Caution in testing phenotypic selection

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Understanding whether genetic drift or natural selection is responsible for the phenotypic difference between two strains is a major goal of evolutionary biology. Building on Orr’s quantitative trait locus (QTL) sign test (1), Fraser developed a clever test of phenotypic selection that requires only a genetic cross between the two strains followed by phenotyping of these strains and their F2 progenies (2). The simplicity of this test makes it applicable to a wide variety of traits of many species. Here we draw attention to two caveats.

That we are investigating the role of natural selection in the divergence of a trait between two strains, S1 and S2, implies that they likely have different natural environments, which we name E1 and E2, respectively. Under which environment (E3) should phenotyping be conducted? This is a relevant question, because the outcome of Fraser’s test varies with E3 as a result of widespread genotype–environment interactions (GxE) in phenotype determination (3–5). That is, moving from one E3 to another may differentially influence the phenotypic effects of different QTLs. Consequently, whether the phenotypes of S1 and S2 represent extreme values in the F2 phenotypic distribution, which would indicate directional selection by Fraser’s test, depends on E3. If the environment (E0) of the common ancestor of S1 and S2 equals E1, the test environment should be E2, because the potential directional selection would have occurred in E2. If E0 is distinct from both E1 and E2, or any of E0, E1, and E2 is unknown, an appropriate E3 cannot be determined without additional information. Using an inappropriate E3 could produce false negatives or false positives in detecting selection, although false negatives seem more likely if GxE is as idiosyncratic as found in epistasis (6). This is because, under the correct E3, directional (or stabilizing) selection would push Fraser’s v statistic to unusually large (or small) values; upon the change of E3 to an arbitrary environment, idiosyncratic GxE adds random effects to individual QTLs, which tends to render the difference between S1 and S2 relative to the F2 phenotypic distribution mediocre and v less significant.

Even when v significantly exceeds 1 in Fraser’s test performed in the correct E3, directional selection may not be the cause, because lineage-specific relaxation of selection, coupled with mutational bias, can also create this signal. For instance, if a trait that has been selectively constrained at a low level in E1 is subject to relaxed selection in E2, mutational bias could move the trait value higher in S2 than in S1 and lead to a significant v. Evidence such as sequence-based selective sweep signals may distinguish directional selection from relaxed selection (7), but collecting such evidence requires genotyping and QTL mapping.

The first of the above two caveats is likely of concern to most tests of phenotypic selection (1, 2, 8–10), while the second applies to Orr’s (1) and Fraser’s (2) tests. We hope that understanding these caveats helps one use and interpret Fraser’s and other tests of phenotypic selection more judiciously.

5 J. M. Flynn et al., Comprehensive fitness maps of Hsp90 show widespread environmental dependence. eLife 9, e53810 (2020).

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