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INFERENCES ABOUT QUANTITATIVE INHERITANCE BASED ON NATURAL POPULATION STRUCTURE IN THE YELLOW MONKEYFLOWER, *MIMULUS GUTTATUS*

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Abstract.—We used a nonmanipulative, marker-based method to study quantitative genetic inheritance in two habitats of a common monkeyflower population. The method involved regressing quantitative trait similarity on marker-estimated relatedness between individuals sampled in the field. We sampled 300 adult plants from each of two transects, one along a stream habitat and another through a meadow habitat. For each plant we measured 10 quantitative characters and assayed 10 polymorphic isozyme loci. In the meadow habitat, relatedness of plants within 1 m was moderate (r = 0.125, corresponding to half-sibs) as was actual variance of relatedness (V_r = 0.044). Significant heritabilities of 50–70% were found for corolla width and the fitness characters of flower number and plant weight. Genetic correlations were strongly positive, but sharing of environmental effects within 1 m was weak. In the stream habitat, levels of relatedness were lower and similar heritabilities were indicated. To detect dominance variance and the correlation of phenotypes due to shared inbreeding, we also estimated higher-order coefficients of relationship and inbreeding, but these did not significantly differ from zero. Laboratory-based estimates of heritability in the field were lower than the marker-based estimates, indicating that natural heritabilities and genetic correlations may be stronger than indicated by controlled studies.

Key words.—Genetic markers, heritability, *Mimulus*, quantitative genetics, relatedness.

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Knowledge of heritability under natural conditions is of two-fold significance. First, the genetic response to selection, or the change of mean phenotype between generations, is determined by the product of heritability h^2 with phenotypic selection intensity S (Falconer 1989). Accurate measures of heritability are thus a prerequisite to predictions of evolutionary response to selection. However, while the methodology for inferring phenotypic selection is well developed and applied (Lynch and Walsh 1996), field studies of heritabilities are generally limited to organisms that can be manipulated in some way (Riska et al. 1989). Second, theoretical models for the maintenance of polygenic variation make specific predictions about levels of heritability in natural populations (Barker and Thomas 1987). Models of polygenic selection (Turelli 1988) indicate an inverse relation between heritability and the equilibrium genetic variance, so that observed levels of heritability provide indirect evidence for the nature of selection upon the trait (Prout and Barker 1989).

A recurring problem in evolutionary biology is the relationship of parameter estimates derived from experimental manipulations to the actual values in undisturbed, natural situations (Riska et al. 1989). This applies especially to quantitative traits, whose expression depends on the environment. Although most workers have estimated heritability in the uniform environmental conditions of the laboratory, greenhouse, growth chamber or garden, several workers have used innovative methods to estimate heritability "in the field." Most notable are the cross-fostering studies in birds (e.g., Boag and Grant 1978; van Noordwijk et al. 1980; Dhondt 1982). More recently, workers on *Drosophila* have estimated heritability in the field by regressing lab-grown progeny on field grown parents (Coyne and Beecham 1987; Prout and Barker 1989; Riska et al. 1989). Plant workers have predominantly relied upon hand-planting sibships in the field (c.f. Mitchell-Olds 1986; Shaw 1986).

The advent of molecular markers has made possible genetic studies in many species, and not just those that could be crossed and raised under laboratory conditions (Avise 1994). Nevertheless, marker-based analyses of quantitative traits has been largely devoted to humans and domesticated animals and plants (Lander and Schork 1994). Less attention has been given to marker-based inferences about quantitative traits in nondomesticated species, particularly in their native habitats. Ritland (1996b) presented a marker-based method to estimate heritability and other quantitative genetic parameters "in the field." It is based on inferring levels of relatedness between individuals using genetic markers, and analyzing the covariation of this relatedness with phenotypic similarity for a quantitative trait.

In this paper, we use this marker-based method to estimate quantitative genetic parameters in two habitats of a common monkeyflower population. We also regress lab-grown progeny on field-grown parents to judge the difference between lab versus field of heritability. We address the following questions: (1) What is the magnitude of heritability in the field, does it differ between life history versus morphological characters, and do marker-based estimates differ from lab-based estimates? (2) What is the magnitude of genetic correlations between characters, and do fitness characters show negative correlations? (3) What is the magnitude of the environmental correlation between nearby plants? (4) Do dominance variance and shared inbreeding levels contribute to the correlation of phenotypes in the field? In performing this analysis, several types of coefficients of relationship are estimated at marker loci, some of which have never been estimated before, and which provide glimpses of new facets of population genetic structure.

A major limitation of this approach is the need for significant levels of both mean and variance of actual relatedness between individuals. Plants, with their sedentary habit and

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passive dispersal of seeds, and hence their tendency to form spatial clusters of relatives or show "isolation by distance" (Wright 1946), seem at first glance well suited for this joint analysis of relatedness and quantitative trait similarity. Individuals within a certain distance may show significant levels of both mean and variance of actual relatedness.

However, upon closer inspection, when restricting comparisons between individuals to those residing within a defined physical distance, there are two other factors (besides genetic similarity) that may contribute to the correlation of phenotypes. First, environments may be shared between proximate individuals, inflating the phenotypic correlation. This sharing may decrease with physical distance, confounding the decrease of genetic correlation with distance. Second, inbreeding may contribute to the correlation between relatives when inbreeding varies on a spatial scale. Both of these factors need to be taken into account during inferences about "heritability in the field" based on genetic markers. Furthermore, any proper interpretation of biological results warrants strict considerations of the assumptions and statistical behavior of estimation procedures (Mitchell-Olds and Bergelson 1990). It is with these cautions that we apply this procedure to the common monkeyflower.

