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POPULATION STRUCTURE AND MORPH-SPECIFIC FITNESS DIFFERENCES IN TRISTYLOUS *LYTHRUM SALICARIA*

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Abstract.—In tristylous plant populations, style-morph frequencies are governed by an interaction between frequency-dependent selection due to disassortative mating and stochastic processes. Provided that there are no inherent fitness differences among morphs, frequency-dependent selection should result in equal morph frequencies at equilibrium. Stochastic models indicate that the short-styled morph has the highest and the long-styled morph the lowest probability of being lost from local populations as a result of random processes. We surveyed the morph composition of 82 populations of the tristylous, self-incompatible herb *Lythrum salicaria* in two archipelagos, one in central and one in northern Sweden, located close to the range-margin of the species. To examine whether deviations from even morph frequencies can be explained by among-morph differences in reproductive success, we quantified flower and seed production in six and three populations in the northern and southern archipelago, respectively, and we recorded segregation ratios in offspring produced in six trimorphic populations in the northern area. Seed germination and offspring growth were studied in the greenhouse.

Ninety percent of the populations in the southern archipelago ($N = 31$) and 69% of the populations in the northern archipelago ($N = 35$) were trimorphic; the remaining populations were dimorphic (only populations consisting of at least three flowering plants considered). Dimorphic populations were smaller than trimorphic populations, as predicted by stochastic models. There was a striking difference in the morph composition of *L. salicaria* populations between the two archipelagos. In the southern archipelago, there was a slight excess of the long-styled morph and a corresponding deficiency of the short-styled morph. In contrast, the northern populations were characterized by a marked deficiency of the mid-styled morph: the average frequency of the mid-styled morph in trimorphic populations was 0.21, and nine of eleven dimorphic populations lacked the mid-styled morph. In both archipelagos, the long-styled morph (the most common morph) produced about 20% fewer seeds per fruit than the other morphs. The long-styled morph also tended to produce fewer seeds per plant. A hand-pollination experiment performed in two of the northern populations indicated that seed production per flower was pollen-limited in the long-styled morph but not in the other two morphs. Seed germination and offspring size after 24 weeks of growth did not differ among morphs. The mid-styled morph tended to have a higher representation in the offspring than in the parental generation in all six trimorphic populations studied further indicating that the deficiency of the mid-styled morph in the northern archipelago does not represent an equilibrium. Taken together, the results do not support the hypothesis that morph-specific differences in reproductive success can account for deviations from even morph frequencies in *L. salicaria.* It is suggested that among-morph differences in other components of fitness and historical factors may contribute to the current morph structure.

Key words.—Founder effect, frequency-dependent selection, genetic drift, *Lythrum salicaria,* population structure, sexual polymorphism, tristyly.

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A fundamental problem in population biology is to determine the relative importance of selection and stochastic processes for the genetic structure of natural populations. Reciprocal transplant experiments have demonstrated local adaptation at different spatial scales in several plant species (e.g., McGraw and Antonovics 1983; Schemske 1984; Schmidt and Levin 1985; Galen et al. 1991; Sork et al. 1993; but see Plantenkamp 1990; Rice and Mack 1991). At the same time, population genetic theory (Wright 1931, 1938, 1969) and empirical estimates of the effective population size (e.g., Heywood 1986; Levin 1988; Husband and Barrett 1992a) indicate that genetic drift may be an important factor for the structure of many natural plant populations. Tristyly is a sexual polymorphism that offers a valuable system for investigating the mechanisms behind genetic differentiation within and among populations (Eckert and Barrett 1992). This is because, first, tristylous species produce three floral morphs that can readily be identified in the field, and, second, the inheritance of the polymorphism is relatively simple, and specific predictions about morph frequencies at equilibrium can therefore be made (Charlesworth 1979; Heuch 1979a, 1979b, 1980; Barrett et al. 1989; Barrett 1993).

The three floral morphs of tristylous species, the long-styled (L), the mid-styled (M), and the short-styled (S), differ in the relative positioning of anthers and stigma. In each flower of a given morph, two anther whorls are produced at levels corresponding to the positions of the stigmas in the flowers of the other two morphs (reciprocal herkogamy). In many tristylous species, the reciprocal herkogamy is associated with a self- and intramorph incompatibility system (Ganders 1979; Barrett 1993). Both the reciprocal arrangement of anthers and stigma and the incompatibility system should promote disassortative mating (mating among different morphs).

The morph ratio in populations of tristylous species is governed by an interaction between frequency-dependent selection due to disassortative mating and stochastic processes. Provided that there are no inherent fitness differences among morphs (differences that are expressed independent of the morph composition of the population), frequency-dependent selection should result in equal morph frequencies at equilibrium (Heuch 1979a, 1979b). The rate at which populations reach equilibrium will depend on the life-history of the plant, the extent of clonal growth, the outcrossing rate and the ratio between random and disassortative mating (Morgan and Barrett 1988; Eckert and Barrett 1992).

Anisoplethic population structure (uneven morph frequencies) could represent a transient condition in a population evolving towards equilibrium or could result from inherent fitness differences among morphs (e.g., Charlesworth 1979; Heuch 1979b; Barrett et al. 1983; Weller 1986). In addition, stochastic models indicate that both founder effects and genetic drift in small populations can produce skewed morph ratios (Heuch 1980; Heuch and Lie 1985; Morgan and Barrett 1988; Barrett et al. 1989; Eckert and Barrett 1992; Husband and Barrett 1992b). Based on the inheritance of tristyly, it can be shown that, if the initial morph frequencies are at equilibrium, then the S morph has the highest and the L morph the lowest probability of being lost from populations because of stochastic processes. Stochastic models predict that the frequency of morph loss should decrease with increasing population size. They further predict that morph frequencies in trimorphic populations should not be consistently biased towards any particular morph (but see Heuch 1980; Morgan and Barrett 1988).

The purple loosestrife, *Lythrum salicaria* L. (Lythraceae), is a tristylous perennial herb that is native to Eurasia and that has been introduced to northeastern North America. Surveys of *L. salicaria* populations in Europe indicate that the morph ratio may vary considerably among populations, but also that, on a regional scale, there is consistently an excess of the L morph and a deficiency of the S morph (Haldane 1936; Schoch-Bodmer 1938; Halkka and Halkka 1974). Based on the results of computer simulations, Heuch (1980) suggested that this could be due to the stochastic loss of the S and sometimes the M morph from local subpopulations or genetic neighborhoods. However, this hypothesis was not supported by a recent analysis of the spatial distribution of morphs within a large *L. salicaria* population in southern Sweden (Andersson 1994), nor could it by itself explain deviations from even morph frequencies in trimorphic *L. salicaria* populations in Ontario, Canada (Eckert and Barrett 1992). The alternative hypothesis that uneven morph frequencies are due to inherent fitness differences among morphs has not been examined empirically. Darwin (1877) found differences in seed number per fruit among the three style-morphs after controlled crosses on experimentally grown *L. salicaria,* but there are few quantitative data on fecundity and other life-history components in natural populations. Nicholls (1987) found indications of among-morph differences in germination rate in two populations in Switzerland, whereas O'Neil (1992) documented a lower seed production by the S morph than by the other morphs in two populations in northeastern United States.

In this study, we documented morph frequencies in *L. salicaria* populations in two archipelagos, one in northern and one in central Sweden, and we compared components of male and female fertility in the three style morphs to test whether inherent differences in reproductive success can explain deviations from even morph frequencies. The southern archipelago is located in a region where *L. salicaria* is common in a variety of wetland habitats, whereas the northern archipelago is located close to the northern range margin of the species in Fennoscandia. Both areas are characterized by a substantial land uplift; the rate is about 0.9 meter per century in the northern archipelago and about 0.7 meter per century in the southern archipelago (Ericson and Wallentinus 1979). This contributes to the dynamic nature of the *L. salicaria* populations growing on the seashores. Moreover, as a result of this land uplift, new islands are continuously formed. Many of the small populations in these archipelagos are young, and founder effects may be expected to be important for their genetic structure.

