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Natural selection in *Potentilla glandulosa* revisited

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ABSTRACT

Clausen, Keck and Hiesey's studies of clonal transplants during the 1930s and 1940s are famous for demonstrating ecotypic variation. Less appreciated are their studies on natural selection, even though at the time there was little hard evidence of its operation in nature. Here we present new analyses of their study of natural selection in *Potentilla* (Clausen *et al.*, 1940; Clausen and Hiesey, 1958). Although they concluded from their data that natural selection was responsible for the phenotypic differences between altitudinal races of *Potentilla glandulosa*, many of their conclusions lacked statistical support. We present the results of analyses of data for 107 F_2 clones planted at each of three elevations, indicating significant support for their inference of natural selection in the wild, as well as revealing new findings. Survival at the high-altitude Timberline station was highest for those F_2 clones with multivariate phenotypes most similar to the local alpine subspecies and lowest for clones with phenotypes most similar to the mid-elevation parent. There is evidence of strong directional selection on characteristics of plant size, but targets and intensity of selection varied among stations. At Stanford, more and longer stems were favoured together with later flowering. At Mather, selection was mainly on rosette width. At Timberline, selection was strongest and had varied targets: directional selection favoured larger rosettes, more stems and later flowering, but there was strong correlational selection among these traits as well. We also present evidence for selection on phenotypic plasticity: stabilizing selection on plasticity of flowering time, as well as selection favouring decreased plasticity of stem length and stem number.

Keywords: adaptation, ecological genetics, local adaptation, natural selection, population differentiation, *Potentilla glandulosa*, reaction norms.

INTRODUCTION

Natural selection emerged as the main causal process of evolutionary change during the period in which the evolutionary synthesis was forged (Mayr and Provine, 1980). However, the enthusiasm for selection must have been driven largely by its theoretical appeal, because at

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the time of the synthesis there were few actual demonstrations of natural selection in the wild (Endler, 1986; Antonovics, 1987). This apparent dearth of evidence prompted Gould and Lewontin (1979) to deliver their famous polemic, warning of the dangers of the 'adaptationist program' and 'pan-selectionism' (Selzer, 1993; Pigliucci and Kaplan, 2000). Antonovics (1987) referred to this in reference to the legendary Unicorn – many claimed to observe it, but few had 'dissected' it.

The importance of natural selection and its role in adaptive evolution was cemented by the study of Kettlewell (1955) on *Biston betularia* (Cook, 2000), but this was a rare example of an investigation of selection in the wild. Although the explicit study of natural selection can be traced to the turn of the twentieth century (e.g. Weldon, 1898, 1901–2; Bumpus, 1899; DiCesnola, 1904, cited in Endler, 1986), the vast majority of studies demonstrating selection in the wild have been carried out since 1965 (see Endler, 1986; Núñez-Farfán, 1993; Kingsolver *et al.*, 2001).

To validate inferences regarding the existence and prevalence of natural selection, the study of variation in natural populations is necessary. Early studies of selection were performed almost exclusively on polymorphic traits controlled by a single locus (see Ford, 1964). There were significant difficulties in analysing polygenic traits, including the often substantial influence of the environment on such traits. The work by Jens Clausen's group, initiated in the 1930s, is notable for combining three avenues of investigation: employing surveys of natural variation, using experimental approaches, and applying Mendelian analyses of quantitative characters. The studies of Clausen *et al.* tackled these problems during the consolidation of the evolutionary synthesis, well before the recent resurgence of interest in the study of natural selection in the wild. Furthermore, they had access neither to the massive computing power nor to the statistical, methodological and experimental tools for analysing multiple, continuous traits (e.g. Lande and Arnold, 1983; Janzen and Stern, 1998; Scheiner *et al.*, 2000).

Although Clausen, Keck and Hiesey (henceforth CKH) are known largely for their spectacular demonstration of ecotypic differentiation and phenotypic plasticity in the plant species *Achillea* (Clausen *et al.*, 1948; see Lubchenco and Real, 1991), their research programme was far broader (Schlichting and Pigliucci, 1998; Núñez-Farfán and Schlichting, 2001). One of the specific goals was to demonstrate how adaptive variation in, and genetic structure among, wild populations of plants could be brought about by natural selection (Clausen *et al.*, 1938). Specifically, they attempted to show that population differentiation in *Potentilla glandulosa* was the result of selection. We review their original results, and present a set of new analyses of their data from their monumental *Experimental Studies on the Nature of Species*, Volumes I (Clausen *et al.*, 1940) and IV (Clausen and Hiesey, 1958), to examine this issue.