Materials and Methods

Field Sampling

The common yellow monkeyflower, *Mimulus guttatus* (Scrophulariaceae), is a herbaceous annual or perennial plant that occurs in moist meadows and small streams throughout western North America. It has small seeds (ca. 10^{-5} g) dispersed by wind or water primarily (Waser et al. 1982). Initially, we sampled eight linear transects from five locations in Lake and Shasta Counties, CA. Each transect consisted of 300 adult plants genotyped for 8–12 polymorphic isozyme loci. However, only two transects showed significant variation of relatedness, a prerequisite for estimating heritability with genetic markers. Both were from a population near the northwest shore of Indian Valley Reservoir (Lake Co., CA). This population is predominantly outcrossing ($t = 0.7$) with slightly smaller flowers than typical for *M. guttatus,* and occurs on serpentine soil. The population is annual and plants produce 1–10 flowers and 20–200 seeds per flower.

In this population, plants were sampled along two linear transects, separated by ca. 20 m. The first transect was through a meadow and the second was along the banks and gravel of a small, vernal stream. Along each transect, 300 plants were chosen in groups of 2–4 neighboring plants, separated by random intervals encompassing a range of plant density and size. The distance between plants is plotted in Figure 1. Plants were marked with labeled tape wrapped around the stem, their distance along the transect recorded, and one corolla was removed from each plant. Five corolla characters were measured (see Ritland and Ritland [1989] for details): corolla width and length, calyx width and length, and stigma-anther separation. The corollas were then put into labeled 1.5 cc microcentrifuge tubes, placed on ice, and transported back to Toronto. Isozyme extracts were obtained using the procedures of Ritland and Ganders (1987), then frozen at −60° C until use.

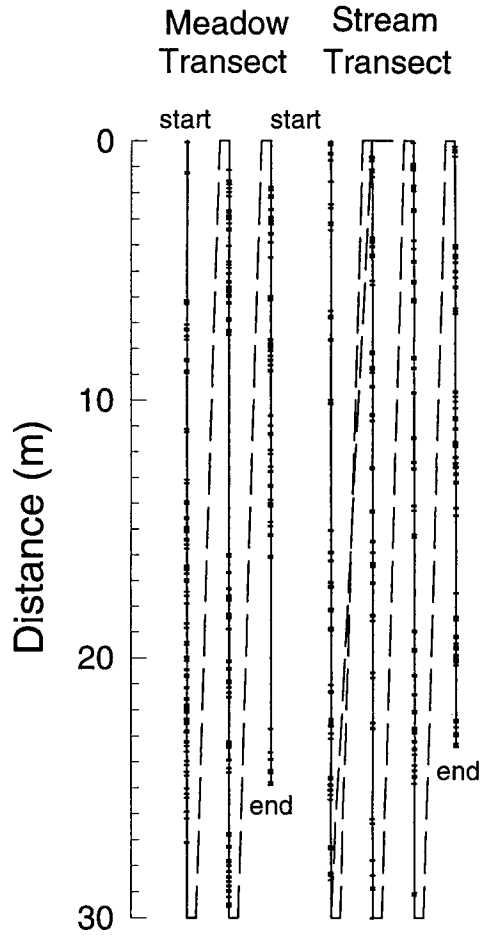

FIG. 1. The distribution of plants, indicated by hash marks, sampled along each transect. Dotted lines indicate continuation of the transect. The meadow transect was 85 m long and the stream transect was 114 m long.

Four weeks later, after senescence but before significant seed loss, the marked plants were harvested and dried. Four meristic and dry weight characters were measured on individual plants: flower number, plant weight, weight of all capsules (capsule weight), and the weight of seeds and the enclosing pod (pod weight). Capsule and pod weight were measured only on plants from the stream transect. The following polymorphic enzyme systems were assayed in two running buffers as described in Ritland and Ganders (1987): isocitrate dehydrogenase, triphosphoisomerase, 6-phosphoglucose dehydrogenase, phosphoglucomutase, phosphoglucoisomerase, aconotase, aspartate aminotransferase, diaphorase (2 loci) and esterase.

From the nine original measurements, ten quantitative characters were computed. These characters fall into four categories of mating system and life history characters: floral size, floral shape, fitness, and allocation to reproduction. They are defined as follows: (1) floral size: corolla width (CORWID), calyx width (CALWID) and stigma-anther separation (STASEP); (2) floral shape: the ratio of corolla length to corolla width (corolla shape, CORSHP) and the ratio of calyx length to calyx width (calyx shape, CALSHP); (3) fitness: flower number (FLRNUM), plant weight (PLTWGT), and weight of seeds and

the enclosing pod (PODWGT); (4) allocation: the ratio of capsule weight to plant weight (flower allocation, FLRALL) and the ratio of pod weight to capsule weight (pod allocation, PODALL). The fitness characters were log-transformed prior to analysis.

