine is approximately 2 km from the Blue Wing Mine and *C. purpureus* grows at both mines. Union mine soil was collected for use in several other experiments and contains high concentrations of copper (3,208 µg/g) and zinc (1,201 µg/g) (Shaw et al. 1987). The Ithaca population was collected later in the season and was not included in this experiment.

Field-collected samples were air-dried, ground to a fine powder, and 70 mg of the powder was sprinkled onto the surface of the appropriate soil mixture in plastic greenhouse pots (Shaw 1986). Three replicates of each treatment were inoculated; the experiment therefore consisted of five populations, four treatments, and three replicates. The experimental design was split block with the populations and treatments completely randomized within the three replicate blocks.

After five months of growth, ten gametophytic stems were selected from each pot and examined for the presence of antheridia or archegonia. Thus, 30 stems per treatment were examined. Each was dissected under a stereomicroscope. Cover area was estimated for each pot by placing a grid, divided into 0.5 × 0.5 cm squares, over the surface. We counted the number of squares under which plants of *C. purpureus* were growing. Although these measurements actually yield estimates of cover frequency, the plants of *C. purpureus* were large enough to virtually fill any square under which they grew. Thus, the measurements can be interpreted as estimates of cover area.

In the second experiment, plants from all six populations were grown on sand. Half the pots received a lead treatment consisting of one ml of 300 µg/g lead chloride applied once each week. The remaining pots received distilled water only. For this experiment, 100 mg of dried moss were inoculated onto each pot. After five months, ten stems from each pot were sampled for the presence of gametangia and cover areas were estimated as described for the first experiment. This experiment consisted of six

TABLE 1. Elemental concentrations in plants from populations of *Ceratodon purpureus* sampled for experimental studies. (ppm = $\mu\text{g/g}$).

	Population					
	Blue Wing Mine	Blue Wing Church	Durham	Baltimore	I-81	Ithaca
Al (ppm)	2,613	5,624	3,180	4,641	4,081	4,517
As (ppm)	0.00	0.00	0.00	0.00	0.00	0.29
B (ppm)	8.11	11.84	7.15	14.39	8.83	23.32
Ca (%)	1.40	0.38	0.48	0.40	0.69	2.78
Cd (ppm)	0.75	0.00	0.37	0.00	0.15	8.29
Co (ppm)	1.34	0.00	4.26	0.00	4.53	0.00
Cr (ppm)	4.00	18.39	24.79	18.71	15.71	43.40
Cu (ppm)	1,605	46	51	160	51	290
Fe (ppm)	5,119	14,250	4,830	17,930	7,739	16,040
K (%)	0.08	0.09	0.14	0.06	0.05	0.28
Mg (%)	0.14	0.06	0.09	0.07	0.10	0.41
Mn (ppm)	212.60	113.50	302.40	512.20	422.90	387.30
Mo (ppm)	0.35	0.00	0.67	0.64	0.96	2.81
Na (ppm)	157.40	165.30	98.30	135.00	228.90	288.60
Ni (ppm)	4.19	5.59	7.91	13.55	9.08	25.19
P (%)	0.07	0.04	0.11	0.06	0.06	0.19
Pb (ppm)	77.69	35.76	205.10	65.56	234.10	931.50
Zn (ppm)	71.80	41.10	491.00	45.30	124.30	1,993.00

populations, two treatments, and four replicates. As in the first experiment, the design was split block with populations and treatments completely randomized within the replicate blocks.

Statistical analyses were performed using the GLM procedure of the SAS statistical package (SAS Institute 1982). Populations and soil treatments were considered random effects as generalizations to other populations and soils were desired. Blocks, however, were considered a fixed effect reflecting the physical layout of pots in the experiment.

