

DENSITY-DEPENDENT MULTILEVEL SELECTION IN THE GREAT LAKES SEA ROCKET

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Abstract. Multilevel natural selection has been demonstrated in natural plant populations, but the ecological conditions that influence the strength of different levels of selection are poorly known empirically. One of the factors most likely to influence the relative strength of individual and group selection is density. If density determines the intensity of interactions among neighbors, one expects that individual selection may be strong at low density but that group selection would be strongest at high density. This study characterized how density influences multilevel selection on size in the Great Lakes sea rocket (*Cakile edentula*). Plants were grown at three different densities in the field, and multilevel natural selection on plant size was estimated. As predicted, only individual selection was detected at the lowest density, but group selection was detected at higher densities. Surprisingly, group selection was strongest not at the highest density, but at intermediate density, where it opposed individual selection; shorter, heavier individuals growing in groups of tall, light plants had the highest fitness. At the highest density, the effect of neighbor phenotype on reproduction differed between central and peripheral group members, leading to no overall group selection detected at high density. The observation that group selection was more prevalent at higher density than at the lowest density supports the general prediction that more intense interactions among group members can lead to stronger group selection. However, the strength and direction of group selection changed unpredictably with increasing density and spatial placement. This result emphasizes the need for spatially explicit investigations of how interactions among individuals influence patterns of multilevel selection in plant populations.

Key words: *Cakile edentula* var. *lacustris*; competition; density; dispersal; group selection; levels of selection; sea rocket; soft selection.

INTRODUCTION

Group selection is known to be theoretically possible (e.g., Willham 1963, Wade 1978, 1985, Cheverud 1985), and it has been shown to contribute to evolutionary responses in experimental populations (Wade 1977, Goodnight 1985). The few empirical studies that have measured the strength of group selection in experimental populations (e.g., Wade 1977) or natural populations (Breden and Wade 1989, McCauley 1994, Stevens et al. 1995, Tsuji 1995, Banschbach and Herbers 1996, Kelly 1996, McCauley et al. 2000) have shown that group selection can work in concert with individual selection or in opposition to it, and that it can have a measurable effect on evolutionary responses even when individual selection is operating as well (Wade 1978, Goodnight et al. 1992, Tanaka 1996). Thus the empirical question becomes not “Does group selection occur?” but “How strong is group selection relative to individual selection?” To address this basic question, empirical studies of multilevel natural selection need to be conducted in ecologically realistic contexts.

Studies that explicitly measure group selection in natural environments are few. Two studies that measured multilevel selection in natural plant populations both found evidence for group-level selection. In one observational study of *Impatiens capensis*, Stevens et al. (1995) used an analytical technique called contextual analysis (Heisler and Damuth 1987, Goodnight et al. 1992) to measure simultaneously the effects of individual and group traits on fitness, or to quantify individual-level and group-level selection. This technique is convenient in observational studies, since the influence of group traits on fitness can be measured using statistical methodology similar to that which is applied to measure natural selection at the individual level (Lande and Arnold 1983). Stevens et al. (1995) found that group selection on size operated with comparable magnitude but in the opposite direction as individual-level selection. In a manipulative study, also of *I. capensis*, Kelly (1996) found that changing the morphology of individuals within groups altered the fitness of other group members. He also observed a plastic response of focal individuals to the morphology of their neighbors. He pointed out that such plasticity reflects a contribution of neighbors to natural selection, since individual phenotype, and hence the opportunity for individual selection, are actually in part due to char-

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acteristics of neighbors. Using path analysis, Kelly's study found evidence for selection at the group level operating both through direct effects of neighbors on plant reproduction and through the effect of neighbors on phenotypes, which in turn influence fitness. Group selection is therefore detectable in natural populations when experiments are designed to measure it.

The ecological conditions that determine the relative importance of group and individual selection are poorly known empirically. Perhaps the most obvious ecological factor likely to influence the relative strength of group selection is the intensity of interactions among individuals within groups (Antonovics and Levin 1980, Kelly 1996). The intensity of interactions often varies strongly with density. At low density, interactions among individuals are expected to be weak, while more intense interactions are expected at high density. Therefore, one predicts that individual selection would be strong at low density but that group selection would be strongest at high density. No study to date has tested this prediction in plants. In the study presented here, I manipulated density in order to test whether multilevel selection on size varies with density, as predicted.

Multilevel selection on size has been hypothesized to influence important population demographic processes such as soft selection and the "law of constant yield" (Harper 1977, Wade 1985, Goodnight et al. 1992, Stevens et al. 1995). Soft selection occurs when groups contribute equally to the next generation regardless of the mean phenotype of the group; the fitness of an individual within the group depends only on its phenotype relative to others within the group (Wallace 1975, Wade 1985, Goodnight et al. 1992). Soft selection often alters evolutionary trajectories and outcomes since it determines whether differential contributions of genotypes to future generations depend on local or global genotype frequencies.

"The law of constant yield" is an example of soft selection. It is observed empirically as reproduction that is not dependent on initial seed densities within a designated unit area (Harper 1977). That is, variable seed inputs yield the same biomass or reproductive output. This can occur when depletion of a limiting resource causes size hierarchies to form such that dominant individuals suppress smaller individuals beneath the canopy, sometimes resulting in self thinning (Schmitt et al. 1987, Weiner 1990). "Constant yield" strongly influences stand productivity and population demography in general.

The degree of soft selection, or the scale of density regulation in a population, is related to multilevel selection (Wade 1985, Goodnight et al. 1992, Kelly 1992, 1994). The efficacy of group selection depends on the differential reproduction of groups, and similarly, whether groups contribute differently to future generations depends on multilevel selection. Kelly (1992, 1994) demonstrated that the spatial scale of density regulation (the degree of soft selection) determines the

degree to which groups of interacting individuals contribute differentially to future generations. If density regulation occurs within groups (strict soft selection), then all groups contribute equally, even if they differ phenotypically. However, if density regulation is at a scale larger than that of the group, then phenotypic differences among groups can contribute to differences in group-level reproduction and therefore can contribute to responses to group-level selection (assuming the phenotypic differences are genetically based).

