

MULTILEVEL PHENOTYPIC SELECTION ON MORPHOLOGICAL CHARACTERS IN A METAPOPOPULATION OF *SILENE TATARICA*

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Abstract.—This study partitions selection in a natural metapopulation of a riparian plant species, *Silene tatarica*, into individual- and patch-level components by using contextual analysis, in which a patch refers to a spatially distinct stand of individual plants. We estimated selection gradients for two morphological characters (plant height and number of stems), their respective patch means, and plant density with respect to reproductive success in a two-year study. The approach was also extended to partition selection separately within habitats with varying degrees of exposure to river disturbances and herbivory. The selection differentials and gradients for plant height were positive at both individual and patch levels, with selection forces highest in the closed habitat with low exposure to disturbance. This pattern suggests that local groups with taller than average plants are more visible to pollinators than to groups that are shorter than average plants; and, within patches, individuals with short stature are visited less often than taller ones. Selection on the number of stems was in opposition at individual and patch levels. At the individual level the character was selected toward higher values, whereas selection at the patch-level favored smaller mean number of stems. The strength of the latter component was associated with the intensity of herbivory in different habitats, suggesting that the patch-level selection against a large number of stems might be due to high attractiveness of such patches to the main herbivore, reindeer. Consequently, direction and strength of selection in spatially structured populations may depend significantly on fitness effects arising at the group level.

Key words.—Contextual analysis, group selection, herbivory, pollination, selection gradient.

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Spatial subdivision of a population into distinct groups or local populations is common especially among rare species (e.g., Frankham et al. 2002). A metapopulation approach that focuses on the effects of suitable habitat area and connectivity between habitat fragments has turned out to be useful when dealing with the management of fragmented populations (e.g., Hanski 1999). Metapopulation models have been used to recognize which local populations are particularly important and how much suitable habitat area is required for metapopulation persistence (Wahlberg et al. 1996; Hanski 1999; Hanski and Ovaskainen 2000).

In addition to identifying the vital parts of a metapopulation it is also important to identify the characters that affect individual fitness at individual and group levels (Heisler and Damuth 1987) and the means by which they do so (Wade and Kalisz 1990). The use of multivariate statistical techniques (see Brodie et al. 1995 for a recent review) for estimating the direction and magnitude of selection from measurements on phenotypes at the individual level has become widespread in field studies (Hoekstra et al. 2001; Kingsolver et al. 2001). These methods are especially valuable when studying closely correlated characters, and increasingly they have been applied to recognize which characters are likely to be relevant to plant fitness in natural populations (e.g., Cambell 1989; Andersson 1992; Totland 1999; Kingsolver et al. 2001).

Despite their usefulness, multivariate statistics are limited to situations with a single population that is internally homogenous with respect to selection acting on individual traits. Even though a statistical approach known as contextual analysis has been proposed to extend the traditional multiple regression method to hierarchically structured populations (Heisler and Damuth 1987; Goodnight et al. 1992; Pedersen

and Tuomi 1995), it has only rarely been applied to natural populations (Stevens et al. 1995; Tsuji 1995; Banschbach and Herbers 1996; Vuorisalo et al. 1997), and, to our knowledge, the estimation of selection gradients with this approach has never been replicated in time or space for any of the studied species.

In structured populations, variation in fitness of individuals is not necessarily only due to properties of the individuals but also to the properties of the groups of which they are members. Contextual analysis extends the traditional multivariate approach by including “contextual traits” or group-level traits (e.g. density, average size) that may influence fitness in the multiple regression analysis (Heisler and Damuth 1987; Stevens et al. 1995). A significant partial regression of relative fitness on a contextual trait provides evidence of a group-level effect on fitness of individuals. This method was originally developed within the social sciences as a tool for interpreting the effects of social, institutional, and political contexts on human behavior (Boyd and Iversen 1979). When contextual analysis is applied to the study of natural selection, it permits one to determine whether group structure has an effect on individual fitness, but it also provides a method for identifying the particular group-level properties that are relevant for fitness variation in spatially structured populations.

Multilevel analyses of selection are potentially valuable in natural populations because in fragmented populations it is often difficult to correctly identify the ecologically appropriate levels of selection (Heisler and Damuth 1987; Tuomi and Vuorisalo 1989; Pedersen and Tuomi 1995, Goodnight and Stevens 1997). Fitness effects can rise from different levels of interaction, and these interactions may have counteracting effects on relative fitness (Heisler and Damuth 1987;

Goodnight et al. 1992, Goodnight and Stevens 1997; Vuorisalo et al. 1997). An evolutionary equilibrium on a quantitative trait may be achieved if individual- and group-level fitness effects are of the opposite signs (Goodnight et al. 1992; Vuorisalo et al. 1997).

The aim of this study is to partition selection in a natural metapopulation of the plant *Silene tatarica*, into patch- and individual-level components by using contextual analysis. Here "patch" refers to a spatially distinct stand of individual plants. We also extended the approach to partition selection separately within habitats with different disturbance regimes. We quantified selection on two morphological traits, plant height and number of stems, which are often correlated to plant reproductive success.

