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EVOLUTION OF THE GENETIC ARCHITECTURE UNDERLYING FITNESS IN THE PITCHER-PLANT MOSQUITO, *WYEOMYIA SMITHII*

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Abstract.—We examined the genetic basis for evolutionary divergence among geographic populations of the pitcher-plant mosquito, *Wyeomyia smithii*, using protein electrophoresis and line-cross analysis. Line-cross experiments were performed under both low density, near-optimal conditions, and at high, limiting larval densities sufficient to reduce fitness (r_c) in parental populations by approximately 50%. We found high levels of electrophoretic divergence between ancestral and derived populations, but low levels of divergence between two ancestral populations and between two derived populations. Assessed under near-optimal conditions, the genetic divergence of fitness (r_c) between ancestral and derived populations, but not between two derived populations or between two ancestral populations, has involved both allelic (dominance) and genic (epistatic) interactions. The role of dominance and epistasis in the divergence of r_c among populations affects its component traits in a pattern that is unique to each cross. Patterns of genetic differentiation among populations of *W. smithii* provide evidence for a topographically complex "adaptive landscape" as envisioned by Wright in his "shifting balance" theory of evolution. Although we cannot definitively rule out the role of deterministic evolution in the divergence of populations on this landscape, ecological inference and genetic data are more consistent with a stochastic than a deterministic process. At high, limiting larval density, hybrid vigor is enhanced and the influence of epistasis disappears. Thus, under stressful conditions, the advantages to fitness due to hybrid heterozygosity can outweigh the deleterious effects of fragmented gene complexes. These results have important implications for the management of inbred populations. Outbreeding depression assessed in experimental crosses under benign lab, zoo, or farm conditions may not accurately reveal the increased advantages of heterozygosity in suboptimal or marginal conditions likely to be found in nature.

Key words.—Dominance, epistasis, heterosis, outbreeding depression, shifting balance, speciation, *Wyeomyia smithii*.

Received February 23, 1996. Accepted November 1, 1996.

Understanding the role on nonadditive genetic variability in the evolution of natural populations remains a fundamental problem in evolutionary biology. While the effects of within-locus interaction (dominance) have received considerable attention (Charlesworth and Charlesworth 1987), the role of between-locus interaction (epistasis) remains controversial and poorly understood (Barton and Turelli 1989; Whitlock et al. 1995). Epistasis plays a central role in Wright's (1977) "shifting balance" theory, which currently provides "perhaps the most popular metaphor for evolutionary change" (Futuyma 1986, p. 172). In the shifting balance process, isolation and drift result first in the fragmentation of coevolved gene complexes and then the independent establishment of new, favorable gene-gene interactions. As a consequence of this process, isolated populations should differ in the contribution of epistasis to fitness (Mayr 1954; Carson 1968; Wright 1977; Templeton 1980; Goodnight 1988, 1995).

Although previous studies have demonstrated the contribution of epistasis to the genetic architecture of both morphological and life-history traits (Vetukhiv 1953; Wallace and Vetukhiv 1955; Templeton et al. 1976; Burton 1990; Brown 1991; Parker 1992), interpreting these results to make inferences about processes of evolutionary diversification has proven difficult for several reasons. First, it is often not known how the characters being studied relate to fitness in natural environments. Second, experiments are usually performed under highly artificial laboratory conditions, again making extrapolation to natural populations tenuous. Third, the historical pattern of the species' range expansion, their evolutionary trajectory, is usually unknown, so that inferences about the transition from ancestral to derived states cannot be made. Finally, few studies have addressed how the

genetic architecture of traits may change under natural levels of environmental variation.

In this study, we investigate the genetic basis of evolutionary divergence among four populations of the pitcher-plant mosquito, *Wyeomyia smithii* (Coq.), and consider the relevance of this genetic divergence to Wright's shifting balance process and to the conservation genetics of small populations. Because biogeographical, physiological, and morphological evidence all indicate that the range expansion of *W. smithii* in North America has proceeded from south to north (Bradshaw and Lounibos 1977; Istock and Weisburg 1987; Bradshaw and Holzapfel 1990), we are able to examine differentiation between ancestral, derived, and ancestral and derived populations. We evaluate a composite index of fitness, Laughlin's (1965) capacity for increase (r_c), under near-natural conditions including a near-optimal density and a limiting density sufficient to reduce fitness in the parental populations by approximately 50%. We also survey electrophoretic variability at eight polymorphic loci to compute Nei's genetic distance among parental populations (Nei 1987).