RESULTS AND DISCUSSION

Elemental analyses.—Concentrations of most macronutrients are comparable among the popu-

lations, although plants from the Blue Wing Mine and Ithaca contained relatively high levels of calcium. There were large differences in iron contents: The Baltimore and Ithaca plants contained three times as much as the Durham and Blue Wing Mine plants (Table 1). In contrast, the Blue Wing Mine plants contained a relatively high concentration of copper. Plants from Ithaca contained surprisingly high concentrations of zinc, copper, chromium, and lead. The Ithaca site is in an urban area, although the city of Ithaca has a population of only about 40,000. Lead enrichment probably results from vehicular traffic. Elevated levels of copper, zinc, and chromium in the Ithaca plants could be due to runoff from metal structures in the downtown area. Chromium, for example, is a common component of many yellow paints. Zinc contamination can result from rainwater carrying leachate from galvanized metals (Harris 1946).

Growth and morphology.—When plants from five populations were grown on sand, mine soil, and two intermediate soil mixtures, the greatest cover was produced in all populations on the two intermediate soil treatments (Fig. 1). Apparently the pure sand provided a suboptimal substrate for *C. purpureus*, possibly because of low nutrient levels. This would account for the greater growth on the two intermediate soils, which were "fertilized" with addition of mine soil (which is at least rich in micronutrients). All experimental populations were inhibited to some extent on the pure mine soil, at least compared to the intermediate soil mixtures.

There were significant differences among the populations in cover area irrespective of soil treatment,

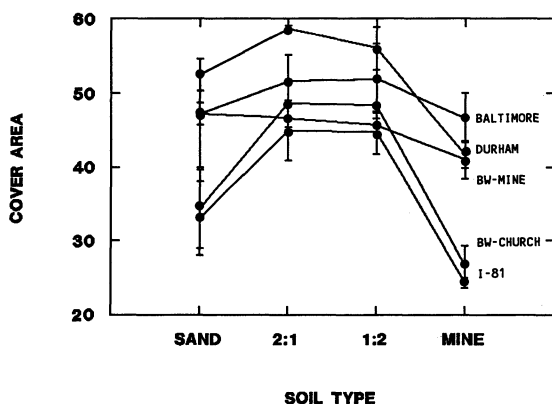


FIGURE 1. Mean cover area (\pm standard errors) in five populations of *Ceratodon purpureus* grown on sand, mine soil, and two intermediate mixtures. See Materials and Methods section for further explanation of cover area estimates. Units on the vertical axis are the number of 0.5×0.5 cm squares occupied by moss. BW-Mine and BW-Church = Blue Wing Mine and Church populations, respectively.

TABLE 2. Analyses of variance for cover area of populations of *Ceratodon purpureus* grown under experimental conditions. In experiment 1, soil treatments were sand, mine soil, and two intermediates. In experiment 2, the treatments were a control and a lead spray. See text for additional information about experimental protocols. * = $p < 0.05$; ** = $p < 0.001$.

	Source			
	SS	df	MS	F
Experiment 1				
Population	1,869.1	4	467.3	12.65**
Soil type	1,923.3	3	541.1	17.35**
Population \times soil	663.9	12	55.3	1.50
Error	1,403.8	38	36.9	
Experiment 2				
Population	4,009.1	6	668.2	66.28**
Lead mist	64.1	1	64.1	6.35*
Population \times mist	40.9	6	6.2	0.67
Error	403.8	40	10.1	

as reflected in the significant population main effect in the analysis of variance (Table 2). The population \times soil treatment interaction was not significant, however, suggesting that populations differ in overall growth rate, or vigor, but not in tolerance of the mine soil per se. Plants from the Blue Wing Mine population did grow better on the mine soil than did those from the adjacent Blue Wing Church population (Fig. 1), but two other nonmine populations (Durham and Baltimore) grew as well as the mine site plants on mine soil. There is therefore no evidence that plants from the Blue Wing Mine represent a genetically specialized, mine site race. Moreover, although the Blue Wing Mine plants grew better than the Blue Wing Church plants on mine soil, the former also grew better on the sand (Fig. 1). Blue Wing Mine plants may be relatively insensitive to suboptimal soils in general but do not appear to be specifically adapted to metal-contaminated soils.

In the second experiment, none of the populations were severely inhibited by the lead treatment (Fig. 2), although the overall treatment effect was statistically significant (Table 2). As in the first experiment, it appears that populations differ in overall vigor, rather than in tolerance of lead per se (i.e., the response curves differed but were parallel). It is possible that tolerance variation would occur if the plants were subjected to more concentrated lead solutions.

