7 Interindividual variability: an underutilized resource

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Introduction

Two principal analytical approaches have been used in studies of organismal physiology. These are represented by the terms "comparative physiology" and "physiological ecology." The former compares functional characters in two or more populations, species, or higher taxa in an attempt to understand mechanism. Biological diversity is used to help understand principles of physiological design. Often the experimental species are chosen specifically because their systems demonstrate an extreme phenomenon or because the experimental preparation is technically accessible. The selection of a species on these grounds is known as the Krogh Principle (Krogh, 1929; Krebs, 1975), which has been very influential and successful in guiding studies in comparative physiology for more than fifty years.

The second approach, physiological ecology or ecological physiology, examines the physiological attributes of a species and interprets them in the context of the natural environment or ecological niche of an animal. These studies concentrate on analysis of adaptive pattern, of how physiology, morphology, and behavior interact to permit survival and reproduction in a given environment. In this approach, emphasis is placed on ecological and evolutionary aspects of physiological function. Monitoring the organism in its natural environment and speculation on selective factors that influenced the evolution of characters are the principal interpretive contexts of these studies.

These two approaches are by no means exclusive and have often proved complementary. They have yielded a substantial understanding of how animals work and function in the natural world. However, my thesis here is that both approaches have overlooked a valuable source of information. In their concentration on population-, species-, or higher-level phenomena, they have failed to analyze and take advantage of biological differences among individuals. As traditionally practiced, physiological studies neglect differences among individual animals and attempt to describe the functional response in the average animal of the group. I believe that this approach has been very short-sighted and that the study of interindividual differences has much to contribute to both comparative physiology and physiological ecology. I will argue that the analysis of the bases and consequences of interindividual variability can provide new tools for both types of physiological analysis. I believe that it is also capable of building new and important bridges to other allied fields of biology, especially ecology, ethology, evolution, and genetics.

The tyranny of the Golden Mean

The framework of physiological studies implicitly emphasizes the description and analysis of central tendency. Depending on the data, this involves the calculation of mean values or the development of least-squares regression equations. After these values are determined, they take on a life of their own and become the only point of analysis and comparison. The complete breadth of biological variation determined in the investigation then is forgotten. Measures of variability (e.g., variance, standard deviation) are calculated and reported only to stipulate confidence limits about the mean or slope of the regression line. Groups are then compared to determine whether they are different from one another or from hypothesized values. The variability inherent in the original data is seen only as "noise," through which the "true" value of the central tendency can be glimpsed with appropriate statistical techniques.

This assumption of a "true" or "real" central tendency, which biological reality only approximates, stems from Platonic philosophical traditions. These maintain that ideal archetypes exist that can be perceived only imperfectly through perceptual sensation. The concept of an ideal form of a structure or process was central to the thinking of medical physiologists of post-Renaissance Europe and heavily influenced the functional biologists of the nineteenth century. These physiologists and morphologists, in their search for proximate causation, maintained a typological approach to experimentation and analysis and were largely unaffected by contemporaneous developments in evolutionary biology and genetics (cf. Mayr, 1982, for a more detailed discussion). Analysis of variability played an important role in these latter fields, but it was ignored by functional biologists at the time and remains largely unexplored by them even today.

To dispel any doubt that analysis of central tendency and neglect of variability is the dominant or exclusive analytical mode in organismal physiology, I reviewed all papers published during 1985 in the *Journal of Comparative Physiology*, the *Journal of Experimental Biology*, and *Physiological Zoology*. These are some of the best and most forward-looking journals in the field. Nearly all the articles reported mean values or regression equations and did statistical analyses. However, less than 5% of the articles even reported the range of values of the data obtained, and out of more than 250

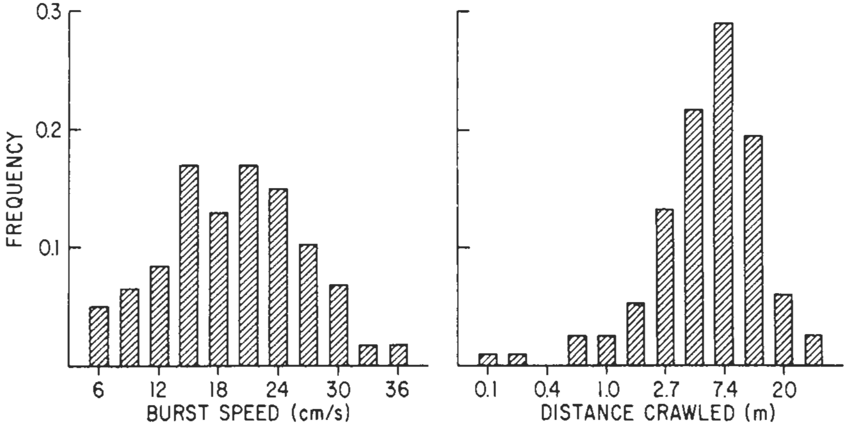

FIGURE 7.1 Frequency distributions of burst speed and total distance crawled under pursuit by individual newborn garter snakes (*Thamnophis radix*). Each individual observation is the mean of two trials conducted on two successive days; individual repeatability is highly significant ($r = 0.60$ for burst speed and 0.55 for distance; $p < .001$). Distance crawled is reported on a logarithmic axis. (Data from Arnold and Bennett, in press.)

articles, only one (Taigen and Wells, 1985) analytically examined the variability in the observations.

