Dominance in Physiological Phenotypes and Fitness at an Enzyme Locus

Abstract. *Aminopeptidase-I allozymes, which are products of the* Lap *locus in the marine mussel,* Mytilus edulis, *differ in their catalytic efficiencies. These biochemical differences result in genotype-specific rates of change in the free amino acid pool, that is, in cell volume regulation, when mussels are subjected to changes in salinity. A high degree of dominance was found among genotypes for these biochemical and physiological phenotypes. Selection models that incorporate dominance adequately predict observed genotypic properties at the* Lap *locus among natural populations that exhibit clinal allele frequency. This suggests that a high degree of dominance for fitness must also occur at this locus in natural populations. These results provide additional evidence that the maintenance of an allele frequency cline is operating by natural selection at the* Lap *locus.*

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Biochemical differences in aminopeptidase-I (AM-I) allozymes (products of the *Lap* locus) in the marine mussel *Mytilus edulis* (*1*) result in genotype-specific rates of change in the free amino acid (FAA) pool when mussels are subjected to changes in salinity (*2*). Estimating fitness differentials associated with loci coding for enzymes is difficult, particularly in natural populations for which, at a minimum, genotype frequencies must be determined over all phases of an organism's life history (*3, 4*). In addition, demonstrating that natural selection influences variation at a specific locus rather than at linked loci requires a mapping of genetic variation through the phenotypic hierarchy and ultimately showing differences in fitness (*5, 6*). Earlier we examined the enzymological and physiological consequences of genetic variation of a lysosomal aminopeptidase (Lap) enzyme that removes NH_2-terminal amino acids from oligopeptides (*1*) in *M. edulis*. We now show that a pattern of biochemical dominance of the Lap^{94} allele over two other alleles is associated with physiological dominance. Hence the genotypic composition of natural populations of *M. edulis* can be best explained by a selection model involving dominance for the Lap^{94} allele.

Aminopeptidase-I genotypes with the Lap^{94} allele exhibit 20 percent greater activity than alternate genotypes without this allele, a difference that can be attributed to the greater catalytic efficiency (k_{cat}) of the Lap^{94} allele. In addition, Koehn and Immermann (*7*) reported that Lap^{94} heterozygotes in a natural population had significantly greater enzyme activity than would be expected of an additive model. We reanalyzed these data with two-way analysis of variance and confirmed the dominance of Lap^{94} (Fig. 1A). Genotypes with the Lap^{94} allele constitute a homogeneous group whether the allele is heterozygous or homozygous. Genotypes without Lap^{94} constitute a second homogeneous group having significantly lower enzyme activity, although analysis of variance does not show a difference between $Lap^{98/96}$ and $Lap^{98/94}$. Nor are $Lap^{96/94}$ and $Lap^{98/94}$ significantly different from one another (Fig. 1A). These data indicate dominance of Lap^{94} over alternative alleles. The degree of dominance (*h*) (*8*) of Lap^{94} over other alleles averages 0.55 for enzyme activity. Genotype-dependent measurements of specific activity for AM-I were repeated and gave identical results; the Lap^{94} allele exhibited a high degree of dominance ($h = 0.88$) (*9*).

Differences in catalytic efficiency (as measured by the ratio of k_{cat} to the Michaelis constant, K_m) among allozymes of AM-I result in genotype-dependent differences in the rates of cell volume regulation in *M. edulis*. When placed in a hyperosmotic medium, genotypes with the Lap^{94} allele are associated with greater rates of intracellular free amino acid (FAA) accumulation (*2, 10*). The statistical differences in FAA accumulation among genotypes also exhibited evidence of dominance, even though the pattern was less definitive of dominance than was enzyme activity. For example, although Lap^{94} genotypes constituted a homogenous group (Fig. 1B), the comparison limits of some genotypes in this group overlapped those of genotypes without Lap^{94}. Nevertheless, while the $Lap^{96/94}$ heterozygotes are statistically indistinguishable from genotypes in either group, the $Lap^{98/94}$ heterozygotes exhibited an FAA concentration that was greater than the midpoint of values expected in an additive model (*11*) ($t = 2.03$, d.f. = 42, $P < 0.05$). The average degree of dominance was 0.97 for the two Lap^{94} heterozygotes. Unfortunately, the three genotypes without Lap^{94} could not be analyzed separately, since these genotypes are uncommon in all samples and were especially rare in this sample.