Goodnight et al. (1992) argued that the scale of density regulation itself, or soft selection and constant yield, is due to an opposition between group and individual selection (see also Wade 1985). Large individuals may have greater reproductive success (individual selection favoring large size), yet groups of small individuals have higher reproductive success (group selection favoring small size). They argue that if the magnitudes of individual and group selection are equal, then groups can contribute equally to the propagule pool whether they comprise large or small individuals. Thus, group contribution is independent of the mean size of individuals in the group, and this is an example of soft selection.

Density becomes a crucial factor when considering constant yield, as group yield must be independent not only of the mean size of individuals in the group but also of the number of individuals in the group. That is, groups with a few large individuals have the same yield as groups with many small individuals. In order for constant yield to be due to a balance between individual and group selection, both individual and group selection must vary with density. Three possible mechanisms exist that could cause a balance in levels of selection across densities. First, individual and group selection could operate and balance each other at all densities. This seems highly unlikely, since group selection is exceedingly difficult to imagine when individuals do not interact in groups. Second, as originally proposed by Wallace (1975), selection could be relaxed at low density, but frequency-dependent selection could be strong at high density; individual and group selection balance each other at high density, but neither is important at low density. The third possibility is that group selection at high density balances individual selection at low density, so that pooled over all densities, group and individual selection may reach a balance. That is, one predicts that individual selection would be stronger at low density and group selection would be stronger at high density. Therefore, measuring density-dependent multilevel selection can provide information on the potential mechanisms of evolutionarily and demographically important processes such as soft selection and constant yield.

This study is an empirical investigation of density-dependent multilevel selection on size in the Great Lakes sea rocket, *Cakile edentula* var. *lacustris* (*C. edentula* hereafter). In previous studies of the ecology of

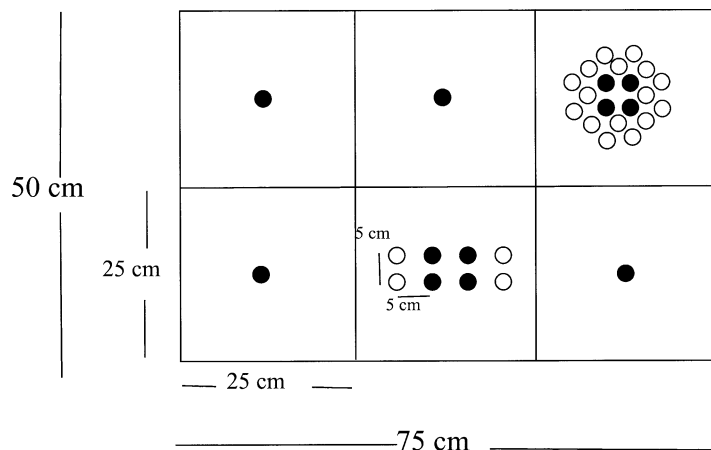


FIG. 1. Experimental layout. Each large plot has six subplots. Four low-density subplots, each with one individual, are shown. The intermediate-density treatment is shown as two lines of plants, and the high-density treatment is shown as four focal individuals surrounded by 16 nonfocal competitors (20 plants total). Black circles represent focal individuals, and white circles represent nonfocal competitors. Three plots were planted for each maternal plant: one at the home site, one 3 m from the home site, and one 15 m from the home site.

seed dispersal in this system, it was shown that density varies greatly in natural populations, and that dispersal efficiency strongly determines the density in which plants grow (Donohue 1998). Hence, dispersal has the potential to influence patterns of multilevel selection by altering the intensity of interactions among individuals. In this study, I test the hypothesis that the strength of individual and group selection depends on density. I do so by using contextual analysis (Goodnight et al. 1992) and path analysis (Kelly 1996) to measure multilevel selection in experimentally manipulated seed dispersion patterns of different densities within a heterogeneous natural landscape. Through this experimental manipulation, I ask the following questions. (1) Does density influence phenotype or reproductive output? (2) Does density influence the strength and/or magnitude of individual and group selection on size? In particular, (3) are both individual and group selection weakest at low density; or (4) is group selection stronger at higher densities and individual selection stronger at lower densities?

METHODS

The study system and experimental design

Cakile edentula var. *lacustris*, an annual mustard (Brassicaceae), grows on the open beach or on primary dunes along the shores of the Great Lakes of North America. It has single-seeded, heteromorphic fruit segments which are dispersed by wind and water (Rodman 1974). *Cakile edentula* often occurs in high-density clumps composed of full or half siblings (Donohue 1998). Intermediate to high densities of seedlings occur along the storm line, where seeds are washed up together. Isolated individuals grow elsewhere on the beach. Thus, density can range from one individual within a several square meter area up to hundreds of individuals within a 100-cm² area (*unpublished data*).

The experiment was conducted at the Indiana Dunes National Lakeshore near Michigan City, Indiana, USA. See Donohue (1997) for more details on the site and experimental design. Ripe fruit segments were col-

lected from 29 maternal plants during the dispersal season in late summer, and the exact location of the maternal plant site was marked with a wooden dowel. Maternal plants were sampled arbitrarily at ~25-m intervals. During March, seeds were weighed, and then forced to germinate by imbibing the seeds and removing the seed coats. Seeds were planted in 96-cell plug trays in a 1:1 mixture of Pro-Mix (an artificial soil; Premier Horticulture, Dorval, Quebec, Canada) and Terragreen (baked clay; Oil Dri, Chicago, Illinois, USA). After the first true leaves appeared, seedlings were transplanted into the field. Seedlings were used instead of seeds because of the extreme instability of the sand substrate, which would have caused the loss of seeds and new germinants.

