Directional selection is the primary cause of phenotypic diversification

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Edited by May R. Berenbaum, University of Illinois, Urbana, IL, and approved July 29, 2002 (received for review June 17, 2002)

Selection is widely accepted as the principal force shaping phenotypic variation within populations. Its importance in speciation and macroevolution has been questioned, however, because phenotypic differences between species or higher taxa sometimes appear to be nonadaptive. Here, we use the quantitative trait locus (QTL) sign test to evaluate the importance of directional selection in phenotypic divergence. If a trait has a history of directional selection, QTL effects should be mostly in the same direction; otherwise QTLs with antagonistic effects should be common. Analysis of QTL effects for 572 traits from 86 studies revealed significantly fewer antagonistic QTLs than expected under neutrality, a result that validates Darwin's claim that phenotypic diversification is caused mainly by selection. Moreover, interspecific trait differences were more strongly or consistently selected than intraspecific differences, strengthening a growing consensus among students of speciation that directional selection is the primary cause of speciation. Contrary to studies of selection in contemporary populations, life history traits appear to be selected more strongly than morphological traits, but traits related to the timing of development are weakly selected relative to most other traits.

It is often lamented that studies of present-day populations provide only the briefest snapshot of evolution and tell us little about the evolutionary forces that have shaped a particular trait or organism in the past (1). Although ancestral phenotypes can be reconstructed with phylogenetic methods (2) or directly determined from fossils (3), neither approach reveals the evolutionary processes that created these phenotypes. Even if these historical data could help, there is considerable uncertainty associated with the reconstruction of ancestral character states (4), and a fossil record is missing for most taxa and incomplete for others. As a result, a direct link between the action of microevolutionary forces detected in studies of contemporary populations and patterns of speciation and macroevolution has been difficult to make (1), yet this is a central problem in evolutionary biology.

A possible method for reconstructing the selective history of complex traits has, however, recently been proposed (5). The method is based on the direction of effects of quantitative trait loci (QTLs) that contribute to phenotypic differences. If a trait has had a continuous history of directional selection, then QTL effects should be in the same direction within a line. In contrast, if a trait has diverged under neutrality, QTLs with opposing or antagonistic effects should be common. This approach has been formalized as the QTL sign test (5), which compares the proportion of antagonistic QTLs in a given line with those predicted under neutral conditions.

In this article, we review the QTL literature to estimate the distribution of plus and minus QTL alleles for different kinds of organisms and for different kinds of traits. Three comparisons were of particular interest to us. Because of the recent emphasis of the speciation literature on the role of divergent selection in speciation (6–12), we asked whether interspecific trait differences are more strongly or consistently selected than intraspecific differences. Likewise, recent reports of stronger selection on morphological than life history or physiological traits in contemporary populations (13) prompted us to test whether conclusions from these short-term studies hold for evolution over the long term. Finally, we asked whether traits that affect the timing of developmental events are as strongly and consistently selected as might be predicted given the emphasis on timing traits in the literature (14, 15).

Methods

Literature Review. We examined the direction of effects of 2,684 QTLs from 572 traits and 84 studies (Tables 4 and 5, which are published as supporting information on the PNAS web site, www.pnas.org). Half of the studies were of wild × domesticated crosses, which were used to test the effectiveness of the QTL sign test for detecting a history of directional selection. The remaining studies involved crosses of natural populations or of laboratory strains that had not been subjected to artificial selection.

Sometimes the same trait was phenotyped in multiple environments or in multiple studies involving the same taxa. Likewise, some studies mapped QTLs onto two coupling phase maps derived from the same segregating population (16). As a result, the QTL ratios reported here are not necessarily independent. If there was little overlap in the QTLs detected in different environments or in different coupling phase maps, we reported the results from each environment or map separately. If most of the QTLs detected mapped to the same position, however, we combined them into a single ratio with each different QTL counted once. We did not combine QTLs for the same trait from multiple studies because different lines and markers often were used, making it difficult to assess whether the QTLs detected were the same or different.

Trait Classification. For crosses between wild and domesticated species, we categorized traits by kingdom, mating system, and whether or not a trait difference results from domestication. Because phenotypic differences between wild and domesticated species are not necessarily caused by artificial selection, particularly for divergent crosses, only the direct products of domestication or most obvious byproducts of unconscious selection (loss of dormancy or dispersal mechanisms) were classified as domestication traits. These included measures of growth rate and body fat in pigs, reduced stinging in bees, large achenes and reduced shattering in sunflowers, increased fruit size, yield, and soluble solids content in tomatoes, increased seed size, yield, and protein content in various grasses, tuber formation in potatoes, and increased shoot growth in lettuce (Table 4).