CLAUSEN, KECK AND HIESEY'S STUDIES ON *POTENTILLA GLANDULOSA*

Potentilla glandulosa (Rosaceae) is a perennial plant of the western USA. In California, its distribution is almost continuous, except for the San Joaquin Valley, from the coast to the Sierra Nevada (Fig. 1). Populations along this altitudinal transect vary substantially in quantitative characters, with some population groups being designated as subspecies (Clausen *et al.*, 1940). All populations, however, have the same chromosome number ($n = 7$) and there are no known reproductive barriers.

The first studies (Clausen *et al.*, 1940) found that clones of each subspecies produced very different phenotypes when grown at each of three experimental transplant gardens, Stanford, Mather and Timberline (S, M and T; Fig. 1). Their analyses (paired *t*-tests)

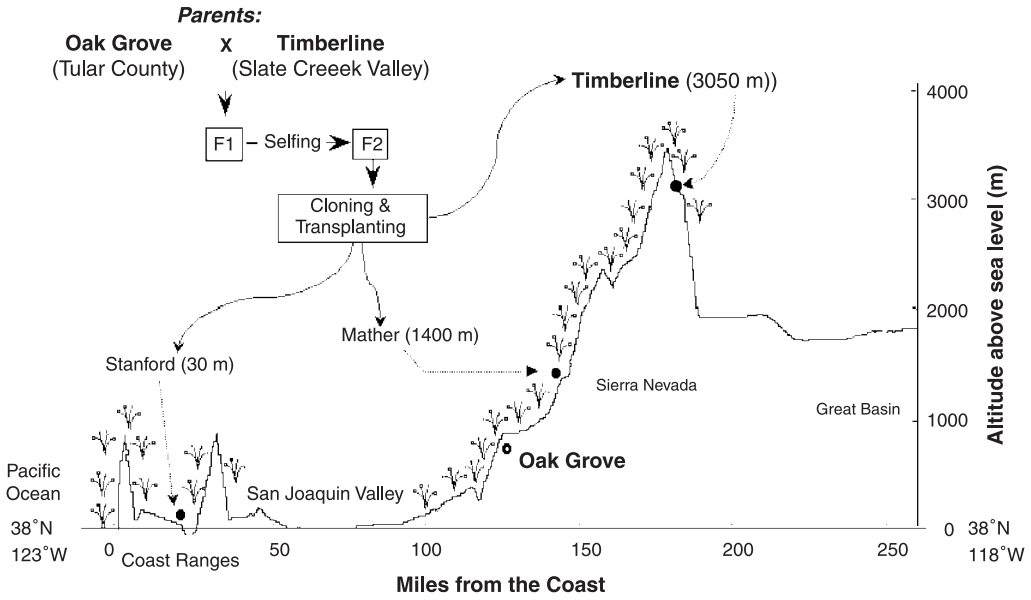

Fig. 1. Altitudinal transect from the coast in Central California up to the Sierra Nevada and Great Basin. The distribution of *Potentilla glandulosa* is indicated, as well as the three field stations, Stanford, Mather and Timberline. Clones of the F_2 progeny of a cross between Oak Grove and Timberline plants were transplanted (see text).

indicated significant phenotypic differences in height and flowering date within and between gardens (Fig. 2A). They also noted that at Timberline clones of *P. g. nevadensis* had the highest survival of all the subspecies, but at Stanford they had the lowest survival (Fig. 2B). Our analyses of data from tables 5–7 of Clausen *et al.* (1940) corroborate these transplant garden and subspecific differences for height (garden: $F = 114.27$, $P = 0.0001$; subspecies: $F = 5.91$, $P = 0.016$; race (subspecies): $F = 38.21$, $P = 0.0001$), and also differences in flowering time between stations (garden: $F = 46.39$, $P = 0.0001$). We also statistically confirm the visual pattern of taxon × environment interaction for height (Fig. 2A; garden × subspecies: $F = 6.24$, $P = 0.0023$) (see Supplemental Table A for details in online version).

The results of these transplant experiments led CKH to concentrate on the genetics of plant adaptation and natural selection (Clausen *et al.*, 1938, 1940). They produced several F_1 progenies among ecotypes, focusing further efforts on the offspring of a cross between ssp. *reflexa* from the foothills (Oak Grove, 760 m altitude) and the subalpine ssp. *nevadensis* from Timberline (Slate Creek Valley, 3050 m; Fig. 1). These subspecies differ in many morphological and physiological characters (e.g. size, leaf morphology, flower size, date of flowering, frost resistance and self-compatibility), and individuals from Oak Grove (*P. glandulosa* ssp. *reflexa*) had relatively poor survival at the Timberline Station (Fig. 2B). CKH took this as evidence that local adaptation was involved in the differentiation of these populations.