The concentration on central tendency has been and will continue to be very useful in testing certain hypotheses, but it has distracted us from an examination of the causes and consequences of biological variability. An example of this variability is given in Figure 7.1, in this case variability in locomotor performance capacity of newborn garter snakes. Maximal burst speed and the total distance crawled under pursuit were measured in nearly 150 laboratory-born animals shortly after birth (Arnold and Bennett, in press). These behaviors are individually repeatable (see below) and represent the breadth of response of the population at birth, before natural selection by the external environment has had the opportunity to act. Both these performance measures show strong central tendencies, but they also show enormous interindividual variability. The fastest snake has a burst speed ten times that of the slowest; the endurance of some individuals is more than twenty times that of others. Assuming for a moment that these individual differences are real (see below), these observations immediately suggest two sorts of questions. First, what is the functional basis of these individual performance differences? Which physiological or morphological factors make a fast snake fast and which account for the relatively low stamina of some other animals?

Second, what are the ecological and evolutionary consequences of these differences? Is there differential survivorship or growth under natural conditions based on locomotor performance capacities? These questions reflect the somewhat artificial dichotomy raised earlier between comparative physiology and physiological ecology, but both of them reflect compelling questions of general biological interest. They are obscured, however, if one concentrates only on central tendency. This is the tyranny of the Golden Mean: it restricts our vision of the data and narrows our conceptual framework so that we cannot take advantage of all the analytical possibilities of biological variability.

The failure to consider interindividual variability is not that of ecological or comparative physiology alone. Almost identical comments and comparisons could be made about any other field of organismal biology.

In our concentration on central tendency, we have failed in several respects:

1. We have ignored interesting biological problems and questions.
2. We have not been particularly interested in the consequences of the data we have gathered for survivorship or fitness.
3. We have failed to utilize the breadth of our data in assessment of physiological hypotheses.
4. We have failed to provide sufficient information in our research reports that would permit others to analyze biological variability.

The reality of interindividual variability

I believe that part of the difficulty that most ecological and comparative physiologists have in reporting and utilizing variability is a suspicion of its reality and information content. Biological measurements are inherently highly variable as compared to those made by physicists or chemists. Coefficients of variation of 20 to 30%, values that would cause a physical scientist to blanch, are routine in most physiological measurements. To what extent, however, is this variability real and useful? It seems to me that there are three potential objections to its use:

1. *Extreme values are atypical or abnormal and do not reflect the true response of most individuals.*

This view is essentially a restatement of the typological concept: the average is the real. Extreme performance certainly is "atypical" and "abnormal" in the strict sense of the words, but that does not mean that it is not real. A physiologist must be sure that experimental animals are in good condition, but it should go without saying that one must have external cause to doubt any data point. It cannot be questioned only because it happens to lie on the extreme of the range.

This view suggests that the experimenter has more confidence in values that lie closer to the mean than those at the extremes. If this is the case, then not all points should receive equal weighting: those closer to the mean should be weighted more highly. The circularity of this logic is apparent. Further, normal parametric statistics are inappropriate in such a circumstance. Either all data points receive equal confidence and equal weight, or the analytical methods we normally use are inapplicable; one cannot have it both ways.

2. *Observed variability is due to instrumentation or procedural error; the observed range does not result from real biological differences but from inaccuracies in experimental setups or procedures.*

According to the type of measurement, this objection may have some validity. However, the precision of modern physiological equipment is typically less than 1% and is consequently a doubtful explanation of much higher apparent biological variability. Further, if such errors are felt to be important, their magnitude must be quantified and analyzed (although they almost never are) even in studies that are interested only in central tendency. If the errors are random, then the mean values will be correct, but the measurements of variance and standard deviation of the means will be inflated. As statistical comparisons between groups are dependent on the extent of intragroup variability, incorrect judgments may be made if experimental or instrumentation error is not analyzed and removed. Consequently, if this type of error is a problem, it is not a special problem in the analysis of variability alone. It also affects any kind of analysis, including that of central tendency.

3. *The variation measured is real but reflects random and unrepeatable responses of individuals; that is, intraindividual variability is so high that there is no significant interindividual component to total variance.*

This is by far the most serious potential objection to the analysis of variability: if the responses are random with respect to individuals, then analyzing the differences among individuals is futile. The measurements required to demonstrate whether this is an important problem are a series of repeated observations on the same individuals and analysis of the significance of the individual component. For instance, if one is interested in oxygen transport capacity, one might measure maximal oxygen consumption in each of several individuals on sequential days to determine whether some individuals have consistently high or low capacities.