Traits from natural populations were classified with respect to kingdom, cross, mating system, trait type, and whether or not the trait relates to the timing of developmental events. Although most traits were easily classified, some traits related to fecundity could logically be defined as either morphological or life history traits. We arbitrarily classified offspring number as a life history

This paper was submitted directly (Track II) to the PNAS office.

Abbreviation: QTL, quantitative trait locus.

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 www.pnas.org/cgi/doi/10.1073/pnas.192360899

Table 1. Proportions of antagonistic QTLs for various categories of organisms and traits in crosses involving wild species, and tests for significant deviations from neutrality

Trait category	No. of antagonistic QTLs	Total QTLs	QTL ratio	Least square means (± SE)†
Animals	73	312	0.234***	0.185 (0.039)
Plants	128	439	0.292***	0.202 (0.025)
Interspecific	47	245	0.192***	0.137 (0.154)
Intraspecific	154	506	0.304***	0.250 (0.243)
Outcross	98	425	0.231***	0.170 (0.174)
Self	103	326	0.316***	0.217 (0.262)
Life history	111	540	0.206***	0.139 (0.175)
Morphology	138	508	0.272***	0.266 (0.255)
Physiology	8	40	0.200**	0.176 (0.125)
Timing	37	124	0.298***	0.236 (0.219)
Total	201	751	0.268***	

**, $P < 0.01$, corrected for multiple tests by sequential Bonferroni (30). ***, $P < 0.001$, corrected for multiple tests by sequential Bonferroni (30).
†Means adjusted for effects of factors in ANOVA model in Table 3.

Table 2. ANOVA of the effects of kingdom, cross type, mating system, and whether or not the trait difference results from domestication on the proportion of QTLs with opposing effects in crosses between wild and domesticated populations

Source	df	SS	MS	*F*	*P* value
Kingdom	1	962.32	962.32	3.68	0.0559
Cross	1	2,002.68	2,002.68	7.66	0.0060*
Mating system	2	1,966.30	983.15	3.76	0.0243†
Domestication trait	1	9,728.08	9,728.08	37.19	<0.0001‡
Total QTLs	1	6,016.61	6,016.61	23.00	<0.0001
Error	348	91,038.72	261.61		

The total number of QTLs detected for each trait was included as a covariate. SS, sum of squares; MS, mean squares.
*The fraction of antagonistic QTLs is lower for *interspecific* than *intraspecific* trait differences.
†The fraction of antagonistic QTLs is lower for traits from crosses between selfers than for traits from crosses between outcrossers. QTL proportions for traits from crosses between selfers and outcrossers are intermediate and not significantly different from crosses involving selfers or outcrossers only.
‡The fraction of antagonistic QTLs is lower for domestication traits than for all other traits.

trait, but characteristics of the offspring (e.g., size, weight, shape, etc.) as morphological traits.

Statistics. Orr (5) provides two versions of the QTL sign test. The first requires knowledge of QTL magnitudes, whereas the second assumes that QTLs have equal effects. Because accurate estimates of QTL magnitudes were not available for many traits, we used the second version (equation 6 in ref. 5). Although this should not be a problem for most comparisons, there may be situations in which evidence for directional selection is missed. For example, the fixation of a major QTL that overshoots the target phenotype might be followed by the evolution of minor QTLs with opposing effects that bring the trait back toward the optimum value (5). In the absence of information about QTL magnitudes, it would not be possible to reject the null hypothesis of neutral evolution.

We first tested the proportion of antagonistic QTLs for each trait for significant deviations from neutrality by using equation 6 in ref. 5. Because at least six QTLs must be detected for a given trait before it becomes possible to reject the null hypothesis of neutral divergence, only 31% of the traits examined could be tested for directional selection (Tables 4 and 5). However, we can also use the QTL sign test to ask whether particular groups of traits have been affected by directional natural selection. This was accomplished by testing the trait categories listed in Table 1 for significant deviations from neutral expectations. Behavioral traits and traits from crosses between selfing and outcrossing populations were excluded from the analysis of natural populations because there were too few for statistical comparisons. Because n was large in all remaining groups (Table 1), the normal approximation (equation 7 in ref. 5) was used for the QTL sign test. Also, traits with only a single detected QTL were excluded from the analyses because, by definition, the proportion of QTL alleles with opposing effects must be zero.