Fast vertical growth is favored especially in dense vegetation where competition for light is intense and where pollinator attraction tends to favor high stature (Aarssen 1995). In stressful and disturbed habitats with more open vegetation these same selection pressures should potentially be weaker. Meristem availability often constrains flower and fruit production (Geber 1990), thus reproductive output should correlate positively with the number of stems (Wyatt 1982; Huhta et al. 1999, 2000). Moreover, pollinators are often attracted to larger inflorescences (Schmid-Hempel and Speiser 1988; Andersson 1991; Eckhart 1991, 1999; but see Harder and Barrett 1995; Robertson and Macnair 1995), and thus a large number of stems may increase conspicuousness of an individual plant to pollinators.

Dense patches may be the most effective at attracting pollinators. This facilitation effect (Rathke 1983; Kwak et al. 1998) can create a positive relationship between population size and/or density and sexual reproductive success in rare plants (Oostermeijer et al. 2000; Mustajärvi et al. 2001). In contrast, mammalian herbivores may be selective, both within and between patches (McNaughton 1978; Danell et al. 1991; Hjäältén et al. 1993), causing fitness consequences in spatially structured plant populations (Tuomi and Augner 1993). We did not have any a priori expectations of the possible causes of the patch-level effect in *S. tatarica*, and our primary interest was to identify whether there were any patch-level fitness effects and whether they tend to strengthen or, alternatively, counteract selection arising from individual-level interactions.

MATERIALS AND METHODS

Study Species

Silene tatarica (L.) Pers. (Caryophyllaceae) is a rare long-lived perennial plant species growing along periodically disturbed riverbanks and shores of two rivers in Finland. *Silene tatarica* grows in habitats ranging from open sand and gravel shores and erosion banks to almost closed vegetated shores and riverbanks. The species flowers between the end of July and the middle of August and is partly self-compatible (A. Jäkäläniemi, unpubl. ms.). Each fertile individual has one to several vegetative and fertile stems with terminal inflorescences. Consequently, fruit production essentially depends on the number and height of flowering stems. Individuals reproduce for the first time in their third summer. Seeds lack special mechanisms for wind or animal dispersal, but their

structure allows them to float and germinate in water. Because *S. tatarica* does not spread vegetatively, establishment of new individuals and patches and expansion of existing patches occur exclusively by sexual reproduction.

Silene tatarica grows in open sand and gravel shore environments to almost completely vegetated shores and riverbanks with varying plant size and fitness properties between habitat types (A. Jäkäläniemi and P. Siikamäki, unpubl. ms.). Most of the fitness properties seem to be connected to the spatial location of the stand. In open habitats near the shoreline, grazing by reindeer is very intense, mortality is high, and the plants are sparsely situated and small. The plants are tall in closed and relatively undisturbed habitats where interspecific competition is presumably more intense than in open habitats. Plant height is intermediate in intermediately disturbed habitats where the intensity of river disturbance and herbivory are lower than in open habitats (A. Jäkäläniemi and P. Siikamäki, unpubl. ms.). Soil moisture, the amount of organic material, and nitrogen are low, but increasing from highly disturbed to undisturbed habitats (N. Tuomi, unpubl. ms.).

The spatially structured populations of *S. tatarica* are consistent with a metapopulation structure (Hanski 1999). The patches are genetically differentiated and gene flow between local populations is restricted (N. Tero, J. Aspi, P. Siikamäki, A. Jäkäläniemi, and J. Tuomi, unpubl. ms.). Local extinctions and colonizations also appear to occur in the study area (A. Jäkäläniemi, P. Siikamäki, and J. Tuomi, unpubl.), and allele frequency distribution of most patches correspond to recently bottlenecked populations (N. Tero, J. Aspi, P. Siikamäki, A. Jäkäläniemi, and J. Tuomi, unpubl. ms.) suggesting recent recolonizations with a few founder members. However, the population may not be primarily structured by the relative availability of disturbed and undisturbed habitats; rather, the patchy distribution may largely reflect a slow rate of recruitment and local dispersal which can create a patchy distribution of plants along a more or less continuous suitable habitat (see e.g. Tilman et al. 1997).

Study Site

The study area is situated in Oulanka River valley in Kuusamo in Northern Finland (66° N and 29° E). Oulanka River is an unregulated river with natural dynamics, including ice jams and dramatic seasonal fluctuations in water level. Flooding creates the opportunity for disturbance by ice scour, floating dead trees, bank slumping, and other erosional events along the riverside. Disturbance is typical not only during flooding but also on a smaller scale afterwards when the sand from river bank moves downwards and aeolian processes dominate on flat sand shores (Koutaniemi 1984).