When bivalves are placed in a hyposmotic medium they reduce their cell volume by releasing FAA into the hemolymph; these are ultimately excreted from the organism as FAA or ammonia (*12*). When *Mytilus* is transferred to low salinity conditions, the ensuing increases in FAA and ammonia excretion rates

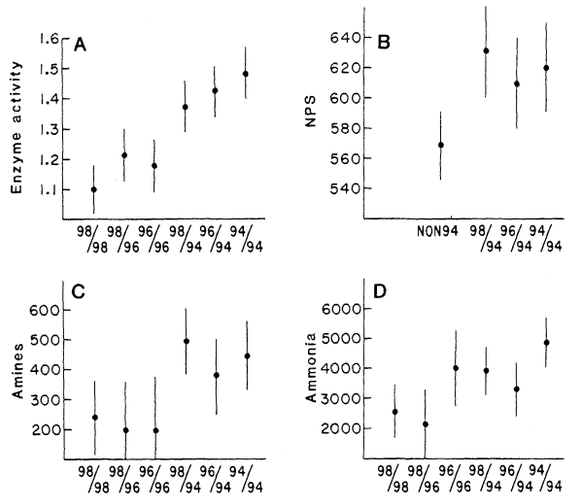

Fig. 1. Genotype-dependent differences in biochemical and physiological phenotypes of aminopeptidase-I in *Mytilus edulis*. Values are means ± comparison limits; if the comparison limits do not overlap the means are significantly different at $P < 0.05$. Except in (B), all analyses were done by two-way analysis of variance, with time in experimental conditions included as a random block. Reported values are unadjusted means averaged across blocks for each genotype. Error mean squares were used to generate comparison limits by the GT2 method (*16*). These statistical procedures were used to elucidate the relations among genotypes rather than to detect the effects of genotype or time alone. In all two-way analysis of variance studies, the effect of genotype was significant ($P < 0.05$). Interaction terms between genotype and time were not significant. (A) Units are enzyme activity per milligram of protein; data are from Immermann (*17*). (B) Units are intracellular concentration of free amino acids measured as ninhydrin-positive substances in micromoles per gram of dry weight; data are from Hilbish *et al.* (*2*). (C and D) Rates of excretion of primary amines and ammonia in micromoles per gram per hour; data are from Deaton *et al.* (*10*).

have a major genotypic component (*10*). Genotypic differences in excretion rates reflect the differential accumulation of FAA while in high salinity. Therefore, on the basis of the enzyme activity and FAA accumulation data, we predicted a high degree of dominance for amine and ammonia excretion rates. Evidence in support of this prediction was found by two-way analysis of variance of genotype and time in the low salinity condition (Fig. 1, C and D). For amine excretion, Lap^{94} heterozygotes and homozygotes formed a homogenous group, and the alternative genotypes also formed a homogenous group. This result is not unambiguous; $Lap^{96/94}$ and $Lap^{94/94}$ are not statistically distinguishable from genotypes without Lap^{94}. The 98/94 heterozygote had amine excretion rates significantly greater than the midpoint of the values expected in an additive model ($t = 2.33$, d.f. $= 110$, $P < 0.05$). The average dominance value for amine excretion rates was $h = 0.96$, indicating a high degree of dominance.

The ammonia excretion data was not definitive, principally because of the high value of $Lap^{96/96}$. The clustered pattern of Lap^{94} genotypes is still apparent, but high variances associated with the measurement of ammonia excretion rates led to extensive overlap among genotypes for this parameter. The dominance values, although small, are in the same direction as in the other analyses; the average degree of dominance of the Lap^{94} allele was $h = 0.06$.

The overall pattern among the four phenotypic parameters indicates that Lap^{94} heterozygotes are phenotypically similar to the Lap^{94} homozygote. Genotypes with the Lap^{94} allele always form a homogenous group, and genotypes without the Lap^{94} allele form another homogeneous group. Some genotypes belong statistically to both clusters. Nevertheless, in seven of eight cases, genotypes heterozygous for Lap^{94} were more similar to the Lap^{94} homozygote than to other homozygotes, such as $Lap^{96/96}$ or $Lap^{98/98}$. This is significant by a sign test at $P < 0.07$. In several cases, the degree of dominance of a phenotype exhibited by the Lap^{94} heterozygote is significantly greater than the midpoint of the values for two homozygotes. This is true for enzyme activity (*7*) and for $Lap^{94/98}$ with regard to accumulation of ninhydrin-positive substances (NPS) and amine excretion. Phenotypic dominance of the Lap^{94} allele is evidenced by this strong tendency for Lap^{94} heterozygotes to be more similar to the Lap^{94} homozygote than would be expected under a hypothesis of strict intermediacy for heterozygotes.

Table 1. Selection models used for generating expected relations between Lap^{94} frequencies and F values in Fig. 2. The column heading "without Lap^{94}" refers to genotypes 96/96, 96/98, and 98/98; "94/−" refers to heterozygotes 94/96 and 94/98; and "94/94" refers to Lap^{94} homozygotes; s is the selection coefficient.

