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Tests for major genes affecting quantitative traits in wild radish, *Raphanus raphanistrum*

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Abstract

The number of gene loci coding for quantitative traits is an important issue in genetics. However, there are still very few empirical data on this point, especially in natural populations. I tested for major gene effects on ten quantitative traits in wild radish, using an indirect method based on the patterns of family means and within and between family variances for traits. This method should reveal whether a single locus is responsible for most of the variation in a trait. Eight of the traits measured were morphological dimensions of leaves and flowers; no strong evidence for major gene effects on these traits was found. In contrast, evidence for major gene effects was found in the other two traits, emergence time and flowering time.

Introduction

A question of current interest in evolutionary genetics is the number of gene loci affecting a given trait (Lande, 1981; Gottlieb, 1984; Coyne & Lande, 1985; Barton & Turelli, 1989; Macnair, 1991; Orr & Coyne, 1992). While classical evolutionary and ecological genetic studies were confined largely to traits with discontinuous phenotypes (see Ford, 1975, for examples), most traits are quantitative, that is, continuously distributed. These quantitative traits are generally assumed to be polygenic (Lande, 1981; Gottlieb, 1984; Falconer, 1989), but there are few convincing studies supporting this assumption (Orr & Coyne, 1992). Since the evolution of a trait can be strongly affected by the number of loci coding for the trait (Lande, 1981; Mitchell-Olds & Rutledge, 1986), it is of interest to test this assumption of polygenic inheritance. While molecular genetic linkage maps are becoming fine-scaled enough in a few intensively studied species to directly estimate the number of loci coding for a trait (e.g. Paterson *et al.*, 1988), the amount of cost and effort required to construct such maps means that indirect methods are still useful for most organisms.

Most previous studies have attempted to determine the number of gene loci responsible for differences between closely related species or divergent lines. These include studies using indirect methods such as segregation analysis of progeny resulting from crosses (the 'biometrical approach', Lande, 1981; Gottlieb, 1984; Orr & Coyne, 1992) and direct molecular mapping studies (e.g. Paterson *et al.*, 1988). This information is useful in understanding the genetic mechanisms underlying past evolution and speciation events. However, these studies do not address the question of whether major genes are responsible for genetic variation in a trait *within* populations (Barton & Turelli, 1989); it is difficult or impossible to use methods that rely on crossing to answer this question. Understanding the genetic basis for variation within populations is crucial for predicting the evolution of a trait in the future.

In this paper I apply a different indirect method (Fain, 1978; Mitchell-Olds & Bergelson, 1990) to determine if a single locus is responsible for most of the genetic variance of ten traits within a single natural population of wild radish plants. This method is based only on the patterns of within- and between-family variances and family means for traits; therefore, it can be used to study the genetic

mechanism of within-population variation in most studies that have full-sibling families. On the other hand, this method only determines whether or not a single major locus is responsible for most of the variability in the trait, and does not provide an estimate of the number of genes segregating for a trait as do linkage studies and the biometrical methods. Nevertheless, given the paucity of information we have on the genetic basis of within-population variation and the difficulty of obtaining this information for natural populations, information gained this way is important in determining how common major genes are across a variety of species and traits.

The conceptual basis for this method is as follows. If a single major locus with two alleles is affecting a given trait, then full-sib families with high or low mean values for the trait should result from matings between parents homozygous for the same allele at the major locus. The offspring of these matings all would be homozygous for the allele, and these families would have low phenotypic variance for the trait. Conversely, some families with intermediate mean values result from matings in which one or both of the parents were heterozygous. In this case, the siblings would be genetically variable so the family would have higher phenotypic variance for the trait. (Assuming no dominance, the other intermediate mean families would result from the mating of alternate homozygotes; the offspring in these families would be all heterozygous and thus not variable.) Therefore, the highest within-family variances should be found in those families with intermediate mean values for the trait, assuming that neither allele is rare.