The apparent absence of a genetically specialized, metal-tolerant mine site race in *C. purpureus* is surprising in light of studies of flowering plants, where genetic specialization appears to be almost universal under similar circumstances. Briggs (1972) and Brown and House (1978) found evidence of metal-

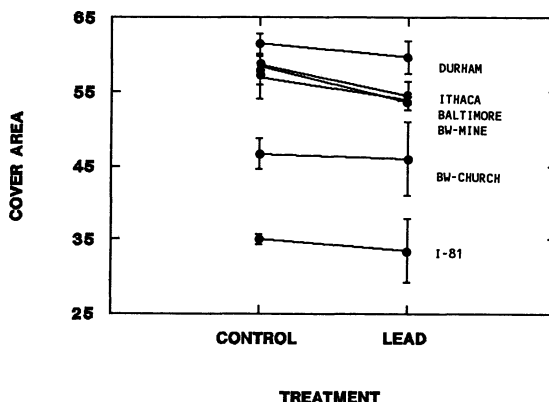


FIGURE 2. Mean cover area (\pm standard errors) in six populations of *Ceratodon purpureus* grown on sand with and without the addition of lead. See Materials and Methods section for further explanation of cover area estimates. Units on the vertical axis are the number of 0.5×0.5 cm squares occupied by moss. BW-Mine and BW-Church = Blue Wing Mine and Church populations, respectively.

tolerant ecotypes in *Marchantia polymorpha* and *Solenostoma crenulatum*, respectively. However, populations of *Bryum argenteum* from contaminated and uncontaminated sites showed indistinguishable responses to metals supplied in axenic culture (Shaw et al. 1989; Shaw & Albright 1990). Furthermore, copper-tolerant races occur in *Fuaria hygrometrica*, but plants appear to exhibit no such adaptive differentiation in response to cadmium or nickel (Shaw 1990). Patterns of metal tolerance evolution that are emerging from studies of bryophytes would not have been predicted from previous work on flowering plants.

Plants from the five populations of *C. purpureus* differed in average leaf length when grown under uniform conditions (Fig. 3). Blue Wing Mine plants were the smallest, whereas plants from the nearby Blue Wing Church population were the largest. The differences among populations were statistically significant (Table 3). In addition to these inherent differences in leaf length among populations, soil treatment also had an effect on leaf size. In general, the leaves formed on mine soil were smaller than those formed on sand or intermediate soil types. In the Baltimore and Interstate-81 populations, average leaf lengths decreased from maxima on sand, through the intermediate treatments, to minima on the mine soil. Leaves of plants from the Blue Wing Church population were larger on the mine soil than on intermediate soils, but in four of the five populations leaves formed on mine soils were smaller than leaves formed on sand or the mixture made up mainly of sand.

It is noteworthy that although inherent population differences in leaf length were obvious under experimental conditions, these differences might not

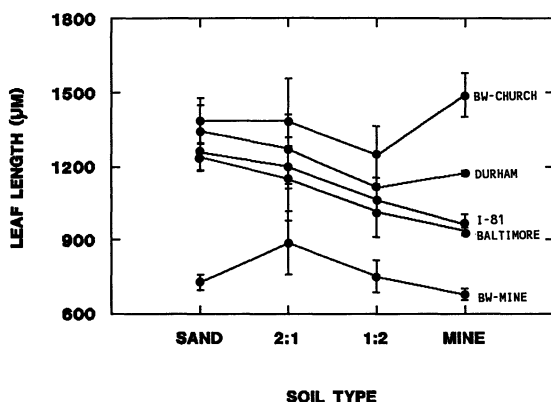


FIGURE 3. Mean leaf sizes (\pm standard errors) in five populations of *Ceratodon purpureus* grown on sand, mine soil, and two intermediate mixtures. BW-Mine and BW-Church = Blue Wing Mine and Church populations, respectively.

be apparent from a study of natural populations. For example, leaf sizes of plants from the Baltimore and Interstate-81 populations grown on the experimental mine soil approached the small sizes of leaves from the Blue Wing Mine population grown on any treatment (Fig. 3). On the other hand, average leaf size was reduced in parallel across the treatments in all populations; the same ranking of populations in terms of leaf size occurred on all four soil treatments. This uniformity of soil effects on leaf size among populations was reflected in the insignificant population \times soil treatment interaction, suggesting an absence of population-specific patterns of phenotypic plasticity.