We used two approaches to test whether differences in reproductive success can explain deviations from even morph frequencies. First, we compared components of male and female fertility in the three style morphs by quantifying flower and seed production in the field and by studying seed germination and offspring growth rate in the greenhouse. If deviations from even morph frequencies are the result of inherent, morph-specific differences in reproductive success, then a positive correlation between the reproductive performance of a morph and its representation in a local population is expected. If, alternatively, skewed morph frequencies represent a transient condition in populations evolving toward equilibrium, then frequency-dependent selection should result in a negative correlation between the average reproductive success of a morph and its frequency in a local population. Second, we scored the segregation ratios in naturally produced offspring. If there are no inherent fitness-differences among morphs, then disassortative mating during the reproductive cycle is expected to result in more even morph frequencies in the progeny than in the parental generation (Fisher and Mather 1943). However, if skewed morph frequencies are a function of morph-specific differences in mating success, then no difference in morph frequency between offspring and parents is expected.

Finally, to test whether among-morph differences in seed production can be attributed to differences in the degree of pollinator limitation, we conducted supplemental hand-pollinations in two populations.

Materials and Methods

The Species

In *L. salicaria,* the style morph is determined by two diallelic loci usually denoted *S* and *M,* with *S* epistatic to *M.* The short-styled form is dominant: It carries the dominant *S*-allele that is missing in long- and mid-styled plants. The dominant *M*-allele is only found in mid- and short-styled plants. These specifics of the inheritance are of importance for the predictions of the stochastic models. In *L. salicaria,* the inheritance of both style morph loci is tetrasomic, with no linkage or dosage effects (Fisher and Mather 1943; Fisher and Martin 1947).

Lythrum salicaria has a heteromorphic incompatibility system: Full seed set is achieved only if pollen is transferred between different morphs and from an anther level that corresponds to the position of the receiving stigma (Darwin 1877; Stout 1923; O'Neil 1994). In Scandinavia, *L. salicaria* flowers for six to eight weeks in July–August. The flowers are visited primarily by bumblebees, but also by honey-bees, syrphids, and lepidopterans (J. Ågren, unpubl. data). The seeds mature six to eight weeks after flowering. In the populations of the two archipelagos studied, reproduction is predominantly from seed. Most reproductive individuals produce one or a few floral shoots; the largest plants may produce more than 20 floral shoots. Flowering individuals are on average 0.5 m tall (J. Ågren, unpubl. data). Chromosome counts in plants from populations in the northern archipelago indicate that those populations are tetraploid ($2n = 60$; R. von Bothmer, pers. comm. 1993). This agrees with counts made in plants from other parts of Europe [Löve and Löve 1961; but see Dulberger (1968) for records of diploid populations in Israel].

Study Sites

Populations of *L. salicaria* were studied in two geographically separated archipelagos in the Gulf of Bothnia of the Baltic Sea. The Skeppsvik archipelago is located about 17 km ESE of Umeå, northern Sweden (63°47′N, 20°37′E), close to the geographic margin of the species' distribution in Fennoscandia (Hultén and Fries 1986). In this area *L. salicaria* is confined to the shores of the Gulf of Bothnia. The Harkskärsfjärden archipelago is located in central Sweden (about 15 km NE of Gävle and 400 km SSW of the Skeppsvik archipelago; 60°47′N, 17°20′E), in a region where *L. salicaria* is common in a variety of wetland habitats (lake- and seashores, riversides, fens).

Population Structure

Style-morph frequencies were scored in 44 populations in the Skeppsvik archipelago and in 38 populations in the Harkskärsfjärden archipelago in 1990–1993. For populations that were scored in more than one year, the first sampling was used in calculations of average morph frequencies. Four populations (two in the Skeppsvik and two in the Harkskärsfjärden area) were located on shores of the mainland bordering the archipelago; the remaining populations were located on islands. A population was operationally defined as all plants on an island, or, when growing on the mainland or an island larger than 6 ha (islands ranged in size from about 200 m^2 to 37 ha), as a group of plants separated from their closest conspecific by at least 300 m. In populations that were sufficiently large, the style-morph was recorded for at least 200 plants. Delimiting individual plants did not present a problem, except in a few cases where *Lythrum* grew in dense patches. In those patches where it was not possible to unambiguously identify individual plants, the style morph was recorded for individual shoots separated by 1 m to avoid scoring the same genet more than once (following Haldane 1936; Halkka and Halkka 1974; Eckert and Barrett 1992). For each population, we estimated the total number of flowering plants. Thirty-five Skeppsvik populations and 31 Harkskärsfjärden populations consisted of at least three flowering plants.

Heterogeneity *G*-tests (Sokal and Rohlf 1981) were conducted to test whether morph frequencies varied significantly among populations within the two archipelagos and to test whether morph frequencies in the pooled data from each archipelago deviated significantly from equal representation of the three morphs. To avoid empty cells (cannot be analyzed with the *G*-test), and for the expected frequency to be at least five in each cell, only data from trimorphic populations with 15 or more flowering plants were included in these analyses.

TABLE 1. Characteristics of the *Lythrum salicaria* populations in the archipelagos of Skeppsvik (northern Sweden) and Harkskärsfjärden (central Sweden) for which data on morph-specific reproductive success were obtained.

Population	Morph frequencies L	M	S	*N*	Total number of flowering plants	Number of plants sampled per style morph
Skeppsvik 1991						
S1	0.427	0.151	0.422	405	6500	30
S2	0.491	0.027	0.482	222	7000	24–41
S3	0.410	0.206	0.384	617	2500	29–30
S13	0.167	0.375	0.458	96	96	13–42
S14	0.409	0.400	0.191	220	800	28–30
S23	0.410	0.087	0.503	161	161	13–33
Skeppsvik 1992						
S1	0.491	0.133	0.376	503	8000	36–57
S2	0.450	0.037	0.513	191	9000	34–35
S3	0.420	0.242	0.338	488	3500	49–53
S13	0.142	0.345	0.513	113	113	15–20
S14	0.425	0.413	0.161	254	1050	26–27
S23	0.410	0.106	0.484	161	200	16–53
Harkskärsfjärden 1992						
H18	0.330	0.355	0.315	200	1400	44–57
H22	0.390	0.315	0.295	200	1500	35–36
H23	0.338	0.343	0.318	198	250	38–42

Flower and Seed Production

Flower and seed production were documented in six trimorphic populations in the Skeppsvik archipelago in 1991 and in 1992, and in three trimorphic populations in the Harkskärsfjärden archipelago in 1992 (Table 1). The six Skeppsvik populations were selected to represent a wide range in morph composition (see Table 1). To estimate flower and seed production, we marked up to 42 (1991) and 57 (1992) haphazardly chosen plants of each morph in each population during flowering. In populations with strongly skewed morph ratios, triplets of plants (including one plant of each morph) were marked by first locating and marking a plant belonging to the minority morph and then marking the two nearest plants of the remaining two morphs. For each plant, we recorded the number of floral shoots and the number of vegetative shoots. At fruit maturation, one floral shoot per plant was collected and brought to the laboratory. For each of the collected shoots, we recorded the number of leaf nodes producing flowers and the total number of fruits produced. To quantify flower production, we determined the number of flowers produced in each of five haphazardly chosen flower-producing leaf nodes per shoot (flower production was determined by adding the number of scars left by aborted flowers and fruits to the number of mature fruits). For each plant, we estimated total flower number as number of floral shoots × number of flower-producing leaf nodes on the sampled shoot × average number of flowers per node. The number of seeds was counted in five intact (non-dehisced) fruits per shoot. For each plant, total seed production was estimated as number of floral shoots × number of fruits on the sampled shoot × average seed production per fruit.

A mixed-model analysis of variance (ANOVA) was used to analyze the influence of style-morph (fixed effect) and population (random effect) on number of floral shoots per plant, flower and fruit number per shoot and per plant, average seed number per fruit and seed production per plant. All variables, except seed number per fruit, were log-transformed prior to analysis to better conform to assumptions of ANOVA. Variation in a total of eight different measures of male and female reproductive success (including germination rate, see below) were subject to ANOVA. To control the type-I error rate, the sequential Bonferroni test (Holm 1979) was used to determine whether results were significant at the 0.05 tablewide level (see Rice 1989). The tablewide significance level was calculated for each archipelago and for each factor or level of variation separately. When the ANOVA indicated significant among-morph differences, the Tukey-Kramer multiple comparison test (Zar 1984) was employed to examine which morphs differed significantly at the 0.05 level.

