Note

Epistasis Underlying a Fitness Trait Within a Natural Population of the Pitcher-Plant Mosquito, *Wyeomyia smithii*

William E. Bradshaw,[1] Brian P. Haggerty and Christina M. Holzapfel

Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, Oregon 97403-5289

ABSTRACT

We selected on divergent photoperiodic response in three separate lines from a natural population of the pitcher-plant mosquito, *Wyeomyia smithii*. Line crosses reveal that there exists within a population, diverse epistatic variation for a fitness trait that could contribute to adaptive potential following founder events or rapid climate change.

A fundamental tenet of evolutionary genetics is that a trait's ability to respond to selection is directly related to the additive genetic variation (or evolvability, *sensu* HOULE 1992) of that trait. In the absence of nonadditive genetic variation, response to stabilizing or directional selection or isolation and drift following a founder event should result in reduced additive genetic variation in the population. However, in the presence of nonadditive genetic variation, selection or isolation and drift can result in increased additive genetic variation, facilitating the evolution of populations having experienced a genetic bottleneck or confronting environmental change (MAYR 1954; TEMPLETON 1980, 1996; GOODNIGHT 1988, 2000; CARSON 1990; CHEVERUD and ROUTMAN 1996; SLATKIN 1996; MEFFERT 1999, 2000; CHEVERUD 2000; NACIRI-GRAVEN and GOUDET 2003).

This generation of additive from epistatic variation has been invoked to explain the increase in additive genetic variation for photoperiodic response among populations of the pitcher-plant mosquito, *Wyeomyia smithii*, dispersing along a latitudinal gradient into postglacial northern North America (HARD *et al.* 1992, 1993). HARD *et al.*'s argument was based on three observations and two fundamental assumptions. The observations were, first, that contrary to the expectations of directional selection on a latitudinal scale and stabilizing selection on a local scale, the additive genetic variance for photoperiodic response increased with latitude. Second, genetic differences in photoperiodic response between southern and northern populations involved differences in epistasis. Third, the contribution of different forms of digenic

epistasis (additive × additive, additive × dominance, dominance × dominance) was unique to each cross. The latter observation was important because it implied stochastic reordering of genic interactions as would be expected as a consequence of random drift following independent founder events. The two assumptions were that postglacial dispersal had taken place by sequential founder events along a latitudinal gradient and that there is actually epistatic variation for photoperiodic response within populations. The first of these assumptions was supported by ARMBRUSTER *et al.* (1998) who showed that average heterozygosity at 10 allozyme loci decreased with latitude from the approximate southern limit of the Laurentide Ice Sheet (∼40° N in New Jersey) northward. The second of these assumptions is the topic of the present article.

Hybridization experiments have revealed genetic differences attributable to epistasis among species (DOEBLEY *et al.* 1995) and among populations within species (HARD *et al.* 1993; LAIR *et al.* 1997; FENSTER and GALLOWAY 2000; GALLOWAY and FENSTER 2000; CARROLL *et al.* 2001, 2003). In theory, one might expect little epistatic variation within populations as selection should favor an optimal combination of alleles (WHITLOCK *et al.* 1995), but crosses between selected lines within populations reveal that such epistatic variation can exist (MATHER and JINKS 1982; CHEVERUD 2000). We use the latter approach to ask whether lines of *W. smithii*, selected for divergent photoperiodic response, differ in epistasis. *W. smithii* enters a larval dormancy that is initiated, maintained, and terminated by photoperiod (day length; BRADSHAW and LOUNIBOS 1977). The critical photoperiod is the length of day at which an individual switches between active development and dormancy and, in direct response to seasonal selection, increases with lati-

[1]*Corresponding author:* CEEB, 5289 University of Oregon, Eugene, OR 97403-5289.   E-mail: wyomya@aol.com

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TABLE 1

Results of the joint-scaling test based on generation means and variances between lines of *W. smithii* selected for long and short critical photoperiods

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Subpopulation	Joint-scaling test[a]			
	A 4 d.f.	AD 3 d.f.	ADM 4 d.f.	ADME 1 d.f.
Streamside				
χ^2	99.29	70.47	80.55	10.54^b
P	<0.001	<0.001	<0.001	<0.001
Backwater				
χ^2	252.90	95.11	91.04	1.49
P	<0.001	<0.001	<0.001	<0.122
Sandy bog				
χ^2	298.66	58.21	86.25	0.04
P	<0.001	<0.001	<0.001	0.844