Oak Grove × Timberline F_1 plants were cloned and again planted at each of the three stations (Clausen *et al.*, 1938, 1940). In 1934, F_1 individuals were self-pollinated to produce a

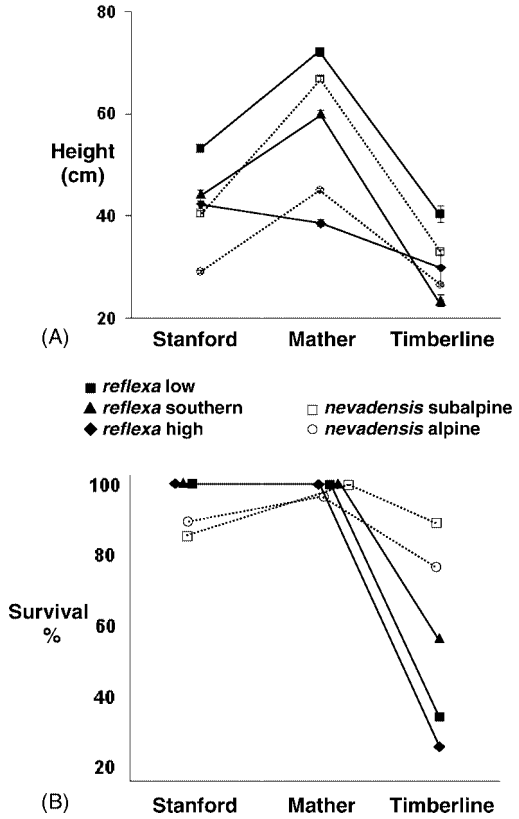

Fig. 2. (A) Reaction norms of plant height of races of *Potentilla glandulosa* ssp. *nevadensis* and *reflexa* (mean ± standard error). Individuals from different populations were cloned and transplanted to each of the three stations: Stanford, Mather and Timberline. Data extracted from tables 5–7 in Clausen *et al.* (1940). (B) Survival of the races of *Potentilla glandulosa* ssp. *reflexa* and *nevadensis* at the three experimental stations. Data calculated from tables 5–7 in Clausen *et al.* (1940).

variable F_2 population (cf. figures 19, 21 and 22 in Clausen and Hiesey, 1958). In total, 549 individuals from this F_2 population were cloned and transplanted at the three stations. (Note that the Stanford station lies outside the normal range of these subspecies.) Plant survival was monitored yearly (until 1943 for Stanford and until 1947 for Mather and Timberline stations). Twelve characters were recorded on each plant until 1943, and plant vigour was assessed visually.

Clausen and Hiesey (1958; henceforth C&H) used several classification methods to reduce the complexity of their data:

1. At each station, all F_2 genotypes were assigned to one of three vigour classes – low (L), medium (M) or high (H) – resulting in a total of 27 vigour classes across stations. Vigour was based on several growth-related traits – the lengths of stems and leaves, number of reproductive stems, and the width of the rosette – and was assessed independently for the clones at each station. Clones with low vigour at all three stations were placed in Group 1 (LLL), and those with high vigour at all three stations in Group 27 (HHH).

2. C&H summarized the phenotypic variability of the parents, the F_1 and each F_2 clone by constructing an index value (*IV*) combining 12 characters that distinguished the parental types: these included petal traits, branching, anthocyanin levels, and leaf and stem lengths. For each trait, the characteristic of the alpine parent was assigned a low value and that of the foothills parent a high value. Those F_2 clones with the lowest *IV* were phenotypically most similar to the alpine parent (*IV* = 19), and those with higher *IV* were more similar to the foothill parent (*IV* = 53). There was substantial variation among *IV* values: the range for 521 F_2 clones was from 20 to 48 (cf. figure 17 in Clausen and Hiesey, 1958). Although all F_2 clones had overall *IV*s between the parental values, some scores for individual traits exceeded the parental values.
3. Plant survival was monitored yearly (until 1943 for Stanford and until 1947 for Mather and Timberline stations). C&H identified each clone's 'survival pattern' based on whether plants survived the entire time or not at each site: *short* (S: 4 years or less at Stanford, 8 years or less at Mather or Timberline) or *extended* (E: 5 years at Stanford, 9 years at Mather or Timberline); this grouping produced eight categories ranging from SSS (short survival at all three stations: Stanford, Mather and Timberline, respectively) to EEE. The parental type from Oak Grove had survival pattern EES, that from Timberline had pattern SEE, and the F_1 had survival pattern EEE.