Individual Sampling and Environmental Factors

To provide data on demographic parameters, 940 and 960 individually marked fertile plants from 15 patches (Fig. 1) were measured from the metapopulation of *S. tatarica* in 1999 and 2000, respectively. A patch was determined as a group of individuals with a maximum (arbitrary) distance between individuals of five meters. The same patches were studied in both study years. The number of marked plants per patch

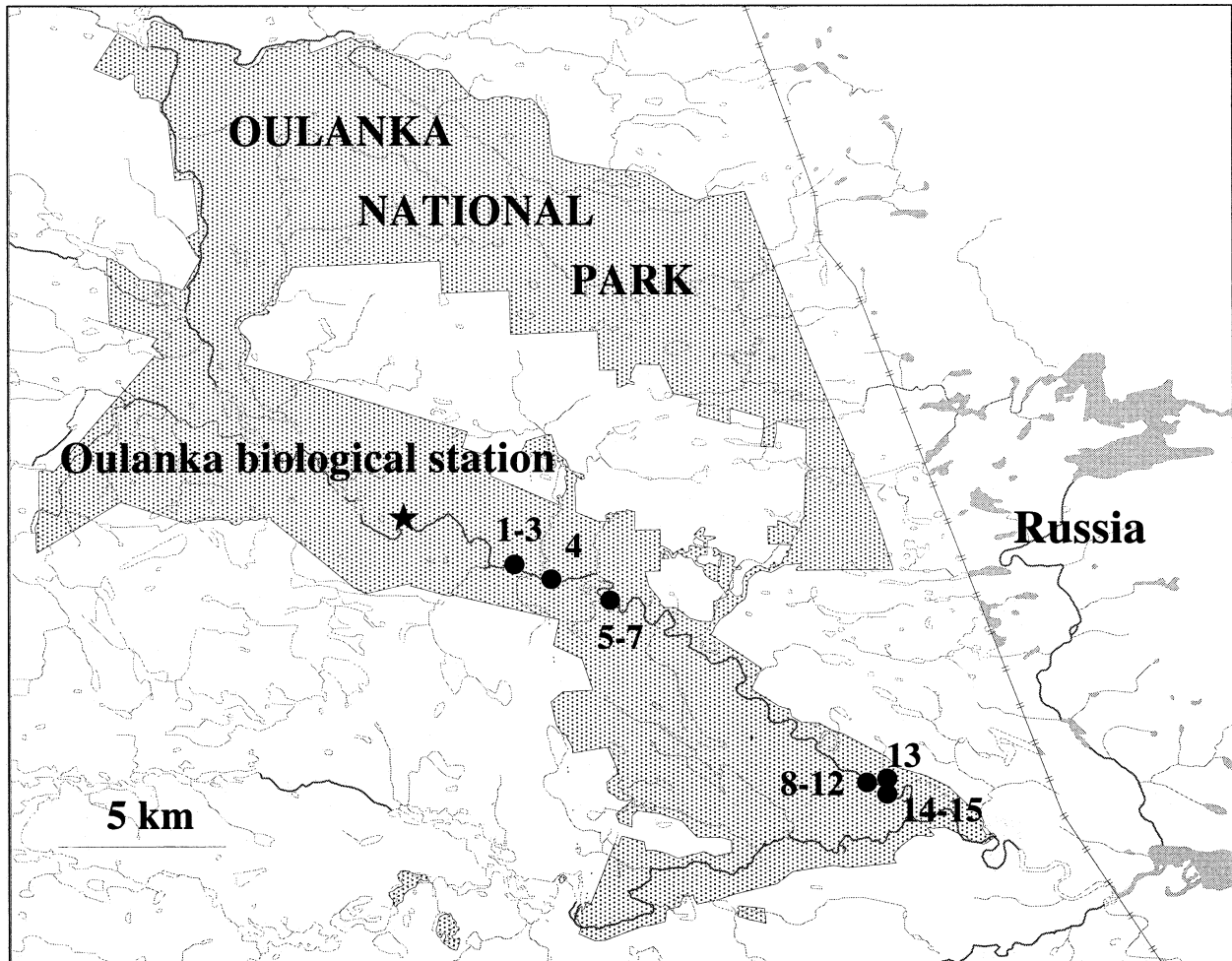


FIG. 1. Geographic location of the investigated patches of the *Silene tatarica* metapopulation.

was six to 134 in 1999 and 24 to 125 in 2000, with 66.9% of the individuals the same in both years.

Several morphological and environmental measurements were made for each individual at the end of the flowering season when the maximum height has been reached, that is, during the first two weeks of August. The height of the tallest stem per individual was measured to the nearest 0.5 cm, and the numbers of sterile and fertile stems were counted. The number of seed-containing capsules was also counted.

We recorded several environmental covariates in the immediate neighborhood of the plant that might affect their performance. The intensity of herbivory was estimated for each individual plant by counting the proportion of shoots eaten. Each measured specimen was used as a central point of a sampling plot (0.25 m²), and the percentage coverage of sand, fine gravel, coarse gravel, herbs, humus, litter, bryophytes, arboreals, and grasses were estimated with an accuracy of 5%.

Statistical Methods

Because the number of stems was not normally distributed in either year, this variable was log-transformed for statistical analysis. The number of seeded capsules per plant was used

as the absolute reproductive fitness value, because it is highly correlated with seed production (1999 sample: $r = 0.902$, $P < 0.001$, $N = 508$). Because the germination capacity of the seeds is very high (90.5% estimated from 1100 seeds from 34 individuals), the number of capsules is a good estimator of the number of offspring produced. To allow comparison across characters the absolute fitness values were transformed to relative fitness (w) values by dividing each absolute measure by the mean absolute fitness in the population (Lande and Arnold 1983). The morphological characters were standardized before estimating selection coefficients; that is, the selection coefficients are given in units of standard deviation to provide a standard metric for comparison among traits and years.