MATERIALS AND METHODS

The distribution of *W. smithii* follows that of its host plant, the carnivorous purple pitcher-plant *Sarracenia purpurea* L., from the Gulf of Mexico north to Labrador and westward to Saskatchewan. Throughout their range, the water-filled leaves of *S. purpurea* provide a remarkably consistent habitat for populations of *W. smithii*, wherein larval development takes place utilizing the resources provided by host prey capture. *Wyeomyia smithii* overwinter as diapausing larvae. The onset, maintenance, and termination of diapause is mediated by pho-
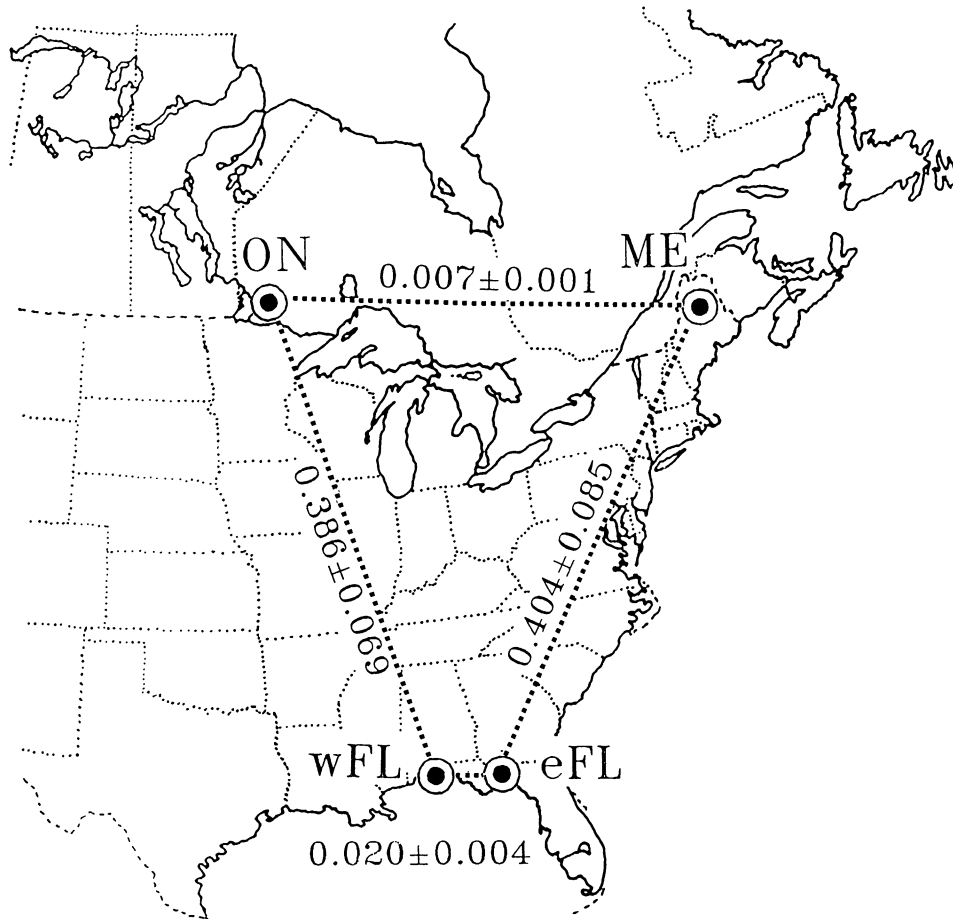
FIG. 1. Origin of parent populations in Ontario (ON), Maine (ME), western Florida (wFL), and eastern Florida (eFL), populations DL, KC, CR, and WI, respectively of earlier studies (Bradshaw and Holzapfel 1989, 1996; Hard et al. 1992, 1993a,b). Dotted lines between populations show crosses performed. Numbers on lines indicate Nei's genetic distance, D ($\pm$SE).

toperiod, and critical photoperiod increases linearly with latitude and altitude (Bradshaw 1976).

Our experimental design incorporates two features that enable us to run experiments under near-natural conditions. First, we run our experiments in the mosquito's natural habitat, the water-filled leaves of intact pitcher plants on a fixed diet of whole prey (freeze-dried adult *Drosophila melanogaster* Meigen) provided over a three-week period to simulate the prey-capture pattern of the host plant (Bradshaw 1983). Second, in a controlled-environment room, we are able to recreate a summer physical environment common to all populations. *Wyeomyia smithii* normally experience a fluctuating daily thermoperiod and fluctuating temperatures sustain a higher fitness than constant temperatures (Bradshaw 1980). Consequently, we rear *W. smithii* and perform all experiments on a warm:cool = 29:13°C ($\bar{x}$ = 21°C) smooth, sine-wave daily thermoperiod. Intermediate day lengths affect the rate of development in *W. smithii* (Bradshaw and Lounibos 1972). To avoid the three-way interaction among population, photoperiod, and development time, we run all experiments at a light:dark = 18:6h photoperiod incorporating two, half-hour twilights that provide unambiguous long days promoting direct (nondiapause) development in all populations (Bradshaw and Lounibos 1977). Since the phase angle difference between the light and temperature cycles can affect the interpretation of photoperiod, the thermoperiod is programmed to lag the photoperiod by three hours, mimicking their phase relationship during the summer in nature (Bradshaw 1980). To mimic the wet bogs and pine savannahs where pitcher plants grow, we provide a constant, high (80%) relative humidity.