THEIR RESULTS

Survival

C&H grouped clones into three classes of index values (20–29, 30–39, 40–49), and the percentages of survivors of each group of plants at the three stations were compared. If clone survival were random with respect to phenotype (i.e. there was no selection), proportions of survivors in each *IV* class at each station should be the same as the overall proportions. Their data suggested that the clones with lower *IV*s (i.e. resembling the alpine parent) had the highest survival at the Timberline station; clones with intermediate and high *IV*s had low survival at Timberline. This relationship between survival and phenotype (Fig. 3A) constituted their strongest evidence of genetic change and local adaptation promoted by natural selection. We expand this analysis below.

C&H then examined the survival of the clones with respect to the 27 vigour classes. Although Group 1 (LLL) with low vigour at all sites had generally low survival, vigour patterns of many other groups showed no close relationship to their survival (e.g. Group 2, LLM, with low vigour at both Stanford and Mather, had relatively high survivorship at all stations). C&H also found that the rank order of survival of the different vigour groups changed dramatically across stations. A group's relative average survival at one location appeared almost independent of its survival at other locations. C&H interpreted these results as an indication of differential selection on the F_2 clones.

Finally, they compared the clones' 'survival patterns' to the parental and F_1 patterns. Over half of the F_2 belonged to the parental (Oak Grove – EES, 18%; Timberline – SEE, 10%) or F_1 categories (EEE – 27%). However, about one-third of clones fell into categories that transcended 'predictable' combinations of the parental types: ESS (12%), SSE (5%), SSS (11%) and ESE (5%).

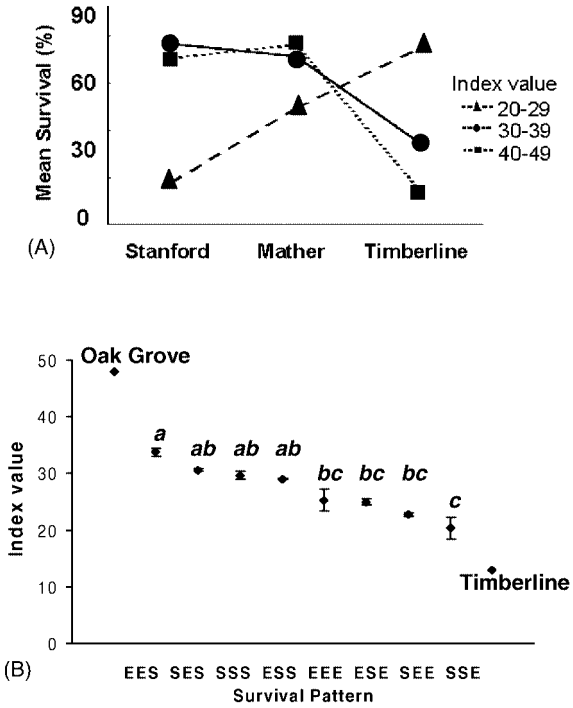

Fig. 3. (A) Mean survival of *Potentilla glandulosa* F_2 clones, grouped by index value (*IV*; see text), at Stanford, Mather and Timberline. Data from table 47 of Clausen and Hiesey (1958). (B) Index values for the eight survival classes (mean ± standard error); categories not sharing a letter are significantly different. S = short survival, E = extended survival; thus EES indicates extended survival at Stanford and Mather and short survival at Timberline.

Vigour and reaction norms

C&H addressed the issue of whether the response of a genotype in one environment was a predictor of its responses in the others. They used the 27 vigour categories discussed above: a plant with low vigour at Stanford had three vigour possibilities at Mather and three more at Timberline. Under the hypothesis that 'vigours' measured at each station were independent, they predicted that the 27 classes should be equally represented. The F_2 displayed a tremendous array of reaction norms, and a few genotypes (15 of 505) possessed high vigour at all three stations. However, C&H rejected the hypothesis of independence because only 24 of 27 possible classes were present (three combinations were absent: HLH, HLM and HLL), and because there were statistically significant correlations of performance at different stations (C&H, table 46). Overall, their analyses led C&H to conclude that the patterns of character relationships represented by the summary variables *IV* and vigour were the signature of selection against 'alien' phenotypes in the local environments.