To compare the strength of selection in the two study years we estimated the opportunity of selection (Wade and Arnold 1980), defined as the variance in relative fitness:

$$I = \text{Var}(w) = \frac{n}{n-1}(\bar{w}^2 - 1), \quad (1)$$

in which n is the number of individuals.

The statistical analysis of correlation between morphological characters and number of capsules, our measure of fitness, involved calculations of two selection coefficients

which allow an estimation of directional phenotypic selection and selection for correlated characters (Lande and Arnold 1983; Arnold and Wade 1984a,b) at both the individual and patch levels. Selection differentials are the differences between the mean value or variance of a trait before and after selection and represent the total effect of selection.

The total selection differential can be partitioned (Price 1970):

$$S_T = S_W + S_B, \quad (2)$$

where S_W is the selection differential within patches and S_B is the observed selection differentials on the patch means themselves. Selection differentials within patches (S_W) were calculated as the average covariance between the measured morphological characters and the relative number of capsules within patches. Selection differentials among patches (S_B) were calculated as the covariance between the patch phenotypic means and patch mean relative fitness. Significance of the selection differentials was tested by using Spearman's rank correlations after sequential Bonferroni correction (Rice 1989).

Selection gradients measure the direct effects of selection on each trait. This measure is independent of any indirect effects caused by selection on the traits with which this trait is correlated. Thus, they show the target and the direction of selection, and allow a comparison of the magnitude of selection among several different traits. Sometimes an environmental factor may affect both the variable under consideration and fitness, and selection gradient analysis can be inaccurate (Mitchell-Olds and Shaw 1987, Rausher 1992, Stinchcombe et al. 2002). The analysis can be improved by adding the environmental factors as covariates in the model (Mitchell-Olds and Shaw 1987). Accordingly, we tested the possible effect of the measured environmental factors by adding them as factors in the regression model. Only the intensity of herbivory in the latter study year marginally increased the power of the statistical model. Since the regression coefficient for the intensity of herbivory was not statistically significant, it was not included in the final models.

Analysis of covariance (ANCOVA) was used to test whether patch membership accounts for some variance in fitness beyond what can be explained by the regressions of fitness on the measured morphological traits (Heisler and Damuth 1987). Because the ANCOVA suggested that patch membership significantly affected variance in fitness in both study years (see Results) the selection gradients were estimated by using contextual analysis as described in Heisler and Damuth (1987).

Two types of group characters may be used in contextual analysis. The first are aggregate characters created by combining measurements made on individuals (e.g., mean height in a patch). The second are global characters, which cannot be obtained solely from measurements of group members (e.g., density). Selection gradients in *S. tatarica* for individual and contextual characters were estimated from the slopes of linear multiple regressions in which individual plant height, number of stems, respective patch means, and density (the percentage coverage of *S. tatarica* in the neighborhood of each individual) were included as independent characters and the relative number of capsules as the dependent variable.

The total selection gradient B_{Ti} for a character i was estimated as:

$$\beta_{Ti} = \beta_{ij} + \sum_{j=1}^n R_{ij}\beta_{Cj} \quad (3)$$

where β_{Ti} and β_{Cj} are the fitted regression coefficients for the individual (plant height, number of stems) and patch characters (mean plant height, mean number of stems, and density), respectively. R_{ij} is the partial regression coefficient of the group mean for character j on individual values for character i (Heisler and Damuth 1987).

In *S. tatarica* the examination of the partial regression plots did not suggest any nonlinear relationship between the measured and dependent variables. Plotting the residuals against the predicted dependent values did not suggest any heteroscedasticity, and the normal probability plots were normally distributed in both years.

To further analyze the possible difference in selective forces in different habitat types we split the populations into three types (open, intermediate, and closed) based on percentage of vegetation cover. The open (0–20%) habitat type consisted of six patches, the intermediate (31–80%) of five, and closed (81–100%) of four. The selection gradients for both study years were estimated separately for different habitat types. The null hypothesis that all habitat-specific regression equations estimate the same population regression model was tested according to Zar (1984, p. 347).

RESULTS

Opportunity for Selection

Summer 1999 in our study site was warmer than summer 2000, temperature sums between July 15 and August 15 being 230466 degree days and 22436 degree days, respectively. The mean number of seeded capsules was higher in the warmer summer (Wilcoxon signed rank test: $Z = -7.145$, $P < 0.001$; mean \pm SD for 1999 and 2000: 45.96 ± 82.46 , $N = 946$, and 26.29 ± 57.55 , $N = 940$, respectively). There was more variance in relative fitness ($F = 1.488$, $df = 945, 939$, $P < 0.001$) in 2000, when the opportunity for selection was also higher ($I_{2000} = 4.791$) than in 1999 ($I_{1999} = 3.219$).

Selection at Individual and Patch Levels

There was a significant correlation between the number of stems and plant height (1999: $r = 0.710$, $P < 0.001$; and 2000: $r = 0.764$, $P < 0.001$). On the basis of within-patch selection differentials (S_W) there appeared to be significant selection toward higher plant height and larger number of stems in both study years (Table 1). These coefficients were similar in the two study years, but the selection differential for the number of stems was somewhat higher than the one for the plant total height. Patch-level selection differentials (S_B) contributed little in the total selection differentials (S_T).

