

Testing the Adaptive Significance of Acclimation: A Strong Inference Approach(1).

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Acclimation is a common phenotypic response to environmental change. Acclimation is often thought to enhance performance and thus to be adaptive. This view has recently been formalized as the "Beneficial Acclimation Hypothesis" and predicts that individuals acclimated to one environment perform better in that environment than do individuals acclimated to a different environment. Although Beneficial Acclimation is appealing and widely supported, recent studies with *E. coli* and *Drosophila* have challenged its general validity. Although these challenges could be dismissed as mere exceptions, they encourage a re-evaluation of the adaptive significance of acclimation. Our philosophical approach differs from that of most previous studies of acclimation, in which the prediction derived from a Beneficial Acclimation perspective (e.g., heat tolerance is positively correlated with acclimation temperature) is tested against the null hypothesis ("single hypothesis approach"). Instead, we follow Huey and Berrigan (1996) in advocating a strong inference approach (*sensu* Platt, 1964), which recognizes that Beneficial Acclimation is actually one of a set of competing hypotheses that make different predictions as to how developmental temperature influences the thermal sensitivity of adults ("developmental acclimation"). Using this perspective, Huey and Berrigan proposed a factorial experimental design (3 developmental by 3 adult temperatures) designed to discriminate among all competing hypotheses. We now derive a formal statistical model (ANOVA with orthogonal polynomial contrasts) for this experimental design and use it to evaluate simultaneously the relative impact of each competing hypothesis. We then apply this model to several case studies (*Drosophila*, *Volvox*, *Trichogramma*), and we review also a recent study with *E. coli*. The influence of Beneficial Acclimation is supported (albeit often weakly) in most cases. Nevertheless, other hypotheses (especially the Optimal Developmental Temperature Hypothesis) often have a greater impact. Even so, however, Beneficial Acclimation usually predicts relative performance at extreme test temperatures. We conclude that, although rumors of its death are premature, Beneficial Acclimation cannot be viewed as the dominant expectation, at least with regard to developmental temperature acclimation. Moreover, our findings reinforce the view that a strong inference approach provides a more comprehensive portrait of complex biological responses than do single-hypothesis approaches.

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INTRODUCTION

Acclimation is often defined as a phenotypic alteration in physiology that occurs in response to (or in anticipation of) an environmental change. Studies of acclimation have been central to comparative physiology at least since the days of Claude Bernard (1865, 1949 edition), and acclimation has traditionally been assumed to benefit organisms by helping them compensate for the impact of environmental change. This view was recently codified by Leroi et al. (1994) as the Beneficial Acclimation Hypothesis: "... acclimation to a particular environment gives an organism a performance advantage over another organism that has not had the opportunity to acclimate to that particular environment."

Evidence that acclimation is beneficial comes from a variety of sources, including one's own personal experience. For instance, anyone who has traveled from sea level to moderate elevation in the mountains observes first-hand that the initial physiological stress of high elevation is gradually moderated by acclimation. Nevertheless, such physiological responses to environmental change are not inevitably beneficial. For example, climbers who attempt the highest mountains experience a variety of physiological changes (e.g., muscle wasting and elevated pH), but these changes are pathological, not beneficial! Moreover, performance in one environment may sometimes be enhanced by acclimating to a different environment: for example, some athletes prepare for a low-altitude endurance race by training at moderate elevation.

The physiological literature on acclimation responses--especially those dealing with temperature--strongly reinforces our

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personal impression that acclimation is generally thought beneficial (Hochachka and Somero, 1984; Prosser, 1986; Cossins and Bowler, 1987; Rome et al., 1992). For example, Kinne (1962) wrote: "An evergrowing body of information provides ample evidence for a remarkable capacity of animals and plants to compensate for the ill effects of environmental changes.... Such adjustments tend to bring about an increased efficiency of performance and a greater fitness for continued existence under new conditions." Similarly, Cossins and Bowler (1987, p. 156) noted with respect to acclimatory adjustments: "Usually these adjustments are compensatory in nature but other types of response exist that are no less adaptive within their particular ecological context."

Whether there actually is "ample evidence" for "greater fitness" (c.f., Kinne, 1962, above) is questionable. Even so, voluminous evidence does exist to show that acclimation--especially to temperature--often enhances performance in a changed environment (Prosser, 1986). A classic example shows that the optimal swimming temperature of goldfish increases with acclimation temperature (Fig. 1, Fry and Hart, 1948). Such patterns are common and are usually interpreted as beneficial (but see below).

[Figure 1 ILLUSTRATION OMITTED]

Despite the existence of widespread support for the Beneficial Acclimation Hypothesis, several papers appeared between 1994 and 1996 that challenged the

generality of the hypothesis (Krebs and Loeschcke, 1994; Leroi et al., 1994; Hoffmann, 1995; Huey et al., 1995; Zamudio et al., 1995; Padilla and Adolph, 1996). Leroi et al. (1994) presented the most direct and forthright challenge: "We must ... reject the generality of the beneficial acclimation assumption: a fitness benefit may or may not result from acclimation. In fact, fitness in a particular environment may actually decrease because of acclimation to that environment."

How should these new challenges be treated? Given the mass of evidence supporting Beneficial Acclimation (above), one could dismiss these challenges as trivial exceptions. However, we have instead chosen here and in related papers (Huey and Berrigan, 1996; Kingsolver and Huey, 1998) to interpret these challenges as encouragement to take a fresh look at how acclimation responses are studied and interpreted in an adaptive context. We do so both on empirical and philosophical grounds.