Given the general lack of interest in interindividual variability, analyses of intra- versus interindividual variability are relatively few in ecological or comparative physiological studies. Most of these relate to data on locomotor performance capacity, and many of the examples in this discussion will be drawn from this area. Individual locomotor performance ability has a significant repeatable interindividual component in every study in which it has

TABLE 7.1 Studies demonstrating significant interindividual variability in locomotor performance

Group	Performance	No. of species
Lizards		
	burst speed	6[a]
		1[h]
		2[c]
		2[d]
		1[e]
	stamina	6[a]
		1[f]
		1[d]
	defensive behavior	1[b]
Snakes		
	burst speed	1[g]
		1[h]
	stamina	1[h]
	defensive behavior	1[i]
Anurans		
	stamina	2[j]

[a]Bennett (1980). [b]Crowley and Pietruszka (1983).
[c]Huey and Hertz (1984). [d]Garland (1984, 1985).
[e]Crowley (1985). [f]John-Alder (1984).
[g]Garland and Arnold (1983). [h]Arnold and Bennett (in press).
[i]Arnold and Bennett (1984). [j]Putnam and Bennett (1981).

been examined (Table 7.1). An example of individual constancy of day-to-day differences in locomotor performance is given in Figure 7.2 (Bennett, 1980). Maximal burst speed was measured in fifteen adult fence lizards on five sequential days. Rank order of performance was conserved through the repetitive trials ($p < .001$). These individual differences in burst speed capacity were independent of both sex and body mass. Similarly, individual performance rank is stable even when the internal environment of the animals is grossly altered, as during changes in body temperature. Individual rankings of burst speed performance of alligator lizards at different body temperatures are given in Table 7.2. Again, individual differences are highly significant ($p < .001$): some animals are fast and some are slow at all body temperatures (see also Huey and Hertz, 1984).

I believe that locomotor measurements would a priori be among the *least* repeatable of any of the potential spectrum of "physiological" measurements. They may be influenced by a great many motivational and psychological factors, as well as differences in underlying physiological or morphological

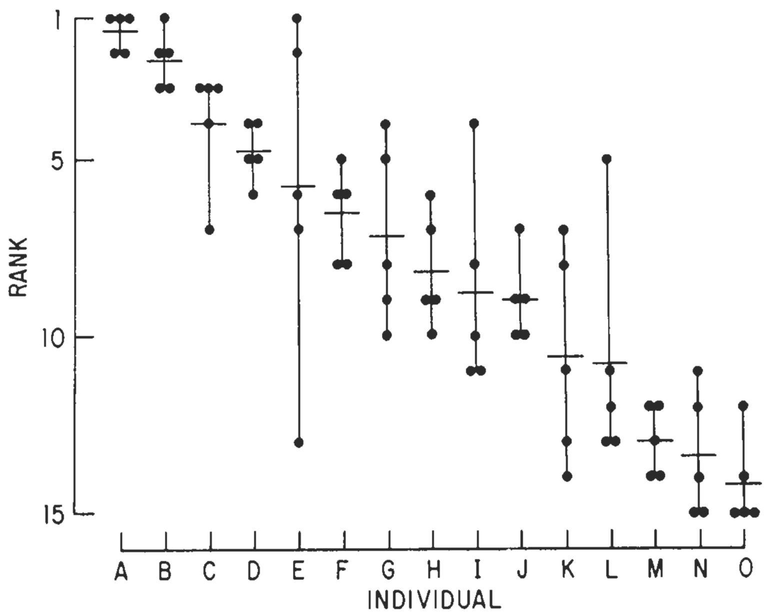

FIGURE 7.2 Rank order performance of burst speed in fifteen adult fence lizards (*Sceloporus occidentalis*) measured on five successive days. Rank 1 is the fastest animal, rank 15 is the slowest. Dots indicate rank performance on each day; vertical bars, range; horizontal bars, mean rank. Individual ranking effects are highly significant ($p < .001$ by Kendall's coefficient of concordance). (Data from Bennett, 1980, and unpublished observations.)

capacity. From that viewpoint, a significant interindividual component in measurements of locomotor capacity might suggest that many other physiological variables would also have individual fidelity.

Significant interindividual differences have been demonstrated in such diverse systems and measures as maximal oxygen consumption in amphibians and lizards (Pough and Andrews, 1984; Wells and Taigen, 1984; Sullivan and Walsberg, 1985), enzymatic activities in fruit flies (Laurie-Ahlberg et al., 1980), cuticular water loss in cicadas (Toolson, 1984), muscular morphology of birds (Berman, Cibischino, Dellaripa, and Montren, 1985), skeletal morphology of salamanders (Hanken, 1983), kinematics and muscle activity patterns during feeding in salamanders (Shaffer and Lauder, 1985a, 1985b), foraging tactics in fish (Ringler, 1983), food preferences in snakes (Arnold, 1981), and regulated body temperatures of lizards (Christian, Tracy, and Porter, 1985).