Each subpopulation was transported to the lab, reared through three lab generations to minimize field effects, divergently selected for critical photoperiod for 13 generations with cumulative inbreeding <5% (Bradshaw *et al.* 2003), reared through 9 subsequent generations with $N > 1000$ to permit recombination, and then crossed to test for epistatic differences between lines within subpopulations. The two parent populations were hybridized to produce 14 "generations": the two parents, F_1, F_2, both backcrosses, and all of their possible reciprocals. The experimental generations were reared on short days to induce dormancy and to synchronize development. Day lengths were then increased by 3 min·day^{-1}, ultimately inducing development and pupation in each individual. The day length on the day of pupation was then scored as the critical photoperiod of the pupating individual. Since development under these conditions was log-normally distributed, individual critical photoperiods were $\log_{10}$ transformed prior to analyses. The number of parents of the experimental larvae averaged 85 females and 115 males; the number of experimental larvae per "generation" averaged 206.

[a] The joint-scaling test (Lair *et al.* 1997) tests sequentially for goodness of fit to successively more inclusive models: A, additive; AD, additive-dominance; ADM, additive-dominance-maternal (including both additive-maternal and dominance-maternal effects); ADME, additive-dominance-maternal-digenic epistasis models. Mather and Jinks' (1982) F-∞ parameterization was used to calculate expected generation means. A high value of χ^2 and a significant P-value indicate inadequacy (poor goodness of fit) of the model and that more inclusive genetic effects need to be considered. Note that in the presence of substantial epistasis, estimates of additive and dominance effects are difficult to interpret (Hayman 1958, 1960). A copy of our joint-scaling test written in Mathcad 4.0 is available on request.

[b] Heterogeneity chi square (Zar 1996: $\chi^2 = 11.95$, d.f. $= 2$, $P = 0.003$) indicates significant differences among subpopulations in rejection of the ADME model.

FIGURE 1.—Critical photoperiod of long and short selected lines and their F_1 and F_2 hybrids and backcrosses (B_1, B_2). The plots show mean $\log_{10}$(critical photoperiod, hr $\pm$ 2SE). The solid line shows the weighted least-squares expectation of an additive model. The results of the joint-scaling test (see also Table 1) are shown for the additive-dominance-maternal (ADM) and the ADME models; ***$P < 0.001$; $^{NS}P > 0.05$. The symbols in the boxes refer to the sign of digenic epistasis (+, significant and positive; −, significant and negative; 0, not significant) in the order A $\times$ A, A $\times$ D, D $\times$ D from the coefficients in Table 2.

tude and altitude of population origin (Bradshaw and Lounibos 1977; Bradshaw *et al.* 2003). The range of *W. smithii* extends from the Gulf of Mexico to northern Canada. We collected mosquitoes from a mid-latitude locality along a stream meandering through a cedar swamp in the New Jersey Pine Barrens (40° N, locality
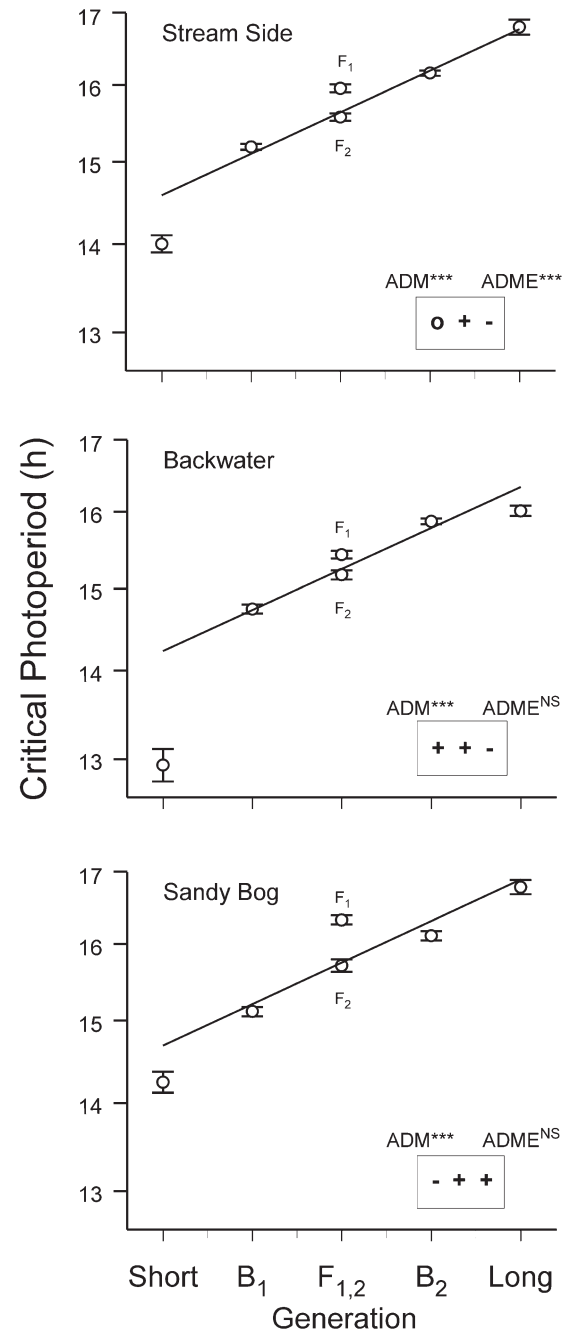
"PB" of earlier studies from this lab). To increase independence of the replicate lines, we collected from three subpopulations within a 200-m radius of each other (Bradshaw *et al.* 2003): "streamside" from along the