NEW ANALYSES

Compared with modern methods of selection analysis, the analyses and results of C&H would be considered preliminary at best. Fortunately, their monograph (Clausen and Hiesey, 1958) contains a surprising amount of semi-raw data on the characteristics of 107 F_2 clonal genotypes resulting from the Oak Grove × Timberline cross. Their table 44 presents categorical data for each clone for 14 traits, as well as the vigour and survival at each station and the overall index value. These categories are strictly qualitative for some traits (e.g. petal traits and frost resistance), but for others the categories are based on actual quantitative measurements. To carry out robust statistical analyses, we back-transformed data for five traits (rosette width, flowering stem number, days to flowering, stem length and leaf length) to their class medians (see Supplemental Table B in the online version for details and an example). We calculated the plasticity of each trait as its coefficient of variation across the three stations.

Vigour at the three stations

We note that C&H's null hypothesis of equal distribution across the 27 classes was too simplistic. The actual null hypothesis is that, given a particular vigour at one station, vigour at the other two stations should be random. We analysed the contingency table (Clausen and Hiesey, 1958, figure 31) with PROC CATMOD (SAS, 1996) (Table 1). This analysis revealed that vigour at Timberline was generally independent of vigour at Mather and Stanford (Table 2). However, vigour at Stanford was not independent of that at Mather, and vigour at Mather was not independent of vigour at either of the other sites.

Survival pattern versus index value

We evaluated the relationship between the index value (*IV*) and survival pattern in two ways. Initially, however, because survival had been one of the original 12 traits used to calculate *IV*, we subtracted the mean survival from the total *IV* to obtain IV_{adj}. If the hypothesis that the local phenotype is favoured is true, we expect the clones with the lowest *IV*s to have extended survival at Timberline and, conversely, clones with the highest *IV*s should have extended survival at Mather. The clones with either short or extended survival at all stations should have intermediate *IV*; their success will depend on having the right combination of traits for wide adaptability.

Table 1. A contingency table of expression of joint vigour across the three transplant stations

	Stanford Low			Stanford Medium			Stanford High		
	Mather			Mather			Mather		
	L	M	H	L	M	H	L	M	H
Timberline Low	31	59	17	22	72	40	0	7	38
Timberline Medium	8	27	14	9	59	51	0	8	27
Timberline High	2	6	5	1	10	11	0	2	15

Table 2. Results of PROC CATMOD (SAS, 1996)

MODEL: 'Vigour at Mather' = S T S*T

Source	d.f.	σ^2	Pr > σ^2
Intercept	1	1564.33	<0.0001
S	2	65.98	<0.0001
T	2	8.32	0.016
S*T	4	8.10	0.088

MODEL: 'Vigour at Stanford' = M T M*T

Source	d.f.	σ^2	Pr > σ^2
Intercept	1	234.55	<0.0001
M	2	45.91	<0.0001
T	2	2.71	0.26
M*T	4	4.48	0.34

MODEL: 'Vigour at Timberline' = M S M*S

Source	d.f.	σ^2	Pr > σ^2
Intercept	1	99.03	<0.0001
M	2	3.13	0.21
S	2	4.34	0.11
M*S	4	2.81	0.59

Note: S, M and T refer to 'vigour at' Stanford, Mather and Timberline, respectively.

First, we analysed IV_{adj} with analysis of variance using C&H's survival classes. IV_{adj} was very strongly related to survival pattern, with significant differences apparent among survival classes (Fig. 3B). Groups with extended survival at Mather and short survival at Timberline (i.e. −ES) had higher IV_{adj}, whereas those with extended survival at Timberline (−−E) had lower IV_{adj} (<26). The group with the highest IV_{adj} (EES) is significantly larger (33.7) than all classes with extended survival at Timberline, and the IV_{adj} of the lowest group (SSE) is significantly smaller than that of all classes with short survival at Timberline (Fig. 3B). As expected, survival groups EEE and SSS have intermediate IV_{adj}.

Second, we regressed IV_{adj} on the actual number of years the 107 clones survived at each station. There were positive slopes but no significant regressions of survival on IV_{adj} at either Mather or Stanford. However, at Timberline, survival was strongly negatively related to IV_{adj}: survival = $-0.311\ IV_{adj} + 15.71$; $P < 0.0001$; $R^2 = 0.46$). Thus, on average, an additional year of survival was gained for each decrease in IV_{adj} of 3. These results further support the conclusion of C&H that *IV* incorporates some characters that contribute to or mirror local adaptation.

Selective deaths

We calculated the number of deaths due to selection (i.e. death above background mortality) by comparing the survival of each group (s_o) to the fractional survival of the whole population (S). Using the proportional survivorship at each station (Fig. 3A), the intensity of selection ($= \ln s_o - \ln S$) and selective mortalities ($= 1 - \{[1 - s_o]/[1 - S]\}$ (Haldane, 1954) estimate the additional mortality due to natural selection in each environment. Intensities of selection for Stanford, Mather and Timberline were 0.16, 0.08 and 0.69, respectively, corresponding to 33%, 20% and 60% selective mortalities. As Fig. 3A shows, the selective deaths at Stanford and Timberline stations came from different IV classes.