TABLE 7.2 Rank order of burst speed at different body temperatures in twelve individual alligator lizards (*Gerrhonotus multicarinatus*)

Temperature	Individual											
(°C)	A	B	C	D	E	F	G	H	I	J	K	L
10	8	7	3	11	4	9.5	1	9.5	2	5	12	6
15	8	5	1	11	3	6	2	10	7	9	12	4
20	7	6	2	11	3	8.5	5	8.5	1	4	12	10
25	9	8	2	11	3	4	6	7	1	12	10	5
30	6	9	2	12	7.5	5	4	10	1	7.5	11	3
35	8	10	7	11	5.5	2	4	9	1	5.5	12	3
37.5	9	8	7	11	6	3.5	1	10	2	3.5	12	5

Note: Rank 1 = fastest; $p < .001$ by Kendall's coefficient of concordance.
Source: Bennett (1980).

In my opinion, the large majority of physiological variables that can be sampled repeatedly will show real and significant interindividual variation. The question then becomes how we can utilize this variability to our benefit in asking analytical questions.

The analytical utility of interindividual variation

I suggest four different types of studies in which the exploration of interindividual variability might play a crucial role. Some represent new sorts of investigations for ecological or comparative physiology. Others permit a new approach to both current and classical questions in the fields.

The testing of correlative hypotheses

A common analytical approach in comparative physiology is to measure the correlation between two or more variables in two or more groups (e.g., populations, species) and to infer mechanistic relationships if significant correlations exist. For example, if positive associations are found between the length of the loops of Henle in kidneys of various mammals and their ability to concentrate urine, one might conclude that these may be functionally linked. These correlational examinations have been central in building the field of comparative physiology. They have, however, been criticized for their failure to take into account the phylogenetic history of the experimental animals involved (Gould and Lewontin, 1979; Felsenstein, 1985; Chapter 4).

A companion approach to interspecific analyses is the examination of interindividual correlations of variables within a species. This approach maintains the benefits of comparative analysis without some of the objections associated with using organisms that are distantly related phylogenetically (see Chapter 4). If two factors are functionally related, they should be significantly correlated among individuals within a species. In fact, if evolutionary or functional trends are proposed, the argument is strengthened if intraspecific associations can be demonstrated, because selection on traits within populations must be the ultimate source of adaptation.

The experimental protocol required to investigate interindividual variability is similar to that of interspecific comparative studies, except that observations on functional traits of interest must be made on the same individuals and analyzed on that basis. The researcher then correlates one trait with the other to determine whether they are positively or negatively associated. If so, the hypothesis of functional relationships among the traits is supported, and further experimentation can be planned to explore the nature of the relationship (see Huey and Bennett, 1986). If no significant association is found, then the traits are not functionally linked and the hypothesis is rejected.

One important step in this analysis is the determination of the dependence of the traits in question on body size (mass) and the elimination of such a dependence in the analysis. So many morphological, physiological, and behavioral traits are dependent on body size (see Calder, 1984; Schmidt-Nielsen, 1984) that it is very easy to obtain positive correlations among otherwise unrelated traits because of their mutual dependence on mass (see Appendix for a further discussion and example). Allometric analyses should be performed (see Chapter 10) and, if mass effects are significant, the mass-corrected residuals should be analyzed for correlation.

An illustrative example of the use of interindividual variability in testing correlative hypotheses may be beneficial here. These data are drawn from some observations on the skeletal muscle physiology and locomotor performance of tiger salamanders (Else and Bennett, 1987, and unpublished observations). Close (1964, 1965) proposed a correlation between the speed of isometric and isotonic contractions of skeletal muscle: the maximal velocity of shortening (isotonic) is supposed to be positively related to the rate of tension development in an isometric twitch or tetanus. This proposal is a straightforward mechanistic linkage that is supported by interspecific comparative studies. We can test this hypothetical connection by making observations of all these factors on individual animals and determining whether they are associated within individuals. A further correlation that might also be investigated is the association between muscle contractile speed and locomotor speed: are the animals that have the greatest intrinsic speed of muscle contraction also the fastest? First, all variables are mass-corrected and the residuals are then correlated with each other in Table 7.3. Correlations are significant among isometric variables and between isotonic variables, but no associations are significant between any isotonic and isometric variable nor between burst speed and any measure of muscle contractile performance.