Marker-Based Estimation of Quantitative Inheritance

The method of Ritland (1996b) for inferring quantitative inheritance was applied to the joint measurements of quantitative traits, physical distance between plants, and isozyme genotype. This method is based upon first defining a similarity, Z, for quantitative traits between pairs of individuals as

$$Z_i = \frac{(Y_i - U)(Y_i' - U)}{V} \quad (1)$$

where Y_i is the trait value for the first individual, Y_i' the value for the second, and V the trait variance in the population. This is done for all pairings of individuals, then these similarities are regressed on measures of relatedness between members of the pairs, using a standard linear regression model with the qualification that "actual" variances and covariances of relatedness are used. For example, in the simplest case where only additive genetic variance and shared environment contributes to the Z_i, then $E[Z_i] = a_e + 2r_i h^2$, and the heritability is estimated as

$$\hat{h}^2 = \frac{C_{ZR}}{2V_r} \quad (2)$$

where C_{ZR} is the covariance between the Z_i and estimated relatedness, and V_r is the actual variance of two-gene relatedness among all pairs (as opposed to the variance of estimated relatedness). A similar approach is used to find genetic correlations.

The full model, which incorporates narrow and broad-sense heritability (h^2, H), sharing of environments that decline linearly with distance (d) as $a_e - d_i b_e$, and correlation of phenotypes due to shared levels of inbreeding b_f^2, is described by the expectation of Z for the ith pair of relatives,

$$E[Z_i] = a_e - d_i b_e + 2r_i h^2 + 2r_{2i}(H - h^2) + f_{2i} b_f^2 \quad (3)$$

(Ritland 1996b). In this model, three coefficients of relationship are involved: (1) Wright's (1969) two-gene coefficient of relationship r (the probability that two alleles, one sampled from each individual, are identical-by-descent [ibd]); (2) the four-gene coefficient of relationship r_2 (the probability that both genes of one individual are ibd to both genes of the other individual), and (3) the shared level of inbreeding $f_2 = (f - u_f)(f' - u_f)$, where f and f' are the estimated inbreeding coefficients of the two individuals compared, and u_f is the mean inbreeding coefficient. If any of these three coefficients are zero or are constant among pairs, or if the parameters they index can be assumed to be zero (e.g., $H = h^2$), then a reduced model excluding these coefficients is sufficient.

In our monkeyflower study, all three relatedness coefficients (r, r_2, and f_2) were estimated using the linear approach of Ritland (1996a). This procedure, compared to maximum likelihood, gives nearly unbiased estimates with small sample sizes (1–20 loci). We also estimated the actual variances of these coefficients, denoted respectively as V_r, V_{r_2}, and V_{f_2}, using the weighted ANOVA procedure given in Ritland (1996b).

Prior studies had indicated high enzyme polymorphism in this population. We found that over both transects, the average expected heterozygosity was 0.41, with many loci showing three alleles (gene frequencies available upon request). This ensured good power for marker-based estimates of heritability.

When relatedness declines with physical distance, it is optimal to restrict comparisons to within certain distances (Ritland 1996b). Thus, to maximize our power to detect heritability, we estimated these coefficients and their actual variances in each of several distance cutoffs: 0.5 m, 0.75 m, 1.0 m, 1.5 m and 2 m. We found 1.0 m to give the greatest variation of relatedness; thus the analysis of Ritland (1996b) was performed on our data for all pairs of plants within 1 m of each other. Estimates were obtained for each habitat. Standard errors were computed with the bootstrap method, where individual plants were the unit of resampling (pairs of identical plants in bootstrap datasets were excluded from analyses). A computer program implementing this procedure is available from KR.

Nonmarker Estimates of Heritability

Three progeny from every second plant in each transect were planted in 1.5-in pots with ProMix soil mixture, and grown to maturity in growth chambers with 16 h day/8 h night at 18°C/12°C. A total of 330 progeny from the meadow, and 305 progeny from the stream, were scored (incomplete germination and mortality resulted in less than 450 scored). The same characters were measured, excluding capsule and pod weight (these are pollinator dependent and therefore difficult to replicate).

Two methods were then used to estimate heritability in the field: (1) simple regression of lab-progeny on maternal field-parent ($2b_{op}$); and (2) the Riska estimator (Riska et al. 1989), which equals $4b_{op}^2(V_{PN}/V_{AL})$, where b_{op}^2 is the regression of lab-progeny on field-parent (maternal), V_{PN} is the phenotypic variance in the field, and V_{AL} is the additive genetic variance in the lab (obtained by within-family ANOVA of lab-raised plants; block effects due to shared trays were excluded). These two estimators have expected values $\gamma\sqrt{h_L^2 h_N^2}$, and γh_N^2, respectively, where h_N^2 is the heritability in the field, h_L^2 is the heritability in the lab, and γ is the additive genetic correlation between the trait in the field and in the lab. The Riska estimator is the closest to the heritability in the field, but is downwardly biased by any partial, positive genetic correlations.

RESULTS

The means and coefficients of variation for all quantitative characters in the field are given in Table 1. Meadow plants, compared to stream plants, showed greater stigma-anther separation, less narrow corollas and more flowers per plant. Plants from both habitats showed similar corolla size, calyx size, calyx shape and plant weight. Interestingly, the coefficients of variation were the same across habitats for all

TABLE 1. Means and coefficients of variation (cv) of character, for the meadow and stream habitats (SE in parentheses; see text for abbreviations).