We begin by describing traditional acclimation experiments, which involve testing whether observed responses correspond with those predicted by Beneficial Acclimation. We then argue that such single-hypothesis approaches are inappropriate in many contexts, because Beneficial Acclimation is in fact only one in a set of possible phenotypic responses to acclimation. The recognition of multiple competing hypotheses leads naturally to a "strong inference" approach (Platt, 1964; Feder, 1987; Huey and Berrigan, 1996) that attempts to evaluate the relative importance of multiple competing hypotheses, not just the importance of a single hypothesis. To implement this approach, we review a factorial experimental design (Huey and Berrigan, 1996) and then develop a formal statistical model that can simultaneously evaluate all competing hypotheses. Finally, we use this approach to analyze acclimation in several taxa (*Drosophila*, *Volvox*, *Trichogramma*). Our analyses often support Beneficial Acclimation. However, our analyses also show that competing hypotheses usually have an even more profound impact on performance than does Beneficial Acclimation, except at extreme test temperatures. We conclude that while Beneficial Acclimation may not be dead, it is no longer the only dog on the block.

We note that "acclimation" is sometimes viewed as a reversible phenotypic response of an individual organism. Even so, the term is often used somewhat more generally for any non-genetic, physiological adjustment, even if irreversible in a given individual (Cossins and Bowler, 1987; Leroi et al., 1994; Huey and Berrigan, 1996; herein). In fact, most of the studies we discuss below have examined "developmental acclimation," which is the phenotypic response of adults to conditions experienced during development (specifically for insects, from egg to adult). Note, however, that development at different temperatures will induce irreversible as well as reversible changes. Consequently, some readers may prefer the generic phrase "phenotypic plasticity" over "developmental acclimation."

RECENT EMPIRICAL CHALLENGES TO BENEFICIAL ACCLIMATION

The first major challenge to Beneficial Acclimation came from studies of *E. coli* by Leroi et al. (1994). These workers

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acclimated bacteria at two temperatures (32 [degrees] C and 41.5 [degrees] C) for several generations and then measured fitness at 32 [degrees] C and 41.5 [degrees] C and also survival at 50 [degrees] C. Leroi et al.'s (1994) index of fitness was relative population growth of clones in a competition assay. As predicted by Beneficial Acclimation, the 41.5 [degrees] C-acclimated bacteria showed enhanced survival of a severe heat stress (50 [degrees] C), and the 32 [degrees] C line had higher relative fitness at 32 [degrees] C than did the 41.5 [degrees] C lines. However, contrary to Beneficial Acclimation, the 41.5 [degrees] C lines actually had relatively low fitness at 41.5 [degrees] C in competition with the 32 [degrees] C-acclimated lines. On the basis of this observation, Leroi et al. (1994) rejected the generality of beneficial acclimation (see quote above).

The Leroi et al. (1994) study (plus a major extension by Bennett and Lenski, 1997) has to be taken seriously because it is based on a measure of fitness per se rather than of performance or some presumed correlate of fitness. Thus the results are unambiguous with respect to evaluating the net evolutionary benefits of acclimation. Of course, the study itself can be debated on other grounds (for example, on the relevance of the temperatures used [see Bennett and Lenski, 1997] or for studying lines that were adapted to constant temperature [J. Kingsolver, personal communication] or for using the term "acclimation" when the experiments actually involve across-generation responses [see Bennett and Lenski, 1997]). Nevertheless, Leroi et al. (1994) and Bennett and Lenski (1997) are the only studies to date that have directly measured the fitness consequences of acclimation to temperature.

The Leroi et al. (1994) result is not unique in challenging Beneficial Acclimation. For example, two studies in our lab tested whether "developmental acclimation" (i.e., rearing *D. melanogaster* from egg to adult at 18 [degrees] C vs. 25 [degrees] C) influenced male dominance (Zamudio et al., 1995) or female fecundity (Huey et al., 1995). These studies took an explicit strong-inference approach. Neither study supported Beneficial Acclimation (but see Nunney and Cheung, 1997), but did support competing hypotheses (see below).

Beneficial acclimation has been challenged on conceptual grounds as well. Virtually all studies focus exclusively on the gross benefits of acclimation, ignoring the associated costs (see DeWitt et al., 1998). However, Hoffmann (1995) called attention to the potential importance of considering the net benefits of acclimation. Further, Padilla and Adolph (1996) argued that plastic responses can be disadvantageous when the activation of the plastic response lags an environmental change. Finally, Kingsolver and Huey (1998) outlined a variety of issues that are relevant to analyses of the evolution of acclimation responses and noted that environmental unpredictability can limit the adaptedness of acclimation responses.

Concerns as to whether acclimation is invariably beneficial are not new. For example, Precht et al. (1973) noted that the metabolic responses of some ectotherms to decreased temperature sometimes exacerbated the effects of temperature rather than ameliorated them. So Precht et al. (1973) referred to such non-homeostatic responses as "paradoxical" compensation. (Later workers changed the term to "inverse" compensation, and argued that it was adaptive in conserving energy.) Further, Precht et al. (1973, p. 325) noted that the words "acclimation or acclimatization" imply a selective advantage, but that the identification of such usefulness was largely unstudied. Nevertheless, our reading--as well as that of Leroi et al. (1994)--of the standing literature is that acclimation responses are assumed beneficial until proven otherwise.

SINGLE-HYPOTHESIS VERSUS STRONG INFERENCE APPROACHES

Most analyses of the adaptive significance of acclimation are examples of what might be called "single-hypothesis" hypothesis testing. For example, a researcher might test whether acclimation to high temperature (or long photoperiod) enhances heat tolerance, as predicted from a Beneficial Acclimation perspective. If the null hypothesis of no acclimation effect is rejected, then the results are interpreted as supporting Beneficial Acclimation.