TABLE 7.3 Correlation coefficients (r) among mass-corrected residuals of locomotor performance and muscle contractile factors in the salamander *Ambystoma tigrinum nebulosum* at 20 °C ($n = 20$)

		Isometric muscle factors				Isotonic muscle factors	
	Locomotion (burst swim speed)	Tetanic force	Twitch force	Tetanic contraction rate	Twitch contraction rate	Maximal rate of shortening	Maximal power output
Burst run speed	.13	−.27	−.38	−.52	−.42	−.05	.31
Burst swim speed		.14	−.24	−.11	−.09	−.41	−.29
Tetanic force			.79*	.67*	.72*	−.67*	−.47
Twitch force				.85*	.86*	−.43	−.31
Tetanic contraction rate					.97*	−.27	−.27
Twitch contraction rate						−.38	−.24
Maximal rate of shortening							.71*

Note: Asterisks indicate significant correlations ($r > 0.56$, $p < 0.01$).
Source: Unpublished data of A. F. Bennett, P. L. Else, and T. Garland.

These results argue against any necessary mechanistic association among these factors.

This is only one example of an approach that can be utilized in many different physiological or functional studies. For instance, the role of maximal heart rate in limiting maximal oxygen consumption or that of a particular muscle in generating force during feeding or locomotion could be investigated using an appropriate analysis of interindividual variability.

Examining the functional bases of organismal or physiological variables
Another use that can be made of interindividual variability is the determination of which of a potential suite of characters might influence performance at a higher level of biological organization. This is a multivariate statistical approach based on an array of characters measured in identified individuals of a species. The researcher measures a performance variable, such as burst speed or lower critical temperature, and a number of morphological and/or physiological predictor variables that might reasonably be associated with it (e.g., limb length and maximal velocity of muscle shortening, or fur density and body temperature, respectively). All these measurements are made on the same series of individuals. Mass dependence of any of the factors is analyzed and removed, as discussed previously. Then stepwise multiple regression analysis (or another appropriate technique, such as canonical correlation) is used to determine which, if any, of the predictor variables are associated with the performance variables.

An example of this approach is provided by the study of Garland (1984) on locomotor performance by a lizard, *Ctenosaura similis*. Endurance, burst speed, and maximal distance run under pursuit were measured in a series of individuals, along with a variety of physiological and morphological variables, including body mass and length; standard and maximal rates of oxygen consumption and carbon dioxide production; mass of thigh muscle, heart, and liver; hematocrit and hemoglobin concentration of the blood; myofibrillar ATPase activity of thigh muscle; and activities of three selected metabolic enzymes in heart, liver, and skeletal muscle tissue. Body mass effects were removed by regressing all variables on mass and analyzing only mass-corrected residuals. Each measure of locomotor performance was then regressed as a dependent variable on the suite of morphological and physiological characters as independent variables. The results of these analyses are given in Table 7.4. Nearly 90% of the mass-corrected interindividual variation in endurance could be attributed to four predictive factors, including maximal oxygen consumption, skeletal muscle and heart mass, and hepatic aerobic enzyme activity. This is a remarkable amount of predictive power. More than half the variation in maximal distance run is correlated with maximal carbon dioxide production and anaerobic enzyme activity of the skeletal muscle. None of the variables measured in this study was significantly associated with

TABLE 7.4 Stepwise multiple regression analysis of locomotor performance of the lizard *Ctenosaura similis*

Performance	Variable	Partial R^2	
Endurance	Thigh muscle mass	0.540	
	Maximal oxygen consumption	0.187	
	Heart mass	0.086	
	Liver aerobic enzyme activity	0.080	
	Total	0.893	($p < .0001$)
Distance run	Maximal carbon dioxide production	0.405	
	Thigh anaerobic enzyme activity	0.177	
	Total	0.582	($p = .0022$)
Burst speed	None	N.S.	

Source: Garland (1984).

burst speed. Thus, a multivariate statistical approach does not necessarily find a significant association among any set of variables. It may uncover strong correlations (as in the case of endurance) or no correlation (as with burst speed). A subsequent investigation on another species of lizard found significant interindividual correlations between burst speed and glycolytic enzymatic activity of skeletal muscle and an inverse relationship between burst speed and muscle fiber diameter (T. Gleeson, unpublished data).

A multivariate statistical approach can be particularly powerful when numerous underlying variables might be expected to influence higher-level performance. It can help to single out the most significant factors from an entire array and allow a researcher to concentrate further on those. The result of the analysis may serve to confirm a priori associations or may suggest entirely unexpected linkages that can be explored further. This multivariate analysis should be regarded as a first-stage approach, to be followed by more detailed comparative and experimental research on the factors identified with this technique. These further studies may also take advantage of interindividual variability.

Measurement of selective importance of traits under field or experimental conditions

Physiological ecologists and comparative physiologists usually assume that the traits that they study are of adaptive significance, that is, that they enhance survivorship and reproductive potential. This assumption is, however, almost never tested directly (Arnold, 1983; Endler, 1986). Using interindividual variability, one can evaluate whether performance of any given physiological or organismal trait is in fact correlated with differential survivorship under natural conditions. The observations required involve scoring a trait on a large number of individual animals, releasing them into their natural environment, and recapturing the survivors after exposure to this environment. The survivors are then examined to determine whether they are drawn from any subset of the original distribution.

