

Evolutionary analyses of morphological and physiological plasticity in thermally variable environments.

by Joel G. Kingsolver and Raymond B. Huey

Morphological and physiological plasticity is often thought to represent an adaptive response to variable environments. However, determining whether a given pattern of plasticity is in fact adaptive is analytically challenging, as is evaluating the degree of and limits to adaptive plasticity. Here we describe a general methodological framework for studying the evolution of plastic responses. This framework synthesizes recent analytical advances from both evolutionary ecology and functional biology, and it does so by integrating field experiments, functional and physiological analyses, environmental data, and genetic studies of plasticity. We argue that studies of plasticity in response to the thermal environment may be particularly valuable in understanding the role of environmental variation in the evolution of plasticity: not only can thermally-relevant traits often be mechanistically and physiologically linked to the thermal environment, but also the variability and predictability of the thermal environment itself can be quantified on ecologically relevant time scales. We illustrate this approach by reviewing a case study of seasonal plasticity in the extent of wing melanization in Western White Butterflies (*Pontia occidentalis*). This review demonstrates that 1) wing melanin plasticity is heritable, 2) plasticity does increase fitness in nature, but the effect varies between seasons and between years, 3) selection on existing variation in the magnitude of plasticity favors increased plasticity in one melanin trait that affects thermoregulation, but 4) the marked unpredictability of short-term (within-season) weather patterns substantially limits the capacity of plasticity to match optimal wing phenotypes to the weather conditions actually experienced. We complement the above case study with a casual review of selected aspects of thermal acclimation responses. The magnitude of thermal acclimation ("flexibility") is demonstrably modest rather than fully compensatory. The magnitude of genetic variation (crucial to evolutionary responses to selection) in thermal acclimation responses has been investigated in only a few species to date. In conclusion, we suggest that an understanding of selection and evolution of thermal acclimation will be enhanced by experimental examinations of mechanistic links between traits and environments, of the physiological bases and functional consequences of acclimation, of patterns of environmental variability and predictability, of the fitness consequences of acclimation in nature, and of potential genetic constraints.

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INTRODUCTION

"... a frog or a toad is by no means the same thing in summer as in winter."

Claude Bernard, 1865 (1949 edition)

One way that individual organisms may respond to variable environmental variation is by altering their phenotypes. Traditionally this response has been termed "plasticity" if it involves morphological traits but "acclimation" if it involves physiological traits. The basic phenomenon is, however, the same in either case: an individual's phenotype changes in response to (or in anticipation of) environmental conditions in a way that may alter that individual's fitness.

Acclimation to temperature in ectotherms has long been a central topic of physiological research (Bernard quote above; Prosser, 1955; Fry, 1958). The literature is now immense (Prosser, 1986) and has focused primarily on two issues: (i) the physiological mechanisms underlying acclimation and (ii) the consequences of acclimation for organismal function or performance. Thermal acclimation in ectotherms affects diverse physiological traits, including shifts in enzyme kinetics, membrane fluidity, lethal and optimal temperatures, metabolism, etc. (Prosser, 1986; Somero, 1978; Hochachka and Somero, 1984; Cossins and Bowler, 1987).

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The adaptive significance of thermal acclimation has, however, received relatively limited attention: typically, any observed effect of acclimation on organismal function has been interpreted uncritically (i.e., without a formal test) as the adaptive result of evolution by natural selection in response to variable environmental conditions (cf. Feder, 1987; Leroi et al., 1994; Huey and Berrigan 1996; Bennett and Lenski, 1997). Consequently, several crucial questions have received limited empirical or theoretical attention. For example, does a given pattern of acclimation actually enhance fitness in nature? Is genetic variation in acclimation response sufficient to permit rapid evolutionary responses to selection on the direction and magnitude of the response? Are environmental cues (e.g., developmental temperature, photoperiod) reliable predictors of environmental shifts (Levins, 1968)? If not, might this unreliability limit the degree of adaptation of plasticity (Moran, 1992)? Answering these and related questions is a significance challenge to evolutionary physiologists (Leroi et al., 1994; Travis, 1994; Huey and Berrigan, 1996; Bennett and Lenski, 1997).

The analytical challenges of studying the evolution of plasticity can be reduced by merging recent developments made in evolutionary ecology with those in evolutionary physiology. For example, the necessary theoretical framework for conceptualizing and analyzing selection and evolution of plasticity in quantitative traits has recently been developed in detail by evolutionary ecologists (e.g., Via and Lande, 1985; Stearns, 1989; Gomulkiewicz and Kirkpatrick, 1992; Moran, 1992; Scheiner, 1993). Similarly, the important mechanistic links between specific trait values and environmental conditions are often accessible via the tools of functional biologists. In addition, thermal and hydric aspects of the environment are often amenable to quantitative analyses of patterns of environmental variability and predictability. Thus, progress towards an understanding of the evolution of plasticity can be accelerated by combining strengths of evolutionary and of functional ecology.

In this paper we examine several aspects of selection on and the evolution of morphological and physiological plasticity. We focus on plastic responses to variation in the thermal environment as a model system because of the demonstrable importance of temperature to the physiology and ecology of animals, because variation and predictability in the thermal environment can be readily quantified, and because the mechanistic links between performance and the thermal environment can often be specified.