Regression analysis of selection

We estimated the mode and magnitude of natural selection for traits affecting survival using the trait values of the 107 F_2 clonal genotypes (from table 44 in Clausen and Hiesey, 1958). The number of years of survival was standardized for each group so that $\bar{w} = 1$, and traits were standardized to $\bar{x} = 0$, $\sigma^2 = 1$ to facilitate comparison of the selection gradients (in units of standard deviation). We regressed relative survival against the individual traits using a variety of models (SAS: PROC GLM, type III sums of squares). Directional (β') and stabilizing/disruptive and correlational (γ') selection gradients were estimated separately through the linear, quadratic and interaction regression terms, respectively (Lande and Arnold, 1983).

For the individual traits, the evidence for significant linear selection gradients varied across transplant sites (Table 3 abstracts the significant values; for full analyses, see Supplemental Table C in the online version). Rosette width was under positive selection at Mather and Timberline, and days to flowering and stem number at Stanford and Timberline (Fig. 4). Stem length was positively selected at Stanford (Fig. 4), but negatively selected at Timberline. Quadratic terms that were significant for simpler models were typically due to saturating effects (i.e. survival reached a maximum before the trait values), and became non-significant in more complex models. Days to flowering at Stanford was an exception, with a significant negative quadratic term in the most complex model (Fig. 5). Selection at Timberline involved more traits in all models, and there was significant correlated selection on traits as well. There was negative correlational selection of rosette width with both stem number (Fig. 5) and days to flowering, and synergism between days to flowering and stem length. The intensity of selection on stem number and stem length varied significantly across sites (analysis of covariance, trait × site interactions; Supplemental Table D in online version).

Selection favoured lower plasticity of both stem number and stem length (Table 4, Fig. 6). Stabilizing selection was found for plasticity of flowering time (Fig. 6) and plasticity of leaf length. Plasticity of rosette width and stem number were also correlated with IV_{adj} ($r = 0.524$ and 0.446, respectively), suggesting reduced plasticity of clones with Timberline-like morphology.

DISCUSSION

Knowing the characteristics that strongly differentiated the foothill and alpine populations of *Potentilla glandulosa*, Clausen *et al.* were hoping to observe a 'repetition' of the evolutionary process by exposing the variability among the F_2 genotypes to three distinct

Table 3. Summary of significant results from different multiple regression analyses of selection on morphological traits versus survival at three sites in Central California for F_2 clones of *Potentilla glandulosa*

	MODELS											
	Linear only			Linear + quadratic			Linear + trait interactions			All		
Selection at		β' or γ'	*P*		β' or γ'	*P*		β' or γ'	*P*		β' or γ'	*P*
Stanford	$R^2 = 0.222$			$R^2 = 0.302$			$R^2 = 0.311$			$R^2 = 0.381$		
	Ros	0.092	0.018	St#	0.083	0.051	St#	0.068	0.011	St#	0.083	0.062
	DF	0.043	0.091	DF	0.052	0.039	DF	0.045	0.01	DF	0.046	0.09
				StLn	0.085	0.021	StLn	0.067	0.08	StLn	0.100	0.012
				$StLn^2$	–0.035	0.056				DF^2	–0.068	0.023
				$St\#^2$	–0.042	0.07						
Mather	$R^2 = 0.317$			$R^2 = 0.519$			$R^2 = 0.540$			$R^2 = 0.545$		
	StLng	0.049	0.045	Ros	0.057	0.024	Ros	0.044	0.078	Ros	0.051	0.071
				Ros^2	–0.035	0.008	*Ros*StLn*	–0.060	0.078			
				$StLn^2$	–0.033	0.003						
Timberline	$R^2 = 0.43$			$R^2 = 0.489$			$R^2 = 0.543$			$R^2 = 0.561$		
	Ros	0.209	<0.0001	Ros	0.159	0.002	Ros	0.136	0.011	Ros	0.133	0.017
	St#	0.091	0.01	St#	0.182	0.0002	St#	0.216	<0.0001	St#	0.217	<0.0001
	DF	0.081	0.0045	DF	0.079	0.008	DF	0.083	0.028	DF	0.075	0.062
				StLn	–0.089	0.039	*StLn*	–0.077	0.086	*StLn*	–0.092	0.055
				$LfLn^2$	–0.045	0.083	*Ros*St#*	–0.178	0.0013	*Ros*St#*	–0.159	0.032
				$St\#^2$	–0.051	0.009	*Ros*DF*	–0.133	0.016	*Ros*DF*	–0.118	0.049
							DF*StLn	0.098	0.018	DF*StLn	0.078	0.11

Legend: DF = days to flower; Ros = rosette width; St# = stem number; StLn = stem length; LfLn = leaf length. *Italicized* traits have a negative selection gradient.