Such approaches interpret experimental data in the light of only one hypothesis at a time. They have a great and proven tradition in acclimation studies in particular and in science in general. Nevertheless, single-hypothesis testing has long been known to have significant limitations. For example, in a classic paper, Chamberlain (1890) argued in favor of "the method of multiple working hypotheses." He felt that having multiple hypotheses would not only reduce the likelihood of observer bias associated with testing a single (often "pet") hypothesis, but that it would also illuminate complex problems from new perspectives. Platt (1964) extended Chamberlain's advance by formalizing a "strong inference" approach (see below), and argued that such approaches should speed scientific progress as well as promote creativity relative to

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single-hypothesis approaches.

Platt (1964) conceived of strong inference as involving three sequential (and repeating) steps: 1) devise multiple (or alternative) hypotheses, 2) devise a "crucial" experiment that will exclude one or more of the multiple hypotheses, and 3) execute the experiment as cleanly as possible. Then, based on the outcome, reiterate the above procedure by deriving "subhypotheses or sequential hypotheses." Platt argued that progress would be speeded by testing multiple hypotheses simultaneously rather than sequentially.

Platt (1964) likened strong inference to the act of climbing a tree. At each fork, one uses the results of a crucial experiment to determine whether to follow the left or the right branch (assuming only two mutually exclusive hypotheses). One then climbs to the next fork and performs another crucial experiment. In programming jargon, strong inference is analogous to a series of CASE statements (in case of condition 1, do X; condition 2, do Y).

Feder (1987) argued for the incorporation of strong inference approaches in studies of evolutionary physiology. Zamudio et al. (1995), Huey et al. (1995), and especially Huey and Berrigan (1996) applied this approach to the special case of acclimation to temperature. They developed a set of alternative hypotheses (next section), and described "crucial" experiments to distinguish among them (below). However, they did not propose a formal statistical model, which we do below.

MULTIPLE HYPOTHESES OF ACCLIMATION RESPONSES

Beneficial Acclimation is one possible phenotypic pattern of acclimation, but it isn't the only possible response. In fact, several competing hypotheses have been proposed for the specific case of the effects of developmental temperature on thermal sensitivity of adult performance ("developmental acclimation": Zamudio et al., 1995; Huey et al., 1995; Huey and Berrigan, 1996; Herron, 1996). All these hypotheses are diagrammed in Figure 2 and are briefly described (below).

[Figure 2 ILLUSTRATION OMITTED]

Beneficial acclimation hypothesis

In Beneficial Acclimation, acclimation to one temperature confers a performance advantage at that temperature but a performance disadvantage at different temperatures (Leroi et al., 1994). In a basic 2 by 2 (two acclimation, two test treatments) factorial design, the expected pattern of performance is straightforward (Leroi et al., 1994; Zamudio et al., 1995). However, when more complex designs are required (Huey and Berrigan, 1996; see DEVELOPING A CRUCIAL EXPERIMENT), the interaction of acclimation and test temperature on performance becomes complex (Fig. 2a): in fact, reaction norms for different acclimation treatments can cross and even be non-linear.

Optimal developmental temperature hypothesis

Cohet and David (1978) first raised this hypothesis based on a study of the effects of developmental temperature on various life history traits of *D. melanogaster*. They noted that flies reared at intermediate temperatures (22 to 25 [degrees] C for *D. melanogaster*) were vigorous and performed well in a variety of adult thermal environments. Presumably, development at such intermediate to warm temperatures is harmonious and results in adults with well integrated physiology that do well in diverse thermal environments (Fig. 2B). This hypothesis was formally suggested as an alternative to Beneficial Acclimation by Zamudio et al. (1995), Huey et al. (1995), and Huey and Berrigan (1996).

Note, however, that this hypothesis must be applied with care. For example, a study using two extreme and one intermediate developmental temperatures will almost certainly generate results consistent with this hypothesis, simply because development at extreme temperatures is pathological. Consequently, the key issue is not whether an optimal developmental temperature exists (it must), but rather how broad the optimal temperature range is (A. E Bennett, personal communication).

Colder ("bigger") is better

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Ectotherms are larger in size if they develop at low temperatures (Atkinson and Sibly, 1997), and large size is often an ecological advantage (Partridge et al., 1987; Barbault, 1988). Consequently, the large size of ectotherms reared at low temperature might give them a performance advantage--in any adult thermal environment (Fig. 2c)--over ectotherms reared at high temperature. Zamudio et al. (1995) referred to this as the "Bigger is Better Hypothesis," though Herron (1996) retitled it as "Colder is Better" (because colder developmental temperatures could enhance performance for reasons other than, or in addition to, size per se). The implicit assumption of this hypothesis is that large size is sufficiently advantageous to outweigh any effect of Beneficial Acclimation.

Warmer is better

This hypothesis (Fig. 2d) is the reciprocal of Colder is Better and applies to species in which small adults have ecological and behavioral advantages (e.g., McLachlan and Allen, 1987; McLachlan and Neems, 1989; Aspi and Hoikkala, 1995). Alternatively, warmer could appear to be better if low-temperature acclimation leads either to diapause or to a general reduction in physiological activity (see below).

Developmental buffering

By this hypothesis developmental temperature does not affect adult temperature sensitivity at all (not shown). This could reflect past selection having favored developmental mechanisms that effectively buffer temperature perturbations, or it could simply reflect the null hypothesis of no effect (Huey and Berrigan, 1996).