Sexual expression.—The field-collected populations differed in the frequency of male, female, and sterile stems and none contained male and female stems in equal proportions (Table 4). Field-collected plants from the Ithaca population were uniformly sterile, and in four of the remaining five populations sterile stems strongly outnumbered sexual (male or female) stems. Male plants were absent in the Durham, Blue Wing Church, and Baltimore populations, and were far less abundant than female plants in the population from Interstate-81 (Table 4).

In the first experiment, there was no demonstrable effect of soil treatment on sex ratio, although there was an effect on overall sexual expression (Fig. 4; Table 5). The greatest number of sexual stems occurred on the sand and mine soil, with lower levels of sexual expression on the two intermediate soils. These latter are the two treatments with the highest cover area, so the relatively low frequency of gametangial formation does not appear to be related to a general lack of vigor. There may be a relationship between stem density and sexual frequency, but that could not be tested with our data. The addition of lead at weekly intervals appeared

TABLE 3. Analysis of variance for leaf lengths of plants of *Ceratodon purpureus* grown on sand, mine soil, and two intermediate mixtures. See text for additional details about experimental protocols. * = $p < 0.05$; ** = $p < 0.001$.

	Source			
	SS ($\times 10^{-3}$)	df	MS ($\times 10^{-4}$)	F
Population	23.81	4	59.51	22.13**
Soil type	2.89	3	9.63	3.58*
Population \times soil	2.71	12	2.26	0.84
Error	10.22	38	2.68	

to have no effect on sex ratio, but the overall level of sexual expression was decreased (2.3 ± 0.5 vs. 1.4 ± 0.3 sexual stems out of each sample of ten in the control and lead mist treatments, respectively). As in the first experiment, both the population and treatment effects were significant for sexual expression, although differences among the populations accounted for most of the variation (Table 6). Because treatment had no effect on sex ratios in either experiment, the data were pooled across treatments in order to estimate the average frequencies of male, female, and sterile stems in each population (Table 4). Log-likelihood ("G") tests were performed on the frequencies in Table 4 in order to test the null hypothesis that ratios of reproductive status (male : female : sterile) did not differ between field-collected plants and samples from the same populations regenerated in the two experiments. The statistical tests (not shown) indicated that in every population, reproductive behavior of regenerated plants differed statistically from field samples. However, there was general agreement between experimental and field estimates. For example, the Blue Wing Mine population contained no females, and no females were observed among regenerated plants. The actual percentages of plants in the three reproductive categories differed between field and experimental samples but the experimental results corroborate the impression that female plants are either absent or very infrequent in this population.

The other populations had a preponderance of females over males, and this was also corroborated by the experimental results. The Blue Wing Church population had no males among field samples, but one was observed in the second experiment (Table 4). Thus, an interpretation that the Blue Wing Church population is unisexual and female based on field data would be erroneous. However, the strong female bias in this population is supported by both field and experimental observations. The percentage of sterile plants in the Blue Wing Church population was higher under experimental than field conditions, in contrast to the Durham population, where regenerated plants were more sexually active

TABLE 4. Percentages of male, female, and asexual gametophytic stems in field-collected and experimentally regenerated samples from six populations of *Ceratodon purpureus*. Fifty stems were sampled from the field collections; 120 stems were sampled from each population in experiment 1, and 80 stems from each in experiment 2. The Ithaca population was sampled too late for inclusion in this analysis.