Analysis of covariance indicated that patch membership significantly affected variance in fitness in both study years (patch effect in 1999: $F = 10.148$, $df = 14$, $P < 0.001$; in 2000: $F = 5.293$, $df = 14$, $P < 0.001$). Thus selection cannot be solely explained by the regressions of fitness on the measured morphological traits of individual plants. Accordingly,

TABLE 1. Standardized within-patch (S_W), patch-level (S_B), and total (S_T) selection differentials, and individual ($\beta_I \pm SE$), patch-level ($\beta_C \pm SE$), and total (β_T) standardized selection gradients for different morphological characters in the two study years.

Trait	S_W	S_B	S_T	β_I	β_C	β_T
Year 1999 (N = 922)						
Height	0.485***	0.025 ns	0.510	0.589 ± 0.083 ***	0.187 ± 0.060 **	0.684
Stem number	0.855***	-0.006 ns	0.849	0.653 ± 0.065 ***	-0.209 ± 0.055 ***	0.674
Density					0.631 ± 0.038 ***	0.631
Year 2000 (N = 864)						
Height	0.481***	0.112 ns	0.593	0.800 ± 0.117 ***	0.471 ± 0.082 ***	0.955
Stem number	0.838***	-0.015 ns	0.823	0.742 ± 0.086 ***	-0.697 ± 0.086 ***	0.505
Density					0.682 ± 0.053 ***	0.682

ns, not significant; ** $P < 0.01$; *** $P < 0.001$.

there was obviously something in the patch membership that must be taken into account in explaining the pattern of selection.

The proportion of variance in relative fitness explained by the contextual multiple regression model including the individual morphological characters, patch means, and density in the neighborhood was high and highly significant in both study years (in 1999: $R^2 = 0.504$, $F = 188.381$, $df = 5, 916$, $P < 0.001$; in 2000: $R^2 = 0.411$, $F = 121.264$, $df = 5, 858$, $P < 0.001$). To test the robustness of the regression models we split the data randomly into two parts: an estimation subsample for creating separate regression models for both study years, and a validation subsample to test the equations (e.g. Hair et al. 1998). The regression model based on parameters of the estimation subsample in 1999 explained as much of the variation in the relative fitness in the validation subsample as did the model estimated from the validation subsample itself ($R^2 = 0.522$ and $P < 0.01$ in both cases). The model

based on the estimation subsample for the year 2000 explained slightly less ($R^2 = 0.340$, $P < 0.01$) than the model based on validation subsample ($R^2 = 0.522$, $P < 0.01$), but was still a good overall predictor of individual fitness, suggesting that the estimated selection gradients were robust.

The total selection gradient (β_T) for both plant height and number of stems was significant and positive demonstrating that total selection favored larger plant size (Table 1). The selection gradients were large and similar in both study years. The sign and magnitude of individual-level selection gradients (β_I) for both characters were positive and about the same magnitude as the corresponding within-patch selection differentials, suggesting that both morphological characters are direct targets of selection (Table 1, Fig. 2). The individual-level standardized selection gradients were slightly larger in 2000 when the opportunity for selection was also higher. However, the difference in the gradients was not significant for either plant height ($t = -1.471$, $df = 1781$, $P = 0.142$)