DEVELOPING A CRITICAL EXPERIMENT

The first step in testing the validity of Beneficial Acclimation and of its competitors (next section) requires developing a suitable experimental protocol. Initial attempts to test Beneficial Acclimation employed variants on a 2 by 2 (or 2 by 3) factorial design; namely, individuals were subjected to one of two developmental temperatures and tested at one of two adult temperatures (Leroi et al., 1994, Zamudio et al., 1995, Huey et al., 1995). Unfortunately, this design is inadequate to simultaneously test all competing hypotheses. For example, Zamudio et al. (1995) found that flies reared at 25 [degrees] C were dominant over flies reared at 18 [degrees] C, whether tested at either 18 or at 27 [degrees] C. This pattern rejects Beneficial Acclimation and also Colder is Better; however it is consistent with (and does not distinguish between) both Optimal Developmental Temperature and Warmer is Better.

In response to limitations of the 2 by 2 design, Huey and Berrigan (1996) noted that a 3 by 3 factorial design (3 acclimation crossed with 3 test temperatures) would allow for the simultaneous evaluation of all competing hypotheses. More elaborate versions of this model are feasible. For example, Herron (1996) used a 3 by 6 factorial design, thereby gaining a more complete portrait of the thermal sensitivity of adult performance.

DEVELOPING A STATISTICAL MODEL

Our next challenge was to develop a statistical model that uses data from a 3 by 3 (or similar) factorial design and then tests simultaneously Beneficial Acclimation and other competing hypotheses. Because the various hypotheses are unlikely to be mutually exclusive (i.e., more than one hypothesis could be supported), we sought a statistical model that would enable us to test simultaneously the relative impact of all hypotheses.

Before developing the actual model, we digress for a moment to describe why a complex model is necessary. Reconsider the classical data (Fig. 1) of Fry and Hart (1948) on the thermal dependence of swimming performance of goldfish acclimated to a variety of temperatures. Fish acclimated to warmer temperatures have higher "optimal" temperatures for swimming than do fish acclimated to cooler temperatures, consistent with Beneficial Acclimation. However, note that the maximal performance of fishes at their optimal temperature increases markedly as acclimation temperature increases from 5 [degrees] to 25 [degrees] C, and then declines at 30 [degrees] C, the highest acclimation temperature (Fig. 1). Thus even though fish acclimated to low temperature swim faster at low temperature than do fishes acclimated to higher temperatures, they can't swim nearly as quickly as fish acclimated to higher temperatures. This pattern is reminiscent of an "optimal" acclimation temperature, and perhaps a "warmer acclimation temperature is better." So even though beneficial acclimation is evident in these data, other patterns also appear present. Obviously, we need a way to partition

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the relative impact of each.

Our experimental design (3 by 3 factorial) lends itself to a standard ANOVA. However, simple ANOVA models ignore the inherent ordering of predicted effects of developmental temperatures, and consideration of order is crucial to testing several of the acclimation hypotheses. (For example, "Bigger is better" predicts that the relative performance rank for the three developmental temperatures is low [is greater than] intermediate [is greater than] high.) Polynomial regression (Gavrilets and Scheiner, 1993; Delpuech et al., 1995) incorporates order of factors, but is inappropriate for a fixed-factor design with only three levels. Isotonic regression (Gaines and Rice, 1990) and other ordered heterogeneity tests (Rice and Gaines, 1994) also incorporate order, but are currently designed to evaluate only a single hypothesis, not to evaluate the relative importance of several competing hypotheses.

Fortunately, however, ANOVA with ordered factors (developmental temperature in this case) is a suitable approach. In particular, the single degree-of-freedom, orthogonal polynomial contrasts dissect the linear and curvilinear (=quadratic) influences of developmental temperature. Detecting curvilinearity is crucial, for example, to a test of the Optimal Developmental Temperature Hypothesis. The basic model we use is:

$$P \sim [T.sub.test] + [T.sub.dev(l)] + [T.sub.dev(q)] + ([T.sub.test] \times [T.sub.dev(l)]) + ([T.sub.test] \times [T.sub.dev(q)]) + [Epsilon],$$

where P refers to the performance (or fitness) measure, [T.sub.test] is the adult test temperature, [T.sub.dev(l)] is the linear effect of development temperature, [T.sub.dev(q)] is the quadratic effect of development temperature, and the remaining two terms describe the interactions between test and development temperatures. This model allows us to assess the significance of both the linear and quadratic influences of developmental temperature, with the proviso that the linear and quadratic coefficients are fully orthogonal and thus independent.

Using the above model to evaluate competing hypotheses is straightforward (see Fig. 2). Colder is better (Fig. 2C) or Hotter is better (Fig. 2D) will be supported if the linear effect of developmental temperature ([T.sub.dev(l)]) is significant and if the coefficient is negative or positive, respectively. Optimal Acclimation Temperature (Fig. 2B) will be supported if the quadratic effect of acclimation temperature ([T.sub.dev(q)]) is significant and if the curvature is concave downward. Beneficial Acclimation (Fig. 2A) requires that the interactions are significant: in particular, it requires that the significance of the linear and quadratic effect of development temperature should vary with test temperature (see Fig. 2A). Developmental Buffering (not shown), which is also the null hypothesis (see above), will be supported if only [T.sub.test]--but none of the terms involving developmental temperature--is significant.

Although orthogonal polynomial contrasts are amenable to our purposes, these analyses demand that the data rather strictly meet the general assumptions of ANOVA. Moreover, estimation of the orthogonal components requires a balanced design; and homogeneity of variances within cells and normally distributed residuals are also assumed. These assumptions were met for most of the experiments we review (below), except for *Trichogramma*, in which all individuals from two developmental temperatures failed to produce any eggs at one test temperature. Accordingly, we chose not to do a formal analysis of the *Trichogramma* data.