Approximately 2000 larvae were collected from each of four localities (Fig. 1) during early spring when 100% of the genotypes in the population were available for sampling as diapausing larvae. Stocks consisted of the unselected control lines of Hard et al. (1993b) and Bradshaw and Holzapfel (1996), and were maintained for 20 generations prior to the initiation of this experiment, as in Bradshaw and Holzapfel (1996). Each generation had been composed of at least 250 adult breeding mosquitoes. Assuming a 60:40 = $\male$:$\female$ sex ratio, that all females mate, and that all females are monogamous (Clements 1963), the average male mating success is 0.66. If mating is random, then $1 - e^{-2/3} = 0.487$ of the males will mate. For a census population size of 250 individual adults, the expected effective population size (N_e) will be given by:

$$\frac{1}{N_e} = \frac{1}{(4)(150)(0.487)\male} + \frac{1}{(4)(100)\female}$$

or N_e = 169 (Wright 1969). After 20 generations, these populations are expected to have lost $1 - (1 - 1/2N_e)^{20}$ = 5.8% heterozygosity or additive genetic variance prior to the beginning of our experiments (Lande and Barrowclough 1987; Falconer 1989).

To obtain estimates of biochemical divergence between parental populations, we scored electrophoretic variability at the following eight polymorphic loci in each of the four parental populations using standard histochemical techniques (Steiner and Joslyn 1979): isocitrate dehydrogenase, hexokinase 2, hexokinase 4, phosphogucomutase, phoshoglucose isomerase, aldehyde oxidase, adenylate kinase, and malic enzyme. We computed Nei's genetic distance (D) and its standard error for each pair of populations crossed in our experiments (Nei 1987). Nei's D is zero when populations have identical gene frequencies and infinity when populations share no alleles, but generally ranges from 0 to 2 among species of *Drosophila* that are not completely reproductively isolated (Coyne and Orr 1989).

For each of the four crosses indicated in Figure 1, we generated hybrid lines while maintaining the parental stocks as follows. At the start of the experiment, parental stocks in the F_{20} lab generation were split into two halves: one half was mass swarmed to regenerate the parental lines and the other half was outcrossed to generate the F_1 and reciprocal F_1 hybrid lines. In the next (F_{21} lab) generation, parental stocks were again split and mass swarmed to regenerate the parental lines and outcrossed to generate the F_1 and reciprocal F_1 hybrid lines. The original F_1s and reciprocal F_1s were each mass swarmed to generate the F_2 and reciprocal F_2 hybrids. In generating the hybrid lines, between 32 and 96 adults of each sex were used.

We measured fitness (r_c) in five replicate cohorts for all experimental lines simultaneously at a density of 20 larvae and 200 *D. melanogaster* (food) per leaf, a culture regimen that provides near optimal-conditions (Bradshaw and Holzapfel 1989). After regenerating all parent and F_1 and F_2 hybrid lines from F_{23} parental stock, we again measured fitness (r_c) for all lines at a density of 40 larvae and 200 *D. melanogaster* per leaf, thereby imposing density sufficient to reduce fitness in the parental lines by approximately 50% (Bradshaw and Holzapfel 1989, 1990).

Details of the procedure of measuring r_c are given in a previous paper (Bradshaw and Holzapfel 1996). Briefly, eggs of each generation were collected from large adult cages and scored for fertility (% hatch). On the day of hatch, first instars from parent or hybrid lines were used to establish cohorts consisting of 20 larvae in the near-optimal treatment, and 40 larvae in the density-limiting treatment. Five cohorts per line were established for each density treatment. For each treatment, each cohort was placed in 15 mL distilled water in the leaves of intact pitcher-plants. To mimic the natural prey-capture cycle of leaves, developing larvae were fed whole, freeze-dried *D. melanogaster*. Twenty-five flies were added to the leaf on the day of mosquito hatch and 100, 50, and 25 flies were added at successive weekly intervals. Upon pupation, mosquitoes were sexed, and transferred to adult cages. Pupal exuviae were removed every Monday, Wednesday, and Friday, and used to calculate survivorship (% adult eclosion). Eggs were collected every Monday, Wednesday,