We begin by outlining an experimental field and laboratory approach that can help evaluate the nature and extent of adaptive plasticity and the intrinsic and extrinsic limits to such adaptation. Then we illustrate this approach by reviewing a case study of morphological plasticity of wing melanin pattern in pierid butterflies in seasonally fluctuating thermal environments. Our discussion emphasizes the importance of environmental unpredictability in limiting adaptive plasticity, an issue relatively unexplored in most empirical studies of plasticity. Finally, we highlight some understudied aspects of thermal acclimation of ectotherms. Specifically, we sample published studies of ectotherms to explore the magnitude ("flexibility") of thermal acclimation responses, and also review what little is known about genetic variation in thermal acclimation. Our discussion emphasizes that an understanding of the evolution of thermal acclimation--and of plasticity in general--will be furthered via studies that combine the mechanistic strengths of evolutionary physiology with genetical and field approaches of evolutionary ecology (see also Travis, 1994; Huey and Berrigan, 1996).

ANALYZING ADAPTIVE PLASTICITY

A reasonably complete understanding of microevolutionary issues of adaptive plasticity requires explicit consideration of six main issues (Travis, 1994; Huey and Berrigan, 1996). The first two issues explore basic functional aspects of acclimation (e.g., the physiological bases and the functional consequences of a plastic response) and represent the "how" of acclimation (Mayr, 1961). These two issues have long been the focus and strength of physiological studies (Prosser, 1986), and so we will discuss them only in passing. The remaining four issues explore the "evolutionary" aspects of adaptation, selection, and response to selection. These issues, which represent the "why" of acclimation (Mayr, 1961), are only beginning to be explored by evolutionary physiologists and hence require more attention.

Functional issues

1) Describe the induction, direction, magnitude, and functional consequences of phenotypic plasticity.--This involves exploring the environmental conditions (or cues such as temperature or photoperiod) that induce the plastic response as well as elucidating the functional consequences of expressing different phenotypes in different environments. (Note: information on functional consequences is crucial to understanding both the ecological context and the adaptive

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significance of plasticity.)

2) Explore the physiological mechanisms underlying the observed plastic response.--For example, determine whether the observed changes are a consequence of lower-level changes in membrane structure, enzyme activities, expression of stress proteins, etc. (Note: This information provides insights not only into "how" plasticity is achieved mechanistically, but sometimes as to "why" plasticity might be functionally significant.)

Evolutionary issues

3) Evaluate whether plasticity is in fact adaptive in nature.--In other words, do alternative phenotypes actually increase fitness in their appropriate environmental conditions in the field? Effective protocols for testing various aspects of the adaptive significance of acclimation in the laboratory currently exist (Krebs and Loeschcke, 1994; Leroi et al., 1994; Huey et al., 1995; Zamudio et al., 1995; Huey and Berrigan, 1996; Krebs and Feder, 1997), but the ultimate tests of adaptation must be done in the field. For example, the adaptiveness of plasticity (e.g., seasonal change in a phenotype) can be tested by experimentally generating these alternative phenotypes, releasing them into the field, and then determining their relative fitnesses in each season (see Karban and Carey, 1984; Harvell, 1986; Schmitt et al., 1995; Dudley and Schmitt, 1996).

4) Determine whether selection in nature on the observed degree of plasticity is stabilizing or directional.--For a quantitative trait, even if plasticity generates phenotypic trait values that increase fitness in each environment (e.g., if increased heat tolerance in summer phenotypes increases survival), the degree of plasticity may not yield trait values that are in fact "optimal" for the environmental conditions. Moreover, by estimating how selection on existing variation in a plastic trait varies with time (or with habitat), we can determine whether selection is acting on the actual magnitude of plasticity (Weiss and Gorman, 1990; Kingsolver, 1995a). Looked at in a different way, evidence for directional selection on plasticity provides a quantitative measure of maladaptation--i.e., of the degree to which plasticity fails to generate the optimal phenotype in each environment.

5) Determine whether unpredictability in environmental conditions may limit the degree to which plasticity can adapt organisms to variable environments. (Hoffman, 1978; Moran, 1992).--Consider a plastic trait that is determined by developmental temperature, presumably as an adaptive cue of temperatures to be experienced by adults. However, developmental temperature may be an unreliable predictor of adult temperature, because of the inherent time lag between larval and adult stages. As a result, environmental unpredictability may cause a mismatch between the trait value expressed and the environmental conditions during which selection actually operates. Environmental unpredictability--if marked--may potentially limit the degree to which adaptive plasticity may evolve.

6) Determine the extent of genetic variation in plasticity.--Even if selection on plasticity is strong, evolutionary changes in the degree of plasticity cannot occur without appropriate genetic variation (Via and Lande, 1985; Scheiner and Lyman, 1991). Genetic variation in plasticity may be limited by mechanistic limitations on plasticity and by physiological costs of maintaining or employing plastic responses. Many studies have documented substantial genetic variation in plasticity of morphological and life history traits (see Scheiner, 1993), but studies of the genetic basis of physiological acclimation are still rare (see below).