Other approaches are possible. Noting that the various hypotheses (Fig. 2) are predictions concerning the relative performance of individuals with different developmental histories at various test temperatures, Herron (1996) developed an analysis based on multiple regressions of rank performance on test temperature. Both analyses yield similar patterns for *Volvox* (see below).

CASE STUDIES

In selecting studies to demonstrate our approach, we generally restricted our sample to studies that used a 3 by 3 (or bigger) experimental design. Note that two of these studies (Barnes and Laurie-Ahlberg, 1986; Zwaan et al., 1991) used a 3 by 3 factorial design, but their motivation for so doing was unrelated to testing Beneficial Acclimation. Nevertheless, these experiments meet our requirements and are directly suitable for re-analysis in the present context. In all cases described below, test temperature (not surprisingly) has a much greater influence on performance than does development temperature. However, our focus here is on the patterns of deviation imposed by developmental temperature.

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Longevity of *Drosophila*

Zwaan et al. (1991) measured longevity of *D. melanogaster* in a 3 by 3 design (20 [degrees], 25 [degrees], 29 [degrees] C). Their purpose was to test a developmental theory of aging (Lints and Lints, 1971), but their data are suitable for analyses here. (For illustrative purposes only, we assume that longevity is correlated with fitness.)

Longevity was influenced by developmental temperature (Fig. 3A). In particular, longevity was greatest for flies from an intermediate (25 [degrees] C) developmental temperature. Longevity was generally intermediate for flies from 29 [degrees] C developmental temperature, and shortest for flies from 20 [degrees] C.

[Figure 3 ILLUSTRATION OMITTED]

ANOVA with orthogonal polynomial contrasts clarifies the impact of developmental temperature (Table 1). The quadratic effect for developmental temperature ([T.sub.dev(q)]) for was highly significant (P [is less than] 0.001) and concave downward, and the linear effect of developmental temperature ([T.sub.dev(l)]) was marginally significant and positive (P = 0.029). None of the interactions approached significance.

TABLE 1. ANOVA with orthogonal polynomials for effects of test ([T.sub.test]) and of the linear ([T.sub.dev(l)]) and quadratic ([T.sub.dev(q)]) contrasts for developmental temperature on performance (longevity or power output) in *Drosophila melanogaster*.(*)

Population (trait)	Factor	df	Sum sq
D. melanogaster (longevity)	Sex	1	0.20
	[T.sub.test]	1	1,933.44
	[T.sub.dev(l)]	1	7.65
	[T.sub.dev(q)]	1	27.24
	[T.sub.test] x [T.sub.dev(l)]	2	3.90
	[T.sub.test] x [T.sub.dev(q)]	2	3.80
D. melanogaster A (power output)	[T.sub.test]	2	68.84
	[T.sub.dev(l)]	1	0.56
	[T.sub.dev(q)]	1	3.53
	[T.sub.test] x [T.sub.dev(l)]	2	3.71
	[T.sub.test] x [T.sub.dev(q)]	2	0.92
D. melanogaster AB (power output)	[T.sub.test]	2	22.22
	[T.sub.dev(l)]	1	0.02
	[T.sub.dev(q)]	1	0.79
	[T.sub.test] x [T.sub.dev(l)]	2	1.14
	[T.sub.test] x [T.sub.dev(q)]	2	0.41
D. melanogaster R (power output)	[T.sub.test]	2	63.56
	[T.sub.dev(l)]	1	0.72
	[T.sub.dev(q)]	1	5.17
	[T.sub.test] x [T.sub.dev(l)]	2	5.12
	[T.sub.test] x [T.sub.dev(q)]	2	1.34
D. melanogaster N (power output)	[T.sub.test]	2	7.92
	[T.sub.dev(l)]	1	0.01
	[T.sub.dev(q)]	1	0.44
	[T.sub.test] x [T.sub.dev(l)]	2	0.35
	[T.sub.test] x [T.sub.dev(q)]	2	0.17
Population (trait)	Factor	F-value	Pr(F)
D. melanogaster (longevity)	Sex	0.10	0.719
	[T.sub.test]	601.99	0.000
	[T.sub.dev(l)]	4.77	0.029
	[T.sub.dev(q)]	16.96	0.000
	[T.sub.test] x [T.sub.dev(l)]	1.20	0.299
	[T.sub.test] x [T.sub.dev(q)]	1.20	0.306
D. melanogaster A (power output)	[T.sub.test]	1,322.45	0.000
	[T.sub.dev(l)]	21.47	0.000

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	[T.sub.dev(q)]	135.77	0.000
	[T.sub.test] x [T.sub.dev(l)]	71.37	0.000
	[T.sub.test] x [T.sub.dev(q)]	17.67	0.000
D. melanogaster AB (power output)	[T.sub.test]	477.52	0.000
	[T.sub.dev(l)]	0.77	0.381
	[T.sub.dev(q)]	34.10	0.000
	[T.sub.test] x [T.sub.dev(l)]	24.54	0.000
	[T.sub.test] x [T.sub.dev(q)]	8.89	0.000
D. melanogaster R (power output)	[T.sub.test]	1,504.53	0.000
	[T.sub.dev(l)]	34.21	0.000
	[T.sub.dev(q)]	244.92	0.000
	[T.sub.test] x [T.sub.dev(l)]	121.13	0.000
	[T.sub.test] x [T.sub.dev(q)]	31.70	0.000
D. melanogaster N (power output)	[T.sub.test]	130.68	0.000
	[T.sub.dev(l)]	0.43	0.512
	[T.sub.dev(q)]	14.51	0.000
	[T.sub.test] x [T.sub.dev(l)]	5.78	0.004
	[T.sub.test] x [T.sub.dev(q)]	2.75	0.069

(*) The factor involving [T.sub.dev] with the greatest F-value for each population is boldfaced. Longevity data reanalyzed from Zwaan et al. (1991), and power output data from Barnes and Laurie-Ahlberg (1986). The letters for Populations in the power output analyses refer to source localities in Barnes and Laurie-Ahlberg (1986) (A and AB are populations near Sydney, Australia; R is Raleigh, North Carolina, and N is Groningen, The Netherlands).