Character	Meadow habitat		Stream habitat	
	Mean	cv	Mean	cv
CORWID (mm)	18.41 (0.18)	0.17 (0.01)	19.04 (0.21)	0.17 (0.01)
CALWID (mm)	5.66 (0.05)	0.18 (0.01)	5.69 (0.06)	0.18 (0.02)
STASEP (mm)	1.00 (0.07)	1.05 (0.08)	0.74 (0.04)	0.94 (0.06)
CORSHP	0.90 (0.01)	0.16 (0.01)	0.99 (0.01)	0.11 (0.01)
CALSHP	2.03 (0.02)	0.16 (0.01)	2.02 (0.02)	0.14 (0.02)
FLRNUM	3.43 (0.07)	0.38 (0.02)	2.08 (0.06)	0.41 (0.02)
PLTWGT (ln mg)	5.19 (0.06)	0.24 (0.01)	5.22 (0.07)	0.25 (0.01)
PODWGT (ln mg)	2.08 (0.04)	0.40 (0.02)	—	—
FLRALL	0.44 (0.02)	0.91 (0.17)	—	—
PODALL	0.47 (0.01)	0.22 (0.02)	—	—

characters except corolla shape, whose CV was greater for the meadow habitat.

Table 2 gives estimates of the marker-locus parameters required under the full model (eq. 1). The number of pairs considered was 1657 in the meadow and 1043 in the stream. Plots of the empirical distribution of bootstrap estimates indicate these estimates are normally distributed, so the standard errors in Table 2 give a good indication of confidence limits.

Mean levels of two-gene relatedness were quite high in the meadow habitat ($r = 0.125$, corresponding to half-sibs) but much lower in the stream habitat ($r = 0.052$, corresponding to first cousins). Mean levels of four-gene relatedness r_2 were lower, but significant. The mean shared level of inbreeding, f_2, did not significantly differ from zero, indicating no spatial variation in the level of inbreeding. This occurred in spite of the high average level of inbreeding ($f = 0.20$ and 0.12 in the meadow and stream, respectively).

The variance of two-gene relatedness V_r was higher for meadow plants than in stream plants (0.044 versus 0.027), and both were significantly greater than zero. However, the variance of four-gene relatedness V_{r_2} did not significantly differ from zero in either habitat. This was not due to decreased power to detect V_{r_2} as opposed to V_2, but rather to much lower values of the V_{r_2}, being one-fifth to one-tenth the value of V_r. The variance of shared inbreeding, V_{f_2} was essentially zero. This is expected because its mean level was also zero. Because V_{r_2} and V_{f_2} are probably zero, we cannot use the full model for estimating heritability (eq. 1), but instead a reduced model that excludes broad-sense heritability and shared levels of inbreeding.

TABLE 2. Estimates of coefficients of relationship and inbreeding and their actual variances, by habitat, for individuals within 1 m (SE given in parentheses; * = $P < 0.05$, ** = $P < 0.01$ as determined by percentiles of bootstrap distribution). Also given is mean and variance of distance between relatives, the inbreeding coefficient f.

	Meadow habitat		Stream habitat	
Coefficient				
r	0.125**	(0.018)	0.052**	(0.014)
r_2	0.044**	(0.013)	0.027*	(0.019)
f_2	0.000	(0.009)	0.010	(0.013)
d	0.464**	(0.014)	0.453**	(0.016)
f	0.201**	(0.022)	0.121**	(0.019)
Actual variances				
V_r	0.033**	(0.007)	0.012**	(0.005)
V_{r2}	0.0025	(0.0035)	0.0023	(0.0328)
V_{f2}	0.000	(0.004)	0.002	(0.006)
V_d	0.300**	(0.014)	0.299**	(0.017)

Estimates of heritabilities in the field, by habitat, are given in Table 3. These estimates are based on the reduced model incorporating just narrow-sense heritability and shared environments. Before we discuss estimates, we raise three points of statistical interest. First, the errors of these estimates are markedly skewed to the right. An example of error distribution is given in Figure 2, which gives the distribution of bootstrap estimates for heritability of corolla width. Because of this right skewness, asterisks are used in Table 3 to indicate values significantly greater than zero, as determined by the percentiles of the bootstrap distribution. Second, note that in Table 3, significant estimates of heritability were obtained only for those characters showing significant phenotypic correlations (rightmost column). This is expected, since it is probably impossible for the shared environmental correlation to be negative. Third, while almost all estimates of heritabilities were positive, only four estimates were significantly positive, and three of these four were for the meadow habitat. The low levels of relatedness and its variance in the stream habitat is the cause of this nonsignificance.

In the meadow habitat, significant heritabilities were found for corolla width, and for two of three fitness characters (flower number and plant weight). These characters showed heritabilities of 50–70%. In the stream habitat, only calyx width showed significant heritability, although the same characters that were significant in the meadow habitat showed near significance ($P < 0.1$) in the stream habitat. The inferred slope of the environmental correlation, b_e, was usually negative, but not significantly negative (Table 3). The intercept of the environmental correlation, a_e, while generally positive as expected, was significant only for pod weight in the meadow habitat. It was also near significant ($P < 0.1$) for corolla shape and flower number in the meadow habitat. These results suggest that environmental patchiness is rather weak, at least at the scale of 1 m, and that a further reduced model may be appropriate.