These six issues provide a comprehensive--if ambitious--agenda for studying adaptive plasticity from a microevolutionary perspective. Nearly all of the empirical literature on evolution of plasticity has focused on Issues 1-3 (environmental determinants and functional and fitness consequences of plasticity) and Issue 6 (genetic variation for plasticity). In contrast, we are aware of only two published studies that have estimated natural selection on the magnitude of plasticity within populations in the field (Issue 4: Weiss and Gorman, 1990; Kingsolver, 1995a). Further, we know of no study that explores quantitatively how environmental unpredictability may limit the degree of adaptive plasticity in field populations (Issue 6: but see Hoffman, 1978, and also next section); as we shall show, studies of the thermal and hydric environment may be particularly amenable to such analyses of environmental unpredictability.

SEASONAL WING MELANIZATION IN PIERID BUTTERFLIES

We will illustrate the above issues by describing a case study of seasonal shifts in the degree of wing melanization in

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Pierid butterflies. Wing melanization is of physiological and evolutionary interest because of its influence on the thermal biology of these butterflies (see below). We concentrate on the Western White Butterfly, *Pontia occidentalis*, which is widely distributed at high latitudes and elevations throughout the western U.S. and Canada. Many populations have multiple generations each year, and the different generations develop and live under quite different thermal regimes. These butterflies are thus suitable for studying plasticity across seasonal thermal environments.

Issue 1: Environmental determinants and functional consequences of plasticity

In *P. occidentalis* as well as in many other butterfly species (Watt, 1969; Brakefield, 1987), the amount of melanin pigment in different areas of the wings of adults is a plastic trait. Developmentally, the degree of melanization is set by photoperiodic (and to a lesser extent, temperature) cues experienced late in larval development (Watt, 1969; Shapiro, 1976). In *P. occidentalis*, the greatest shift in wing melanin occurs in the ventral hindwings and in the bases of the dorsal hindwings (Shapiro, 1976; Kingsolver and Wiernasz, 1991). In both these regions, melanization is significantly darker and more extensive in spring than in summer (or following development in short rather than long photoperiods in the laboratory).

Shifts in wing melanization can have diverse functional consequences (Kingsolver and Wiernasz, 1991), but one obvious consequence is for heat exchange and thermoregulation. Thermoregulation is important to fitness because pierid butterflies require elevated body temperatures (28-40 [degrees] C in many species) for vigorous and sustained flight. In cool conditions, *Pontia* butterflies can achieve elevated temperatures by basking with their dorsal wing surfaces oriented to solar radiation; conversely in hot conditions, many butterflies stop flying, close their wings, and orient wings and body to minimize their radiative heat load (heat-avoidance). The degree of wing melanin in certain wing regions can affect the amount of solar radiation absorbed and thus the body temperatures achieved. For example, classic studies by Watt (1968, 1969) showed that the intensity of melanin on the ventral hindwings (VHW) of *Colias eurytheme* butterflies positively affected body temperature during basking. Consequently, even under identical environmental conditions, seasonal phenotypes that differed in VHW melanin had different equilibrium basking temperatures. Analogous studies with *P. occidentalis* show that the intensity of melanin on the basal dorsal hindwings (bDHW) directly affects body temperatures achieved during basking (unlike *Colias*, *P. occidentalis* butterflies bask with their wings open), whereas melanin on the VHW affects body temperatures during heat-avoidance (Kingsolver, 1987). These functional analyses suggest a simple adaptive hypothesis: seasonal plasticity in wing melanin is an adaptation enhancing thermoregulation in seasonally varying thermal environments (Watt, 1968, 1969; Hoffman, 1978).

Issue 2: Mechanistic bases of plasticity

The biochemistry of melanin wing pigments is well described for butterflies, but relatively little is known about the mechanisms underlying plasticity in melanism (Nijhout, 1991). Although pigment deposition occurs during the pupal stage, the actual determination of melanin plasticity is set largely by photoperiod and occurs earlier during the larval stage. Photoperiodic cues alter titers of juvenile hormone (JH) during larval development, and JH in turn later affects the amount of pigment deposition in the pupal stage (Nijhout and Wheeler, 1982); however, quantitative relations between JH titers in larvae and melanin pattern in adults are still poorly known. A recent study suggests that gene expression at the distalless locus controls plasticity in wing melanin in *Byclicus* butterflies (Brakefield et al., 1996).

Issue 3: Is plasticity adaptive?

The "adaptive plasticity" hypothesis predicts that "summer" phenotypes, which have relatively less wing melanin, should have higher fitness during summer conditions than should "spring" phenotypes, which are darker. The reverse should be true during spring conditions.

Following the pioneering field studies by Shapiro (1976), Kingsolver (1995b) conducted field experiments to test these predictions in a population of *P. occidentalis* in southeastern Washington state. This population has four to five adult flight seasons (generations) between April and August. The degree of melanization in many wing regions is distinctly different (but somewhat overlapping) in adults from summer and spring generations. Kingsolver collected eggs from females and reared the eggs (split-family design) in the laboratory in either Long-day (16 hr light, 8 dark) "Summer" or Short-day (10L:14D) "Spring" treatments. The resulting adult butterflies differed significantly in wing melanin traits (degree of

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melanin was measured as grey-level intensity using a video analysis system: see Kingsolver, 1995a, b). For example, Long-day individuals had significantly less melanin on the bDHW and VHW than did Short-day individuals. Shortly after emergence, each butterfly was marked individually and released at the field site. Daily recapture-release sampling and mark-recapture statistical methods were used to determine whether the rearing treatment affected survival (as distinct from recapture) probabilities (Kingsolver, 1995a, b).