Supplemental Pollination

To test whether morph-specific differences in seed production could be attributed to differences in the degree of pollinator-limitation, supplemental hand-pollinations were performed in two populations in the Skeppsvik archipelago in 1992 (populations S2 and S3; see Table 1). For the experiment, 30 triplets (one plant of each style-morph growing within 1 m of each other) were marked in the S2 population and 21 triplets were marked in the S3 population. On the experimental plants, pairs of newly opened flowers (one flower in each of two adjacent leaf nodes) were marked and assigned to two different treatments. One of the flowers received supplemental pollination, whereas the other served as a control. When control flowers and flowers receiving supplemental pollination are located on the same plant, the effect of hand-pollination may be inflated by reallocation of resources from control flowers to flowers that receive a surplus of pollen (Zimmerman and Pyke 1988). The strength of such an effect should increase with decreasing distance between control and experimental flowers (cf. Watson and Casper 1984). To determine whether control flowers on treated shoots suffered a reduction in seed production, we marked, for each pair of hand-pollinated and control flowers, a second open-pollinated flower on a different shoot on all plants with more than one floral shoot. Three to four triplets (or pairs) of flowers were marked on each plant, and all hand-pollinations were performed within 10 days during the central part of the flowering season. Pollen was transferred by brushing dehiscing anthers across the stigma until it was densely covered by pollen. "Legitimate" outcross pollen (pollen from anthers at the corresponding anther level of another morph) produced by a plant 5–10 m away from the focal plant was used in all hand-pollinations. Fruits were collected as they matured and were brought to the laboratory. For each plant, the number of seeds per fruit was counted in up to two fruits per treatment under a stereo-microscope. For each plant and treatment, the average number of seeds per fruit was multiplied by the proportion of flowers producing a fruit to obtain the average number of seeds per treated flower. For each morph and population, the effect of supplemental pollination was tested with paired *t*-tests (hand-pollinated flowers vs. control flowers on the same shoot, control flowers on shoot with hand-pollinated flowers vs. open-pollinated control flowers on another shoot).

Offspring Comparisons

To compare the offspring produced by the three style-morphs, we quantified seed germination and offspring growth in the greenhouse. We determined the germination rate of seeds collected in two trimorphic and one dimorphic (LS) population in the Skeppsvik archipelago in 1991 and in the nine trimorphic populations sampled for flower and seed production in the Skeppsvik and Harkskärsfjärden archipelagos in 1992 (see above). In each population, at least five mature fruits were collected from each of up to 30 maternal plants per style-morph (except for the dimorphic population S18, these were plants for which data on flower and seed production were obtained; see above). To break seed dormancy, the seeds were stored in paper bags at 5°C for five months before planting. For each maternal plant, we planted 10 seeds in each of three pots (seeds from at least three different fruits were used). The pots were arranged in a randomized array in the greenhouse. The cumulative number of germinating seeds was scored every 10 days for seven weeks, after which no further seed germination was observed.

For seeds collected in 1991, offspring quality was further assessed by quantifying growth in the greenhouse. Seven weeks after planting, seedlings were thinned to one plant per pot. All plants were checked for flowering start and style-morph once a week. Twenty-four weeks after planting, the plants were harvested. At harvest, the plants were cut at the soil level, dried at 70°C to constant weight, and weighed to the nearest 0.01 g.

Plants were grown in 15 cm wide and 14 cm tall plastic pots filled with an 85:15 mixture of fertilized peat and perlite. The plants were fertilized twice a week with a weak solution of complete fertilizer (Superba S 65-10-47, at a concentration corresponding to approximately 325 ppm elemental nitrogen).

A mixed-model ANOVA was used to analyze the effects of maternal style-morph and population on the proportion of germinating seeds. The analysis was performed on maternal-plant means (arcsine square-root transformed prior to analysis).

Segregation Ratios

To examine whether frequency-dependent selection during the mating cycle resulted in more even morph frequencies in the offspring than in the parental generation, we compared the morph ratios in naturally produced progeny with those of flowering plants in two trimorphic (populations S2 and S3) and one dimorphic (population S18) population in the Skeppsvik archipelago in 1991 and in the six trimorphic populations sampled for flower and seed production in the Skeppsvik archipelago in 1992 (see above). We scored the floral morph of 2–4 (1991) and 2–8 (1992) offspring from each of the maternal plants contributing seeds to the greenhouse study described above (a total of 656 offspring of 197 maternal plants were scored in 1991 and 1418 offspring of 360 maternal plants in 1992). Segregation ratios were calculated for each population and maternal style-morph. To get

TABLE 2. Morph structure in *Lythrum salicaria* populations sampled in the archipelagos of Skeppsvik (northern Sweden) and Harkskärsfjärden (central Sweden) in 1990–1993. Tri, Di, and Mono are the proportions of populations that are trimorphic, dimorphic and monomorphic, respectively. The morph structure of dimorphic populations is denoted by letters representing the morphs present (L long-, M mid-, S short-styled morph). Only populations consisting of at least three flowering plants are included.

	Proportion of total sample			Proportion of dimorphic populations			Number of populations sampled
	Tri	Di	Mono	LM	LS	MS	
Skeppsvik	0.69	0.31	0.00	0.09	0.82	0.09	35
Harkskärsfjärden	0.90	0.10	0.00	0.67	0.33	0.00	31
Total	0.79	0.21	0.00	0.21	0.71	0.07	66

an estimate of the overall morph frequencies in seeds produced in the population, the observed ratios were weighted by the morph frequencies in the source population in 1991 and 1992, respectively. The difference between the weighted segregation ratio and the morph ratio of flowering plants in the source population was evaluated with the *G*-test (Sokal and Rohlf 1981). In this test, the number of observations contributing to the weighted segregation ratio was set to be the number of maternal sib-ships observed rather than the total number of offspring scored, because observations of plants belonging to the same sib-ship are not strictly independent. Because the style morphs tended to differ in seed production (see below), we also compared the morph ratios in the offspring and parental generations after having weighted the observed segregation ratios by both the morph frequencies and the morph-specific average seed production in the source population. Including the average seed production in the weighting procedure affected the results only marginally, and this analysis will not be reported below.

RESULTS

Population Structure

In the Skeppsvik archipelago (northern Sweden), 24 out of 35 populations were trimorphic and the remaining were dimorphic (only populations with three or more flowering plants considered; Table 2). Nine of the dimorphic populations lacked the mid-styled morph, one population lacked the short-styled morph and one lacked the long-styled morph (Table 2; Fig. 1). Five populations consisted of two flowering plants, and four populations of a single flowering individual. The long-styled morph was represented in six, the mid-styled morph in two, and the short-styled morph in five of these populations.

In the Harkskärsfjärden archipelago (central Sweden), 28 out of 31 populations with at least three flowering plants were trimorphic and the remaining three were dimorphic (of the latter, two lacked the S morph, one lacked the M morph; Table 2). Two populations in the sample from the Harkskärsfjärden archipelago included two flowering plants, and five populations consisted of a single flowering plant. The long-styled morph was represented in three, the mid-styled morph in five and the short-styled morph in one of the populations that consisted of fewer than three flowering plants.