In the case where one allele is rare, the highest within-family variances would be in families with mean values at one extreme of the phenotypic distribution (Fain, 1978). This occurs because matings between two individuals homozygous for the rare allele are extremely uncommon, so families consisting of only this homozygote (and thus having low variance and extreme mean) are essentially absent.

Under the assumption of polygenic inheritance, that is, traits that are affected by many loci each of small effect, no differences in variances among families are expected because the loci segregate independently.

Materials and methods

Wild radish, *Raphanus raphanistrum*, is a hermaphroditic annual weed naturalized in North America. To generate the families for this study, 350 plants were grown in the greenhouse from field-collected seeds; these plants constituted the parental generation. Fifty of these plants were randomly selected to be sires (pollen donors), and each sire was used to pollinate six different randomly selected dams. This created 300 full-sibling families. Four offspring were planted from each full-sib family for a total of 1200 offspring planted; 67 of these did not germinate. Eleven of the full-sib families produced less than three offspring; these were eliminated from these analyses because within-family variances could not be estimated accurately. Therefore, the final sample size was 1125 offspring in 289 full-sib families. Ten traits were measured on each offspring: emergence time in days, time from emergence to first flower in days, length and width of one of the first pair of true leaves, length and width of the distal, showy part of one of the flower petals (the 'claw'), the length of the inner part of the petal (the 'limb'), the lengths of one short and one long filament, and the length of the entire pistil. (For further details of this experiment see Conner & Via, 1993).

If a single locus is responsible for most of the genetic variance in a trait, then two predictions can be made (Fain, 1978; Mitchell-Olds & Bergelson, 1990). First, within-family variances should differ among families. This was tested using Levene's test, in which an analysis of variance was done using absolute values of the deviations from the full-sib family means as the independent variable (Snedecor & Cochran, 1989, pp. 252-253). The deviations were natural log-transformed to stabilize variances. Variance components were computed by equating observed and expected mean squares (PROC VARCOMP, SAS Institute Inc., 1985) to determine the proportion of variance that is accounted for by among-family differences in variance.

Second, the highest within-family variances should be found in those families with intermediate (in the case of equal allele frequencies) or extreme (unequal allele frequencies) means values for the trait. This was tested with (1) quadratic regression of natural log-transformed within-family variances

on family means (Fain, 1978) and (2) lowess (locally weighted regression scatter-plot smoothing; Chambers *et al.*, 1983) curve fits of the same variables. Lowess provides a better graphical representation of the shape of a bivariate distribution than does quadratic regression, because it fits the points locally instead of globally. Similar results were obtained by regressing absolute deviations from family means against family means (cf. Garland, 1988; Mitchell-Olds & Bergelson, 1990). Fain (1978) found that quadratic regression gave reasonable power with 50 full-sib families of four offspring each, so this analysis with 289 families should have excellent power, although there are some conditions under which the power of this test is reduced (Fain, 1978; Mayo, Hancock & Baghurst, 1980).

Residual plots of all regression and ANOVA analyses did not indicate any strong heteroscedasticity. KaleidaGraph on a Macintosh computer was used for the lowess plots, and SAS (SAS Institute Inc., 1985) was used for statistical analyses.

Results

The Levene's tests for heterogeneity of full-sib family variances were significant for all traits except petal, tube, and long filament lengths (Table 1A). However, among-family variance in the deviations from family means accounted for less than 15% of the total variance for all traits except emergence time and flowering time (Table 1A). The quadratic regressions of within-family variances on family means revealed significant regression coefficients for only three traits: leaf length, emergence time, and flowering time. The linear term of the leaf length regression was just significant at the 0.05 level while the quadratic term was not significant (Table 1B). The lowess curve fit for this trait (Fig. 1A) also supports the interpretation of a roughly linear relationship with a weakly positive slope. In the two life-history trait regressions the linear terms were significantly positive and the quadratic terms were significantly negative (Table 1B), suggesting a positive decelerating function. This function shape was further supported by lowess curve fits (Fig. 1, B and C), particularly for emergence time. In no case is there evidence for maximum variance at intermediate family means.