The adaptive plasticity hypothesis was supported by release experiments conducted during two summers (July 1991 and 1992): in both years, Long-day (summer phenotype) individuals had significantly greater survival than did Short-day (spring phenotype) individuals. Interestingly, the effect of developmental treatment on survival was stronger in 1992 than in 1991, perhaps indicating an enhanced advantage for the summer phenotype during the relatively hot summer of 1992.

The adaptive plasticity hypothesis was, however, not supported by a release experiment conducted in one spring (1991): Long-day and Short-day individuals had similar survival rates during this season (Kingsolver, 1995b). In combination, these field studies provided only partial support for the hypothesis of adaptive plasticity: Plasticity appeared adaptive during summer conditions, but not during spring. Moreover, even in the summer studies, the strength of selection varied between years.

These results show that rearing treatment can affect both wing melanin phenotype and survival in the field. However, the rearing environment will influence many important aspects of the adult phenotype, not just wing melanin pattern. Consequently, the above field tests do not necessarily imply a causal link between wing phenotype and survival. To determine whether plasticity in wing pattern and survival are in fact causally linked, Kingsolver (1996) conducted companion experiments that directly manipulated melanin on the posterior VHW (the wing region showing the greatest degree of melanin plasticity in *P. occidentalis*) into one of three treatment groups: "Blackened" individuals in which black pigment was added along each vein on the posterior half of the VHW; "Yellowed" individuals served as a control group in which yellow pigment was added along each vein on the posterior half of the VHW; and an "Unpainted" group. If seasonal plasticity is adaptive, then Blackened individuals should show reduced survival in summer relative to Yellowed or Unpainted butterflies.

Mark-release-recapture experiments were conducted in the summers of 1991 and 1993. Blackened males indeed had significantly reduced survival probabilities relative to Yellowed and Unpainted males, consistent with the adaptive plasticity hypothesis. However, paint treatment had no effect on females, contrary to the hypothesis. Females may be insensitive here because they are less active than males, and so may be less exposed to stressful summer temperatures. (Because VHW melanin does not affect mate choice in this species [Wiernasz, 1989], the different effects of the manipulations on males and females are unlikely to be related to effects of mating on survival.) Taken as a whole, the above studies support the hypothesis that plasticity in wing melanin pattern is sometimes--but not always--adaptive for seasonally varying thermal conditions. Obviously, plasticity isn't always adaptive and its importance varies between seasons, years, and sexes.

Issue 4: Selection on the magnitude of plasticity

Even if plasticity in wing melanin is adaptive in this system, selection may still favor changes in the degree of plasticity. For example, selection could potentially favor greater (or lesser) plasticity than is actually observed.

To explore selection on the magnitude of plasticity, Kingsolver (1995a) conducted mark-release-recapture studies (two MRR in spring and two in summer) with wild-caught individuals, and measured directional selection coefficients for several wing traits (including bDHW and VHW melanin) during both spring and summer generations. For most of the wing traits, directional selection was generally weak or absent. However for melanin at the bases of the dorsal hindwings (bDHW melanin), which influences thermoregulation during basking (Kingsolver, 1987), significant directional selection was detected in all four studies. Most importantly, the direction of selection alternated between seasons: selection was positive for enhanced bDHW melanin in spring but negative in summer. These results are strongly consistent with expectations based on thermoregulatory considerations.

Recall, however, that bDHW melanin is phenotypically plastic, so the mean degree of bDHW melanin differs significantly between spring and summer generations (Fig. 1). As a result, the observed pattern of seasonally alternating directional

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selection implies that selection favors increased plasticity in this trait--that is, selection currently favors an increase in the slope of the "norm of reaction" relating bDHW melanin to environmental rearing conditions (in this case, photoperiod). Thus, even though plasticity is generally adaptive, even more plasticity in this trait would further increase survival.

[Figure 1 ILLUSTRATION OMITTED]

Issue 5: Environmental unpredictability

Whether plasticity is adaptive will depend in part on the predictability of the environment (Levins, 1968). In other words, do environmental conditions experienced by butterfly larvae adequately predict conditions experienced by adults? The thermal environment is particularly suitable for exploring this question, as long-term weather records are often available. To address this issue, we therefore examined long-term weather records (1941-1992) of daily maximum air temperatures ([T.sub.max]) from a weather station near Kingsolver's (MRR) study site.

Temperature variation at three different scales is relevant to the short-term evolution of plasticity in this system. First, are between-season differences in [T.sub.max] predictable, such that photoperiodic cues during larval development would be good predictors of seasonal temperatures experienced by adults? Not surprisingly, the answer is yes (Fig. 2): monthly average [T.sub.max] in April is on average 13 [degrees] C lower than in July, and in fact average [T.sub.max] is always lower in April than in July. Interestingly, there is a significant position correlation ($[r_{sup.2}] = 18\%$) between mean April and July [T.sub.max]: years that are on average warmer in April are also usually warmer in July. Second, are within-season differences in [T.sub.max] minor, which would reinforce the usefulness of photoperiod as an environmental cue? Here the answer is no (Fig. 3): the weekly average [T.sub.max] during (for example) the first week of July each year was surprisingly variable and ranged from 23 to 38 [degrees] C. [Note: because adults butterflies usually live for about one week, a weekly average reflects the temperature conditions experienced during the time scale of an individual butterfly's life span.] Because [T.sub.max] in early July is strongly related to solar radiation intensity, a [T.sub.max] value of 23 [degrees] C represents cool, cloudy conditions in which flight activity may be limited by low body temperatures; whereas a [T.sub.max] of 38 [degrees] C represents hot, sunny conditions in which flight activity may be limited by high body temperatures. Thus, even in a given season (and hence at a given photoperiod), adults in different years may see very different temperature conditions.