Each maternal plant site was designated as a plot of 75 cm × 50 cm, with the maternal plant site located at its center (Fig. 1). This plot was divided into six 25 cm × 25 cm subplots. At each maternal site, three density treatments were imposed. Four focal individuals were randomly assigned to each of the density treatments. In the low-density treatment, single individuals were planted in four of the subplots. This treatment represents the density experienced by isolated individuals on the beach. In the intermediate-density treatment, eight seedlings were planted in one subplot in two rows, 5 cm apart, with the central four seedlings as the focal individuals. This treatment represents the spatial arrangement of seeds that were washed up along the storm line; such seeds are frequently linearly arranged. In the high-density treatment, 20 seedlings were planted within a single subplot as closely together as possible. The focal seedlings were in the center of the group. This treatment represents the high-density clumps caused by nondispersal from the maternal plant. All seeds at a given site were half to full siblings ("families," hereafter) collected from the maternal plant that grew at that site during the previous season. The placement of the three treatments was randomly determined at each maternal site. This arrangement of densities was replicated at 0 m, 3 m, and 15 m from the original

maternal plant site in a direction parallel to the water line, in order to control for the elevation on the dune.

Censuses were conducted on focal seedlings every two weeks throughout their life. During censuses, the number of flowers and fruits was recorded, as was the date of death. At the time of death, a final fruit count was conducted, and the plant was collected. Height and stem mass of dead plants were determined. Fitness was estimated as the total number of fruit segments. Some focal plants blew away before collection. Their fitness was estimated as the number of segments during the census immediately preceding disappearance. Some nonfocal individuals also blew away, but they were not censused biweekly. This resulted in missing values for both phenotype and fitness for nonfocal plants. If more than one third of the individuals in a group disappeared before collection, that group was not included in the contextual analysis (see *Statistical analysis* section) since it was assumed that the remaining sample might not accurately represent group composition experienced during the majority of the experiment.

Statistical analysis

Natural log transformation improved normality of fitness data and achieved normality of height and stem mass. To test for the effect of density and distance from the home site, a mixed-model analysis of variance (PROC GLM; SAS 1990) was performed on transformed data using the SAS (1990) statistical package. Only focal plants were used in this analysis to insure a more balanced design. Sample sizes were smaller than expected since only plants in families that had representatives at each density and distance combination could be included in the full model. Sample sizes for fruit production were larger than those for size characters because plants that blew away had information on fitness but not size. Density and distance treatments were fixed factors, family was a random factor, and seed mass was a continuous covariate. Main effects of density and distance were tested over their interactions with family. Many plants did not reproduce at all, so segment number data were non-normal even after transformation. Therefore, probabilities associated with the analysis of fitness are only approximate. See Donohue (1997) for a more complete nonparametric analysis of treatment effects on fitness.

To test whether density or distance changed size or fitness hierarchies within groups, the variance in natural log-transformed height, stem mass, and fitness was calculated for each group at each density and each distance (Tonsor 1989) using focal and nonfocal individuals. These variances were compared across density and distance treatments using analysis of variance, with density and distance as fixed effects. Planned contrasts were conducted using the "contrast" statement in SAS (PROC GLM).

Because distance from the maternal home site did not influence phenotypes or fitness, all subsequent anal-

yses compared only density treatments. To compare total fruit production by groups that were initiated at different densities, or group-level reproduction, the total number of fruits produced by each low-, intermediate-, and high-density group was determined. Although this experiment cannot test for the law of constant yield directly because the areas of planting were not precisely controlled, some patterns would be consistent with it. Low-density groups occupied ~ 1.9 m², intermediate- and high-density groups occupied ~ 625 cm², with high-density groups possibly occupying a smaller area depending on how closely packed the plants were. With constant yield, one therefore predicts that low-density groups would have the most reproduction and intermediate- or high-density groups would have the least, since stand-level reproduction is independent of density but dependent on area. Fruit production by whole groups was compared across density treatments using a Kruskal-Wallis test. In order to factor out variation in environmental quality across sites, an additional Kruskal-Wallis test was conducted on the residual variation in group fitness after factoring out effects of location. To obtain residuals, each site and distance combination (each plot) was treated as having a different categorical "location." Residuals from an analysis of variance of fruit number, with "location" as the fixed effect, were used in the Kruskal-Wallis test. To test for soft selection, Spearman rank correlations were calculated between fruit production by groups and mean phenotypes of individuals within groups. Soft selection occurs when the reproductive contribution of groups does not depend on the mean phenotype of individuals in the group. A significant correlation between group fitness and the mean phenotype of group members would therefore be evidence that soft selection is not present. Correlations were calculated for each density separately and for all groups pooled over density. They were also calculated using the residual variation in fitness after factoring out effects of location, as just described.

To estimate the strength of multilevel natural selection on the size characters of height and stem mass, a contextual analysis (Heisler and Damuth 1987, Goodnight et al. 1992) was performed. Family was initially included as a covariate in all selection analyses. Its inclusion did not alter results appreciably, so these results are not presented. For comparative purposes, the first contextual analysis was performed on data pooled over all density treatments and with density as a covariate, as was done in the Stevens et al. (1995) paper on multilevel selection in *Impatiens capensis*. Next, a separate contextual analysis was performed separately within each density treatment. For both contextual analyses, focal and nonfocal plants were used in the intermediate and high-density treatments so that all group members could be included. The mean height and stem mass were calculated for each group, and these mean phenotypes were included in the regression

analysis with the individual phenotypes for each member of the group. In this manner, the influence of the group mean phenotype can be estimated independently of the influence of the individual phenotype. The regression coefficient for the individual phenotype is interpreted as the strength of individual-level selection, and the regression coefficient for the group mean phenotype is considered an estimate of the strength of group selection. Because plants grown at low density were not expected to be interacting, the low-density treatment also serves as an internal experimental control for spurious correlations that could be manifest as group selection. If group selection is not detected at low density, then group selection that is detected at the other densities is not caused by experimental artifacts, but is true group selection. For the low-density treatment, a standard phenotypic selection analysis without group traits was performed for comparison. The strength of selection was compared across treatments using analysis of covariance with relative fitness as the dependent variable; a significant interaction between the trait and density treatment indicates that selection on that trait differs across treatments. Because residuals of the selection analyses were not always normally distributed, jackknife resampling was performed to estimate standard errors, using the program Free-stat (Mitchell-Olds 1989).