In Table 4, we present estimates of heritabilities under these further reduced models. In the model incorporating just h^2

TABLE 3. Estimates of heritabilities h^2 in the field, and the slope b_e and intercept a_e of the environmental correlation in the field, based upon the genetic marker method (SE given in parentheses; * = $P < 0.10$, ** = $P < 0.05$, *** = $P < 0.01$ as determined by percentiles of bootstrap distribution). Also given is the total phenotypic correlation r_p.

Character	h^2	b_e	a_e	r_p
Meadow habitat				
CORWID	0.73*** (0.62)	−0.23 (0.16)	0.00 (0.18)	0.08** (0.05)
CALWID	0.53* (0.53)	−0.18 (0.16)	0.18 (0.15)	0.23*** (0.06)
STASEP	0.22 (0.47)	0.12 (0.16)	−0.06 (0.12)	0.05 (0.05)
CORSHP	0.32 (0.52)	−0.09 (0.14)	0.25* (0.16)	0.29*** (0.07)
CALSHP	0.65 (0.74)	−0.22 (0.18)	0.12 (0.20)	0.18*** (0.07)
FLRNUM	0.78** (0.61)	−0.30 (0.20)	0.23* (0.16)	0.28*** (0.06)
PLTWGT	0.63** (0.54)	−0.18 (0.16)	0.17 (0.15)	0.24*** (0.06)
PODWG	0.39 (0.48)	−0.11 (0.15)	0.24** (0.14)	0.28*** (0.06)
FLRALL	−0.09 (0.22)	−0.02 (0.07)	0.00 (0.07)	−0.03 (0.04)
PODALL	0.41 (0.56)	0.05 (0.16)	−0.11 (0.17)	0.02 (0.05)
Stream habitat				
CORWID	1.51* (3.22)	−0.06 (0.42)	0.03 (0.45)	0.16*** (0.07)
CALWID	1.93** (3.20)	−0.08 (0.41)	0.03 (0.51)	0.19*** (0.09)
STASEP	0.13 (1.21)	0.01 (0.19)	0.00 (0.18)	0.01 (0.06)
CORSHP	0.09 (1.67)	−0.04 (0.26)	0.10 (0.27)	0.09* (0.06)
CALSHP	0.45 (1.38)	−0.08 (0.21)	0.01 (0.21)	0.02 (0.05)
FLRNUM	2.04* (3.27)	−0.29 (0.45)	0.08 (0.47)	0.16*** (0.08)
PLTWGT	1.34* (2.43)	−0.27 (0.34)	0.13 (0.36)	0.15*** (0.06)

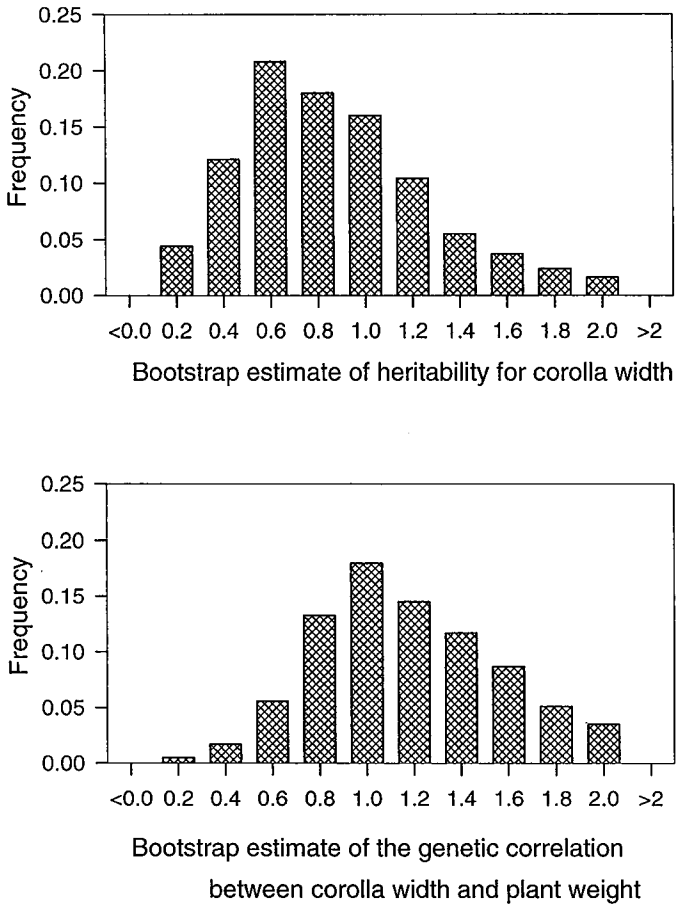

FIG. 2. The distribution of bootstrap estimates for heritability in the meadow habitat of corolla width and for the genetic correlation between corolla width and plant weight, for the meadow habitat. Both distributions are skewed to the right.

and r_e, estimates of h^2 were practically identical to those of Table 3, and almost the same pattern of statistical significance was found, the difference being that corolla width instead of calyx width was significant in the stream habitat. However, the estimates of r_e were not the same as those of a_e in Table 3. This is expected, as r_e is the average environmental correlation between individuals regardless of distance, whereas a_e is the environmental correlation at near-zero distance. One expects r_e to be less than a_e, and indeed this is found. Consequently, the estimates of r_e tended to show less statistical significance, even though their standard errors were less than those for a_e.