[Figures 2-3 ILLUSTRATION OMITTED]

Third, can between-year unpredictability of photoperiodic cues alone be ameliorated if butterflies were able to integrate both photoperiodic and temperature cues (Hoffmann, 1978; Shapiro, 1976; Kingsolver, 1995b) from their rearing environment? If so, the butterflies might adjust their developmental trajectories for wing melanization in response to immediate weather conditions as well as to photoperiod. For *P. occidentalis* in the field, adult butterflies emerge about two weeks after melanin pattern is determined developmentally in the larvae. How well do current temperature conditions predict temperatures two weeks later? Data on weekly average [T.sub.max] for the first and third weeks of July yield a clear, negative answer (Fig. 3): average [T.sub.max] in the first week of July explains less than 7% of the variation in [T.sub.max] two weeks later. Thus, the thermal environment (and photoperiod) during the larval stage is a poor predictor of the thermal environment adults will face, at least within seasons.

This explicit consideration of the predictability--or lack thereof--of the thermal environment adds some insights to our analysis of adaptive plasticity. Between-season patterns in temperature are very predictable, at least on a gross scale (Fig. 2); and the magnitude of the between-season shifts in the thermal environment favor the evolution of plasticity. However, if we shift the time scale to variation between years or to within the lifetime of an individual, the thermal environment is clearly unpredictable (Fig. 3). This short-term unpredictability likely prevents plasticity from evolving a precise match between mean phenotypic trait values and the fluctuating thermal environment.

Issue 6: Genetic variation in plasticity

Even if selection currently favors increased plasticity in bDHW melanin (see Issue 4, above), genetic variation in plasticity is a prerequisite for an evolutionary response to this selection. One common way of identifying genetic variation is to examine variation in the slopes of reaction norms for a set of genotypes or families sampled from the population of

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interest (e.g., Via and Lande, 1985). By measuring reaction norms of wing melanization for a set of full-sib families of *P. occidentalis*, Kingsolver and Wiernasz (1991) identified significant (broad-sense) genetic variation in the plasticity of the extent of melanin in many wing regions. This suggests that the evolution of plasticity in these traits is not strongly constrained genetically.

ADAPTIVE PLASTICITY OF WING MELANIN: A SUMMARY

This case study provides some provisional understanding of the existence, degree, and limits to adaptive plasticity in thermally variable environments. First, experimental analyses show that plasticity of wing melanin increases one component of fitness (adult survival) during summer but not spring conditions. Experimental manipulations of wing pattern verify that this effect on survival that can be attributed to plasticity of wing pattern per se, rather than some other effect of rearing conditions. Second, selection analyses of natural variation detect consistent selection favoring increasing plasticity in one wing trait that is known to be important for thermoregulation. Thus, the observed plasticity was not at an evolutionary optimum. Third, short-term unpredictability of the thermal environment within a season is surprisingly large. This unpredictability likely limits the capacity for adaptive plasticity in wing melanin traits, simply because larval conditions do not adequately predict adult ones. In fact, in many temperate regions short-term weather variation is dominated by weather systems that occur on the time scale of 4-7 days, such that weather variation on a scale of a week or more is typically highly unpredictable (e.g., Kingsolver 1981)--this is why weather forecasts beyond a few days are notoriously unreliable. As a result, short-term unpredictability in weather may be limiting to adaptive plasticity for many other temperate-zone species with life cycles on this time scale. Fourth, there is sufficient genetic variation in plasticity of wing melanin to permit evolutionary responses to selection on plasticity per se.

PHYSIOLOGICAL ACCLIMATION AS ADAPTIVE PLASTICITY

The above section uses research on butterfly wing melanin to illustrate our proposed agenda for studying the evolution of adaptive plasticity. To apply this agenda to the study of physiological acclimation, however, we need to broaden the type of traits we review. For most cases of morphological plasticity, including butterfly wing melanin, the trait value is determined during development and may be fixed throughout much or all of adult life. In contrast, many physiological traits have values that vary dynamically with the recent and present state of a given individual. Such traits are more challenging to study, if only because they can not be characterized by a single measurement.

The paradigmatic example of a multi-value, labile trait would be thermal performance curves, which describe the relationship between some aspect of physiological or organismal performance (or fitness) and temperature for an individual. An individual's performance curve can be characterized by quantifying upper and lower temperatures for performance (including survival), thermal performance breadth, and the optimal temperature (Huey and Stevenson, 1979). [Alternatively, these curves can be analyzed as "infinite-dimensional traits" (Kirkpatrick and Heckman, 1989).] The impact of acclimation of a thermal performance curve will be manifest as a change in position or shape of the performance curve, as evidenced for example by a shift in the optimum temperature.