Nonlinear (stabilizing or disruptive) selection and correlational selection were estimated by including second order interactions in a separate regression model. Significant effects of squared terms would indicate significant stabilizing (if negative) or disruptive (if positive) selection on the characters. Significant interactions between characters would indicate that the effect of one character depends on the other character, and the coefficients for these interactions are estimates of correlational selection.

Two different methods of standardization were used. In the first method, traits were standardized within each density treatment to have a mean of zero and standard deviation of one. Relative fitness was calculated as the fitness of each individual divided by the mean fitness of all individuals within each density treatment. In the second method of standardization, traits were standardized across all density treatments to have a mean of zero and standard deviation of one. Relative fitness was calculated as the fitness of each individual divided by the mean fitness of all individuals in all density treatments. Contextual analysis was performed as previously described. This second method of standardization differs from the first in that it permits the variance among groups to differ across treatments. If density influences the degree of variance among groups, and therefore the opportunity for between-group selection, then such differences will be manifest using the second method of standardization but not the first, in which the total variance is constrained to be one in all treatments (see Donohue 2003). This method of

standardization gave results that were very similar to those from the first method of standardization, so these results are not presented in the tables. Therefore, any density-dependent selection observed in this experiment was not due to the effect of density on among-group variation.

Environmental heterogeneity can cause spurious correlations between phenotypes and fitness (Mitchell-Olds and Shaw 1987, Rausher 1992). In order to control for potential environmentally induced correlations, selection analyses were also performed on the residual variation in fitness after factoring out variation due to the spatial location of the groups (see earlier in this section). Residuals were used in a contextual analysis with phenotypes standardized within and across each density treatment. Results based on residual variation in fitness were similar to those from the analyses of the phenotypes themselves. These results are therefore not presented in the tables, but any differences are indicated in the table legends.

Path analysis (Kingsolver and Schemske 1991, Kelly 1996, Scheiner et al. 2000) was used to interpret results of the contextual analysis for intermediate and high density. With path analysis, causal pathways known a priori can be included in the analysis of selection. In the case of multilevel selection, the influence of group phenotypes on individual phenotypes are included as separate paths (Kelly 1996). Path coefficients were estimated on standardized traits and fitness using SAS (PROC CORR and PROC REG). In contrast to the standard contextual analysis, group mean traits were calculated only from nonfocal individuals so that the phenotype of focal individuals would not contribute to a spurious correlation with group mean phenotype. Effects of neighbor traits on the phenotype and fitness of focal individuals were estimated. Path coefficients between phenotypes and fruit segment production were estimated using the total number of segments and the residual variation in segment number after factoring out effects of location, as described earlier in this section.

RESULTS

Density influenced the fitness of focal individuals, but not plant size (Table 1), with higher density being associated with less fruit production (mean number of fruit segments ± 1 untransformed SD = 4.6 ± 19.8 , 5.0 ± 20.8 , and 1.3 ± 7.6 for low, intermediate, and high density, respectively). Families differed in phenotypes and fitness, as indicated by the significant main effect of family. The phenotypes of families responded differently to distance from the home site, as indicated by a significant interaction between family and distance. This interaction can be explained by variation in the attributes of the maternal sites alone; families from good sites, for example, may grow large at their home site, whereas families from poor sites grow larger elsewhere. Seed mass influenced height and stem mass

TABLE 1. Results of analysis of variance to test for treatment effects on plant characters and fitness.

Source	Height		Seed mass		Fitness	
	df	F	df	F	df	F
Seed mass	1, 240	33.66***	1, 241	17.18***	1, 749	0.57
Density	2, 53	0.74	2, 49	0.26	2, 59	7.13**
Distance	2, 33	1.63	2, 31	1.55	2, 57	0.62
Family	21, 19	5.40***	21, 20	3.54**	28, 15	8.50
Density × distance	4, 38	0.64	4, 37	0.93	4, 106	0.14
Density × family	37, 38	0.66	37, 37	0.69	56, 106	0.51
Distance × family	28, 43	2.48**	28, 40	2.83**	56, 105	1.29
Density × distance × family	35, 240	1.24	35, 241	1.99**	105, 749	3.14***
N	372		373		1004	

Notes: F ratios are given for each trait by column. Results are based on Type III sums of squares.
 ** $P < 0.01$; *** $P < 0.001$.

but not fruit production by individuals. A significant three-way interaction between density, distance, and family indicates that the stem mass and fruit production of families responded differently to different combinations of density and distance. For more discussion on treatment effects on individual fitness, see Donohue (1997).

Neither density nor distance influenced the variance in characters among group members (results not shown but available upon request). Thus, there is no evidence of size hierarchies at higher densities in this system. When the total fruit production by an entire group was compared across density treatments, low-density groups (mean fruit production \pm 1 SD: low density = 12 ± 32 segments) produced fewer fruits than higher density groups (intermediate density = 23 ± 61 segments, high density = 27 ± 77 segments), but the variation among density treatments was not significant ($\chi^2 = 0.73$, $P > 0.05$, $df = 2$, $N = 87$ for all groups). This result is in contrast to the prediction based on constant yield; low-density groups are expected to have higher yield since they occupied a greater area. When the effect of environmental heterogeneity on fitness was controlled for, groups at intermediate density had the highest fruit production (5 ± 40 residual units), and groups at high density had the lowest (-7 ± 45 residual units; low density = 2 ± 26 residual units),

TABLE 2. Spearman rank correlations between group mean phenotype and group-level reproduction at three densities and pooled over all densities.