We then asked whether there were differences among categories of traits for the proportion of QTLs with opposing effects. For studies of wild × domesticated populations, we used a multifactor ANOVA to test whether the fraction of antagonistic QTLs was lower for domestication traits than for all other traits. Main effects included in the model were kingdom, cross type, mating system, and whether or not the trait difference resulted from domestication. Traits with a single detected QTL were excluded from the analyses. The total number of QTLs detected per trait was Box–Cox-transformed before analysis (17) and included as covariate, whereas the proportions of QTLs with opposing effects were arcsine-square root transformed (18). All main effects were tested over the residual error. Natural populations were analyzed as above, except that trait type and whether or not the trait affected the timing of developmental events were included as main effects, behavioral traits and traits from crosses between selfers and outcrossers were excluded because of small sample sizes, and domestication traits were excluded by definition. The analysis of natural populations was repeated by using a data set in which all potentially redundant traits/QTLs were removed. Because there were no substantive changes in our results, only the original analysis of the full data set is discussed below. All analyses were performed by using JMP (Version 4, SAS Institute, Cary, NC).

Results and Discussion

Domestication Traits. To verify the QTL sign test, we first examined the fraction of QTLs with antagonistic effects in 42 studies of wild × domesticated populations (Table 4). According to this approach, traits under selection during domestication should have fewer antagonistic QTLs than traits less clearly linked to the domestication process. Of 54 domestication traits with six or more detected QTLs (the minimum required for significance by using the QTL sign test), 35 (64.8%) had proportions of antagonistic QTLs that deviated significantly ($P < 0.05$) from neutral divergence. This finding is well in excess of the three significant tests expected by chance, so selection during domestication appears to have had a substantial impact on QTL proportions. In contrast, only 14 of 84 traits (16.7%) not directly associated with domestication had QTL proportions that differed significantly ($P < 0.05$) from neutral divergence, compared with four expected by chance. A comparison of these ratios revealed a significant difference between domestication traits and all others ($\chi^2 = 21.4$, df = 1, $P < 0.001$). QTL ratios were also analyzed by ANOVA and, after accounting for the effects of kingdom, cross type, mating system, and the total number of QTLs (Table 2), there was a highly significant effect of selection during domestication ($P < 0.0001$). As expected, domestication traits exhibited a significantly lower proportion of antagonistic QTLs (0.059 ± 0.028, least square mean ± SE) as compared with other traits (0.181 ± 0.027). These results validate the QTL sign test as a means of detecting directional selection.

There is a caveat associated with this conclusion, however. It might be that all traits diverge under selection, but that the

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Table 3. ANOVA of the effects of kingdom, cross type, mating system, trait type, and whether or not the trait relates to timing of developmental events on the proportion of QTLs with opposing effects in crosses involving wild species

Source	df	SS	MS	*F*	*P* value
Kingdom	1	28.80	28.80	0.11	0.7411
Cross	1	1,482.49	1,482.49	5.64	0.0189*
Mating system	2	354.19	354.19	1.35	0.2476
Trait type	2	2,877.60	1,438.80	5.47	0.0051†
Timing	1	1,231.26	1,231.26	4.68	0.0321‡
Total QTLs	1	4,324.14	4,324.14	16.45	0.0001
Error	147	38,645.86	262.90		

The total number of QTLs detected for each trait was included as a covariate.
SS, sum of squares; MS, mean square.
*The fraction of antagonistic QTLs is lower for interspecific than intraspecific trait differences.
†The fraction of antagonistic QTLs is lower for life history than morphological traits. QTL proportions for physiological traits are intermediate between, and not significantly different from, life history and morphological traits.
‡The fraction of antagonistic QTLs is greater for traits relating to the timing of developmental events than for all other traits.

divergence of nondomestication traits more often involves the fixation of QTLs that overshoot the optimum trait value, followed by the evolution of minor QTLs in the opposite direction. Although it seems unlikely that entire categories of traits would differ substantially in this way, this possibility cannot be ruled out for the comparison of domestication and nondomestication traits or for the cross-trait comparisons described below.

Wild Species' Traits. To examine the role of directional selection in the wild, we tabulated the direction of QTL effects in 42 studies of wild animals and plant species (Table 5). Of 42 traits with six or more QTLs, six (14%) had QTL proportions that deviated significantly from neutral expectations ($P < 0.05$), which exceeds the two significant proportions expected by chance. However, tests of individual traits underestimate the prevalence of directional selection because too few QTLs were detected to reject the null model of neutral divergence for most traits (4.2 QTLs per trait on average). We overcame this difficulty by assessing QTL proportions for groups of traits (Table 1). All trait categories had QTL proportions that deviated significantly from neutral expectations (Table 1), implying that directional selection is a major contributor to phenotypic differentiation in essentially all kinds of organisms and traits.