TABLE 4. Marker-based estimates of heritabilities in the field under simpler models: (a) heritability and environmental correlation only, and (b) heritability only (SE given in parentheses; * = $P < 0.10$, ** = $P < 0.05$, *** = $P < 0.01$ as determined by percentiles of bootstrap distribution).

Character	(a) h^2	r_e	(b) h^2 only
Meadow habitat			
CORWID	0.73*** (0.57)	−0.10 (0.15)	0.53*** (0.24)
CALWID	0.53* (0.54)	0.10 (0.14)	0.71** (0.28)
STASEP	0.22 (0.48)	−0.01 (0.13)	0.20 (0.24)
CORSHP	0.32 (0.51)	0.21* (0.14)	0.73*** (0.30)
CALSHP	0.65 (0.70)	0.01 (0.16)	0.68** (0.37)
FLRNUM	0.78** (0.59)	0.09 (0.14)	0.95*** (0.33)
PLTWGT	0.63** (0.49)	0.08 (0.12)	0.79*** (0.28)
PODWG	0.39 (0.46)	0.19* (0.11)	0.74*** (0.29)
FLRALL	−0.09 (0.20)	−0.01 (0.04)	−0.11 (0.14)
PODALL	0.41 (0.52)	−0.08 (0.13)	0.25 (0.24)
Stream habitat			
CORWID	1.53** (2.16)	0.00 (0.24)	1.53** (2.18)
CALWID	1.95* (2.75)	−0.01 (0.32)	1.92*** (2.11)
STASEP	0.13 (1.01)	0.00 (0.12)	0.13 (0.65)
CORSHP	0.10 (1.20)	0.08 (0.14)	0.28 (1.04)
CALSHP	0.47 (1.32)	−0.03 (0.14)	0.41 (0.90)
FLRNUM	2.10* (3.07)	−0.06 (0.34)	1.97** (2.63)
PLTWGT	1.40* (2.15)	0.00 (0.24)	1.41** (1.74)

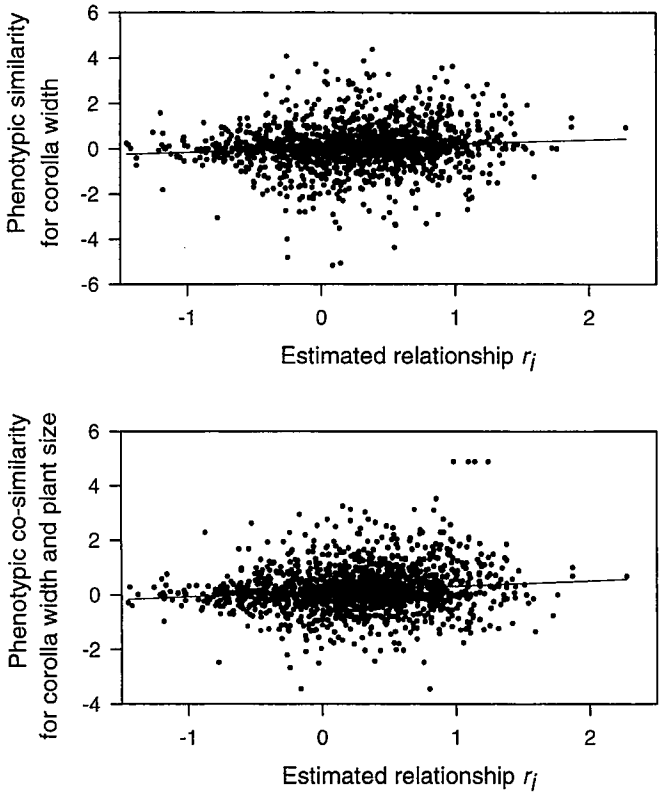

FIG. 3. A graphical portrayal of how heritability is related to the slope of the regression of character similarity on relationship, and how the genetic correlation is likewise related (fitted regressions are indicated by the lines). If relationship is known, the heritability equals twice the slope of the regression. In our case, the slope is less because relationship is inferred with some error, which tends to flatten the slope.

Table 4 also gives estimates under a model incorporating only h^2. Under this assumption, heritabilities were ca. 20% higher than those given in Table 3, and their standard errors were substantially reduced as well. This resulted in a large number of significant heritability estimates. Only the allocation characters and stigma-anther separation consistently showed nonsignificant heritability in both habitats.

Figure 3 graphically portrays the nature of the data, and how heritability is related to the slope of the regression of character similarity on relatedness. It also shows the relationship for the genetic correlation. A large scatter in the estimates of relatedness is evident, with many negative values (truncating these to zero would cause bias of this relationship between relatedness and similarity). A large scatter in the phenotypic similarities is also evident, but due to the large number of points, a relationship is evidenced by the positive slope of regression. If relationship is known, the heritability equals twice the slope of the regression. In our case, the slope is less because relationship is inferred with some error, which tends to flatten the slope.

The estimates of heritabilities in the field using nonmarker methods are given in Table 5. With regression of lab-progeny on field-parents, significant heritabilities were found for all floral characters in at least one habitat. Their values were substantially less than those estimated with the marker method, in line with the expectation that this method underestimates heritability in the field. The Riska method suffered from larger errors, due to the requirement of estimating additive genetic variation in the lab. Only stigma-anther separation and corolla shape were significant for the stream habitat. Generally, the Riska estimates are smaller than the marker-based estimates. The results of both methods suggest that heritabilities in the stream habitat are greater than in the meadow habitat.