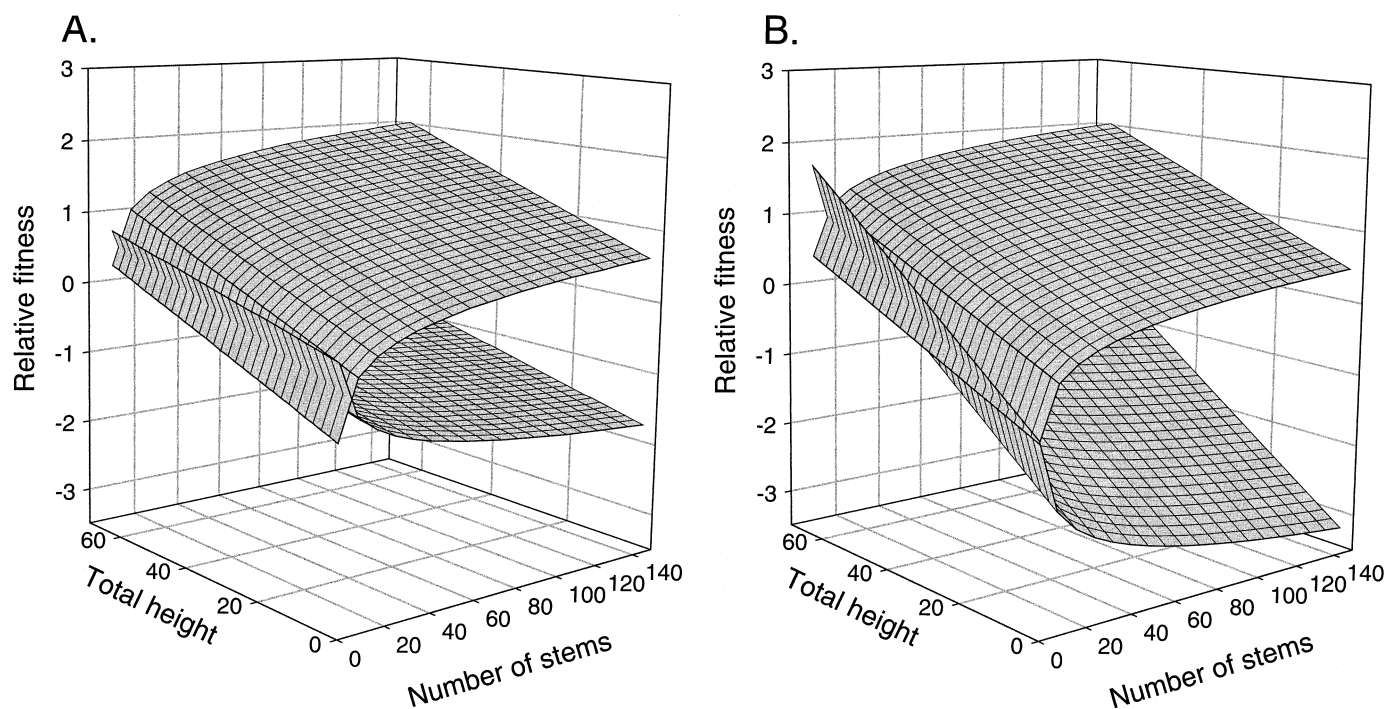


FIG. 2. Relative fitness surfaces with respect to plant total height and number of stems at individual (upper surface) and patch level (lower surface) in (A) 1999 and (B) 2000.

or the number of stems ($t = -0.826$, $df = 1781$, $P = 0.507$) between the study years. On average the increase of one standard deviation in total plant height would have increased relative fitness by about 59% in 1999 and 80% in 2000, whereas increase of one standard deviation in the number of stems would have increased relative fitness by about 65% in 1999 and 74% in 2000 (Table 1).

Even though none of the patch-level selection differentials (S_B) for the measured characters was significant (Table 1), the signs of all patch-level selection gradients (β_C) were similar to those of the corresponding patch-level selection differentials, and all patch-level selection gradients were significant in both years when the individual level characters were controlled (Table 1, Fig. 2). The magnitude of the patch-level selection gradient in 1999 was significantly lower for both plant height ($t = -2.795$, $df = 1781$, $P = 0.0052$) and number of stems ($t = 4.784$, $df = 1781$, $P = 0.001$) when compared to those in 2000.

Individual- and patch-level selection gradients for plant height were both positive, even though the magnitude of individual-level selection seems to be much higher than the patch-level selection. In contrast, the signs of the selection gradients for the number of stems at individual and patch levels were in opposition, suggesting opposite selective forces at the individual and patch levels (Table 1). Selection at the individual level favored plants with a large number of stems, whereas at patch level high average number of stems was maladaptive to members of the patch (Fig. 2). The magnitude of individual selection seemed to overshadow the patch-level selection in 1999, but in 2000 the magnitude of the patch-level gradient was almost as high as the individual-level coefficient. However, the total selection gradients (β_T) for the number of stems were also positive in both years suggesting that the total selection is acting toward a higher number of stems (Table 1).

The effect of plant density on relative fitness was positive in both years (Table 1) and there was no significant difference in the magnitude of the selection gradient between the study years ($t = 0.782$, $df = 1781$, $P = 0.434$).

Variation in Selection between Habitats

When the data was split into three groups on the basis of the habitat type, there appeared to be significant differences in the number of capsules produced (Kruskal-Wallis test: $\chi^2 = 24.26$, $df = 2$, $P < 0.001$), and also significant heterogeneity in variances (Levene statistics test value = 16.309; $df = 2$, 938; $P < 0.001$) between habitat types in 1999. Reproductive performance and its variance in 1999 was highest in the open habitat type (mean number of capsules 61.56; $SD = 105.5$). The means and variances for the intermediate (34.80; $SD = 61.39$) and closed (37.82; $SD = 64.74$) habitat types were similar. In 2000 there were no significant differences in the mean number ($F = 1.218$; $df = 2$, 943; $P = 0.296$) or variances (Levene statistics = 0.211; $df = 2$, 943; $P = 0.810$) of capsules produced between different habitat types.

The intensity of herbivory (measured as the percentage of the stems eaten) was different between habitats in both study years (Kruskal-Wallis test: $\chi^2 = 252.43$ and 216.49; $df = 2$ and $P < 0.001$ in both cases). Herbivory was more intense

in the latter year (9% in 1999 compared to 18% in 2000) and greatest in the open habitat in both years (16.4% and 24.2%), followed by the intermediate (4.3% and 11.4%) and closed habitats (4.5% and 8.5%).

The selection gradients were estimated separately for each habitat type. Because the intensity of herbivory was not similar between habitats, it was also added into the regression models as a covariate. Adding level of herbivory in the models increased the explanatory power of the model in each habitat type.

The selection gradients for different habitat types are presented in Table 2 (note that the number of plants in different habitats did not sum up to the total number of plants in Table 1 since herbivory data was missing in some cases and these plants were omitted from the analysis). The contextual regression equations were different between habitats in both study years (1999: $F = 8.529$; $df = 14$, 897; $P < 0.001$ and 2000: $F = 2.860$; $df = 14$, 762; $P < 0.001$) suggesting that the magnitude of the selective forces is different in different habitats. In general, the signs of the individual-level selection gradients in both years were similar to those for combined data, even though the magnitude of the selection gradients estimated separately for different habitats tended to be greater.