Style-morph frequencies differed markedly between the two archipelagos. In trimorphic populations in the Skeppsvik archipelago, there was an excess of the long-styled and the short-styled morphs and a marked deficiency of the mid-styled morph (Table 3; Fig. 1). As most dimorphic populations in the Skeppsvik archipelago lacked the mid-styled

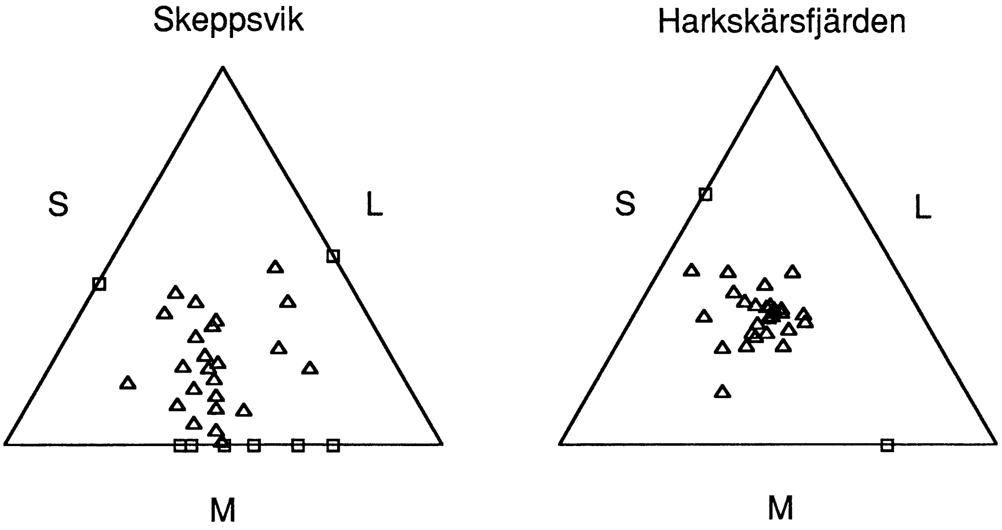

FIG. 1. Style morph frequencies in *Lythrum salicaria* populations consisting of at least three flowering plants in the archipelagos of Skeppsvik (northern Sweden; N = 35) and Harkskärsfjärden (central Sweden; N = 31). Trimorphic and dimorphic populations are represented by triangles and squares, respectively. The relative frequencies of the different morphs (L, M, and S) in a population are indicated by the distances of the symbol from the three axes.

TABLE 3. Style morph frequencies in *Lythrum salicaria* populations sampled in the archipelagos of Skeppsvik (northern Sweden) and Harkskärsfjärden (central Sweden) in 1990–1993. Frequencies (± SD) have been averaged for all populations that consisted of at least three flowering plants, and for trimorphic populations only. Data from earlier surveys of morph frequencies in trimorphic *L. salicaria* populations are included for comparison.

Sample	Morph frequencies			Number of populations sampled	Number of plants sampled	Reference
	L	M	S			
All populations						
Skeppsvik	0.40 ± 0.14	0.17 ± 0.16	0.42 ± 0.16	35	6251	This study
Harkskärsfjärden	0.37 ± 0.07	0.35 ± 0.12	0.28 ± 0.13	31	4347	This study
Trimorphic populations only						
Skeppsvik	0.41 ± 0.12	0.21 ± 0.12	0.37 ± 0.10	24	5891	This study
Harkskärsfjärden	0.37 ± 0.07	0.34 ± 0.07	0.29 ± 0.07	28	4337	This study
Tvärminne, Finland	0.36 ± 0.05	0.34 ± 0.08	0.30 ± 0.09	16	1823	Halkka and Halkka 1974
England	0.35 ± 0.05	0.33 ± 0.06	0.32 ± 0.04	5	2365	Haldane 1936
Zürich-Lausanne, Switzerland	0.36 ± 0.03	0.33 ± 0.03	0.30 ± 0.03	8	6169	Schoch-Bodmer 1938
Ontario, Canada	0.38 ± 0.13	0.30 ± 0.15	0.33 ± 0.16	79	14,147	Eckert and Barrett 1992

morph, the deficiency of the mid-styled morph was even more pronounced if these were included in the calculation of average morph frequencies (Table 3). In trimorphic populations in the Harkskärsfjärden archipelago, morph frequencies were more even; there was an excess of the long-styled morph and a corresponding deficiency of the short-styled morph (Table 3). In both archipelagos, the overall morph frequencies deviated significantly from even (Skeppsvik $G_P = 820.0$, df = 2, $P < 0.001$; Harkskärsfjärden $G_P = 30.3$, df = 2, $P < 0.001$; analyses based on pooled samples of trimorphic populations with at least 15 flowering plants). Moreover, morph frequencies varied substantially among populations within each archipelago (Skeppsvik $G_H = 684.3$, df = 42, $P < 0.001$; Harkskärsfjärden $G_H = 158.4$, df = 48, $P < 0.001$). In the Skeppsvik archipelago, the frequency of the L morph ranged from 0.15–0.64, and the frequency of the M morph from 0.01–0.47 in trimorphic populations with at least 15 flowering plants ($N = 22$). In the Harkskärsfjärden archipelago, the corresponding ranges were from 0.24–0.56, and from 0.14–0.46 ($N = 25$).

Trimorphic populations were larger than dimorphic populations (only populations consisting of at least three flowering plants considered). In the Skeppsvik archipelago, the number of flowering plants ranged from 5–8000 (median = 175, $N = 24$) in trimorphic populations, and from 3–229 (median = 7, $N = 11$) in dimorphic populations (Mann-Whitney U-test, $P < 0.001$). In the Harkskärsfjärden archipelago, the corresponding figures were from 4–1500 (median = 250, $N = 28$) in trimorphic populations and from 3–4 (median = 3, $N = 3$) in dimorphic populations (Mann-Whitney U-test, $P < 0.01$). In the combined sample from the two archipelagos, 50% of the populations with 3–50 flowering plants were dimorphic ($N = 24$), whereas only 5% of the populations with more than 50 flowering plants were dimorphic ($N = 42$; $G = 18.9$; df = 1, $P < 0.001$).

Flower and Seed Production

The number of floral shoots, flowers and fruits did not differ significantly among style-morphs but, with the exception of flower number in the Harkskärsfjärden archipelago, did vary significantly among populations (Table 4; Fig. 2).

In the Skeppsvik archipelago, the style-morphs differed significantly in seed number per fruit (Table 4; Fig. 2; Tukey-Kramer multiple comparison, L < S with the M morph intermediate and not significantly different from either of the two other morphs in 1991, L < M = S in 1992). The morphs also tended to differ in seed production per plant (single-test significance $P = 0.01$ in 1991, and $P = 0.08$ in 1992; Table 4). On average, the long-styled morph produced 13% (1991) and 20% (1992) fewer seeds per fruit, and 26% (1991) and 20% (1992) fewer seeds per plant than the short-styled morph, with the mid-styled morph intermediate (Fig. 2; note the $\log_{10}$-scale for seed number per plant). The L morph had the lowest average seed number per fruit in all Skeppsvik populations except S14 (Fig. 2). Moreover, the rank order of the three morphs based on average seed number per fruit was consistent in the two years in all populations except S23, in which the highest seed number per fruit was produced by the M morph in 1991 and by the S morph in 1992 (Fig. 2).

TABLE 4. Effects of style-morph and population on fitness components in *Lythrum salicaria* analyzed as mixed model ANOVAs. The analyses included six populations sampled in the Skeppsvik archipelago in 1991 and 1992, and three populations sampled in the Harkskärsfjärden archipelago in 1992. Each population included at least 13 plants of each morph. Single-test significance values are indicated with asterisks. *F*-values significant at the 0.05 table-wide level (sequential Bonferroni test) are indicated in boldface.