and Friday and used to calculate fecundity (total eggs per ecdysed female) and mean generation time, $T = \Sigma x E_x / \Sigma E_x$ where E_x is the number of eggs produced by the cohort at age x in days since oviposition of the cohort. Development time (DT) was calculated as the mean number of days from oviposition to pupation, and maturation time as $MT = T - DT$. Then, for each cohort of larvae raised in an individual leaf, we calculated cohort replacement rate as R_0 = (fertility)(survivorship)(fecundity) and capacity for increase as $r_c = ln(R_0)/T$. We calculated r_c instead of r from the Euler equation for two reasons. First, r_c can be decomposed into its component life-history traits more directly than r. Second, fecundity is computed as total eggs per ecdysed female in r_c. Thus, random variation in sex ratio, which may be substantial in cohorts of 20 individuals, does not contribute directly to variation in r_c as it would to variation in r from the Euler equation.

We tested the generation means for goodness of fit to genetic models incorporating composite additive and additive-dominance effects using the joint scaling test of Mather and Jinks (1982) as in Hard et al. (1992). In brief, the mean and error variance of performance measures for four experimental generations (two parents, F_1, and F_2) resulting from crosses between any two parental populations were used to derive estimates of the composite additive and dominance effects affecting this trait. If differences in performance were determined additively, by genes acting independently without dominance or epistasis, performance in both the F_1 and F_2 hybrids should equal the midparent value (average performance of the two parent populations). If the parental populations differ in dominance, the F_1 hybrids acquire increased heterozygosity, and due to the masking of deleterious recessive alleles, should show increased performance relative to the midparent value. Because of independent assortment, heterozygosity will decline in the next generation and, in the absence of epistasis, fitness in the F_2 should be intermediate between the F_1 and midparent value. If epistasis has been important in population divergence, independent assortment and recombination in the F_1 will cause favorably interacting gene combinations present in the parental populations to be disrupted in the F_2. In this case, performance in the F_2 should be lower than the average of the F_1 and midparent values.

Goodness of fit of the generation means to the additive model was tested with the χ^2 statistic of Hayman (1958) with two degrees of freedom. Acceptance of the additive model ($P \geq 0.05$) indicated that composite additive effects alone were adequate to explain genetic divergence of the parent populations. Rejection of the additive model ($P < 0.05$) lead us to test for goodness of fit to an additive-dominance model with one degree of freedom. Acceptance of the additive-dominance model ($P \geq 0.05$) indicated that composite additive and dominance effects were sufficient to account for genetic divergence of the parental populations. Rejection of the additive-dominance model ($P < 0.05$) indicated that genetic divergence between the parental populations had also involved at least epistatic interactions and potentially linkage (Mather and Jinks 1982). In the presence of significant epistasis, estimates of the additive and dominance effects are unreliable (Hayman 1958, 1960).

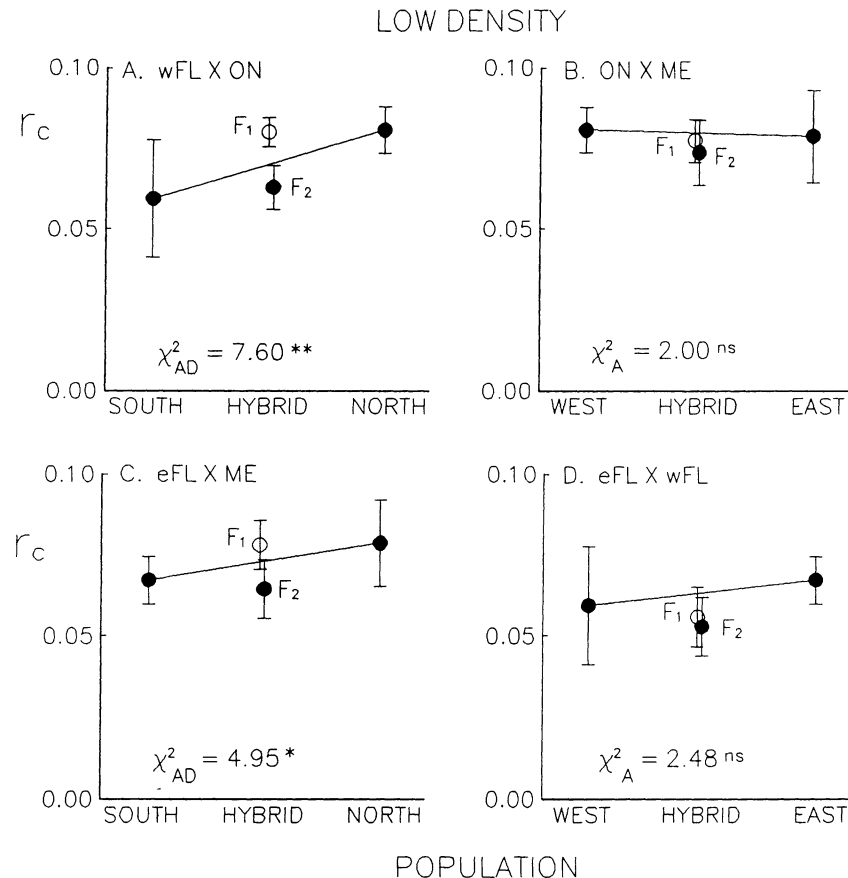Because of similar performance, we pooled reciprocal F_1