In contrast to the individual-level selection gradients, the patch-level selection gradients were exceedingly different between habitat types in both study years. The selection gradient for the mean number of stems was significantly negative only in the open habitat in 1999, whereas in 2000 increasing the mean number of stems decreased fitness at patch level in each habitat. The selection gradient for the mean height in a patch was consistently significant only in the closed habitat. The patch-level selection gradient for height was significantly positive also in the open habitat in 1999, whereas it was not significant in 2000.

Fitness of individuals tended to increase with increasing density in all habitats, and importance of density appeared to be greatest in the open habitat.

DISCUSSION

Stevens et al. (1995) used contextual analysis combined with path analysis to examine multilevel selection in *Impatiens capensis*. In this species, selection was acting at both individual and patch levels. The largest individuals in the smallest neighborhoods had the highest survival, and produced the most cleistogamous (self-pollinated) flowers. Intraspecific competition appeared to be an important factor affecting individual fitness in *I. capensis*.

In contrast, intraspecific competition does appear to affect individual fitness in *S. tatarica*, since increasing density increased reproductive success. In habitats typical to *S. tatarica*, where light is abundant, factors other than intraspecific competition seem to be important determinants of individual success. The positive density effect on reproductive success was most pronounced in the open habitat. This pattern is consistent with intraspecific facilitation for pollinator attraction, which is expected to be strongest at low densities (Rathke 1983). Our field observations suggest that there may be pollen limitation of female fertility in *S. tatarica*. The proportion of flowers that set fruit was estimated in a sample of flowers in

TABLE 2. Standardized individual ($\beta_I \pm SE$), patch-level ($\beta_C \pm SE$) and total (β_T) standardized selection gradients for different morphological characters in the different habitat types.

Trait	1999			2000		
	Open (N = 351)	Intermediate (N = 254)	Closed (N = 314)	Open (N = 281)	Intermediate (N = 251)	Closed (N = 296)
	β_I	β_C	β_T	β_I	β_C	β_T
Height	0.796 \pm 0.138**	0.742 \pm 0.190***	0.622 \pm 0.136***	0.604 \pm 0.150***	-0.228 \pm 0.137	0.203 \pm 0.076*
Stem number	0.822 \pm 0.148**	-0.656 \pm 0.155***	0.777 \pm 0.104***	0.847 \pm 0.137***	-0.085 \pm 0.148	0.014 \pm 0.095
Density		0.613 \pm 0.059***	0.532 \pm 0.072***	0.438 \pm 0.085***		
Height	0.981 \pm 0.269***	-0.015 \pm 0.300	1.054 \pm 0.223***	0.538 \pm 0.197**	0.043 \pm 0.120	0.260 \pm 0.115*
Stem number	0.302 \pm 0.269	-0.358 \pm 0.166*	0.959 \pm 0.147***	1.010 \pm 0.146***	-0.224 \pm 0.123**	-0.223 \pm 0.107*
Density		1.459 \pm 0.120*	0.455 \pm 0.081***	0.291 \pm 0.089**		

* $P < 0.01$, ** $P < 0.01$, *** $P < 0.001$.

2000, and it appeared to be rather low (52.6%; $N = 41$). Thus, intraspecific competition for pollinator attraction has a high potential for being an important component of fitness in this species.

In general, the selection differentials and gradients for plant height appeared to be positive both at individual and patch levels, indicating that both individual- and group-level selective forces favor tall plants. This may also be associated with the pollination biology since taller plants often attract more pollinators (e.g., Peakall and Handel 1994; Donnelly et al. 1998; Lortie and Aarsen 1999), and dense local groups with taller than average plants may also be more visible to pollinators than sparse groups with shorter than average height (Heisler and Damuth 1987). However, when the different habitat types were analyzed separately the pattern appeared to be more complicated. Even though there was no counteracting selection on plant height at individual and patch level in any habitat, the patch-level gradients were consistently significant only in the closed habitat. The results revealed that the importance of patch-level attraction is greatest in the closed habitat. Perhaps the visibility of flowers is more dependent on the plant height in the closed habitat among dense vegetation than in the other habitat types. However, this cannot be the sole explanation, since there was also significant positive patch-level selection gradient on plant height in the open habitat in 1999. Furthermore, competition for light may not explain selection for taller plants in *S. tatarica* because individual-level selection gradients were consistently positive in all habitat types.

The selection gradients for the number of stems appeared to be in opposition at individual and patch levels when the combined data was considered. Within groups, selection favored higher character values, whereas groups with low average number of stems had higher fitness than groups with high averages. When the data were analyzed separately for each habitat, the patch-level selection against large number of stems was evident in the open habitat in both years and in other habitats in the second year only. The strength of this selection tended to decrease when the closeness of the habitat increased.

This pattern suggests that the patch-level selection against large number of stems was associated with herbivory by reindeer (even though it was statistically controlled at the individual level in the model). In the former year, level of herbivory was high only in the open habitat, and in the latter year it was relatively high in each habitat, even though it was still highest in the open habitat. Similarly, the patch-level selection gradient was again highest in the open habitat, and somewhat lower in the other habitats. Consequently, local groups with a large average number of stems may have been more attractive to reindeer especially in the open habitat, and thus a small number of stems at the patch level were favored. Reindeer grazing results in a loss of fruit-bearing stems and hence in reduced reproductive output of individual plants.