		Skeppsvik 1991			Skeppsvik 1992			Harkskärsfjärden 1992		
Fitness component	Source of variation	df	MS	*F*	df	MS	*F*	df	MS	*F*
Number of floral shoots[1]	Morph	2	0.326	1.3	2	0.087	0.2	2	0.331	5.2
	Population	5	6.521	**12.9******	5	5.034	**9.5******	2	2.194	**9.0*****
	Morph × Population	10	0.252	0.5	10	0.266	0.5	4	0.064	0.3
	Error	523	0.507		627	0.527		369	0.244	
Number of flowers per shoot[1]	Morph	2	2.213	5.2*	2	0.103	0.1	2	1.219	5.7
	Population	5	37.774	**35.8******	5	23.913	**27.2******	2	0.912	2.1
	Morph × Population	10	0.423	0.4	10	0.723	0.8	4	0.215	0.5
	Error	523	1.056		627	0.881		369	0.432	
Number of flowers per plant[1]	Morph	2	3.711	4.7*	2	0.239	0.2	2	1.314	7.6*
	Population	5	70.215	**34.8******	5	43.152	**22.0******	2	1.048	1.3
	Morph × Population	10	0.787	0.4	10	1.418	0.7	4	0.172	0.2
	Error	523	2.017		627	1.962		369	0.809	
Number of fruits per shoot[1]	Morph	2	2.782	5.3*	2	1.317	2.0	2	0.640	2.9
	Population	5	31.409	**33.9******	5	25.564	**31.4******	2	11.375	**22.5******
	Morph × Population	10	0.522	0.6	10	0.670	0.8	4	0.219	0.4
	Error	523	0.927		627	0.815		369	0.506	
Number of fruits per plant[1]	Morph	2	4.368	5.6*	2	1.118	0.8	2	1.850	10.5*
	Population	5	61.740	**33.4******	5	43.741	**23.6******	2	12.403	**13.8******
	Morph × Population	10	0.774	0.4	10	1.374	0.7	4	0.176	0.2
	Error	523	1.849		627	1.853		369	0.899	
Seed number per fruit	Morph	2	6715.6	**12.1****	2	11,800.9	**12.8****	2	2237.2	1.4
	Population	5	4514.5	**9.8******	5	10,229.4	**21.0******	2	5786.8	**16.3******
	Morph × Population	10	555.4	1.2	10	919.5	1.9*	4	1634.6	**4.6****
	Error	523	461.1		627	486.3		369	355.8	
Seed number per plant[1]	Morph	2	11.230	7.6**	2	7.611	3.3	2	6.408	2.3
	Population	5	73.052	**29.8******	5	64.819	**25.3******	2	38.369	**25.7******
	Morph × Population	10	1.483	0.6	10	2.284	0.9	4	2.812	1.9
	Error	523	2.455		627	2.563		369	1.494	
Proportion of seeds germinating[2]	Morph	2	0.445	2.3	2	0.001	0.1	2	0.033	0.8
	Population	1	0.144	1.4	5	0.554	**12.3******	2	0.022	0.7
	Morph × Population	2	0.197	2.0	10	0.023	0.5	4	0.044	1.3
	Error	161	0.100		341	0.045		189	0.032	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ (single-test significance levels).

[1] Log-transformed prior to analysis.

[2] Arcsine square-root transformed prior to analysis.

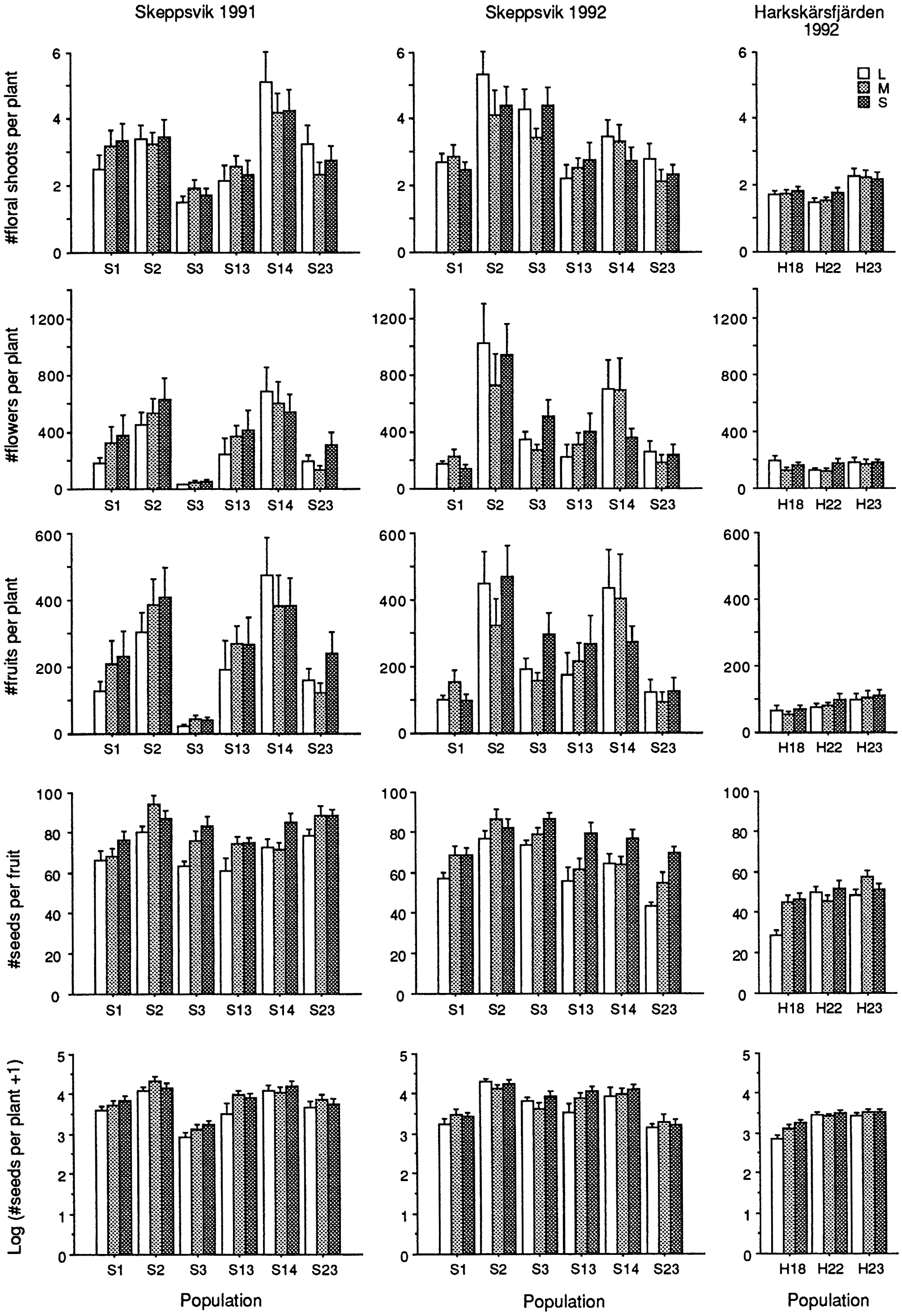

FIG. 2. Components of reproductive success of the three style morphs in six *Lythrum salicaria* populations in the Skeppsvik archipelago, and in three *L. salicaria* populations in the Harkskärsfjärden archipelago. Means (± SE) were calculated based on plant means. Population characteristics and sample sizes are given in Table 1.

In the Harkskärsfjärden archipelago, there was a significant morph-by-population interaction for seed number per fruit, indicating that differences among morphs varied among populations (Table 4). Analyses performed on the three populations separately showed that the mid- and short-styled morphs produced about 50% more seeds per fruit than the long-styled morph in population H18 ($F_{2,150} = 12.9$, $P < 0.0001$; Tukey-Kramer, L < M = S), whereas the mid-styled morph produced about 20% more seeds than the long-styled morph, with the short-styled morph intermediate, in population H23 ($F_{2,116} = 3.0$, $P = 0.05$; Tukey-Kramer, L < M; Fig. 2); seed number per fruit did not differ between morphs

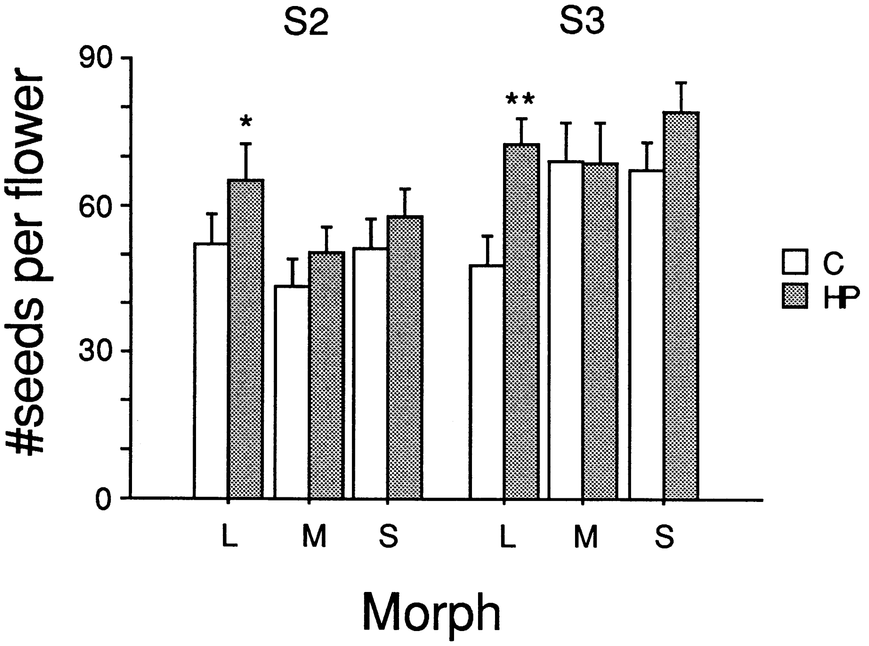

FIG. 3. Number of seeds (mean ± SE) produced by open-pollinated control flowers (C) and by hand-pollinated flowers (HP) in two populations (S2 and S3) in the Skeppsvik archipelago in 1992. The data are based on plant means (S2, L $N = 29$, M $N = 28$, S $N = 29$; S3, L $N = 21$, M $N = 20$, S $N = 18$). Paired t-tests were performed to test the effect of supplemental pollination. * $P < 0.05$, ** $P < 0.01$.

in the third population (H22, $F_{2,103} = 1.1$, NS). Population-wise analyses further indicated that seed production per plant was about 50% higher in the short-styled than in the long-styled morph, with the mid-styled morph intermediate in population H18 ($F_{2,150} = 6.3$, $P < 0.01$; Tukey-Kramer, L < S) but did not differ significantly between morphs in the other two populations.