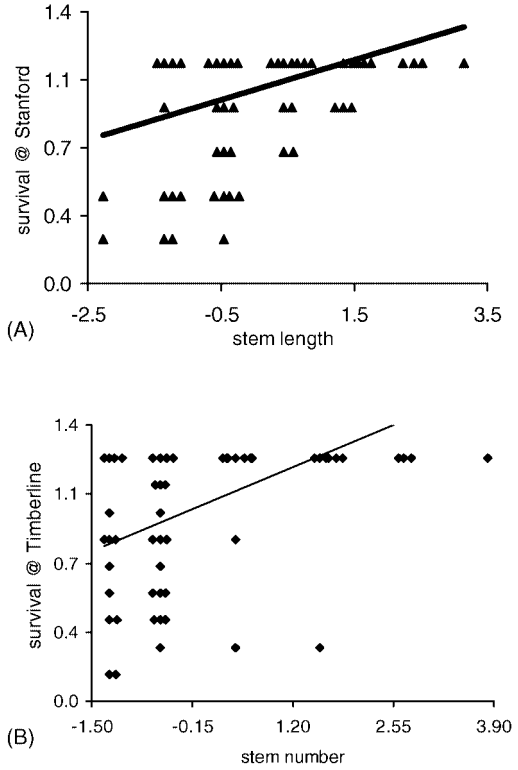

Fig. 4. Traits with patterns of directional selection: (A) stem length at Stanford, (B) stem number at Timberline.

environments. Their ability to detect the signature of natural selection was made difficult due to the large number of genes controlling the characters of interest, the strong plasticity of phenotypic expression across the altitudinal gradient, and the correlation among various traits. We have shown, however, that most of the claims of C&H are supported by detailed statistical analyses.

Each experimental population started with the same amount of genetic variability among the F_2 clones. Our results verify C&H's conclusion that the distinctive climatic differences among locations (e.g. mean temperature, water availability, day length, frost incidence) selected for those phenotypes characteristic of the 'home' populations. In addition, our new analyses show that the stations also differed in the strength and mode of natural selection on phenotypic variation: viability selection on size-related traits is stronger at Timberline, and selection affects more traits there as well. Furthermore, even though some clones were capable of surviving at all three stations, a large proportion of the deaths appear to be selective: genotypes with 'Timberline' phenotypes survived very poorly at Stanford, and those with 'Oak Grove' phenotypes survived poorly at Timberline (Fig. 3A). Thus the initial broad genetic variation of the F_2 had been reduced, with survivors at each station representing different fractions of the original variation. These results clearly support C&H's claims that population structure and local adaptation could be produced by natural

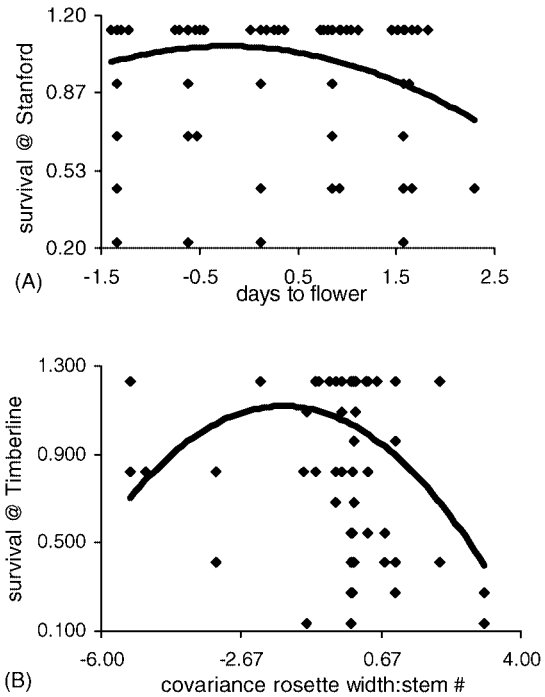

Fig. 5. Traits with patterns of stabilizing selection (A: days to flower at Stanford) or correlational selection (B: rosette width and stem number).

selection. Later studies using C&H's methodology (reciprocal plantings of F_2 offspring) have also detected local adaptation (e.g. Jordan, 1991; Nagy, 1997).