LOW DENSITY

FIG. 2.   Capacity for increase ($r_c \pm 2SE$) of parent, F_1, and F_2 hybrid lines at low, near-optimal density (20 larvae/leaf). The values of χ^2 represent tests for goodness of fit to an additive (χ^2_A) or additive-dominance (χ^2_{AD}) model; n.s. = $P \geq 0.05$, $^*P < 0.05$, $^{**}P < 0.01$.

and F_2 lines with their respective reciprocals. This pooling is conservative with respect to identifying dominance and epistatic effects because, if anything, it inflates the error variance for the F_1 and F_2 generation means. We tested for the importance of nonadditive effects in overall fitness (r_c) and its component life-history traits; replacement rate (R_0), mean cohort generation time (T), development time (DT), maturation time (MT), survivorship (% eclosion), fertility (% hatch), and fecundity (eggs/eclosed female) for each of the four crosses. Since these comparisons among the components of r_c were suggested a posteriori by the results in Figures 2 and 3, we used a sequential Bonferoni (Rice 1989) applied tablewide to the probabilities associated with the 56 combined χ^2 tests for goodness of fit to the additive model (4 crosses × 7 components × 2 densities).

RESULTS

Genetic distance (Nei's D) was greater between both north-south population pairs than either east-west population pair (Fig. 1), even though the geographic distance between the northern east-west cross (1945 km) approximated that between either north-south cross (2203 km and 2306 km).

Figure 2 shows capacity for increase (r_c) in parental and hybrid lines at near-optimal, low density. The joint scaling test rejects both the additive and the additive-dominance models in both north-south crosses, but in neither east-west

cross. Hence, under near-optimal conditions, the evolutionary divergence of r_c between southern (ancestral) and northern (derived) populations of W. smithii has involved both dominance and epistasis while any east-west differences in fitness may be ascribed to additive effects. The lower standard errors in the F_1 and F_2 hybrid lines relative to the parental lines reflects the larger sample size subsequent to the pooling of reciprocals.

Figure 3 shows capacity for increase (r_c) in parental and hybrid lines at high density. As in previous experiments (Bradshaw and Holzapfel 1989), the higher larval density reduced r_c by 50% or more in each parental population. The joint scaling test did not reject the additive-dominance model for r_c in any of the four crosses, but did reject the additive model in both north-south crosses and in the northern (ON × ME) but not the southern (wFL × eFL) east-west crosses. Hence, under density-limiting conditions, the effects of dominance in the evolutionary divergence of r_c between southern (ancestral) and northern (derived) populations or between the two northern (derived) populations became more prevalent than under near-optimal conditions, while the influence of epistasis was diminished. Any evolutionary divergence of r_c between the two southern (ancestral) populations may be ascribed to additive effects.