Table 6 gives estimates of phenotypic correlations (above diagonal) and genetic correlations (below diagonal) for plants in the meadow habitat. Generally, the genetic correlations were large and positive (the exception involving a shape character, whose sign is arbitrary). As in Table 3, the estimates show positive skewness (Fig. 2), so the percentiles of the bootstrap distribution were used to assign significance. Phenotypic correlations were also generally positive, but not as large. Although there was no case of a significant phenotypic correlation being of opposite sign from a significant genetic correlation, greater values of genetic correlations suggest that environmental correlations are negative.

DISCUSSION

This is the first study to use inferred relatedness in natural populations to study quantitative inheritance in the field with nonmanipulative techniques. This is also the first attempt to characterize genetic correlations as well as environmental effects using inferred relatedness. In this process, several facets of population structure have been estimated for the first time. Overall, the results suggest that heritabilities in the field estimated by this nonmanipulative marker method are higher than those estimated by other methods. Marker-based estimates of heritability were usually greater than lab-

TABLE 5. Estimates of heritabilities in the field using two other methods: (1) regression of lab-grown progeny on field collected parents, and (2) the Riska method (SE given in parentheses; * $P < 0.05$ based on bootstrap percentile).

	Meadow habitat		Stream habitat	
	Regression	Riska method	Regression	Riska method
CORWID	0.34* (0.16)	0.16 (3.64)	0.42* (0.15)	0.37 (2.65)
CALWID	0.10 (0.17)	0.01 (0.11)	0.36* (0.13)	0.31 (1.83)
STASEP	0.12 (0.18)	0.02 (0.05)	0.42* (0.15)	0.16* (0.12)
CORSHP	0.20* (0.09)	−0.26 (2.62)	0.36* (0.20)	0.12* (0.16)
CALSHP	0.23* (0.15)	−0.54 (1.09)	0.00 (0.17)	0.00 (0.81)
FLRNUM	0.05 (0.10)	−0.02 (2.22)	−0.06 (0.12)	−0.03 (0.43)
PLTWGT	0.04 (0.11)	0.02 (0.79)	−0.04 (0.10)	−0.01 (0.45)

TABLE 6. Matrix of phenotypic (above diagonal) and genetic (below diagonal) correlations in the meadow habitat (* $P < 0.05$). Genetic correlations were inferred with the marker method assuming the presence of spatial environmental correlations.

	CORWID	CALWID	STASEP	CORSHP	CALSHP	FLRNUM	PLTWGT	PODWG	FLRALL	PODALL
CORWID		0.60*	−0.03	−0.51*	−0.03	0.57*	0.37*	0.48*	−0.01	0.19*
CALWID	1.34*		0.16*	−0.37*	−0.44*	0.59*	0.42*	0.52*	−0.04	0.18
STASEP	0.52	−0.17		−0.17*	−0.13*	0.17*	0.17*	0.22*	−0.05	0.18
CORSHP	−1.91*	−1.54	−0.27		0.30*	−0.22*	−0.21*	−0.18*	0.04	−0.21*
CALSHP	−0.44	−0.29	−0.23	1.20*		0.03	0.01	0.01	0.01	−0.13
FLRNUM	0.83*	0.84*	0.31	−0.45	−0.32		0.73*	0.85*	−0.09	0.22
PLTWGT	1.13*	0.96*	0.14	−0.56	−0.18	1.30*		0.79*	−0.04	0.11
PODWG	1.85*	1.59*	0.36	−0.87	−0.55	2.10*	1.45*		−0.03	0.36*
FLRALL	0.62	0.13	1.05	−1.20	0.48	2.96	1.47	1.05		−0.42*
PODALL	0.09	0.18	0.25	−0.10	−0.87	0.25	−0.05	0.19	−0.40	

oratory-based estimates of heritability (Tables 3–4 versus Table 5).

However, the potential biases of this method must always be kept in mind. In addition, given the relatively high level of isozyme polymorphism found in the population of *Mimulus guttatus* we studied, the power to detect heritability with this method is somewhat disappointing. Thus, while this study suggests that heritabilities may be higher than previously thought, the major significance of our study is to open the possibility of using markers in other studies, using the insights we have gained in this first attempt at measuring heritability with an entirely nonmanipulative technique.

Plants, with their sedentary nature and passive dispersal of seeds, may be more appropriate than other organisms for this marker-based approach for estimating heritability and environmental effects. This method may be particularly suited for long-lived plants such as conifers, as well as for other short-lived plants that are difficult to culture because of unusual germination, soil, or other requirements. However, until the patterns of relatedness are known in more species, particularly animals, one cannot ascertain the generally applicability of our approach.

Levels of "Natural" Heritability

The goal of our study was to measure heritability in undisturbed, natural populations. The major result is rather large estimates of heritability, larger than those found in other studies of natural populations (see Mousseau and Roff 1987). Of particular note for comparison are the results of Carr and Fenster (1994), who found heritabilities for corolla width and length in greenhouse-raised *Mimulus guttatus* of 30–40%, which match closely our laboratory estimates, but are less than our marker-based estimates, of heritability in the field for these characters.