Population	Field			Experiment 1			Experiment 2		
	♀	♂	Asexual	♀	♂	Asexual	♀	♂	Asexual
Baltimore	20	0	80	0	0	100	0	0	100
Blue Wing Church	32	0	68	3	0	97	1	1	98
Blue Wing Mine	0	76	24	0	31	69	0	35	65
Durham	6	0	98	30	0	70	60	0	40
Interstate-81	28	2	70	2	1	97	8	2	90
Ithaca	0	0	100				10	0	90

than they were in the original population. In the Durham population, female plants were five and ten times more abundant, respectively, in experiments 1 and 2 than in the original population. Nevertheless, the Durham population consisted only of female and sterile gametophores under both field and experimental conditions, and may be unisexual.

In general, the level of sexual expression (formation of gametangia) differed between field and experimental samples of the same population, yet such differences were consistent from population to population. Nevertheless, the relative frequency of plants bearing male or female gametangia was fairly consistent between experimental and field conditions, and it appears that most populations were actually biased in favor of genetically female plants.

Although the basis for sex determination in bryophytes is not well understood, sexuality is generally thought to be under genotypic control (Ramsey & Berrie 1982). The genotypic (i.e., cytological) control of sex determination suggests that male and female plants should occur in equal numbers among

the meiotic progeny of diploid sporophytes, as the X and Y chromosomes of sporophytes segregate among spore progeny at meiosis. In the absence of differential mortality between sexes, bryophyte populations should therefore consist of approximately 50% males and 50% females. Even if a population has a skewed sex ratio when founded (by unequal numbers of male and female spores), the ratio should approach 1:1 over time if each generation of spore progeny is 50% male and 50% female.

Strongly biased sex ratios are known in many populations of bryophytes and appear to be a consistent feature of some species. Female-biased sex ratios have been reported in a number of species of mosses (Bedford 1938, 1940; Lacey 1950; Newton 1972; Reese 1984; Stoneburner 1979). Male-biased sex ratios are much less common, but are known in *Racomitrium lanuginosum* (Tallis 1957). Our study demonstrates that sex ratios can be highly variable among conspecific populations. Thus, the Blue Wing Mine population of *C. purpureus* is strongly male-biased whereas other populations of this species are

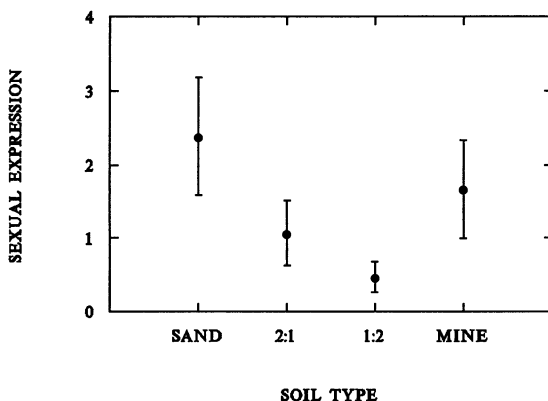


FIGURE 4. Sexual expression (total number of males plus females out of ten, \pm standard errors) in populations of *Ceratodon purpureus* grown on sand, mine soil, and two intermediate mixtures. Average differences between soil treatments include extensive variation in response to the treatments among the populations, but show general environmental effects summed over the six populations.

TABLE 5. Analyses of variance for sexual expression (total number of males plus females) and sex ratio (number of males/number of males + number of females) in five populations of *Ceratodon purpureus* grown on sand, mine soil, and two intermediate soil mixtures. Sex ratios were arcsine transformed prior to the analyses. * = $p < 0.05$; ** = $p < 0.001$.

	Source			
	SS	df	MS	F
Sexual expression				
Population	130.9	4	32.7	17.19**
Soil type	35.8	3	11.9	6.28**
Population \times soil	82.8	12	6.9	3.62**
Error	72.3	38	1.9	
Sex ratio				
Population	1.33	4	0.33	33.28***
Soil type	0.02	3	0.01	0.81
Population \times soil	0.38	12	0.02	2.21*
Error	0.38	38	0.01	

TABLE 6. Analyses of variance for sexual expression (total number of males plus females) and sex ratio (number of males/number of males + number of females) in six populations of *Ceratodon purpureus* grown on sand with or without lead applications. * = $p < 0.05$; ** = $p < 0.001$.