Some of our results require further consideration. For instance, we found that there was selection at both Stanford and Timberline on days to flowering, despite having no *a priori* reason to suspect that the onset of flowering *per se* should affect long-term survival. There may, however, be indirect selection on flowering time as a correlated response to selection on overall phenology. For example, there may be selection on the timing of initiation of the regrowth phase after winter dormancy at Timberline, which has by far the shortest 'window' for growth, with only 36 frost-free days on average, compared to 271 for Stanford and 149 for Mather. Another finding that might seem surprising is the general lack of strong selection at Mather, but this site had the highest overall survivorship and the smallest differences among *IV* groups (Fig. 3A).

We detected strong selection on the plastic responses of the clones across all three stations. The strong selection against plasticity of stem-related traits was not surprising, given the overall directional selection for larger values of these traits. More interesting are the results indicating selection for intermediate plasticity of flowering time and leaf length. We suspected that these patterns might be due to the inclusion of the data for the Stanford station, because it lies outside the natural range of the parental subspecies. However, the patterns remain even if only survival at Mather and Timberline are examined (data not presented).

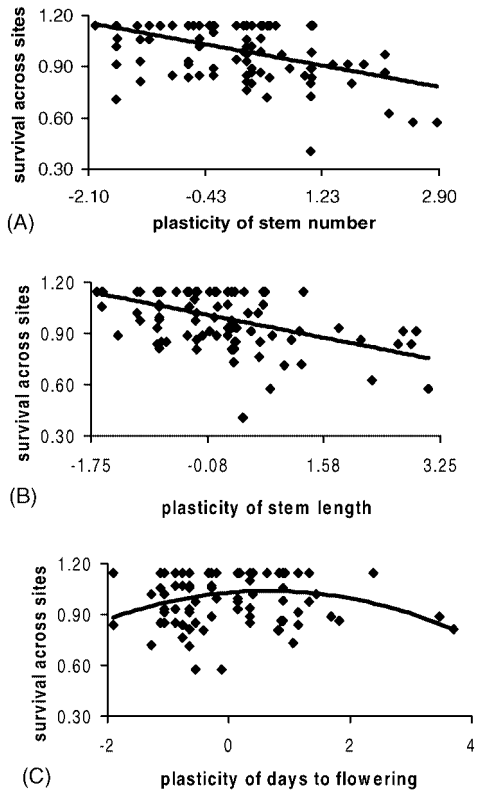

Fig. 6. Selection on phenotypic plasticity across sites: (A) plasticity of stem number, (B) stem length and (C) days to flower.

Table 4. Standardized linear (β') and non-linear (γ') gradients from multiple regression analysis of trait plasticities versus mean survival across three sites in Central California for F_2 clones of *Potentilla glandulosa* ($R^2 = 0.44$)

Plasticity of:	β' or γ'		P
Rosette width	–0.080		0.46
Rosette width^2		0.039	0.52
Leaf length	0.160		*0.052*
Leaf length^2		–0.111	**0.040**
Stem number	–0.343		**0.0004**
Stem number^2		–0.076	0.21
Stem length	–0.264		**0.008**
Stem length^2		–0.030	0.55
Days to flower	0.110		0.21
Days to flower^2		–0.092	**0.036**

Despite the fact that natural selection had been affirmed as the major evolutionary process during the 'synthesis', few studies of selection had been performed, particularly in plants (see Morley, 1959; Antonovics, 1987), and indeed few experimental methods were available (see Futuyma, 1988). The limited methods available for analysis led most researchers to avoid polygenic traits when looking for evidence of selection, but CKH were convinced of the importance of studying such traits (Núñez-Farfán and Schlichting, 2001).

The efforts of CKH to demonstrate the action of natural selection as the process responsible for adaptive physiological differences between climatic races of *Potentilla glandulosa* were ahead of their time. The approaches they took convincingly supported the view that population differentiation (and local adaptation) is the product of natural selection in *P. glandulosa*, and their inquiries into reaction norm evolution through the use of cloned genotypes were pioneering. However, despite the comprehensive nature of the research programme of CKH on plant evolution, recent surveys of natural selection have not mentioned the study of *P. glandulosa*. The results of Clausen and Hiesey (1958) constitute a keystone for the study of natural selection in the wild. This is a strong demonstration of local adaptation by natural selection, made especially significant by the timing and approach of the study. Clausen and co-workers were actively 'dissecting the unicorn' right as the modern 'synthesis' was coalescing (Antonovics, 1987; Futuyma, 1988; Núñez-Farfán and Schlichting, 2001).

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