In contrast, individual-level selection seemed to consistently favor plants with a large number of stems. This is expected for conditions in which there is no trade-off between the number of stems and the number fruits and seeds that, on average, mature on a stem (Geber 1990). Even though selection forces at different levels counteracted each other, the total selection gradients and differentials for the number of stems was positive in both years suggesting that overall

selection acts toward a higher number of stems in this species. However, if there is significant temporal and spatial variation in predation risk and pollinator availability, there may be significant consequences on selective plant morphology in *S. tatarica*. For example, Galen and Cuba (2001) have recently addressed how a conflict between pollinator attraction and avoidance of predation influences the geographical variation and evolution of a morphological character in the alpine sky-pilot, *Polemonium viscosum*.

Our results suggest that selection was acting both at the individual and patch level in *S. tatarica*. Patch-level selection gradients were significantly larger in 2000, suggesting that the group in which an individual belongs may play a more important role in harsher years. Because there were significant differences in selection equations between habitats, the habitat in which a specific individual grows could explain some of the differences in selection forces among patches. However, note that the contextual analysis is a correlative method and cannot demonstrate causality (Heisler and Damuth 1987; Wade and Kalisz 1990). Experimental studies of association between selection gradients and environmental characters are necessary to identify the agents of natural selection (Wade and Kalisz 1990).

Selection gradients estimated by using the Lande-Arnold method can be biased if covariances between traits and fitness are caused when each is influenced by the same or correlated environmental factors (Mitchell-Olds and Shaw 1987; Rauscher 1992; Stinchcombe et al. 2002). Stinchcombe et al. (2002) estimated that in their dataset nearly 25% of the phenotypic selection gradients estimated were biased by environmental covariances. In most cases the sign of the selection gradient was correct, and the bias caused by environmental covariances affected only the quantity of the selection gradient estimates. It is also possible that in our study there might have been some unmeasured environmental covariates that may have affected the magnitude of the individual-level selection gradient estimates. However, there are no theoretical predictions about the effect of unmeasured environmental covariates on patch-level selection gradients.

Recently, Kingsolver et al. (2001) and Hoekstra et al. (2001) reviewed studies that estimated the strength of linear selection in quantitative characters for field populations. Estimates of the magnitude of directional selection $|\beta|$ were exponentially distributed, with few estimates greater than 0.50 and most estimates less than 0.15 (Hoekstra et al. 2001). For sample sizes less than 1000, most cluster between -0.1 and 0.1 (Kingsolver et al. 2001). According to Kingsolver et al. (2001), the selection studies were seldom replicated in time or space. In our case the sample sizes were reasonable, and still the selection differentials and gradients were high, suggesting that a significant gradient may be a biological rather than statistical artifact. Our study was also replicated in time and space, and the individual selection gradients appeared to be consistent between the two study years. In *S. tatarica* opportunities for selection were $I_{1999} = 3.219$ and $I_{2000} = 4.791$. The most that any mean can be shifted is \sqrt{I} (Wade and Arnold 1980), which was 1.794 for 1999 and 2.188 for 2000. The total selection differentials were about one eighth to one fourth of the maximum absolute change and were of a similar magnitude in the two study years: 15% and 12% for plant height (0.510 and 0.593), and 26% and

17% for the number of stems, respectively (0.849 and 0.823). Thus, even though the selection gradients were reasonably high, the total selection differentials were far from the maximum possible.

In Kingsolver et al.'s (2001) review, large selection gradients were often associated with small sample size. Our results may suggest an answer to this phenomenon. When we split our total dataset with respect to different habitats, the magnitude of the selection gradients appeared to increase. This was because the mean fitness was different in different habitats. When this was taken into account the selection gradients increased, and this may also be true in more general contexts. Large samples may be collected from more heterogeneous environments than the small ones, and the heterogeneity may lower the estimates of the selection gradients.

Our results suggest that environmental heterogeneity, as well as spatial population structure, may significantly contribute to the selection forces encountered by individuals. With respect to plant height, patch-level fitness effects tend to strengthen selection arising from individual level. In contrast, the patch-level selection on the number of stems counteracted the individual-level selection—at least under some circumstances. It has been suggested that between-group selection may override within-group selection in conditions when group and individual level selection gradients are of opposite signs, and between-group variance accounts for a sufficiently large part of total genetic variance in the population (Price 1970; Goodnight et al. 1992; Vuorisalo et al. 1997). In *S. tatarica* relatedness within patches is likely to be high. New patches are established by a few founding individuals (A. Jäkäläniemi, P. Siikamäki, and J. Tuomi, unpubl. ms.), and investigation of genetic structure using AFLP-markers suggested a reasonably high level of differentiation between patches ($F_{ST} = 0.29$; N. Tero, J. Aspi, P. Siikamäki, A. Jäkäläniemi, and J. Tuomi, unpubl. ms.). This kind of population genetic structure may lead to high between-group genetic variance. In such conditions multilevel selection may have evolutionary implications in *S. tatarica*, and also in other plant species with spatially subdivided and genetically structured populations.

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