We will explore two questions dealing with acclimation and performance curves. First, how much do performance curves shift with acclimation temperature, and are shifts in position accompanied by shifts in height (i.e., in maximal performance)? Second, what is known about the magnitude of genetic variation in acclimatory plasticity in thermal performance curves (or in parts thereof)? The first question extends some of our above comments on Issues 1 and 4, whereas the second question addresses Issue 6.

Because of space limitations, we will not review laboratory tests of the "beneficial acclimation hypothesis," which proposes that acclimation enhances fitness in the environment that induced it (Leroi et al., 1994; Zamudio et al., 1995; Bennett and Lenski, 1997). Several of these tests are elegant (e.g., Bennett and Lenski, 1997) and complement field tests of the "adaptive plasticity hypothesis." Fortunately, these laboratory tests have recently been reviewed (Hoffmann, 1995; Huey and Berrigan, 1996; Bennett and Lenski, 1997). Those reviews suggest two key points: (1) the laboratory environment offers a superb--if arguably artificial--venue for unambiguous tests of the adaptive significance of acclimation, and (2) many of these laboratory tests either fail to support Beneficial Acclimation or sometimes actually contradict it (Leroi et al., 1994; Zamudio et al., 1995; Huey and Berrigan, 1996; Bennett and Lenski, 1997).

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Flexibility of acclimation

A fundamental issue in acclimation studies is how much the phenotype shifts in response to acclimation. This issue is relevant not only to discussions of physiological "compensation" (Precht, 1949), but to general discussions of physiological adaptation to tropical versus temperate zones (e.g., Dobzhansky, 1950; Feder, 1977, 1982; Tsuji, 1988) or to any constant versus fluctuating environment (Levins, 1968; Brown and Feldmeth, 1971).

In a classic study of thermal acclimation in *Drosophila*, Richard Levins (1969) used the term acclimation "flexibility" with reference to the magnitude of an acclimation-induced shift in a phenotypic trait: he quantified flexibility as the magnitude of the difference in heat tolerance of individuals acclimated to two temperatures. Similarly, the difference in wing melanism could index the flexibility of response to a photoperiod treatment. Note, however, that for the special case of performance curves or related traits (e.g., optimal, lethal, or preferred temperatures), both the trait of interest and the acclimation treatment itself are measured in the same units (temperature). For such traits, acclimation flexibility can be calculated as the slope of the regression of the trait value on acclimation temperature, such that flexibility is a dimensionless number. A flexibility of 1 indicates a positive, 1 [degrees] C shift in the trait for each 1 [degrees] C shift in acclimation temperature, suggesting complete compensation for acclimation temperature. In contrast, a flexibility of 0 indicates that the trait is unaffected by the acclimation treatments. [Slopes can also be negative, indicating an "inverse" response (Forster-Blouin, 1989).]

How flexible, in fact, are thermal performance curves of ectotherms? Are plastic traits flexible enough to compensate fully for temperature shifts? The literature here is immense (Prosser, 1986), and we have only made a cursory search for examples. Brett (1956) tabulated acclimation responses of upper lethal and for lower lethal temperatures for a variety of fish species; and we calculated flexibilities for both (Fig. 4; see Table 1). Both traits respond positively though weakly (i.e., low slopes) to acclimation temperature, but the flexibility of lower lethal temperatures is greater than that of upper lethal temperatures (paired t-test, P [much less than] 0.001). In general, flexibilities of various thermal traits for insects are also relatively small (Table 1).

TABLE 1. Acclimation flexibilities for various traits and organisms. Flexibilities (non-dimensional) are calculated as slopes of trait values on acclimation temperature, where 0 = no acclimation response and 1 = complete (i.e., compensatory) acclimation response.

Trait	Taxon	Flexi- bility
Thermal preference	<i>Drosophila</i> spp.	0.11
Lower lethal [T.sub.b]	fishes	0.55
	insects	0.21
Upper lethal [T.sub.b]	fishes	0.24
	Insects	0.22
Knock-down [T.sub.b]	<i>Drosophila melanogaster</i>	0.12
Lipid melting T	grasshopper (<i>Melanoplus</i>)	0.37
Optimal swim. [T.sub.b]	<i>Volvox aureus</i>	0.18
Optimal running [T.sub.b]	<i>Drosophila melanogaster</i>	0.05
Average		0.23

Trait	Range	N
Thermal preference	-0.40 to 0.58	12
Lower lethal [T.sub.b]	0.30 to 0.78	11
	0.13 to 0.36	11
Upper lethal [T.sub.b]	0.08 to 0.41	20
	0.07 to 0.29	3
Knock-down [T.sub.b]	0.07 to 0.16	8 (treatments)
Lipid melting T	0.07 to 1.00	13 (populations)
Optimal swim. [T.sub.b]	0.00 to 0.49	8 (clones)
Optimal running [T.sub.b]	-0.22 to 0.35	8 (treatments)
Average		

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Trait	Reference
Thermal preference	Yamamoto and Ohba, 1984; McDaniel et al., 1995
Lower lethal [T.sub.b]	Brett, 1956 Mellanby, 1939; Calhoun, 1960
Upper lethal [T.sub.b]	Brett, 1956 Baldwin, 1954; Melanby, 1939
Knock-down [T.sub.b]	Crill et al., 1996
Lipid melting T	Gibbs et al., 1991
Optimal swim. [T.sub.b]	Herron, 1996
Optimal running [T.sub.b]	Crill et al., 1996
Average	