Supplemental Pollination

Supplemental pollination significantly increased seed production per flower in long-styled plants (paired t-test, population S2 $t = 2.3$, df = 28, $P = 0.03$; S3 $t = 3.7$, df = 20, $P = 0.002$), but not in mid-(S2 $t = 1.2$, df = 27, $P = 0.26$; S3 $t = 0.1$, df = 19, $P = 0.92$) or short-styled plants (S2 $t = 1.3$, df = 28, $P = 0.20$; S3 $t = 1.9$, df = 17, $P = 0.07$; Fig. 3). In long-styled plants, hand-pollinated flowers produced on average 25% more seeds than control flowers in the S2 population and 51% more seeds than control flowers in the S3 population. In the S3 population, the seed production of control flowers was about 30% lower in the long-styled morph than in the mid- and short-styled morphs ($F_{2,56} = 3.5$, $P < 0.05$), whereas the seed production of hand-pollinated flowers did not differ among morphs ($F_{2,56} = 0.6$, $P = 0.53$; Fig. 3). In the S2 population, no significant among-morph differences in seed production per flower were detected in control flowers ($F_{2,83} = 0.7$, $P = 0.52$) or in flowers receiving supplemental pollination ($F_{2,83} = 1.4$, $P = 0.11$).

There was no difference in seed production per flower between open-pollinated control flowers on shoots with hand-pollinated flowers and open-pollinated flowers on shoots with no hand-pollinated flowers (S2, L $t = 0.1$, df = 21, $P = 0.94$, M $t = 0.7$, df = 18, $P = 0.52$, S $t = 1.8$, df = 21, $P = 0.08$; S3, L $t = 1.3$, df = 17, $P = 0.21$, M $t = 0.9$, df = 16, $P = 0.38$, S $t = 0.5$, df = 17, $P = 0.61$).

Offspring Quality

The proportion of seeds germinating varied significantly among populations in the sample from the Skeppsvik archipelago in 1992 that included six trimorphic populations, but not in the smaller samples from Skeppsvik in 1991 and from Harkskärsfjärden in 1992 (Table 4; Fig. 4). No significant among-morph differences in the proportion of germinating seeds were detected in the trimorphic populations (Table 4, Fig. 4), or in the dimorphic population S18 that was sampled in 1991 ($t = 0.05$, df = 33, $P = 0.96$; Fig. 4).

There was no significant among-morph variation in offspring size (population S2, $F_{2,217} = 0.43$, $P = 0.65$; S3, $F_{2,230} = 1.59$, $P = 0.21$; S18, $t = 0.48$, df = 102, $P = 0.64$; dry mass of above-ground parts square-root transformed prior to analysis) or in the proportion of the offspring that had produced flowers at 24 weeks (S2, $G = 0.6$, df = 2, $P = 0.73$; S3, $G = 3.1$, df = 2, $P = 0.22$; S18, $G = 0.1$, df = 1, $P = 0.80$; Table 5).

Segregation Ratios

Differences in morph ratios between offspring and flowering plants in the source populations were relatively small, and statistically significant in only two of the six trimorphic populations studied (S2 and S23). In these two populations, there was an excess of the L and M morphs and a deficiency of the S morph in the offspring produced in 1992 (Fig. 5). Although differences in morph frequencies between the offspring and parental generations were not statistically signif-

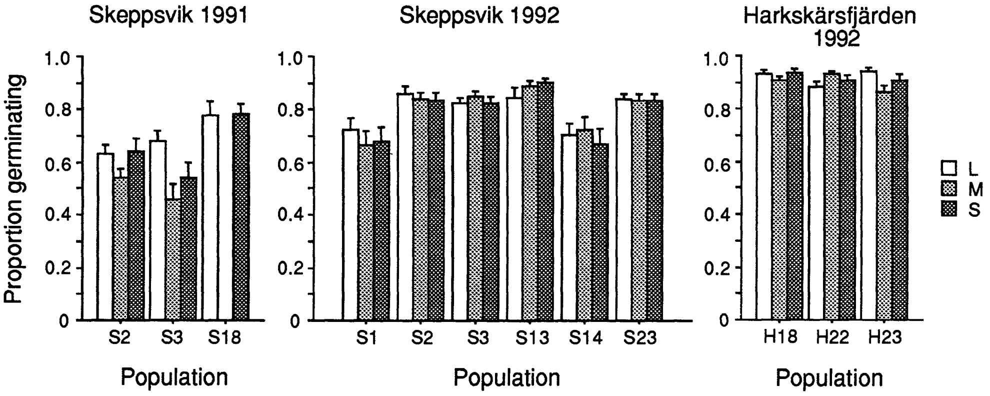

FIG. 4. Proportion of seeds germinating in tests of naturally produced seeds from *Lythrum salicaria* populations in the Skeppsvik and Harkskärsfjärden archipelagos. Means (± SE) were calculated based on maternal plant means [in each population, seeds were sampled from between 11 and 30 (median = 22) maternal plants per style morph]. Population characteristics are given in Table 1.

icant in the other populations, the power of the test employed was limited by the relatively low number of independent observations (maternal sib-ships sampled) in the offspring generation. If the documented differences were real, then 200–250 independent observations in the offspring generation would have been required for significance at the 0.05 level in the S3 and S13 populations in 1992 (J. Ågren, unpubl. data). There was no clear trend for morph ratios to be more even in the offspring than in the parental generation, and differences in morph frequencies between generations were not fully consistent among years. The M morph tended to increase in the offspring generation in all trimorphic populations (except population S3 in 1992; Fig. 5). The S morph had a higher frequency in the offspring than in the parental generation in both trimorphic populations studied in 1991 (S2 and S3) but tended to decrease in these and in the four other populations observed in 1992 (including population S14, in which the S morph was the minority morph; Fig. 5). No mid-styled plant appeared in the progeny produced in the dimorphic population S18 (a total of 120 offspring scored).

TABLE 5. Offspring size (dry mass of aboveground parts; mean ± SE) and proportion of offspring that had produced flowers at harvest (after 24 wk of growth in the greenhouse). Seeds for this experiment were collected in two trimorphic and one dimorphic *Lythrum salicaria* population in the Skeppsvik archipelago in 1991.

Maternal style morph	Offspring dry mass (g)	Proportion of plants flowering	*N*
Population S2			
L	11.3 ± 0.72	0.86	83
M	10.4 ± 0.98	0.80	51
S	10.7 ± 0.70	0.83	86
Population S3			
L	10.7 ± 0.63	0.80	83
M	12.7 ± 0.87	0.77	70
S	11.7 ± 0.59	0.88	80
Population S18			
L	9.9 ± 1.01	0.81	32
S	10.7 ± 0.78	0.83	72

DISCUSSION

This study revealed a marked difference in the morph composition of *L. salicaria* populations between two archipelagos in the Baltic Sea. The overall morph frequencies in the Harkskärsfjärden archipelago in central Sweden were remarkably similar to those documented in *L. salicaria* populations in an archipelago in southern Finland (Halkka and Halkka 1974), and in other parts of Europe (England, Haldane 1936; Switzerland, Schoch-Bodmer 1938; Spain, J. Ågren and M. Fuentes, unpubl. data). All these surveys have detected a slight excess of the L morph and a corresponding deficiency of the S morph (Table 3). In contrast, the populations in the Skeppsvik archipelago in northern Sweden were characterized by a marked deficiency of the M morph. In the Skeppsvik archipelago which is located close to the northern range margin of *L. salicaria* in Europe, the average frequency of the M morph in trimorphic populations was 0.21. A deficiency of the M morph, although less marked, has previously been documented in populations in the adventive range in Ontario, Canada (Eckert and Barrett 1992), i.e., in another marginal area of the distribution of this species.