Density	Trait	Correlation	P
Low	mean height	0.69†	0.001
Low	mean stem mass	0.66	0.001
Intermediate	mean height	0.71	0.001
Intermediate	mean stem mass	0.59	0.001
High	mean height	0.69†	0.001
High	mean stem mass	0.57	0.001
Pooled over densities	mean height	0.69	0.001
Pooled over densities	mean stem mass	0.59	0.001

† Not significant when based on residual variation in fitness after factoring out effects of location.

and these differences were significant based on Kruskal-Wallis analysis of the residuals ($\chi^2 = 8.34$, $P = 0.02$, $df = 2$, $N = 87$ for all groups). While the law of constant yield does predict that high-density groups would have the least reproduction since they probably occupied the smallest area, it also predicts that low-density groups would have the highest, in contrast to these observations. The limited reproduction by low-density plants is apparently due to factors unrelated to competition for resources. Therefore, the law of constant yield does not appear to be operating in this system, despite significant effects of density on individual reproduction at higher densities. The use of seedlings instead of seeds necessarily prevents examination of density-dependent effects operating at the early seedling stage, including early soft selection via self thinning. However, self thinning has not been observed in this species at any stage (*personal observation*), so the basic dynamics observed here are not likely to be substantially altered during the early seedling stage.

Soft selection occurs when all groups contribute equally to the propagule pool, regardless of the mean size of individuals within groups. Different groups contributed differently to the propagule pool, with wide ranges of group-level reproduction at all densities (low-density range = 0–218; intermediate-density range = 0–274; high-density range = 0–502). At all densities, the mean phenotypes of group members were strongly correlated with group-level reproduction, such that groups composed of small individuals had lower reproductive output than groups composed of large individuals (Table 2). Strong correlations were also detected when groups were pooled over all densities. Thus, soft selection does not appear to be operating in this system.

When a contextual analysis was performed with data pooled over all density treatments, and with density included as a covariate, group selection was in the opposite direction of individual selection for both characters, although group selection was not significant (Table 3). When residual variation in fitness was analyzed, after factoring out effects of location, group

TABLE 3. Selection analysis when pooled over all density treatments, as in Stevens et al. (1995).

Character	β (1 SE)	β_{resid} (1 SE)
Density	-0.35 (0.26)	-1.53 (0.80) [†]
Height	-2.03 (1.04)*	-6.11 (3.19) [†]
Stem mass	4.61 (1.34)***	14.09 (4.15)***
Mean height	0.73 (0.68)	0.01 (2.08)
Mean stem mass	-0.61 (0.57)	-4.04 (1.77)*
<i>N</i>	1157	1157

Notes: Results are based on analysis of actual phenotypes (β) and on analyses of residual variation in fitness after factoring out variation due to location (β_{resid}). Selection (partial regression) coefficients are presented with jackknife standard errors in parentheses. Significance levels are based on jackknife standard errors.

* $P < 0.05$; *** $P < 0.001$; [†] $P < 0.1$.

selection on stem mass was significant but less than one third as strong as individual selection. Group selection on height was negligible. Shorter, heavier individuals had higher fitness, but plants in groups composed of lighter plants had higher fitness. Therefore, group selection opposed individual selection, but individual selection was stronger.

Density influenced patterns of multilevel selection (Table 4). As expected, no group selection was detected at low density. Height was not significantly associated with fitness at low density, but plants with greater stem mass had higher fitness when only individual traits were analyzed. When residual variation of fitness was analyzed after factoring out variation due to location, the association between stem mass and fitness remained positive and significant, suggesting that the observed relationship is not due simply to environmentally induced correlations between size and fitness due to among-location environmental variation. No nonlinear or correlational selection was detected at low density.

At intermediate density, shorter and heavier plants growing with taller and lighter plants had higher fitness (Table 4). The analysis of residuals did not differ much from the analysis of actual phenotypes, indicating that these relationships are not likely to be environmentally induced. Significant disruptive selection on stem mass was detected (disruptive selection coefficient = 2.48, jackknife SD = 1.32), but it was only significant when residual variation in fitness was analyzed. The phenotype with the minimum fitness was within the range of variation in the sample (as opposed to the relationship being nonlinear but monotonic). Thus, individuals that were smaller or larger than average had higher fitness than individuals of intermediate size, although the largest individuals had the highest fitness. No other nonlinear or correlational selection was detected. The direction of individual selection on stem mass was in the same direction as that observed at low density, and it was of comparable magnitude. Group and individual selection operated in opposite directions at intermediate density for both size characters. Group and individual selection on height were of comparable strength, but individual selection on stem mass was stronger than group selection.

At high density, only the stem mass of the individual influenced fitness (Table 4), with heavier plants having higher fitness. Significant disruptive selection on stem mass was detected (disruptive selection coefficient = 4.18, jackknife SD = 2.06). As in intermediate density, the phenotype with the minimum fitness was within the range of variation in the sample. These results held when residual variation in fitness was analyzed, after factoring out variation due to location. No other nonlinear or correlational selection was detected.

Individual selection on size characters varied with density, as indicated by the significant interactions be-

TABLE 4. Multilevel selection gradients for each of three density treatments when standardized within each treatment.

Character	Low β (1 SE)	Intermediate β (1 SE)	High β (1 SE)	<i>F</i> interaction
Height	0.62 (2.29) 0.08 (1.39)	-1.52 (0.71)*	-3.10 (1.87)	4.28*
Stem mass	3.12 (2.03) 3.95 (1.43)**	3.59 (0.84)***	5.73 (2.39)*	3.71* [†]
Mean height	-0.76 (2.25)	2.11 (0.61)**	0.30 (1.00)	2.16 [‡]
Mean stem mass	1.14 (1.72)	-1.81 (0.57)***	-0.31 (0.82)	2.02 [‡]
<i>N</i>	116	296	745	1157

Notes: Jackknife standard errors are shown in parentheses. Significant differences from zero are indicated and are based on jackknife standard errors. For low density, the second coefficient of selection (beneath) is that estimated when only individual phenotypes were included in the model. When family was included in the model, selection coefficients tended to be larger, but results were qualitatively the same. “*F* interaction” indicates the *F* ratio for the interaction between the trait and density and is based on Type III sums of squares. Significance levels of “*F* interaction” are based on analysis of covariance.