These analyses strongly support the Optimal Developmental Temperature Hypothesis, and also modestly support Warmer is Better. Support for the former hypothesis is probably not an artifact of the low and high developmental temperatures (20, 29 [degrees] C) being stressful, as viability (egg to adult) was independent of developmental temperature (see table 1 in Zwaan et al., 1991). These analyses lend no support to Beneficial Acclimation.

Flight power output of Drosophila

Barnes and Laurie-Ahlberg (1986) conducted an elegant, massive, and complex experiment to explore genetic variation in flight metabolism in *D. melanogaster*. The performance trait of interest was maximal power output, which was calculated using Ellington's (1984) formula. Flies from four localities on three continents (Table 1) were sampled, reared at three developmental temperatures (15 [degrees], 22 [degrees], 30 [degrees] C), and tested for flight power output as adults at each of the three temperatures (Barnes and Laurie-Ahlberg [1986] also scored [G.sub.pdh] allotype, but we ignore these data in our reanalysis.) The original experiment involved repeated measurements on individual flies, but we choose not to use a repeated-measures model here so that we could evaluate the relative importance of the competing acclimation hypotheses (Table 1).

At adult test temperatures of 22 [degrees] and 30 [degrees] C, flies reared at intermediate temperature (22 [degrees] C) produced the highest power out put (Fig. 3B). However, at a test temperature of 15 [degrees] C, flies reared at 15 [degrees] C had the highest power output.

The quadratic contrast for developmental temperature was concave downward and was highly significant (P [is less than] 0.001, Table 1) in all populations, and in fact had the highest F-value in three of the four populations. The linear contrast for developmental temperature was significant and positive in two of four populations. Finally, the test temperature by developmental temperature (both linear and quadratic) interactions were also significant in all cases (Table 1).

Overall, these analyses (major quadratic effect for developmental temperature) most strongly support the Optimal Developmental Temperature Hypothesis (see Fig. 2B). However, the highly significant interactions between test and developmental temperature also support Beneficial Acclimation in all populations (Fig. 2A); and the significant and positive linear effect of developmental temperature supports Warmer is Better in two of four populations (Fig. 2D).

The biological importance of the statistical support for the Optimal Developmental Temperature Hypothesis in this example is somewhat suspect. Both the low (15 [degrees] C) and the high (30 [degrees] C) developmental temperatures

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used in these experiments are approaching the limits for normal development of *D. melanogaster* (David et al., 1983) and thus may have been stressful. Indeed, in a subsequent experiment with a subset of the original lines, Barnes et al. (1989) found that flies reared at 16 [degrees] or at 29 [degrees] C had significantly reduced viability (egg to adult) relative to flies reared at 22 [degrees] C. Consequently, the apparent superiority of 25 [degrees] C-acclimated flies may be an artifact of developmental damage to flies reared at 15 [degrees] or 30 [degrees] C.

Trichogramma parasitism success

Scott et al. (1997) studied the interaction of developmental and adult temperatures on the ability of the wasp *Trichogramma carverae* to parasitize larvae of the light brown apple moth (*Epiphyas postvittana*), a pest of grapes in Australia. Scott and co-workers reared *Trichogramma* at 14 [degrees], 25 [degrees], and 30 [degrees] C, and later tested parasitism rates at the same three temperatures.

At the coldest test temperature, only the wasps reared at 14 [degrees] C were able to parasitize larvae (Scott et al., 1997). At test temperatures of 25 [degrees] C and 30 [degrees] C, developmental temperature had no significant effects on parasitism rates.

Because none of the 25 [degrees] and the 30 [degrees] C wasps were able to parasitize the moths at 14 [degrees] C, the *Trichogramma* data cannot be analyzed by ANOVA (above). Even so, the patterns subjectively support Beneficial Acclimation, at least at 14 [degrees] C. Field releases (see Huey and Berrigan, 1996) are underway to determine whether developmental temperatures of *Trichogramma* influence parasitism rates in nature (A. A. Hoffmann, personal communication).

Swimming speed of Volvox

Herron (1996) studied the effects of developmental temperature (1 1/2 generations at 15 [degrees], 21 [degrees], 27 [degrees] C) on the thermal dependence of swimming speed of several clones of *Volvox aureus* (from Lake Washington) and of several clones of *V. globator* (from Lake Washington and from Sportsman Lake, Washington) at six adult temperatures between 9 [degrees] and 36 [degrees] C. Thus Herron was able to explore the effect of developmental temperatures on adult performance curves. Here we illustrate data for *V. aureus* clones (Fig. 3C), but summarize statistical analyses for all populations (Table 2).