Our results also do not support the hypothesis that traits closely associated with fitness will possess lower heritabilities (Mousseau and Roff 1987). It was expected that life history characters would show less heritability than floral characters because of their closer ties to fitness (Falconer 1989). However, one cannot exclude the possibility that floral characters may also be subject to relatively strong selection (Fenster and Ritland 1994), such that equilibrium heritabilities do not differ between the classes of characters. In general, the high levels of heritability are puzzling, although a variety of mechanisms can maintain significant heritable variation for fitness characters (Barton and Turelli 1989).

Our estimates may also be upwardly biased. Estimates of the higher-order coefficients of relationship (r_2, f_2) and their actual variances (V_{r_2}, V_{f_2}) generally did not significantly differ from zero (Table 2). Only r_2 in the meadow habitat was significantly positive. These nonsignificant estimates do not rule out the possibility of significant variation of these modes of relationship. Such variation would have the effect of inflating character similarity and, if not incorporated in the procedure, would upwardly bias our estimates of heritability and genetic correlations. A companion paper (Ritland 1996b) discusses other statistical issues, including sampling design, the use of maximum likelihood, the use of the bootstrap, the correlation of observations, and general statistical properties of the marker-based method.

In our study, heritability estimates that were based upon regressing laboratory-reared offspring on wild-collected parents were substantially lower than the marker-based estimates. This was particularly dramatic for the two fitness characters, number of flowers per plant, and plant size. Using a similar method, Coyne and Beecham (1987) found substantial "natural" heritabilities in *Drosophila* for two morphological characters, but one character (wing length) showed significantly lower heritability in the field than in the laboratory. An earlier study (Prout 1958) also found lower heritability for a character (wing length) in the field compared to the laboratory.

However, as pointed out by Lande (appendix of Coyne and Beecham 1987), the regression of laboratory-reared offspring on wild-caught parents depends on the genetic correlation of the trait across the two environments. Genotype-by-environment interaction tends to cause the cross-environment offspring-midparent regression to underestimate the heritability in the field. Alternatively, if the norms of reaction are fanlike and do not cross, the additive genetic variance can be larger in the laboratory, resulting in an overestimation of heritability in the field. Lande concluded that Coyne and Beecham (1987) underestimated heritability of wing-length by an unknown amount, while for bristle counts, the estimated "natural" heritability may be accurate. Thus, in our own study, the lower heritabilities found by these nonmarker methods is likely due to genotype-by-environment interaction, where the norms of reactions cross between wild-grown parents and lab-grown progeny.

No evidence for negative genetic correlations between fitness characters was found. Instead, characters showed all positive genetic correlations, probably indicating the presence of pleiotropic genes that generally determine size and fitness. We found genetic correlations to be greater than phenotypic correlations. Likewise, Arnold (1981) found genetic correlations in snakes for chemoreceptive traits to be larger than phenotypic correlations, but precise comparisons were hampered by large sampling errors. Also, the positive skewness of the sampling distribution (Fig. 2) indicates that a positive bias contributes to the large values detected in this study. Finally, we expected heritability to be greater in the meadow, because it appeared to be a less disturbed habitat. However, the opposite was evident, especially for the nonmarker methods: heritability in the stream habitat was greater than for the meadow habitat.

Comparative Population Structures

This study is the first to estimate the four-gene coefficient of relationship, as well as actual variances of relationship, in field-collected material with genetic markers. While the two-gene coefficient of relationship and the inbreeding coefficient are routine descriptors of population structure, these other parameters provide a richer description of the total population structure. In line with our expectations, the two-gene coefficient of relatedness was much higher in the meadow habitat than the stream habitat (0.125 vs. 0.052), as was the four-gene coefficient (0.044 vs. 0.027). This association of relatedness with habitat is almost certainly due to differences in water-mediated seed dispersal. The actual variances of relatedness showed corresponding differences, and in addition, the average inbreeding coefficient was also significantly higher in the meadow versus the stream habitat (0.201 versus 0.121). However, relatives did not share inbreeding coefficients in either habitat, indicating a homogenous inbreeding structure.

Probably the most important new parameter of population structure we have estimated is the actual variance of the two-gene relationship, V_r. This parameter is important because it heavily influences the estimation variance of heritability, and because it measures the heterogeneity of relatedness in the population. Studies are needed in other species on the extent of variance of relatedness, and its dependence upon distance.

Inferences about the biparental inbreeding can also be made from our measures of relatedness. Twice the two-gene coefficient of relatedness equals the selfing rate that one expects to measure with genetic markers, or the "effective selfing rate" (Ritland 1984). Thus one expects that pollen uniformly dispersed within 1 m would cause apparent selfing of 25% in the meadow habitat and 10% in the stream habitat. The relationships of these factors to physical distance are treated in another paper (Ritland et al., unpubl.).

Future Prospects

While providing insights into the pattern of quantitative genetic variation in a natural population, this study has also illustrated the statistical problems with our marker-based approach. Future studies will benefit from three considerations. First, one should screen species or populations for significant micropopulation structure (relatedness between neighbors). Second, more informative classes of marker loci, showing greater polymorphism (e.g., microsatellites) would be useful. Third, the importance of a proper sampling strategy cannot be understated. If relatedness declines with distance, one must sample adequate numbers of individuals within a close distance. All of these actions will decrease error of estimation, and reveal in greater detail the patterns of quantitative genetic variation in natural populations.

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