	Source			
	SS	df	MS	F
Sexual expression				
Population	217.13	5	36.18	77.89**
Treatment	9.02	1	9.02	19.41**
Population \times treatment	7.87	6	1.31	2.82*
Error	18.58	40	0.46	
Sex ratio				
Population	1.97	6	0.33	55.69**
Treatment	0.02	1	0.02	3.59
Population \times treatment	0.08	6	0.01	2.35*

female-biased. Unisexual populations such as those described in the present paper could be the result of the initial colonization by one plant, either as a spore or a gametophytic fragment.

Other studies of sexual expression in bryophyte populations have documented spatial heterogeneity in the frequencies of male and female gametophores both within and between populations. Bedford (1938) found that many populations of *Climacium americanum* were unisexual. Wyatt (1977) reanalyzed Bedford's data and demonstrated a significant regression of the number of females bearing sporophytes on the abundance of male stems in the populations. Longton and Greene (1969), Reimann (1972), Wyatt (1977), and Stoneburner (1979) documented spatial heterogeneity in sex ratios within and among populations of several mosses. In addition, Longton (1985) documented year-to-year differences in one population of *Pleurozium schreberi*. High levels of such environmental pollutants as fluoride (Roberts et al. 1979), sulfur dioxide (Winner & Bewley 1978), acidified rain (Raeymaekers & Glime 1986), and possibly heavy metals (Longton 1985) have been reported to decrease sporophyte frequency in several species of mosses. Most of the evidence, however, comes from qualitative estimates or anecdotal statements about sporophyte abundance, and additional quantitative sampling is necessary. Sergio (1987) observed that urban populations of *Tortula laevipila* produced fewer sporophytes and more asexual gemmae than rural populations. On the other hand, Gilbert (1968) noted that *Funaria hygrometrica* produces sporophytes abundantly under highly polluted conditions. Observations on *F. hygrometrica* (Shaw, unpubl.) in habitats contaminated with heavy metals corroborate those of Gilbert. Fife (1975, quoted in Roberts

et al. 1979) reported that maximum sporophyte abundance in *Pohlia nutans* growing near a fluoride pollution point source in Newfoundland occurred at the most severely enriched sites. Both *Pohlia nutans* and *Funaria hygrometrica* are monoecious, unlike many of the species in which inhibition of successful sex has been reported. Additional experimental studies of environmental effects on gametangium and sporophyte formation in mosses are much needed.

The results of the present study do not provide definitive answers to the question of why some populations of *C. purpureus*, especially those in polluted environments, lack sporophytes. However, several possibilities are suggested by our results. Some populations appear to be unisexual, or nearly so, obviously precluding sexual reproduction. The Blue Wing Mine population of *C. purpureus* may fail to produce sporophytes for this reason. In addition, although our experimental treatments did not affect sex ratios, they did affect the overall level of sexual expression. It is possible that pollutants reduce the level of gametangium formation in plants of both sexes, thereby reducing the probability of successful fertilization. Longton (1985) found that a reduction in sporophyte frequency in plants of *Pleurozium schreberi* growing within a few km of a nickel smelter in Thompson, Manitoba, was correlated with an increase in the frequency of stems devoid of gametangia. His field data also showed that pollution did not appear to affect sex ratios.

Unpublished field studies on *C. purpureus* also suggest a relationship between levels of gametophytic sexual expression and sporophyte formation. This species is common in the vicinity of a zinc smelter in Palmerton, Pennsylvania, yet sporophytes have not been observed in the area. Over 100 gametophytic stems sampled from natural populations within the smelter area lack gametangia of either sex (Jules & Shaw, unpubl.). However, the relationship between sporophyte frequency and pollution in *C. purpureus* is complex. For example, plants growing on metal mine tailings in the Piedmont of North Carolina and around the zinc smelter in Pennsylvania are uniformly sterile, yet plants on copper mine tailings in the Keewanaw Peninsula of Michigan form abundant sporophytes (Shaw, unpubl.). Gilbert (1968) also noted that *C. purpureus* is among few species that can sometimes form sporophytes under highly polluted conditions. Differences in reproductive behavior among populations growing in polluted habitats may reflect differences in tolerance, but additional experimental work is needed.

ACKNOWLEDGMENTS

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