TABLE 2

Components of digenic epistasis contributing to genetic differences among subpopulations of *W. smithii* selected for long and short critical photoperiods

Subpopulation	Digenic epistatic effects[a]		
	A × A	A × D	D × D
Streamside			
Coefficients	0.00733^A	0.253^{MN}	-0.0254^X
t-test[b]	1.71^{NS}	7.90***	4.08***
Backwater			
Coefficients	0.0163^A	0.0289^M	-0.0609^Y
t-test	3.43***	6.68***	8.61***
Sandy bog			
Coefficients	-0.0187^B	0.0158^N	0.0431^Z
t-test	3.09***	4.24***	5.54***

[a] Coefficients for the components of digenic epistasis: A × A, additive by additive; A × D, additive by dominance; D × D, dominance by dominance; within each column, coefficients followed by the same letter do not differ by Wald χ^2 (Fox *et al.* 2004); significant differences: M *vs.* N, $P < 0.05$; A *vs.* B and X *vs.* Y *vs.* Z, $P < 0.001$.

[b] *t*-test for significant effect of the components of digenic epistasis: $^{NS}P = 0.089$, ***$P < 0.001$.

stream itself; "backwater" from a backwater of the stream ~100 m north of the first collection site; and "sandy bog" from a sandy bog ~300 m to the west of the stream and separated from it by dry pine woodlands. We imposed divergent selection for long and short critical photoperiods and then crossed the selected lines from within each subpopulation (Table 1). We tested the specific prediction that, if there were genetic variation at epistatically acting loci in the original population, then genetic differences in photoperiodic response between selected lines derived from the same starting subpopulation should include epistasis.

Response to selection on critical photoperiod consistently revealed substantial epistatic differences between selected lines (Table 1; Figure 1). We interpret these results to mean that there was genetic variation among interacting loci in each of the respective subpopulations in nature. These results are consistent with HARD *et al.*'s (1992, 1993) assumption that within ancestral populations there existed the epistatic genetic variance from which additive genetic variance could have been released during successive, sequential founder events in the northward dispersal of *W. smithii* following recession of the Laurentide Ice Sheet.

The genetic "fingerprint" of epistasis was distinct for each subpopulation (Figure 1). In two of the subpopulations, additive, dominance, maternal, and digenic epistatic effects were sufficient to account for genetic differences in critical photoperiod between the selected lines; but, in one of the subpopulations (streamside), higher-order epistasis or linkage was implicated. Since the se-

lected lines had been maintained for nine potentially recombining generations without selection prior to testing for epistasis, higher-order epistasis was more likely than linkage to be responsible for rejection of the additive-dominance-maternal-digenic epistasis (ADME) model in this cross. In addition, the pattern of coefficients of digenic epistasis was distinct for each subpopulation (Figure 1; Table 2). First, the contribution of additive-by-additive epistasis was negative in the sandy bog subpopulation and positive in the other two. Second, the contribution of additive by dominance epistasis was less in the sandy bog than in the backwater subpopulation. Third, the contribution of dominance-by-dominance epistasis differed among all three subpopulations and was positive in the sandy bog but negative in the other two subpopulations.

These distinct genetic fingerprints indicate that either population subdivision is occurring over a very fine microscale (*e.g.*, FENSTER and GALLOWAY 2000) or unique genetic trajectories underlie similar phenotypic trajectories in response to a uniform selection gradient (*e.g.*, TRAVISANO and LENSKI 1996), or a combination of these processes. Regardless of which of these processes is operating in *W. smithii*, our results show that diverse genetic trajectories of genic interactions are available to respond to short-term selection within a natural population of *W. smithii*. This availability may have contributed not only to the generation of additive genetic variation over millennial time scales since the recession of the Laurentide Ice Sheet, but also to the rapid genetic response of *W. smithii* to recent climate change (BRADSHAW and HOLZAPFEL 2001).

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