Selection might operate in a number of ways to favor different portions of the original distribution of the trait (Simpson, 1953; Lande and Arnold, 1983; Endler, 1986). It might be *directional* and favor individuals at one end of the range of variability. For example, do birds with greater insulation survive better during the winter or do caterpillars that eat more metamorphose more rapidly and successfully? Do newborn snakes that are very fast or have a high endurance (see Figure 7.1) accrue an advantage under natural conditions such that they are more likely to survive to reproductive age? Selection may also be *stabilizing*, favoring animals with modal values for a given trait, thereby reducing variability and reinforcing central tendency in the population. In these cases, both very well and poorly insulated birds, caterpillars with both large and small appetites, and very fast and very slow snakes would be selected against. Selection might also be *disruptive*, favoring animals at both extremes of the distribution and tending to increase overall variability. The null hypothesis against which the presence of selection must be tested is the absence of any detectable effect of the variable on such indices of fitness as survivorship, growth, or reproduction. In the examples above, variability in plumage quality, feeding capacity, or speed would have no detectable influence on fitness under field conditions.

This correspondence between physiological or performance characters and survivorship or fitness under field conditions is termed the "fitness gradient" (Arnold, 1983). Its determination is judged to be essential for the characterization of the ecological and evolutionary implications of any physiological variable. However, comprehensive studies of the fitness gradient have rarely been attempted for any variable. The effects of natural selection on physiological variation generally are unknown (Endler, 1986). A lack of correspondence between maximal oxygen consumption and some measures of reproductive performance has been reported in adult male toads (Wells and Taigen, 1984; Sullivan and Walsberg, 1985), but its effect on differential survivorship up to adulthood has not been measured. Studies on the effects of locomotor performance on postnatal survivorship are currently underway on fence lizards (R. Huey, University of Washington) and garter snakes (my laboratory). The direct measurement of the impact of a character on performance under natural conditions, in spite of its obvious importance to field ecology and evolutionary biology, is almost unexplored. It may be operationally difficult or even impossible on some types of organisms, but I believe it is in fact feasible for many different types of animals in many different environments.

This approach has great potential to measure the importance of selection on traits in natural populations in natural environments. It also can be used in situations in which the environment has been experimentally altered. In this case, the response of the trait in the population can be compared to a priori expectations about the effect of such alteration. For instance, one might remove predators and determine whether burst speed or endurance declines in a population in the absence of this particular selective agent. One might supplement animals living in saline ponds or deserts with fresh water and investigate whether osmotic tolerance or fluid-concentrating capacity changes as a result of altered environmental circumstances. An excellent example of this experimental approach is provided by the study of Ferguson and Fox (1984). A combination of studies, examining responses of populations in both natural and experimentally manipulated environments, has a great deal of potential to help us understand the importance of various physiological processes to total fitness of organisms. This approach presents a protocol for testing assumptions about adaptation, not simply asserting them axiomatically. I believe this is one of the most exciting new developments and directions for physiological ecology as a field.

Determination of heritabilities of organismal or physiological characters

For adaptation and evolution of a trait to occur, it must have a genetic basis. Without a heritable basis, selection on a trait within each generation will not influence the variability or distribution of the trait in ensuing generations. It is necessary, for example, for fast parents to have fast offspring if the population is to respond to a new agent that selects against slower individuals. Studies of the heritability of physiological traits are a valuable supplement to ecological studies because they permit the determination of both the potential of the trait to evolve and the rapidity with which the response can occur.

Some progress has been made in particular systems in identifying effects of individual loci on organismal physiology and performance (e.g., Watt, 1977, 1983; DiMichele and Powers, 1982; Chappell and Snyder, 1984; Barnes and Laurie-Ahlberg, 1986; Chapters 5 and 8). While individual loci may have identifiable effects, many of the traits of interest to a physiological ecologist will be under multilocus control. Consequently, the techniques of quantitative genetics will be the most appropriate for examining the inheritance of these characters (see Falconer, 1981, and Chapter 9 for a general discussion of the field and appropriate methodology). Techniques involve examining the similarities of traits in parents and offspring and/or among the offspring of given parents. They require that the organisms in question can be bred successfully in the laboratory or that gravid females can be obtained that will deliver offspring in the laboratory.