Table 1. Results of the major gene analysis. A. Levene's test for heterogeneity of within full-sib family variances. V_f is the percentage of variability accounted for by among-family differences in variance, and F is the F-ratio from the ANOVA using absolute values of the deviations from the full-sib family means as the independent variable. B. Regression coefficients from quadratic regression of within-family variances on family means; only regressions with significant coefficients are shown. The linear and quadratic terms are shown with their standard errors in parentheses.

A.	V_f	F
Petal Length	1.9	1.08
Petal Width	11.6	1.50***
Tube Length	4.0	1.16
Short Filament	8.6	1.36***
Long Filament	4.1	1.17
Pistil Length	13.8	1.62***
Leaf Length	7.0	1.29**
Leaf Width	7.3	1.31**
Emergence Time	47.4	4.44***
Flowering Time	32.0	2.73***

B.	linear		quadratic	
Leaf Length	0.082*	(0.037)	-3.7×10^{-4}	(2.2×10^{-4})
Emergence Time	0.553***	(0.029)	-0.0084***	(6.4×10^{-4})
Flowering Time	1.201***	(0.342)	-0.017**	(0.0065)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

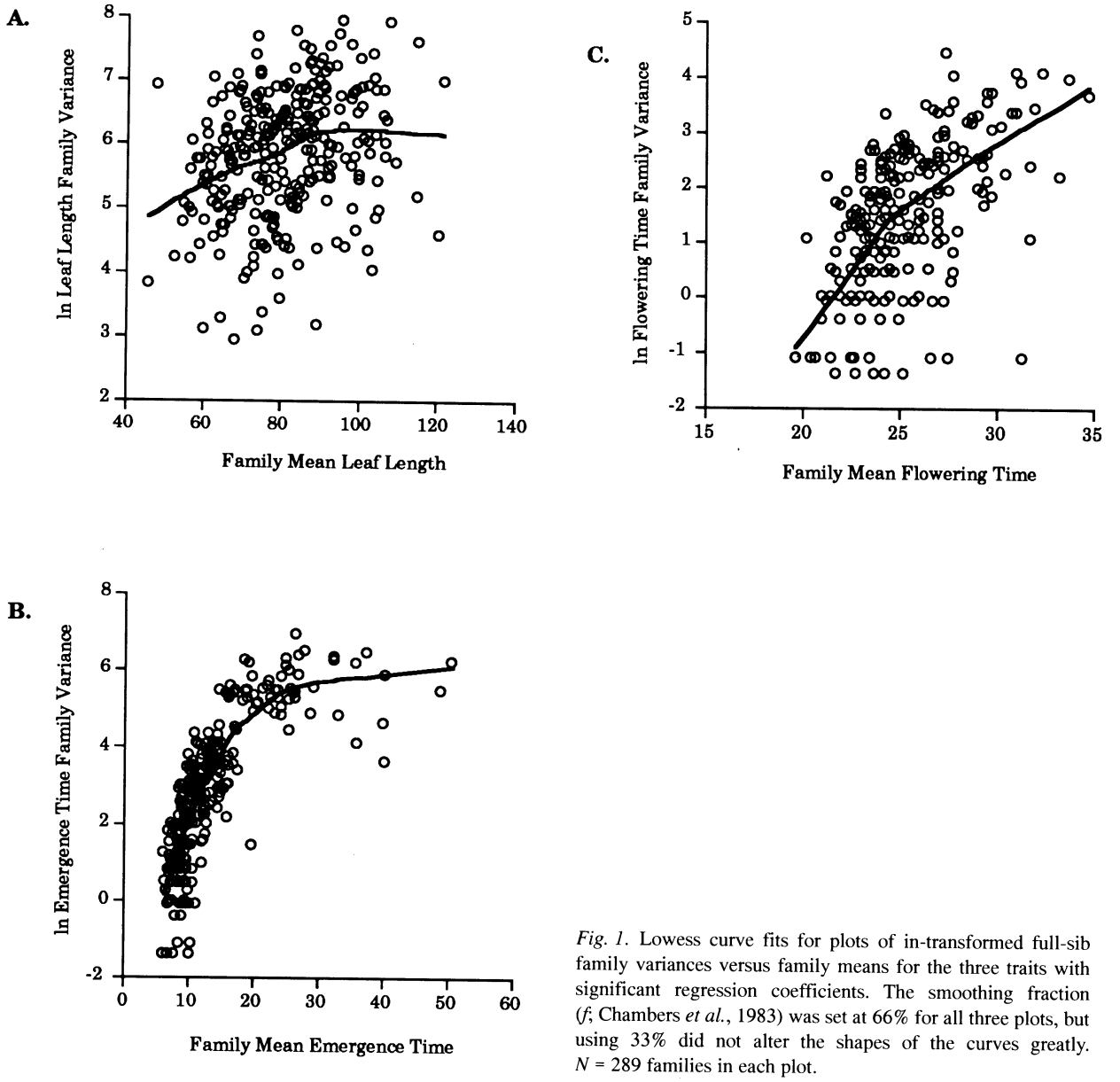

Fig. 1. Lowess curve fits for plots of in-transformed full-sib family variances versus family means for the three traits with significant regression coefficients. The smoothing fraction (*f*, Chambers *et al.*, 1983) was set at 66% for all three plots, but using 33% did not alter the shapes of the curves greatly. $N = 289$ families in each plot.

Discussion

The results of this study provided evidence for major genes affecting the two life-history traits, emergence time and flowering time. The Levene's tests for these traits were strongly significant, with among-family variance accounting for a third to a half of the total variance in the deviations from family means. The regression models for these traits combined with lowess curve fits suggest that within-family variance increases to an asymptote with increasing family mean. This pattern suggests a major locus exhibiting dominance with the allele(s) for longer times being rare (Fain, 1978). Given the indirect nature of this approach, however, further studies are warranted to confirm the existence of a major gene affecting these two life-history traits in wild radish.