We also used a multifactor ANOVA to ask whether certain categories of traits were under stronger or more consistent directional selection than others (Table 3). No differences in QTL proportions were observed between animals and plants or between selfing and outcrossing species. However, phenotypic differences between species had a significantly lower proportion of antagonistic QTLs than did intraspecific trait differences ($P = 0.0189$; Tables 1 and 3), indicating that species differences are more likely to have resulted from directional selection than are differences among conspecific populations. This result supports recent trends in the speciation literature, which include increasing emphasis on divergent selection as a cause of speciation (6–12) and declining support for speciation models that rely on nonselective processes (19, 20). More generally, the greater contribution of selection to interspecific than intraspecific differences opposes a common view among macroevolutionists (21–23) that emphasizes the role of stochastic forces in the evolution of species and higher categories. Rather, these data are most consistent with a model of species' evolution in which the preferential spread of advantageous versus neutral or weakly selected alleles biases the fixed differences between species toward unconditionally advantageous mutations (24).

Comparison of the proportions of antagonistic QTLs among trait categories further implies that life history traits are under stronger or more consistent directional selection than morphological traits (Tables 1 and 3). Physiological traits have QTL proportions intermediate between, and not significantly different from, life history and morphological traits (Tables 1 and 3). The observation that life history traits are selected most strongly (or most consistently) is concordant with numerous previous reports of lower heritability for life history traits than morphological or physiological traits (25–27). For populations at equilibrium, traits most strongly selected on should have the least additive genetic variance (24–26), although lower heritabilities for life history traits could be explained by other factors, such as higher levels of environmental variance (28). Our results appear to conflict, however, with a review of 63 studies of selection in contemporary populations (13), which reported significantly stronger selection on morphological than life history traits. The authors of that review noted that their result could either be real or an artifact of reduced measurement error for morphological traits. Because the direction of QTL effects should not be affected by measurement error, the data presented here suggest that the latter explanation is correct.

Both this study (Table 3) and studies of selection in contemporary populations (13) indicate that selection on traits affecting the timing of developmental events is generally not as strong or consistent as that on other kinds of traits ($P = 0.0321$). These findings are discordant with the low heritability values often associated with timing traits (24–26). As discussed earlier, however, low heritabilities may be caused by increased environmental variance rather than strong selection (28). More broadly, weak selection on timing-related traits appears to contradict prevailing wisdom that emphasizes the importance of changes in developmental timing or heterochrony in speciation and macroevolution (14, 15). Of course, the finding that many timing-related traits are weakly selected does not preclude the possibility that rare heterochronic events have played an important role in phenotypic diversification. Indeed, many of the traits differentiating higher taxa are not variable among sexually compatible species, and thus cannot be tested with the QTL method.

The finding of strong selection for life history traits but weak selection for timing traits might be viewed as contradictory because timing traits represent one kind of life history trait. However, traits affecting reproductive output were also classified as life history traits and were more common in the data set. This finding would suggest that the low proportion of antagonistic QTLs observed for life history traits is primarily caused by reproductive output traits, but this was not tested by ANOVA.

Although our findings point to a strong role for directional selection in phenotypic diversification, there are several caveats associated with this analysis. First, the finding of pervasive directional selection across all categories of traits (Table 1) may be influenced by trait choice. For example, there may be a tendency for researchers to focus on the most important or divergent phenotypes differentiating parental lines. The signature of directional selection might be less strong for arbitrarily chosen traits. Second, as alluded to earlier, the QTL sign test used here accounts for the necessary existence of plus QTLs in the high line and minus QTLs in the low line, but it is not conditioned on the distribution of QTL magnitudes. Given the strong patterns observed here, however, small changes in significance levels for individual traits seem unlikely to affect our overall conclusions. In fact, it seems much more likely that the methods used here underestimate the role of selection in phenotypic evolution, because temporal shifts in the direction of

selection (1), and stabilizing selection, would generate QTL ratios that are likely to be indistinguishable from the null expectation. In addition, as discussed previously, major QTLs fixed during initial bouts of natural selection may overshoot the phenotypic optimum, and minor QTLs with effects in the opposite direction may evolve later to bring the trait back toward the optimum value (5). Finally, the selective fixation of major QTLs that have pleiotropic effects on other traits may generate antagonistic QTLs for the affected traits and inflate QTL ratios.

These latter considerations reinforce our earlier conclusions that Darwinian selection (29) largely accounts for the astonishing diversity of phenotypes we see.

We thank members of the Rieseberg laboratory for their comments on the manuscript. Our research on the genetics of adaptation and speciation has been supported by the National Institutes of Health, the National Science Foundation, the U.S. Department of Agriculture, and the Swiss National Science Foundation.

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