Table 1 shows the results of the joint scaling test for non-additive genetic differences between parent populations ap-
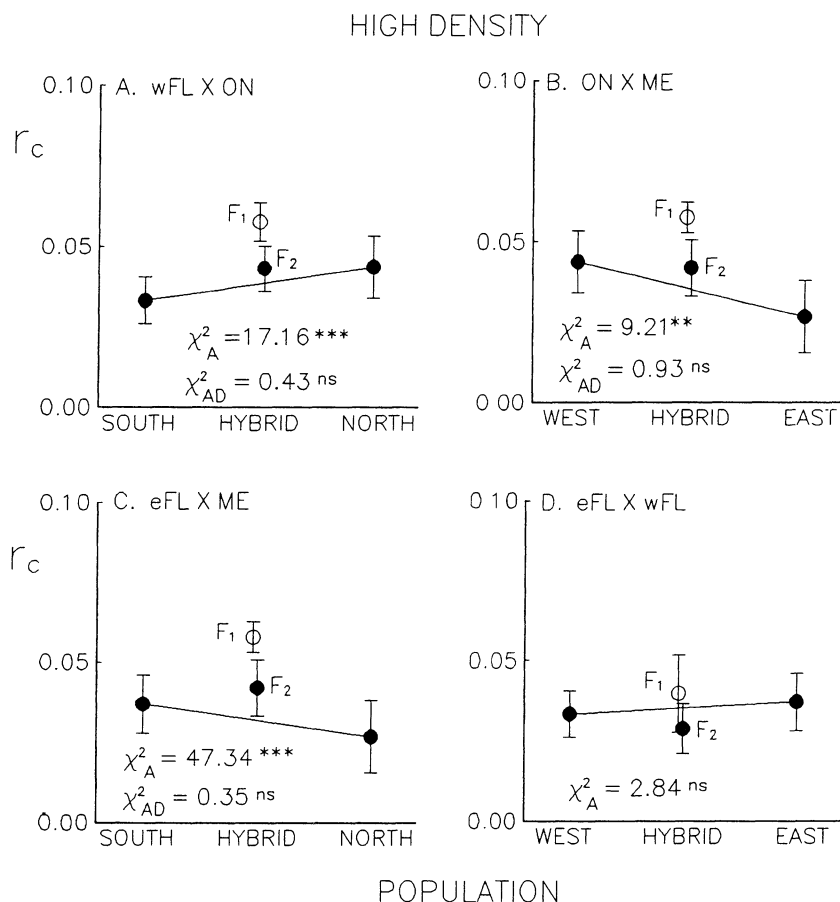
HIGH DENSITY

FIG. 3. Capacity for increase ($r_c \pm 2$ SE) of parent, F_1, and F_2 hybrid lines at high, limiting density (40 larvae/leaf). Abbreviations as in Figure 2.

plied to the components of r_c. Two crosses, both at low density, showed significant epistatic differences in r_c (Table 1A). Epistasis contributed to differences in $\ln R_0$ in the wFL $\times$ ON cross. There were no other significant nonadditive effects contributing to genetic differences in either cross for any other components of r_c.

Three crosses, all at high density, showed significant dominance but not epistatic differences in r_c (Table 1B). Epistasis contributed to differences in development time in the eFL $\times$ ME cross. Dominance contributed to differences in $\ln R_0$ in both the eFL $\times$ ME and wFL $\times$ ON crosses, and in fertility in the eFL $\times$ ME and ON $\times$ ME crosses. Dominance also contributed to differences in survivorship in the ON $\times$ ME cross.

Three crosses, two at low and one at high density, showed no significant nonadditive differences in r_c (Table 1C). Epistasis contributed to differences in generation time, development time, and $\ln R_0$ at low density in the ON $\times$ ME cross. Dominance contributed to differences in fertility at low but not high density in the wFL $\times$ eFL cross.

DISCUSSION

The genetic distance (Fig. 1) between two ancestral (southern) or two derived (northern) populations of *W. smithii* was substantially lower (0.01–0.02) than between ancestral and derived populations (~0.4). Although the geographic distance separating the two derived populations is nearly equal to the distance separating derived and ancestral populations, the northern populations are likely the product of a range expansion that has occurred subsequent to the retreat of the Laurentide Ice Sheet (Pielou 1991), and recently, relative to the differentiation of northern from southern populations. Thus, the more recent common ancestry of northern populations may explain the nonconcordance of geographic and genetic distance (Slatkin 1993). In *Drosophila* (Coyne and Orr 1989), genetic distances of 0.3–0.4 are associated with low levels of hybrid breakdown and we observe the same effect in *W. smithii* at near-optimal density (Fig. 2) where significant epistasis is associated with lower than expected performance (r_c) in the F_2 generation of crosses between southern and northern populations.

While epistasis has been shown to be an important component of the genetic architecture of individual life-history traits in several organisms (Vetukhiv 1953; Wallace and Vetukhiv 1955; Templeton et al. 1976; Burton 1990; Brown 1991; Parker 1992; Hard et al. 1992, 1993a), our results now provide a more direct demonstration that the evolutionary divergence among natural populations of a composite, more inclusive measure of fitness, has involved epistasis. Comparison of Figure 2 with Table 1 reveals that the pattern of

TABLE 1. Summary of nonadditive genetic differences between populations contributing to components of fitness (r_c). Table entries are probability values for χ^2 goodness-of-fit tests to the additive model of population differentiation. Bold type indicates significance at the $\alpha = 0.05$ level of protection for a sequential Bonferoni applied tablewide. If the additive model is rejected, then a subsequent test is made for goodness of fit to the additive-dominance model, and the probability values are provided in brackets below significant (rejected) tests for additivity. A superscript of DOM indicates that the additive-dominance model is not rejected and the source of nonadditivity relates to differences in dominance; a superscript of EPI indicates that the additive-dominance model is rejected and genetic differences between populations involve at least epistasis.