TABLE 2. ANOVA with orthogonal polynomials for effects of test ([T.sub.test]) and of the linear ([T.sub.dev(l)] and quadratic ([T.sub.dev(q)]) contrasts for developmental temperature on swimming speed of *Volvox* spp. (*)

Species (population)	Factor	df	Sum sq
<i>V. aureus</i> (Lake Washington)	[T.sub.test]	5	4.78
	[T.sub.dev(l)]	1	0.27
	[T.sub.dev(q)]	1	0.06
	[T.sub.test] x [T.sub.dev(l)]	5	1.39
	[T.sub.test] x [T.sub.dev(q)]	5	0.12
<i>V. globator</i> (Lake Washington)	[T.sub.test]	5	3.11
	[T.sub.dev(l)]	1	0.63
	[T.sub.dev(q)]	1	0.27
	[T.sub.test] x [T.sub.dev(l)]	5	0.91
	[T.sub.test] x [T.sub.dev(q)]	5	0.05
<i>V. globator</i> (Sportsman's Lake)	[T.sub.test]	5	4.48
	[T.sub.dev(l)]	1	1.20
	[T.sub.dev(q)]	1	0.46
	[T.sub.test] x [T.sub.dev(l)]	5	1.54
	[T.sub.test] x [T.sub.dev(q)]	5	0.03
Species (population)	Factor	F-value	
<i>V. aureus</i> (Lake Washington)	[T.sub.test]	55.7	
	[T.sub.dev(l)]	15.6	

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	[T.sub.dev(q)]	3.4
	[T.sub.test] x [T.sub.dev(l)]	16.2
	[T.sub.test] x [T.sub.dev(q)]	1.4
V. globator	[T.sub.test]	67.0
(Lake Washington)	[T.sub.dev(l)]	68.4
	[T.sub.dev(q)]	28.7
	[T.sub.test] x [T.sub.dev(l)]	19.7
	[T.sub.test] x [T.sub.dev(q)]	1.0
V. globator	[T.sub.test]	82.1
(Sportsman's Lake)	[T.sub.dev(l)]	103.2
	[T.sub.dev(q)]	39.1
	[T.sub.test] x [T.sub.dev(l)]	26.5
	[T.sub.test] x [T.sub.dev(q)]	0.44
Species (population)	Factor	Pr(F)
V. aureus	[T.sub.test]	<0.001
(Lake Washington)	[T.sub.dev(l)]	<0.001
	[T.sub.dev(q)]	<0.067
	[T.sub.test] x [T.sub.dev(l)]	<0.001
	[T.sub.test] x [T.sub.dev(q)]	<0.22
V. globator	[T.sub.test]	<0.001
(Lake Washington)	[T.sub.dev(l)]	<0.001
	[T.sub.dev(q)]	<0.001
	[T.sub.test] x [T.sub.dev(l)]	<0.001
	[T.sub.test] x [T.sub.dev(q)]	<0.42
V. globator	[T.sub.test]	<0.001
(Sportsman's Lake)	[T.sub.dev(l)]	<0.001
	[T.sub.dev(q)]	<0.067
	[T.sub.test] x [T.sub.dev(l)]	<0.001
	[T.sub.test] x [T.sub.dev(q)]	<0.22

(*) The factor involving [T.sub.dev] with the greatest F-value for each population is boldfaced. Data reanalyzed from Herron (1996).

At low test temperatures, performance of *V. aureus* was inversely related to developmental temperature (Fig. 3C); and, at high test temperatures, the opposite was true. At intermediate temperatures, *V. aureus* from intermediate or high developmental temperatures generally swam the fastest.

Several hypotheses are supported by these data (Table 2). The Optimal Developmental Temperature Hypothesis and also Warmer is Better have the strongest support, but Beneficial Acclimation is also highly significant.

In *V. globator* from Sportsman Lake, performance was inversely related to developmental temperature at low test temperature, and positively related at high test temperature (data now shown). Throughout most of the temperature range, *V. globator* from 15 [degrees] C developmental treatment performed poorly compared with *V. globator* from 21 [degrees] C or 27 [degrees] C. Almost identical patterns hold for *V. globator* from Lake Washington (not shown).

Warmer is Better has the strongest support for *V. globator* (Table 2). However, Optimal Developmental Temperature and Beneficial Acclimation are also highly significant. Using a different statistical approach (above), Herron (1996) came to similar conclusions for all three populations.

Beneficial acclimation in bacteria

The original study by Leroi et al. (1994) examined the effect of "acclimation" temperature on relative fitness of *E. coli* that had come from a line that had been evolving at 37 [degrees] C for more than 2,000 generations. New studies by Bennett and Lenski (1997) manipulate the "evolutionary history" of the lines as well as acclimation and test temperature. The protocol used in these studies doesn't lend itself to analysis using the techniques we have described herein, but the studies are nonetheless readily interpretable with respect to Beneficial Acclimation itself.

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Prior to the new experiments, replicate lines of *E. coli* were evolved at 37 [degrees] C for 2,000 generations. Then new replicated lines were established from these "ancestral" lines and cultured at 20 [degrees] C or at 32 [degrees] C for 2,000 generations. Thereafter, these evolved lines were then "acclimated" for eight generations at various temperatures and competed against each other. (Note: because *E. coli* are asexual, little if any evolution would occur over the eight generations of "acclimation" [Bennett and Lenski, 1997, p. 42]. Thus observed differences should reflect phenotypic changes, not genetic ones.)

Beneficial Acclimation was supported in 7 of 15 tests. In most of the other tests, the results were ambiguous; but in a few tests, the results were the exact opposite of those predicted by Beneficial Acclimation. Bennett and Lenski (1997, p. 42) conclude: "Evidently, acclimation may sometimes be beneficial, but it is hardly necessarily so. The general hypothesis of an invariable benefit to acclimation has to be rejected."