[†] Not significant when based on residual variation in fitness after factoring out effects of location.

[‡] Significant when standardized across treatments or when based on residual variation in fitness after factoring out effects of location.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

tween characters and densities (Table 4). The weakest selection on height occurred at low density, and the strongest selection on stem mass occurred at high density. When residual variation in fitness was analyzed, selection on stem mass was of comparable magnitude across all density treatments ($F = 2.45$, $P > 0.05$). Group selection on height and stem mass did not differ significantly when actual fitness was analyzed and when traits were standardized within each treatment (Table 4), but group selection on both characters was significantly stronger at intermediate density when traits were standardized across all density treatments (mean height $F = 3.92$, $P < 0.05$; mean stem mass $F = 3.60$, $P < 0.05$) or when residual variation in fitness was analyzed (mean height $F = 5.62$, $P < 0.01$; mean stem mass $F = 4.25$, $P < 0.05$).

The path analysis revealed that group traits significantly influenced the expression of height and stem mass of focal individuals at both intermediate and high density, with positive correlations among most characters (Fig. 2). The relationships among individual and group phenotypes were similar across intermediate and high density, although the effect of mean neighbor height on height of the focal individuals was significantly stronger at high density ($F = 5.99$, $P < 0.05$). The paths that differed most between density treatments were paths between phenotypes and fitness (height $F = 13.21$, $P < 0.001$; stem mass $F = 34.39$, $P < 0.001$; neighbor height $F = 22.01$, $P < 0.001$; neighbor stem mass $F = 16.99$, $P < 0.001$). At intermediate density, results were very similar to the contextual analysis; more fruit production of the focal individuals occurred when focal individuals were shorter and heavier and when they grew in groups composed of tall, light neighbors. At high density, however, results of the path analysis differed surprisingly from the contextual analysis. Taller focal individuals had higher fitness (nearly significant when based on residual variation in fitness), and individuals growing in groups of shorter and heavier neighbors had higher fitness (nearly significant for stem mass when based on residual variation in fitness). The direction of group selection according to the path analysis is in the opposite direction as the nonsignificant group selection at high density in the standard contextual analysis, and in the opposite direction of the significant group selection detected at intermediate density. This difference may be explained by considering that the focal individuals were in the center of the groups, whereas the nonfocal individuals were at the periphery. Peripheral group members may experience an environment similar to that of the intermediate-density treatment, whereas central individuals may experience a different environment. If, in fact, the effect of neighbor phenotype on individual fitness varies from the periphery to the center of the group, with tall, light neighbors enhancing the fitness of peripheral individuals (as seen at intermediate density) and shorter, heavier neighbors enhancing fitness of cen-

tral individuals (as seen in the path analysis of high-density groups and in a similar study [Donohue 2003]), then no overall group selection would be detected when the entire group is considered. This was the result from the contextual analysis. Therefore, it appears that the effect of neighbor phenotype on individual fitness depends on the location of the individual in the group.

DISCUSSION

Density altered patterns of multilevel selection in this experiment. As predicted, group selection was only detected at higher densities, although it was strongest at intermediate density, not high density. At low density, when plants were not interacting strongly, only the stem mass of the individual influenced fitness, with larger individuals having higher fitness. At intermediate density, both the phenotype of the individuals and the phenotypes of neighbors significantly influenced fitness, with group selection opposing individual selection; short, heavy individuals growing with tall, light neighbors had the highest fitness. Surprisingly, at high density, overall group selection was not significant, although results indicate that the effect of group members on fitness depended on the position of the plants in the group. The observed general relationship between higher density and increased importance of the phenotypes of neighbors is expected, since plants that are interacting closely are more likely to influence the fitness of their neighbors. However, the effect of group members on fitness appears to be complex, and dependent on the particular spatial arrangements of individuals within groups.

Similar patterns of natural selection were found in a different sample of plants planted at low density at native sites for another experiment (*unpublished data*). Similar to the low-density treatment in this study, plants with heavier stems had higher fitness, there was no significant association between height and fitness, and no group selection was detected. Thus, two independent experiments revealed similar patterns of multilevel selection when plants were grown at low density.

In addition, a different study was conducted in which siblings competed with each other at a similar high density as in this experiment (compare "sibling" treatment to "high-density" treatment in this study, Table 4; Donohue 2003). Although no group selection was detected in this study using standard contextual analysis, the direction of group selection based on path analysis in this study was the same as that in the other study when based both on path analysis and standard contextual analysis. Thus, these two independent experiments found similar patterns of group selection when siblings competed at high density, based on path analysis. In both experiments, fitness of centrally located focal individuals was higher when individuals were growing with shorter but heavier neighbors. Such traits may increase the fitness of a neighbor by shading the surface of the sand and helping to conserve water