CROSS[1]	Gen. time	Dev. time	Mat. time	lnR_o	Fertility	Survivorship	Fecundity
A. Nonadditive genetic differences among components of r_c for crosses in which epistasis contributes to differences in r_c:							
wFL × ON^{LD} (Fig. 2A)	0.7985	0.8025	0.7945	**4 × 10^{-5}** [0.0024]^{EPI}	0.0073	0.9851	0.1018
eFL × ME^{LD} (Fig. 2C)	0.4700	0.3809	0.1225	0.1145	0.9103	0.3120	0.7189
B. Nonadditive genetic differences among components of r_c for crosses in which dominance, but not epistasis, contributes to differences in r_c:							
wFL × ON^{HD} (Fig. 3A)	0.0672	0.1148	0.2491	**0.0011**^{DOM} [0.3242]	0.2753	0.0035	0.1119
eFL × ME^{HD} (Fig. 3C)	0.0173	**0.0008** [0.0033]^{EPI}	0.0435	**0.0004**^{DOM} [0.7379]	**0.0005**^{DOM} [0.1261]	0.0885	0.0219
ON × ME^{HD} (Fig. 3B)	0.3413	0.4516	0.2851	0.0093	**0.0002**^{DOM} [0.5485]	**0.0016**^{DOM} [0.8848]	0.2144
C. Nonadditive genetic differences among components of r_c for crosses in which neither dominance nor epistasis contributes to differences in r_c:							
ON × ME^{LD} (Fig. 2B)	**7 × 10^{-12}** [0.0001]^{EPI}	**2 × 10^{-12}** [8 × 10^{-9}]^{EPI}	0.0032	**0.0009** [0.0030]^{EPI}	0.0091	0.1882	0.2091
wFL × eFL^{LD} (Fig. 2D)	0.1130	0.5945	0.6873	0.0605	**0.0002**^{DOM} [0.3222]	0.2851	0.5945
wFL × eFL^{HD} (Fig. 3D)	0.3926	0.1845	0.2923	0.1686	0.1854	0.6750	0.1746

[1] States or provinces by postal code: FL, Florida; ON, Ontario; ME, Maine; w, western; e, eastern. ^{LD} denotes cross performed at low, near-optimal density of 20 larvae/leaf. ^{HD} denotes cross performed at high, limiting density of 40 larvae/leaf. A reference to the figure in which the values of r_c are shown is provided below the label for each cross.

genetic divergence in r_c is not congruent with the pattern of genetic divergence in any of its components. The role of epistasis in the evolution of fitness would be underestimated by evaluation of any single performance measure, except for lnR_0 in the wFL × ON cross. Epistasis would be overestimated as a contributor to the evolution of fitness in derived populations (ON × ME) by evaluating mean generation time, development time, or lnR_0. Our results underscore previous arguments (Charlesworth and Charlesworth 1987; Waser and Price 1994) emphasizing the importance of evaluating composite indices of fitness, rather than individual fitness components or correlates, for testing evolutionary hypotheses.

Our results are especially pertinent to the evolution of natural populations for three reasons. First, in our experiments we assessed fitness under near-natural conditions, so that our results are directly relevant to populations in nature. Second, we have measured a composite index of fitness over the whole life-cycle of experimental cohorts, the most direct estimate of the rate of gene frequency change possible (Fisher 1930; Charlesworth 1980). Finally, along its evolutionary pathway from south to north in North America, W. smithii encounters decreased density-dependent development (Bradshaw and Holzapfel 1986). Hence, the transition in our experiments from high to low density, at which the effects of epistasis become apparent, reflects the conditions under which the evolutionary divergence of these populations has taken place in nature.

Fundamental to Wright's (1977) shifting balance theory

are the joint action of isolation and genetic drift, causing rearrangement of epistatic interactions during phase I, and the subsequent opportunity for derived populations to sample unique fitness peaks on the Wrightian "adaptive landscape." Previous results with W. smithii, in conjunction with those herein, provide evidence for such a rugged adaptive landscape as envisioned by Wright (1977). First, biogeographic, physiological, and behavioral evidence all indicate that W. smithii has evolved from south to north throughout its range in North America (Bradshaw and Lounibos 1977; Istock and Weisburg 1987; Bradshaw and Holzapfel 1990). Figure 2 thus demonstrates that genic interaction (epistasis), fundamental to phase I of Wright's theory, has contributed to the divergence of derived from ancestral populations. Second, during their evolutionary divergence from south to north in North America, W. smithii has experienced a climatic gradient of decreasing favorable season. The photoperiodic response mediating their seasonal development has tracked this climatic gradient very closely (Bradshaw 1976; Bradshaw and Lounibos 1977). But, the consistent increase in the critical photoperiod with latitude has involved the divergence of epistatic interactions. The effects of additive × additive and dominance × dominance interaction appear particularly strong, but the contribution of both these components of epistasis changes in magnitude and sign without regard to each other, or to the evolution of the mean phenotype (Hard et al. 1992, 1993a). Third, the results in Table 1 indicate that the genetic

architecture underlying fitness varies in a pattern that is unique to each cross.