Genotype model	Without Lap^{94}	94/−	94/94
Additive	1	$1 - s$	$1 - 2s$
Multiplicative	1	$1 - s$	$(1 - s)^2$
Dominance	1	$1 - s$	$1 - s$

The average degree of dominance for all phenotypic measures was 0.67, which is fairly high. If data on ammonia excretion are omitted, since this parameter is difficult to measure accurately, the average degree of dominance for other phenotypes is 0.83.

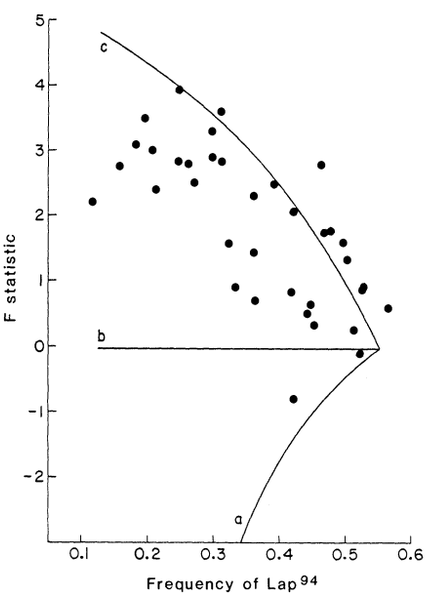

Fig. 2. Observed heterozygote deficiencies as a function of Lap^{94} allele frequency in populations of *M. edulis* from eastern Long Island Sound. Populations were repeatedly sampled from 1980 to 1983. Genotypes were determined by starch gel electrophoresis (*13*) and heterozygote deficiencies are expressed as Wright's F-statistic, $F = (e - o)/e$ where o and e are, respectively, the observed and expected (Hardy-Weinberg) numbers of Lap^{94} heterozygotes. Solid lines are expectations from selection models in which different fitness relations among genotypes are used (a, additive; *b*, multiplicative; and *c*, dominance) (see Table 1). Selection is directed against the Lap^{94} allele among a cohort of zygotes recruited from oceanic populations characterized by a gene frequency of 0.55 for the Lap^{94} allele. Zygotes were assumed initially to be in Hardy-Weinberg equilibrium. Selection was applied by multiplying initial genotype frequencies by the appropriate fitness (see Table 1). Selection coefficients were adjusted to give the observed range of Lap^{94} allele frequency values.

From the phenotypic data we should expect natural populations of *Mytilus* that are influenced by selection to show some evidence of dominance in fitness components. Since natural selection operates on phenotypes rather than the genotype, natural selection should consist of an array of selection coefficients for the various genotypes that reflect a high degree of dominance for Lap^{94}. Estimating selective coefficients in natural populations is onerous, and only in human sickle cell hemoglobin can such estimates be unequivocally attributed to a specific gene by a known functional mechanism. The populations of *Mytilus* in eastern Long Island Sound exhibit a sharp cline in allele frequencies at the *Lap* locus, with the Lap^{94} allele decreasing from an ocean frequency of 0.55 to a Sound frequency of 0.12 over a distance of 30 km (*13*). Koehn *et al.* (*14*) provided evidence for an annual cycle of recruitment relative to selective mortality in the region of this cline. Every year juvenile mussels are recruited from oceanic populations and subsequently exhibit strong genotype-dependent mortality directed against the Lap^{94} allele. This dynamic situation corresponds closely to a single generation of selection and allows an estimate of the relations among selection coefficients affecting *Lap* genotypes.

The frequency of Lap^{94} and heterozygote deficiencies for this allele were computed as Wright's F-statistic (*15*) for populations of *Mytilus* in eastern Long Island Sound from 35 collections made from 1980 to 1983. There is a negative relation between Wright's F-statistic and the frequency of Lap^{94} in clinal populations (Fig. 2). We can estimate the F-statistic that would be generated after one generation of selection sufficient to yield a given allele frequency. We considered three simple selection models; these were additive, multiplicative, and dominance fitness models (Table 1). A range of selection coefficients were used to generate the observed range of allele frequencies. The additive and multiplicative fitness models each yield expected F values that are inconsistent with the observed values (Fig. 2). However, the dominance model produced an expected relation between the F values and the frequency of Lap^{94} that closely parallels the relation observed in natural populations. Although these data could be fitted more closely with more elaborate selection models (*5*), it is noteworthy that a model that assumes complete dominance ($h = 1.0$) explains the observed data extremely well. For a selection model that includes partial dominance, the "best fit" model would be one in which domi-

nance for fitness was $h = 0.90$, or nearly complete dominance.

These data demonstrate that the genotype-dependent patterns of biochemical and physiological phenotypes measured under laboratory conditions are best described by dominance of the Lap^{94} allele. We conclude that there is both fitness dominance and phenotypic dominance for a variety of biochemical and physiological phenotypes of the *Lap* locus.