Few studies have examined the heritability of physiological or performance characters. Most of them have dealt with the inheritance of locomotor performance. Significant heritabilities have been found for speed in race horses (Langlois, 1980; Tolley, Notter, and Marlowe, 1983), speed in humans (Bouchard and Malina, 1983a, 1983b), burst speed and stamina in lizards (van Berkum and Tsuji, in press; R. B. Huey, unpublished data) and snakes (S. J. Arnold and A. F. Bennett, unpublished data; T. Garland, unpublished data). Defensive behaviors in snakes are also heritable (Arnold and Bennett, 1984). In these locomotor studies, a minimum of 30 to 50% of the variability among individuals is genetic. Other types of physiological traits have also been found to be heritable: for example, growth rate and efficiency in pigs (Smith, King, and Gilbert, 1962), reproductive output of chickens (Emsley, Dickerson, and Kashyap, 1977), and thermoregulatory behaviors of mice (Lacy and Lynch, 1979). Observations are so few at this point that a case may be made for a general investigation of the topic of heritability per se of physiological systems in different types of animals. Future studies may concentrate on more specific genetic issues concerning this inheritance, but, given the multilocus nature of these traits, these are bound to be more difficult.

Conclusions

Interindividual physiological variability is rarely studied. However, this variation is real and repeatable in many physiological traits. I believe that the analysis of the causes and consequences of interindividual variability has major promise as an analytical tool in physiological studies. Ecological and comparative physiology have often been characterized as major branches of organismal biology, but their view of the organism has been ideal or typological. It has been that of the nonexistent animal that possesses the average value of all physiological, morphological, and behavioral attributes of the population. Such animals do not exist. Real individuals are unique combinations of traits, some above and some below average. It is time to recognize the uniqueness of the individual and to turn it to our advantage as biologists.

The analysis of variation can be useful in studies on physiological correlation and mechanism, on the importance of the variable to fitness under natural conditions, and on the potential for inheritance of the trait, with the consequent possibility of its adaptation and evolution. I do not suggest that the study of variation should supplant other approaches nor that it is even feasible for all physiological variables. But where such study is applicable, it can be a powerful analytical tool, for analysis of both mechanism and adaptation. Its particular advantage is that it can pull together so many different aspects of biology, not only physiology and ecology, but also behavior, morphology, population biology, and evolution. Biologists often treat these as different areas, but of course individual organisms do not make these arbitrary divisions and distinctions. They react to problems and opportunities as integrated organisms. Appreciating and studying individual differences can be a synthetic approach that puts the individual organism back into organismal biology and gives us a much broader understanding of animals and their evolution.

Appendix

The problem of mass-dependent correlation is so ubiquitous in correlation analysis that I will provide an illustrative example of the utility of the analysis of residuals. In Figure 7.3a, two physiological traits are found to be positively associated when one is plotted as a function of the other. These might, for example, be length of a Malphigian tubule and secretion rate in an insect, or tidal volume and anatomical dead space during ventilation in a mammal. Such a result might lead one to conclude that the traits are positively linked functionally. If, however, both traits are plotted as a function of body mass (Figures 7.3b and 7.3c), each is also found to be strongly and positively mass dependent. Are the traits truly linked to each other or is their apparent association due to their mutual but independent relationship to body mass? This size influence may be removed by examining the deviation of each data point from the mass regression line (i.e., the residuals of the regression). If these mass-corrected residuals are then plotted against each other (Figure 7.3d), their relationship can be examined without the interfering effects of body size. In the case illustrated, the traits are found to be negatively related to each other, which is exactly the opposite of the original conclusion based on Figure 7.3a. Their apparent positive association was due only to their mutual correlation with body mass. This was, of course, a contrived example: the residuals might also have been positively associated or not significantly correlated with each other. The point is that an examination of the original, uncorrected data in Figure 7.3a would not have permitted this assessment.

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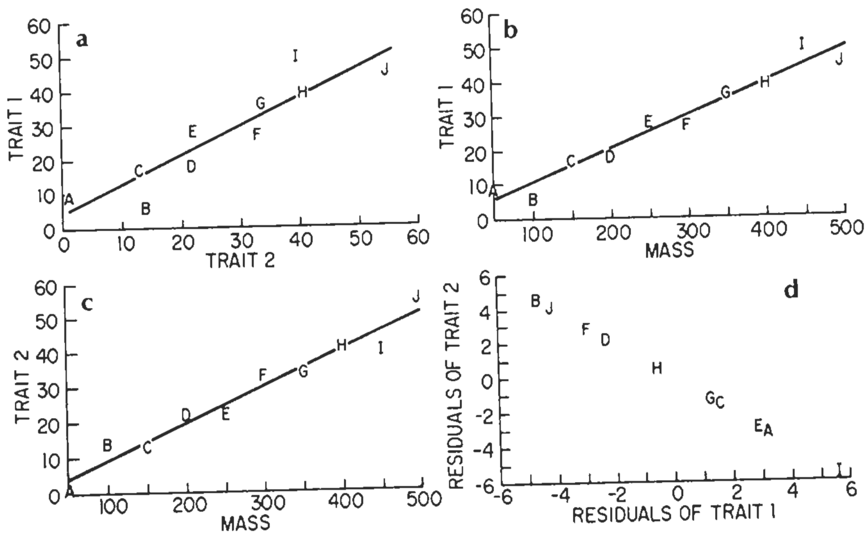

FIGURE 7.3 A hypothetical example of the effect of mass-correlated effects on the apparent association between two traits. Data are reported for two traits and body mass in arbitrary units for ten individuals (A through J) along with least-squares regression lines. (a) The two traits are positively and significantly correlated when they are related to each other directly. (b) and (c) Each trait is positively mass-correlated. (d) Mass effects are removed by calculating residuals, that is, the difference between the observed value for each individual and the value predicted by the mass regression. These are plotted against each other, demonstrating a significant negative association between the traits after the confounding effects of mass are eliminated. Note that opposite conclusions about the relationship between the traits would be derived from (a) and (d).