These and many other data (e.g., Cossins and Bowler, 1987) suggest that thermal traits in fact may show quite limited acclimation flexibility. Performance shifts, but not enough to yield perfect compensation. So why don't traits show greater acclimation responses? After all, if environmental or operative temperature shifts from 10 [degrees] C to 20 [degrees] C, it would seem adaptive for an ectotherm's optimal temperature to shift from 10 [degrees] C to 20 [degrees] C as well. Beyond the obvious but largely untested possibility (see below) that acclimation may have limited impact on fitness in natural populations, we can offer three classes of explanations that require exploration. First, thermoregulation may alter the relationship between gross environmental temperatures and actual body temperatures. If a change in environmental temperature does not cause an equivalent change in body temperature, then the degree of acclimation flexibility might be less than expected. Second, there may be intrinsic constraints on the degree of acclimation response. These constraints could result from a lack of appropriate genetic variation (see below), from the consequences of particular physiological mechanisms of acclimation (e.g., Somero, 1978; Cossins and Bowler, 1987; Leroi et al., 1994), or because the costs of maintaining or deploying acclimation capacity are excessive relative to the benefits (Dykhuizen and Davies, 1980; Scheiner, 1993; Krebs and Loeschke, 1994; Hoffmann, 1995; Krebs and Feder, 1997). Third, environmental unpredictability, as noted above, might in fact select for less than full flexibility, as an animal making a major acclimation response might in fact overshoot the optimal phenotypic shift (Fisher, 1958, pp. 44-45). The asymmetric shape of thermal performance curves may enhance this effect, as the consequences experiencing body temperatures above the optimal temperature are much greater than those of experiencing body temperatures below the optimum.

A related issue is whether acclimation influences the height as well as position of thermal performance curves. Consider, for example, Fry and Hart's (1948) pioneering study of thermal acclimation of swimming speed in goldfish. Between acclimation temperatures of 5 and 35 [degrees] C, these fish show a major acclimation response in the optimal temperature for speed (Fig. 5, flexibility = 0.65), suggesting substantial though not complete compensation. However, the maximum speed of fishes at their optimal temperature was depressed--sometimes severely--if fish were acclimated to temperatures other than 25 [degrees] C (Fig. 5). In fact, after acclimation to 5 [degrees] C, the maximum speed of fishes at their new optimal temperature was only 44% that fish acclimated to 25 [degrees] C. Obviously, acclimation of absolute performance to low temperature changes the height as well as the position of the performance curve; and this may have profound ecological significance.

[Figure 5 ILLUSTRATION OMITTED]

Genetic variation in thermal acclimation

How much genetic variation within populations exists for acclimation response? The fact that acclimation responses often differ among populations and species (Levins, 1969; Prosser, 1986; but see Hoffmann and Watson, 1993) strongly implies that genetic variation in acclimation capacity does exist within populations. Nevertheless, direct evidence of genetic variation in physiological acclimation within populations is remarkably meager (see Huey and Berrigan, 1996). As for other aspects of phenotypic plasticity (see Issue 6 above), genetic variation in thermal acclimation may be explored using studies involving breeding designs or selection experiments in the laboratory.

Studies of genetic variation must distinguish between genetic variation in mean trait value from variation in plasticity per

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se (cf Falconer, 1989; Via and Lande, 1985). For example, Gibbs et al. (1991) sampled families of grasshoppers (*Melanoplus sanguinipes*) from 18 populations, raised them under several seasonal regimes differing in temperature and photoperiod, and then measured melting temperatures of epicuticular lipids, which provide a primary barrier to water loss. Melting temperatures differed significantly among populations, among families within populations, and among seasonal treatments; but were unaffected by interactions between treatment and population or between treatment and family within populations. Thus, despite documenting significant genetic variation in melting temperatures, Gibbs et al. (1991) found no evidence of genetic variation in acclimation ability, either among sites or among families within sites. In a subsequent analysis, Gibbs and Mousseau (1994) found no evidence of genetic variation in the time course of acclimation. Similarly, Loeschcke and Krebs (1994) found no variation in acclimation to high temperature stress among isofemale lines of *Drosophila buzzatii*. However, Krebs and Feder (1997) have recently found variation among isofemale lines of *D. melanogaster* in Hsp70 expression and in inducible thermotolerance of larvae.

Clonal organisms are particularly amenable for studying the genetical architecture underlying acclimation responses. For example, Walton et al. (1995) examined variation in acclimation response to temperature in four strains of a clonal protist, *Euplotes vannus*, each from a different locality in Italy and nearby islands. Their data suggest inter-strain variation in acclimation responses, though it is not clear whether the variation is in the magnitude or in the rapidity of the acclimation response.

Jon Herron and D. Pepin (Herron, 1996) have done the most complete and elegant study to date of the genetic basis of thermal acclimation, using clones of the multicellular alga, *Volvox aureus*, from a single population. They studied genetic variation in the impact of acclimation temperature on the thermal dependence of swimming speed, using light as a stimulus to induce swimming in these positively phototactic algae. Herron and Pepin raised clone-mates at three temperatures and then measured their thermal sensitivity of performance (swimming speed at five temperatures). Because they then replicated the entire experiment, they were able to determine whether the acclimation responses of clones were repeatable (thus genetic). Acclimation flexibility (as defined above) for optimal swimming not only differed significantly among clones, but was also highly repeatable between replicates. Consequently, the sensitivity of thermal performance to acclimation temperatures appears to vary genetically in *V. aureus* (i.e., significant "broad sense" heritabilities).