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The *x* Gene Is Essential for HTLV Replication

Abstract. *The human T-cell leukemia viruses (HTLV) are associated with T-cell malignancies in man and will transform normal human T cells in vitro. The mechanism of malignant transformation by HTLV is unknown but appears to be distinct from that of other classes of retroviruses, which induce malignant transformation through viral or cellular oncogenes. Recently a new gene, termed* x, *was identified in HTLV. This gene has been hypothesized to be the transforming gene of HTLV because of its conservation within the HTLV class of retroviruses. By in vitro mutagenesis of the HTLV-II* x *gene, it is now demonstrated that the presence of a functional* x *gene product is necessary for efficient HTLV transcription. Therefore, these studies provide direct evidence for an important function of the* x *gene in HTLV replication. The functional analogies between the* x *gene and transcriptional regulatory genes of some DNA viruses suggest that these viruses share similar mechanisms for cellular transformation.*

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The human T-cell leukemia viruses (HTLV) are associated with specific T-cell malignancies (*1–3*). Human T-cell leukemia virus type I (HTLV-I) is the likely etiologic agent for adult T-cell leukemia (ATL), which is endemic to parts of Japan, the Caribbean, and Africa (*1, 2*). Human T-cell leukemia virus type II (HTLV-II) was associated with a neoplasm in a single patient with a relatively benign T-cell variant of hairy cell leukemia (*4*). Despite the difference in associated diseases, both viruses will infect normal human T cells in vitro, causing their continued proliferation in the absence of exogenous interleukin-2 (*5–8*). The mechanism by which HTLV induces T-cell malignancies in vivo and T-cell transformation in vitro is unknown. Nucleic acid hybridization to cellular DNA (*9–11*) and nucleic acid sequencing (*12–14*) of the complete HTLV-I and HTLV-II proviral genomes have not revealed sequences in HTLV that are related to classical viral oncogenes. Unlike such replication-competent retroviruses as the lymphoid leukosis viruses (*15–17*), Moloney murine leukemia viruses (*18–20*), and mouse mammary tumor viruses (*21*), the HTLV proviral genome does not integrate near specific cellular sequences in tumors (*22*). This finding argues against the theory of transformation by insertional mutagenesis. Therefore, malignant transformation by HTLV appears to involve a novel mechanism.

The genomic structure of HTLV-I and HTLV-II is different from that of other retroviruses. In addition to the *gag, pol,* and *env* genes, HTLV has a fourth gene between *env* and the 3′ long terminal repeat (LTR), termed *x* (*12*) [also referred to as *lor* (*14*)]. RNA processing generates a subgenomic messenger RNA (mRNA) (*23*) that encodes a protein of 40 kilodaltons (kD) in HTLV-I–infected cells and 37 kD in HTLV-II–infected cells (*24–26*). The function of these proteins is unknown; however, the conservation of the *x* gene in the genomes of both HTLV-I (*12*) and HTLV-II (*14, 27*) suggests a role for the *x*-encoded protein in cellular transformation. It has been proposed that these proteins have a role in the enhancement of transcription by the HTLV LTR's (*28*), which would be analogous to the immediate early gene functions of the adenoviruses (*29, 30*), the papovaviruses (*31*), and some herpesviruses (*32*). We have used a clone of infectious, proviral HTLV-II DNA (*11, 27, 33*) to study the role of the *x* gene of HTLV in viral replication. The results demonstrate that the *x* gene is essential for high levels of HTLV-II transcription. In the absence of a functional *x* gene, approximately 100-fold lower levels of viral mRNA are transcribed.

As with T cells, B cells will also support a productive HTLV infection although HTLV does not transform B cells. Some Epstein-Barr virus (EBV)–transformed B-cell lines infected with HTLV-I have been isolated from ATL patients (*34*) and an EBV-transformed B-cell line infected with HTLV-II was obtained from peripheral blood of the patient Mo (*8*). The kinetics of productive infection by HTLV-II in B-cell lines in vitro is similar to in vitro infection of peripheral blood T cells. Therefore, infection of B-cell lines by HTLV provided a model system in which to study HTLV replication. We have previously described a transfection system for HTLV-II in B-cell lines (*27, 33*).

An activity in HTLV-infected cells has been found which enhances the transcription of HTLV LTR's introduced into the cells by DNA transfection (*28*). It was hypothesized that this transcriptional regulatory function was the product of the *x* gene. To examine these findings with respect to HTLV-II, we made a recombinant construct in which the expression of the gene for chloramphenicol acetyltransferase (CAT) (*35*) was dependent on the function of the HTLV-II LTR. We used an HTLV-II