We found no support for the hypothesis that inherent differences in reproductive success can explain the skewed morph ratios in *L. salicaria* populations in the two archipelagos; there were no statistically significant differences in flower production among morphs. Moreover, there was no indication that seed production would be lower than average in the M morph in the northern archipelago, or higher than average in the L morph in the southern archipelago. Instead, the L morph, which was the most common morph in both archipelagos, produced fewer seeds per fruit than the other morphs in the northern populations and in two of three populations in the southern archipelago. The L morph also tended to produce fewer seeds per plant. Although frequency-dependent selection may have contributed to the lower than average seed production in the L morph in some populations, there was no consistent relationship between the morph ratio and the relative seed production of the morphs in different populations (cf. Table 1 and Fig. 2).

The results of the hand-pollination experiment indicate that

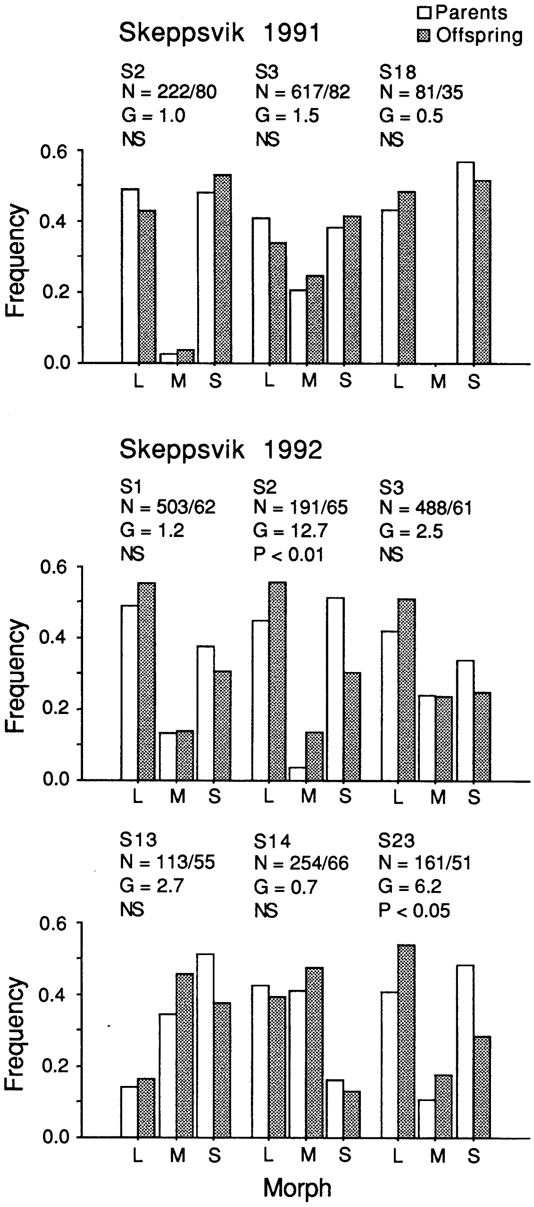

FIG. 5. Style morph frequencies in the parental generation and in the naturally produced offspring in three (1991) and six (1992) *Lythrum salicaria* populations in the Skeppsvik archipelago. The number of plants scored in the source population and the number of maternal sib-ships scored in the offspring generation are indicated (in the *G*-test, the number of independent observations in the offspring generation was set to be the number of maternal sib-ships observed). In total, 656 offspring were scored in 1991 and 1418 offspring were scored in 1992.

differences in the degree of pollinator limitation can explain differences in seed production among morphs. In both populations studied, supplemental pollination resulted in increased seed production in the L morph but not in the other two morphs. Although open-pollinated control flowers on long-styled plants produced fewer seeds than control flowers on mid- and short-styled plants, no such difference was noted among flowers receiving supplemental pollination in population S3. Seed production in the L morph may be limited by the amount of pollen received, by the quality of the pollen received, or by both of these factors. In an experiment with long-styled plants of *L. salicaria,* Nicholls (1987) demonstrated that seed production is reduced if pollination with legitimate pollen is preceded by the deposition of illegitimate pollen on the stigma. The style morphs may differ in their response to the deposition of illegitimate pollen. In a study of the self-incompatible, tristylous *Pontederia sagittata* (Pontederiaceae), the L morph showed a greater reduction in seed set than the other morphs when self-pollen was applied prior to the deposition of compatible pollen (Scribailo and Barrett 1994). In the present study, differences among morphs in the degree of pollinator limitation are unlikely to be due to differences in mate-availability. In both the S2 and the S3 population, the L and S morphs occurred at roughly similar frequencies (see Table 1).

In contrast to the results of the present study, O'Neil (1992) found that the S morph produced fewer seeds than the other morphs in two North American populations of *L. salicaria.* Moreover, hand-pollination experiments conducted in those two populations indicated that seed production was pollinator limited in the S but not in the other two morphs. Bumblebees and honeybees were the most frequent flower visitors in both the North American (O'Neil 1992) and in the Skeppsvik populations (J. Ågren, unpubl. data). Further studies are needed to establish whether among-population differences in the relative efficiency of pollen transfer between anthers and stigmas at different levels can be attributed to differences in behavior and morphology of the major flower visitors. Most studies of tristylous species have not found any morph-specific differences in seed production (Dulberger 1970; Barrett 1977; Weller 1981; Price and Barrett 1982; Barrett et al. 1989). However, a higher degree of pollinator limitation in the L morph may contribute to differences in seed production among style-morphs (L < M = S) in *Decodon verticillatus* (Lythraceae; Eckert and Barrett 1995).

The skewed morph frequencies in the Skeppsvik and Harkskärsfjärden archipelagos were not correlated with morph-specific differences in measures of offspring quality. There were no significant among-morph differences in seed germination or in offspring size after 24 weeks of growth in the greenhouse. This suggests that differences in offspring quality are unlikely to be important for the morph composition of the *L. salicaria* populations. However, these results should be interpreted with caution. Studies of inbreeding depression have shown that differences in offspring fitness that are expressed in the field need not be detectable in the benign greenhouse environment (Schemske 1983; Dudash 1990). We are currently studying the performance of naturally produced progeny from several Skeppsvik populations in a long-term field experiment.

Differences in morph frequencies between the offspring and the flowering plants in the source populations were relatively small and not fully consistent among years. There are a couple of factors that may contribute to a difference in morph ratio between offspring and parents. First, in a disassortatively mating population, differences in mate availability are expected to result in a disproportionate representation of the minority morph in the offspring generation. In this study, the minority morph did have a higher frequency in the offspring than in the parental generation in all trimorphic populations (except in populations S3 and S14 in 1992). However, differences in morph frequencies between generations were sufficiently large to be statistically significant in only two of the populations sampled in 1992 (S2 and S23). Moreover, since the increase of the minority morph occurred at the expense of only one of the two common morphs, there was no obvious trend for morph frequencies to be more even in the offspring than in the parental generation (Fig. 5). Second, differences in the siring ability of different types of outcross pollen could potentially result in morph-specific differences in male reproductive success beyond what is expected from considerations of the relative representation of different mating types in the source population (see Weller 1976; Kohn and Barrett 1992). Darwin (1877) found indications of a lower fertility of pollen from the M morph than of pollen from the L and S morphs in *L. salicaria.* However, no among-morph differences in pollen fertility were detected in controlled crosses on plants from two trimorphic Skeppsvik populations (S2 and S3; J. Ågren, unpubl. data). Moreover, the segregation ratios do not provide any evidence for a lower than average male fitness in the M morph. The frequency of the M morph was higher in the offspring than in the parental generation in all trimorphic populations, except population S3 in 1992. Interestingly, the S morph tended to increase in the offspring generation in both trimorphic populations studied in 1991 but tended to decrease in all six populations studied in 1992. This may reflect among-year variation in the relative male reproductive success of the different morphs.