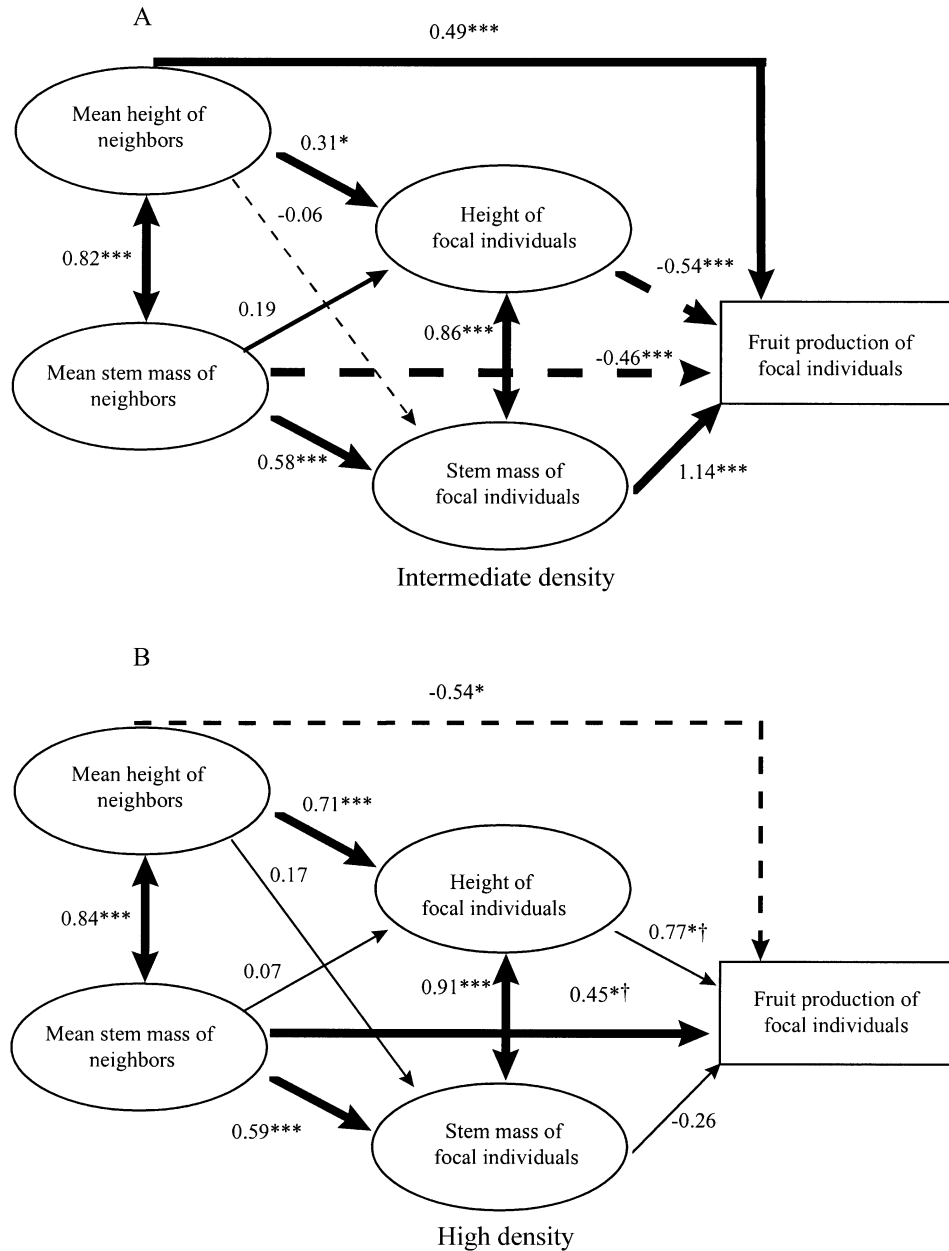


FIG. 2. Path diagram of the relationships between group traits, individual traits, and individual fitness at (A) intermediate density and (B) high density. Single-headed arrows indicate an a priori causal relationship, and double-headed arrows indicate correlation. The thickness of arrows is proportional to the significance of the path coefficient. Solid lines indicate positive path coefficients, and dashed lines indicate negative path coefficients.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Values that are nearly significant when based on residuals after factoring out variation in fruit production due to location are indicated by daggers (†).

while not shading the neighbor itself. Further experimental manipulations of individual phenotype, neighbor phenotype (as in Kelly 1996), and environmental variables are required in order to test these functional hypotheses. One explanation for the difference between the two experiments may be that plants in high-density groups in this experiment were not as close together as in the other experiment due to more shifting of the

sand or other unknown factors. More loosely arranged groups may have resulted in environments that resembled the intermediate density treatment for more peripherally located individuals. In this experiment, group selection at intermediate density was in the opposite direction of group selection on focal individuals at high density. The total effect of neighbor phenotypes on individuals within high-density groups was not sig-

nificant, suggesting that neighbor effects on peripheral group members canceled out neighbor effects on central members.

At intermediate density, shorter and heavier plants had higher fitness. Allocation to lateral as opposed to vertical vegetative growth appears advantageous to these individuals. This result is not uncommon for plants that are not competing for light; plants at intermediate density were not directly shaded by their neighbors on all sides, and the beach is a high light environment. Plants growing with tall and light neighbors rather than short and heavy neighbors had higher fitness. Not only do individual- and group-level selection work in the opposite direction from each other in this treatment, but group selection at intermediate density is in the opposite direction from group selection on focal plants at high density. The effect of neighbor size on reproduction therefore depends on the spatial relationships among neighbors. Wind and disturbance of the sand substrate are two factors that these beach plants contend with chronically. Neighbor morphology may alter exposure to wind and substrate stability in stands with different spatial configurations of plants. Alternatively, the physiological processes of competition for light, water, or other resources may change between the two density treatments, and it is possible that unmeasured correlated variables differed between treatments and caused the different patterns of selection. Again, only experimental manipulations can determine the functional mechanisms of the relationships between size and fitness observed at intermediate density. Explicit manipulations of spatial arrangement in different systems with different seed dispersion patterns, in systems that grow on different substrates, or in those with different limiting resources such as light or water, could identify how abiotic and biotic ecological factors can alter interactions among neighbors as a function of position in the group.