In contrast to the result for the life-history traits, I found little evidence for major gene effects on the eight morphological traits studied. While there were significant differences among within-family variances for five of these traits, most of the variance in the deviations from family means was

within families, not among families. The weakly positive linear relationship for leaf length suggested by the regression analysis and the lowess curve fit might reflect the effects of a major locus with the allele(s) for long leaves being rare, but this evidence is weak.

Garland (1988) and Mitchell-Olds and Bergelson (1990) also reported significant Levene's test results for most traits but found little evidence for major gene effects in graphical and regression analyses. For one trait, however, emergence date in *Impatiens*, families with intermediate means did have the highest variance (Mitchell-Olds & Bergelson, 1990). The results of these two studies combined with our results suggest that Levene's test may not be very useful for discovering major gene effects. Instead, graphical approaches are necessary (see also Mitchell-Olds & Bergelson, 1990).

Using a completely different approach, Shore and Barrett (1990) also found no evidence for major genes affecting stamen length, style length or flower diameter in a greenhouse study of *Turnera ulmifolia*, a tropical weed. Therefore, while there are some well-known examples of major gene effects on floral morphology (e.g. Ford, 1975; Gottlieb, 1984), it may be that in many cases floral traits, especially dimensions, are determined by many genes of small effect (Gottlieb, 1984). It is interesting that both this study and Mitchell-Olds and Bergelson (1990) found the strongest evidence for major genes in emergence time, while neither this study nor Shore and Barrett (1990) found evidence for major gene effects on floral dimensions. Clearly further studies are warranted to see if generalizations can be drawn about the kinds of traits that are likely to be affected by genes of major effect.

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