Morph-specific differences in selfing rate are expected to affect morph frequencies at equilibrium (Charlesworth 1979; Heuch 1979b). Controlled crosses on *L. salicaria* from the Skeppsvik archipelago (J. Ågren, unpubl. data) and from other areas (Darwin 1877; Stout 1923; O'Neil 1994) have demonstrated that the self-incompatibility reaction is weaker in the M morph than in the other two style-morphs. If the M morph has a higher selfing rate than the other morphs and if inbreeding depression is strong, then this could potentially explain a deficiency of the M morph (Charlesworth 1979), as observed in the Skeppsvik archipelago and in trimorphic Ontario populations of *L. salicaria* (Eckert and Barrett 1992). However, contrary to the prediction of this hypothesis, there was no indication that seed production, germination, or offspring growth would be lower than average in the M morph in the present study.

If the skewed morph ratios cannot be attributed to morph-specific differences in reproductive success, what other factors may be responsible for the current population structure? There are two main alternatives. First, it is possible that the morphs differ in some other component of fitness, as, for example, the length of the juvenile period, the frequency of flowering, or mortality. We have initiated long-term demographic studies to address this hypothesis. Second, the skewed ratios could be the result of random processes and historical factors. We will use the rest of the discussion to examine this latter possibility.

In the combined sample from the Skeppsvik and Harkskärsfjärden archipelagos, 21% of the populations that included at least three flowering plants ($N = 66$) were dimorphic. The frequency of dimorphic populations was higher in small (3–50 flowering plants) than in large populations, which is consistent with the results from a survey of *L. salicaria* populations in Ontario (Eckert and Barrett 1992), and with the predictions of the stochastic models (Heuch 1980; Morgan and Barrett 1988; Barrett et al. 1989; Eckert and Barrett 1992). The proportion of nontrimorphic populations documented in this study is similar to that found in Ontario (Eckert and Barrett 1992). In contrast, earlier European surveys did not report nontrimorphic populations (Haldane 1936; Schoch-Bodmer 1938; Halkka and Halkka 1974). The reason is probably that earlier surveys sampled very few small populations. The study by Halkka and Halkka (1974) included five populations with fewer than 50 flowering plants, and that is the only previous European study that has reported data for populations within this size range. Recently, nontrimorphic populations of *L. salicaria* have been documented in a survey in northwestern and southern Spain (in this survey, three out of 27 populations with more than three but fewer than 50 flowering plants lacked at least one morph; J. Ågren and M. Fuentes, unpubl. data), and in southern France (S. C. H. Barrett, pers. comm. 1994). We expect additional dimorphic and monomorphic populations to be identified in the native range as more attention is given to the structure of small populations.

The stochastic models indicate that the S morph has the highest and the L morph the lowest probability of being lost from populations because of founder effects or genetic drift (Heuch 1980; Morgan and Barrett 1988; Barrett et al. 1989; Eckert and Barrett 1992; Husband and Barrett 1992b). The population data from the Harkskärsfjärden archipelago in central Sweden are consistent with these predictions. In that area, two of three dimorphic populations with at least three flowering plants lacked the S morph and one lacked the M morph. Moreover, the S morph was only represented in one of the seven populations that included fewer than three flowering plants (the corresponding figure for the L and M morphs were three and five, respectively). These observations are in line with data collected in Ontario, where most dimorphic *L. salicaria* populations (15 of 20 observed) lacked the short-styled morph (Eckert and Barrett 1992) and with the morph composition of dimorphic populations of the tristylous *Eichhornia paniculata* (Barrett et al. 1989; Barrett 1993). In the southern archipelago, there was an excess of the L morph and a deficiency of the S morph in trimorphic populations. Heuch (1980) suggested that stochastic loss of the S morph, and to a lesser degree of the M morph from local subpopulations or genetic neighborhoods, could potentially explain such a pattern. An analysis of the spatial distribution of morphs within populations (Husband and Barrett 1992c; Andersson 1994), and long-term demographic studies would be useful for an evaluation of this hypothesis.

In contrast to the population data from the Harkskärsfjärden archipelago, the results from the northern area are apparently not consistent with the predictions of the stochastic models. In that area, the M morph was the morph most commonly missing from dimorphic populations. Nine of 11 dimorphic populations lacked the M morph. Moreover, the M morph had the lowest representation in populations consisting of fewer than three flowering plants and was also underrepresented in trimorphic populations. When relating these results to the predictions of the stochastic models it is important to remember the underlying assumptions. In particular, most of those models assume that founders are drawn from a source population with even morph frequencies or that genetic drift occurs in a population that originally had even morph frequencies (see Heuch 1980; Barrett et al. 1989; Eckert and Barrett 1992). However, if the source population has a skewed morph composition, and if the time required to reach equilibrium is long relative to the rate at which new populations are formed, then a historical sampling effect could potentially influence the genotypic identity of founders of new populations over an extended period (Eckert and Barrett 1995). This effect should be particularly strong if the new populations serve as foci for further colonization, i.e., if migration follows a stepping-stone model.

Could the low frequency of the M morph in the northern archipelago represent a historical sampling effect? There are few models that attempt to analyze the effects of perenniality and age structure on the time required to reach equilibrium in tristylous populations. Recent simulations by Eckert and Barrett (1995) indicate that a high survival rate per se is not enough to make a historical sampling effect last for centuries. In one set of simulations, they examined the influence of survival on time to equilibrium in an outcrossing tristylous species with almost complete disassortative mating (i.e., under conditions that should be applicable to populations of *L. salicaria*). They found that, if 90% of the plants survived from one year to another, then the population would reach equilibrium after about 75 years. However, the analyzed model is based on several simplifications and is therefore likely to underestimate the time required to reach equilibrium in a species like *L. salicaria.* For example, the model assumes that there is no juvenile period (established plants begin to flower immediately), that established plants flower each year, that fecundity and mortality are independent of age/size, and that populations are infinitely large (Eckert and Barrett 1995). Demographic studies indicate that *L. salicaria* populations in the Skeppsvik archipelago violate all of these assumptions (J. Ågren and L. Ericson, unpubl. data), but it is not clear to what extent this will influence the expected time to reach equilibrium.

Migration should moderate the influence of founder effects and genetic drift on population structure. Halkka and Halkka (1974) suggested that effective dispersal among populations could explain the lack of nontrimorphic *L. salicaria* populations in their sample from the Tvärminne archipelago in southern Finland. However, there are several observations which indicate that gene flow between populations may be rather restricted in the studied archipelagos. First, there was significant variation in morph composition among-populations within both archipelagos [a heterogeneity *G*-test performed on the data presented by Halkka and Halkka (1974) indicates that this is true also for the Tvärminne populations: $G_H = 59.5$, df = 28, $P < 0.001$]. Second, in 1991, there were 81 flowering plants in the dimorphic (LS) population S18 in the Skeppsvik archipelago. The nearest trimorphic population was located about 300 m away (across water). Despite this relatively short distance of isolation, no mid-styled plant was detected in the progeny that was grown in the greenhouse ($N = 120$ plants representing 35 maternal sib-ships scored), and this population has remained dimorphic over four years of observation (J. Ågren, unpubl. data).

The hypothesis that the low frequency of the M morph in the northern archipelago represents a historical sampling effect deserves further examination. At the same time, the observation of a deficiency of the M morph also in trimorphic populations close to the northern range margin in North America (Eckert and Barrett 1992) motivates additional demographic studies to determine whether the relative fitnesses of the three morphs differ between marginal populations in the north and populations in other parts of the range.

To summarize, this study has established that the population structure of tristylous *L. salicaria* may vary markedly between different areas. We found no support for the hypothesis that inherent, morph-specific differences in reproductive success can explain deviations from even morph frequencies. We suggest that a combination of long-term demographic studies and population modelling will allow an evaluation of the relative importance of selection on the one hand and founder effects and historic factors on the other, for the skewed morph frequencies documented in this species.

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