Of particular interest was the interaction between evolved and acclimation temperatures. Specifically, when acclimation temperatures matched evolved temperatures, the bacteria often had enhanced fitness even at other temperatures. This result is reminiscent of the Optimal Developmental Temperature Hypothesis. (Note: Zwaan et al. [1991] offered a similar argument to account for the greater longevity of flies reared at 25 [degrees] C, which was the ancestral laboratory temperature for the stock they studied.)

Beneficial acclimation at "extreme" temperatures

The above analyses examine the impact of developmental temperatures across a range of adult test temperatures. However, several of the studies that challenged Beneficial Acclimation did find support for that hypothesis at extreme temperature. For example, at an extreme test temperature of 50 [degrees] C, *E. coli* from 41.5 [degrees] C survived longer than did *E. coli* from 32 [degrees] C. Perhaps Beneficial Acclimation might be more influential at extreme than at intermediate temperatures (Leroi et al., 1999). If so, then the rank order of performances for the developmental temperature treatments should be Low [is greater than] Intermediate [is greater than] High when compared at the lowest test temperature, but High [is greater than] Intermediate [is greater than] Low at the highest test temperature. We test whether observed patterns match these predicted patterns using isotonic regression, a confirmatory technique that evaluates whether continuous data fit a specific ordered hypothesis (Gaines and Rice, 1990).

In 6 of 9 cases, the patterns are significantly consistent with Beneficial Acclimation. Consequently, Beneficial Acclimation seems to be more influential at extreme than at intermediate temperature. Note, however, that "extreme" here refers only to the highest and lowest test temperatures in the experiment and hence may not be physiologically "extreme." Consequently, the pattern may not be informative with respect to the distinction between "resistance" versus "capacity" adaptation (Prosser, 1986; Cossins and Bowler, 1987).

If subsequent studies confirm this pattern (namely, Beneficial Acclimation is influential at extreme temperatures), then our interpretation of acclimation responses to temperature may well require reevaluation. Consider a thought experiment in which an organism is acclimated to a low temperature and then transferred acutely to a warm temperature. Perhaps the resulting acclimation response has evolved not to maximize performance at warm temperatures, but rather to protect against the possibility that the environment will become even hotter. In other words, a traditional interpretation of the temperature shift would be to treat it as a steady-state cue ("the environment has become hot"), whereas the latter would treat the dynamic cue ("the environment is becoming hotter--perhaps dangerously so"). If so, then acclimation to temperature shifts might be more geared towards protection against extreme temperatures than to optimizing performance per se.

This view was in fact recognized by Leroi et al. (1994, p. 1920): "... acclimation seems to take the form of hedging a bet that the environment will continue to become even hotter, rather than remaining at a high but tolerable temperature. Perhaps defense against further temperature increments has been more important in the evolutionary history of these bacteria than maintaining competitive ability in stressful but nonlethal temperatures."

CONCLUDING REMARKS

We have advocated a strong inference approach to evaluating the adaptive significance of acclimation. Our approach

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involves three parts: (1) the recognition of multiple competing hypotheses of acclimation responses, (2) the development a "crucial" experimental design (sensu Platt, 1964) that test all hypotheses simultaneously, and (3) the derivation of a statistical model that allows us to evaluate the relative importance of each competing hypothesis. We then exemplify this approach in several case studies.

How do the various hypotheses stack up in the case studies? The relative importance of each hypothesis can be approximated by the relative F-values in Tables 1 and 2. In many of the studies, Beneficial Acclimation receives significant statistical support. However, even in these cases, Beneficial Acclimation is usually not the most strongly supported hypothesis: Optimal Developmental Temperature and Warmer is Better are often more important contributors. Consequently, Beneficial Acclimation may not be dead, but it certainly isn't top dog, at least with respect to developmental acclimation to temperature.

Beneficial Acclimation may, however, be relatively important at "extreme" temperatures, as discussed in the immediately previous section of this paper. It is interesting to note that many early studies of Beneficial Acclimation looked at the effects of acclimation on tolerance to extreme heat or extreme cold. Thus these studies are consistent with the speculation of Leroi et al. (1994) that acclimation responses to a new temperature may be geared primarily to defense against further temperature change rather than to optimizing performance at the new temperature.

Overall, we believe that the results of our analyses highlight the power of a strong inference approach over single-hypothesis testing (above). Note that most of the studies summarized here would have in fact provided significant support for Beneficial Acclimation, were the individual studies analyzed from a traditional, confirmatory hypothesis approach. However, when those same studies are analyzed from a strong inference perspective, the support for Beneficial Acclimation--while still often positive--takes on greatly diminished importance.

Our analyses also highlight a limitation of Platt's (1964) view of scientific progress, in which crucial experiments either reject or support competing hypotheses. As Hilborn (1997) and others have noted (and as Platt was himself surely aware), such a dichotomous view of nature is inappropriate and overly simplistic. Our statistical approach (and also Fig. 1) shows clearly several competing hypotheses can be supported to various degrees--it simply isn't the case that only a single hypothesis is involved. Thus the goal of a crucial experiment should be to elucidate the relative strength of evidence in support of alternatives.

In conclusion, we agree with Leroi et al. (1994), Huey and Berrigan (1996), and Bennett and Lenski (1997) that Beneficial Acclimation is not a necessary outcome of developmental acclimation. Whether this pattern holds for reversible types of acclimation is a question for the future; and we reiterate our call (Huey and Berrigan 1996) for field tests of the adaptive significance of acclimation.

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