Path analysis suggested that the differences between the results from intermediate and high density were not due to differences in the influence of neighbor phenotype on the phenotypes of focal plants, but rather due to differences in the effect of individual and group traits on fitness. At both densities, strong positive associations were observed between traits of neighbors and focal individuals. These positive associations are likely to be the result not only of plastic responses of focal individuals to their neighbors' size, but also of the positive genetic and environmental association between focal plants and neighbors, since the neighbors were siblings growing in the same location as the focal plants. The stronger effect of neighbor height on the height of focal individuals at high density indicates that plasticity is a factor, since the other sources of correlations were the same between the two densities. The extent to which the correlations between group and individual traits are due to plasticity of focal individuals in response to neighbor size could be another con-

tribution to group selection in this experiment; neighbor size can alter individual size and thereby alter the opportunity for individual selection (Kelly 1996).

One other study of multilevel selection in the field found that individual and group selection acted in opposite directions from each other. Stevens et al. (1995) found that *Impatiens capensis* growing in a range of natural densities had higher fitness when they were large, and that they also had higher fitness when they were growing with neighbors that were smaller. In that study, individual and group selection were of the same magnitude, but in the opposite direction; individual selection exactly balanced group selection. Such a balance between levels of selection, they argued, could account for soft selection and the law of constant yield. In the study presented here, group selection was in the opposite direction as individual selection when pooled over all densities (as in their analysis), but individual selection was substantially stronger (Table 3). The reason for stronger individual selection is likely to be because the direction of group selection varied between intermediate and high density and thereby mitigated each other, whereas individual selection (especially on stem mass) acted fairly consistently at all densities. This study found no evidence for constant yield or soft selection. Rather, a strong association was observed between the mean size of group members and the total reproduction of the group at all densities (see also Donohue 2003) even though density did influence the reproduction of individuals and groups. Apparently, some physiological limitation, independent of density, limited the reproduction of plants at low density. The lack of a precise balance between individual and group selection, and the lack of evidence for soft selection, are consistent with the proposition that soft selection is due to oppositions between individual and group selection, as predicted by Goodnight et al. (1992).

The difference between the study of *Impatiens* and this study of *Cakile* is likely due to differences in the ecology of these two species. In *I. capensis*, in which a balance between levels of selection was observed, extreme size hierarchies are common in which dominant individuals suppress smaller individuals beneath the canopy (Schmitt et al. 1987, Weiner 1990). Light is a strongly limiting factor in dense canopies of *I. capensis*, and differences in height are directly associated with the ability to acquire light. Light used by taller individuals is not available to those beneath them, and shorter individuals are thereby suppressed by the growth of their taller neighbors. *Cakile edentula*, in contrast, grows in an environment with abundant light but limited water. Interactions among neighbors are not likely to cause competition for light, which is saturating and reflected off sand. However, such interactions may alter water availability either through competition for water or through the conservation of water by neighbors shading the sand substrate. Interactions among neighbors is also very likely to influence whether plants

become buried by sand. Therefore, neighbor interactions in *C. edentula* may not necessarily lead to the depletion of a limiting resource, such as light for *I. capensis*, or resource depletion may be mediated by other effects of interactions with neighbors. That is, even though high density was associated with less reproduction by individuals, indicating competition, neighbor interactions apparently had effects on the performance of group members other than competitive depletion of resources. Depletion of limiting resources is often manifest as size hierarchies. *Cakile edentula* does not show evidence of size hierarchies in its high-density clumps. Therefore, in systems in which interactions within groups do not lead to depletion of a limiting resource, and those in which size hierarchies do not form, one could expect that individual and group selection on size characters would not be balanced in opposition and would not result in soft selection.

Of the three possibilities discussed previously whereby a balance of levels of selection might lead to constant yield, I found no evidence that individual selection precisely balanced group selection at all densities, nor that selection was completely relaxed, or even weaker, at low density. I did find that the relative importance of individual vs. group selection changed with density; only individual selection was detected at low density, but group selection was apparent at higher density. The results presented here suggest that the pattern of individual selection being important at low density and group selection being important at high density might facilitate constant yield in a general manner. On the other hand, the magnitude and direction of individual and group selection are critical for determining whether constant yield can result from a balance of group and individual selection. No general pattern emerged from this experiment, and in fact a surprising fluctuation in the direction of group selection was detected as density increased. Therefore, depending on how multilevel selection changes with density in different systems, differences in the direction and strength of selection at different levels may or may not lead to the phenomenon of constant yield.

The observation that strict soft selection was not operating at the spatial scale of groups indicates that the scale of density regulation is larger than the scale of interactions among group members. Therefore, phenotypic differences among groups, if genetically based, are likely to contribute to a response to group selection in this system (Kelly 1992, 1994). Because groups are likely to comprise full or half siblings (Donohue 1998), phenotypic differences among groups are likely to be genetically based to some degree. This system of localized dispersal and more global density regulation therefore suggests that the group selection observed here can contribute to evolutionary responses (Donohue 2003).

In conclusion, both individual and group selection varied with density in this natural population, in a man-

ner partially consistent with predictions based on the intensity of interactions among individuals. A general pattern was observed in which individual selection was important at low density and group selection was important only at higher density. The density-dependent changes in the relative importance of group vs. individual selection may have the potential to contribute to important population demographic phenomena such as soft selection and constant yield, but the direction of group selection was not consistent at higher densities, did not necessarily oppose individual selection, and therefore would not lead to constant yield or soft selection in a predictable manner. Thus, patterns of multilevel selection can be highly ecologically variable, since they depend strongly on fundamental ecological factors such as density. The effect of density on patterns of multilevel selection also depends on specific density-dependent interactions among group members. Such interactions can range from competitive depletion of a limiting resource to noncompetitive or facilitative interactions among group members. The apparently changing influence of neighbor phenotypes on reproduction, depending on the position of members within the group, indicates that small-scale spatial arrangements are particularly important influences on group selection. Such species-specific ecology can explain why some species exhibit constant yield while others do not. Further characterization of the ecological conditions that influence the relative contribution of different levels of selection must include explicit consideration of the spatial arrangements of interacting individuals and consideration of specific mechanisms of interactions. Such studies will certainly contribute to our understanding of how multilevel natural selection contributes to important population dynamics.

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