Natural selection in the laboratory has also been used to uncover evidence of genetic variation in plasticity. Cavicchi et al. (1995) detected significant divergence in acclimation responses of heat tolerance and of heat hardening in replicated populations of *D. melanogaster* that had been evolving by laboratory natural selection for 15 years at either 18, 25, or 29 [degrees] C differ in their acclimation responses and in heat hardening (Cavicchi et al., 1995). Similarly, McDaniel et al. (1995) found that apparent adaptation of *D. tripunctata* and *D. robusta* to laboratory conditions resulted in a diminution of their acclimation response.

Bennett and Lenski (1997) have recently demonstrated that the acclimation response of the bacterium *Escherichia coli* depends on the "historical" temperature at which the bacteria have been evolving. For example, bacteria that were evolved at 32 [degrees] C (2,000 generations) and also were acclimated to 32 [degrees] C had higher fitness at 37 [degrees] C than those same bacteria if acclimated to and then tested at 37 [degrees] C. This result clearly contradicts the "beneficial acclimation hypothesis" but is consistent with the "optimal acclimation temperature model," reviewed in Huey and Berrigan (1996).

This brief review demonstrates that our understanding of the magnitude of genetic variation in thermal acclimation responses is strikingly limited: clearly this is an open niche for evolutionary physiologists. Studies with clonal organisms (Walton et al., 1995; Herron, 1996), isofemale lines (Loeschcke and Krebs, 1994; Krebs and Feder, 1997), selected lines (Cavicchi et al., 1995; Bennett and Lenski, 1997), or geographically separated populations (Tsuji, 1968; Hoffmann and Watson, 1993) may be particularly valuable, both to document genetic variation and to isolate strains or lines differing in acclimation (Hoffmann, 1990). Those strains can be exploited not only to begin to elucidate physiological mechanisms underlying variation in acclimation responses, but also to use in studies of the functional consequences of variation in responses (Krebs and Feder, 1997).

FUTURE STUDIES OF EVOLUTION OF PLASTICITY

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Physiologists have provided a detailed understanding of the mechanisms underlying plasticity of many physiological traits (e.g., Somero, 1978). In addition, the consequences of thermal acclimation for performance and even fitness in the lab have been well described in a number of study systems (see Huey and Berrigan, 1996; Bennett and Lenski, 1997). By comparison, the developmental mechanisms underlying plasticity of morphological traits are more poorly known in most instances (e.g., Scheiner, 1993). However, several evolutionary aspects of acclimation seem ripe for study:

First, laboratory tests are an efficient and effective way of exploring the adaptive significance and evolution of acclimation responses (Leroi et al., 1994; Zamudio et al., 1995; Bennett and Lenski, 1997). However, we believe that laboratory results need to be validated in nature. Certainly, as was evident in the example of butterfly melanin patterns (above), the relationship between plasticity and fitness in the nature is rarely as straightforward as functional and laboratory studies might predict. Ultimately, field studies will need to score not only how plasticity influences survival, but also how plasticity influences reproduction, for it is entirely possible that a "costly" trait might increase survival, but simultaneously lower reproduction and hence potentially lower fitness.

Second, additional studies of genetic variation in acclimation are needed. Interestingly, nearly all studies of morphological traits have detected substantial genetic variation in plasticity (Scheiner, 1993), whereas the few studies to date on thermal acclimation are mixed (above). Is this discrepancy merely an artifact of sample size or of relative measurement error, or is it a real difference between morphological and physiological plasticity?

Third, the role of environmental unpredictability for the evolution of plasticity has been widely discussed in theoretical analyses, but remains virtually unexplored in empirical studies (Levins, 1968; Moran, 1992). Physiological and morphological traits may show different responses in this regard. For example, the time lag between environmental determination and expression of physiological acclimation is sometimes short (minutes to days), whereas that involving morphological traits often involves longer time lags. Further, physiological acclimation is usually reversible, whereas morphological plasticity is often much less so. Appropriate use of long-term weather data may be particularly valuable for analysing environmental unpredictability in relation to plasticity of physiological (and certain morphological) traits. For example, G. Davidowitz has explored the relationship between variability and predictability in precipitation to plasticity in age and size at maturity in pallid-wing grasshoppers (*Trimerotropis pallidipennis*) in the southwestern U.S. (G. Davidowitz, unpublished results). In addition, the asymmetric shape of thermal performance curves can have important consequences for the evolution of performance curves (Gilchrist, 1995); mathematical models exploring how these consequences impact the evolution of acclimation may be instructive.

Finally, we need to address the question of why physiological traits appear to show limited flexibility (Table 1). A related issue is why the magnitude of performance can be affected by acclimation (Fig. 5).

These are fundamental--if complex--issues. However, our understanding of the evolution of thermal acclimation, and of plasticity more generally, requires their exploration. We believe that the field of thermal acclimation offers special opportunities and challenges.

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(2) E-mail: kingsolv@zoology.washington.edu

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Joel G. Kingsolver(2) and Raymond B. Huey

Department of Zoology, Box 351800, University of Washington, Seattle, Washington 98195-1800