Although we cannot definitively rule out the role of deterministic evolution in the divergence of ancestral and derived populations of *W. smithii*, ecological inference and genetic data are consistent with a stochastic, rather than deterministic, process operating along the evolutionary trajectory of this species. First, *W. smithii* is a weak-flying mosquito, highly prone to death by desiccation, that lives in discrete patches, restricting gene flow even among nearby bogs (Istock and Weisburg 1987). *Wyeomyia smithii*'s habitat thus provides the necessary isolation for the first phase of Wright's process. Second, results of a more extensive electrophoretic survey in *W. smithii* indicate that average gene diversity at 10 allozyme loci declines with increasing latitude ($R^2 = 0.69$, $P < 0.001$; Armbruster et al., unpubl. data), thus implicating the role of genetic drift during the divergence of northern from southern populations. Finally, photoperiodic response and preadult development time are genetically correlated within populations but these two traits have evolved independently over the range of *W. smithii*. This independent evolution may have been possible due to repeated founder events during the northward migration of *W. smithii*, facilitating the breakdown and independent reestablishment of genetic architectures (Hard et al. 1993b).

We have provided evidence that the adaptive landscape for *W. smithii* is uneven, that the opportunity for isolation exists, and argued that genetic drift has been an important contribution to the genetic differentiation of populations. However, we have not shown that the fitness peaks on this landscape are separated by valleys of lower fitness that constitute a barrier to deterministic evolution and that drift was required to traverse these valleys (Whitlock et al. 1995). We cannot, therefore, definitively conclude or exclude the possibility that Wright's "shifting balance" constituted the mode of population differentiation in *W. smithii*. We have, nonetheless, found that patterns of genetic differentiation among populations of *W. smithii* are consistent with the expected results of a Wrightian process.

As shown in Figure 3, the contribution of epistasis to hybrid fitness depends on larval density. At high density (Fig. 3), fitness in the F_1 is higher than expected from the additive model, indicating the positive contribution of increased heterozygosity to fitness. Fitness in the F_2 almost exactly fits the expectation under an additive-dominance model, indicating little contribution of epistasis to genetic differences among populations in the expression of fitness at limiting density. Thus, under stressful conditions, the advantages to fitness gained by increased heterozygosity clearly outweigh any disadvantages incurred through a breakdown of favorable genic interactions. Previous studies have demonstrated that genetic architectures can change under different environmental conditions (Jinks et al. 1973; Barnes et al. 1989; Blows and Sokolowski 1995). Furthermore, extensive data from the animal breeding literature (Barlow 1981) and evolutionary genetic studies (Hoffman and Parsons 1991) indicate that levels of heterosis tend to increase under increasingly stressful environmental conditions. Our results make it clear that the phenotype of F_2 hybrids between genetically differentiated populations is not just the product of differ-

ences in gene-gene interaction, but rather of the balance between the effects of epistasis and the effects of dominance.

These results bear on the genetics of small populations. With current levels of habitat destruction and population fragmentation, it may become increasingly necessary to manage locally inbred and endangered populations by outcrossing them to other, distant populations (Spielman and Frankham 1992). Outbreeding depression is a consistent concern (Templeton 1986). Just as deciding the conditions under which inbreeding depression should be measured is currently a major question in the field of conservation biology (Soulé and Kohm 1989; Pray et al. 1994), our results indicate that the same question applies to outbreeding depression. Outbreeding depression assessed under benign lab, zoo, or farm conditions may reveal the deleterious effects of outcrossing without recognizing the potentially offsetting advantages of increased heterozygosity in suboptimal or marginal conditions likely to be encountered in nature.

The hybrid vigor and outbreeding depression illustrated in Figures 2 and 3 show that two independent genetic interactions underlie the evolutionary divergence in fitness between ancestral and derived and between derived populations of *W. smithii*. Hybrid vigor results from differences among populations in the interaction of alleles within loci (dominance), while outbreeding depression reflects differences in the interaction between loci (epistasis). Furthermore, the contribution of these interactions to hybrid fitness depends upon the environmental conditions under which they are assessed. The involvement of both types of interaction underscores the importance of nonadditive genetic variation to the evolutionary differentiation of natural populations, and their environmental dependency has important implications for the management of inbred populations.

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