References

Arnold, S. J. (1981) Behavioral variation in natural populations. I. Phenotypic, genetic and environmental correlations between chemoreceptive responses to prey in the garter snake, *Thamnophis elegans*. *Evolution* 35:489–509.

Arnold, S. J. (1983) Morphology, performance and fitness. *Am. Zool.* 23:347–361.

Arnold, S. J., and Bennett, A. F. (1984) Behavioural variation in natural populations. III. Antipredator displays in the garter snake *Thamnophis radix*. *Anim. Behav.* 32:1108–1118.

Arnold, S. J., and Bennett, A. F. (in press) Behavioural variation in natural populations. V. Morphological correlates of locomotion in the garter snake *Thamnophis radix*. *Biol. J. Linn. Soc.*

Barnes, P. T., and Laurie-Ahlberg, C. C. (1986) Genetic variability of flight metabolism in *Drosophila melanogaster*. III. Effects of *Gpdh* allozymes and environmental temperature on power output. *Genetics* 112:267–294.

Bennett, A. F. (1980) The thermal dependence of lizard behaviour. *Anim. Behav.* 28:752–762.

Berman, S. L., Cibischino, M., Dellaripa, P., and Montren, L. (1985) Intraspecific variation in the hindlimb musculature of the house sparrow. *Am. Zool.* 25:21A.

Bouchard, C., and Malina, R. M. (1983a) Genetics for the sport scientist: selected methodological considerations. *Exerc. Sport Sci. Rev.* 11:274–305.

Bouchard, C., and Malina, R. M. (1983b) Genetics of physiological fitness and motor performance. *Exerc. Sport Sci. Rev.* 11:306–339.

Calder, W. A., III. (1984) *Size, Function, and Life History*. Cambridge, Mass.: Harvard University Press.

Chappell, M. A., and Snyder, L. R. G. (1984) Biochemical and physiological correlates of deer mouse α-chain hemoglobin polymorphisms. *Proc. Natl. Acad. Sci. U.S.A.* 81:5484–5488.

Christian, K. A., Tracy, C. R., and Porter, W. P. (1985) Inter-individual and intra-individual variation in body temperatures of the Galapagos land iguana (*Conolophus pallidus*). *J. Therm. Biol.* 10:47–50.

Close, R. (1964) Dynamic properties of fast and slow skeletal muscles of the rat during development. *J. Physiol. (Lond.)* 173:74–95

Close, R. (1965) The relation between intrinsic speed of shortening and duration of the active state of muscle. *J. Physiol. (Lond.)* 180:542–559.

Crowley, S. R. (1985) Insensitivity to desiccation of sprint running performance in the lizard *Sceloporus undulatus*. *J. Herpetol.* 19:171–174.

Crowley, S. R., and Pietruszka, R. D. (1983) Aggressiveness and vocalization in the leopard lizard (*Gambelia wislizennii* [sic]): the influence of temperature. *Anim. Behav.* 31:1055–1060.

DiMichele, L., and Powers, D. A. (1982) Physiological basis for swimming endurance differences between LDH-B genotypes of *Fundulus heteroclitus*. *Science* 216:1014–1016.

Else, P. L., and Bennett, A. F. (1987) The thermal dependence of locomotor performance and muscle contractile function in the salamander *Ambystoma tigrinum nebulosum*. *J. Exp. Biol.*, 128:219–233.

Emsley, A., Dickerson, G. E., and Kashyap, T. S. (1977) Genetic parameters in progeny-test selection for field performance of strain-cross layers. *Poult. Sci.* 56:121–146.

Endler, J. (1986) *Natural Selection in the Wild*. Princeton, N.J.: Princeton University Press.

Falconer, D. S. (1981) *Introduction to Quantitative Genetics*, 2nd ed. New York: Longman.

Felsenstein, J. (1985) Phylogenies and the comparative method. *Am. Natur.* 125:1–15.

Ferguson, G. W., and Fox, S. F. (1984) Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* 38:342–349.

Garland, T., Jr. (1984) Physiological correlates of locomotory performance in a lizard: an allometric approach. *Am. J. Physiol.* 247:R806–R815.

Garland, T., Jr. (1985) Ontogenetic and individual variation in size, shape and speed in the Australian agamid lizard *Amphibolurus nuchalis. J. Zool. (Lond.)* 207:425–440.

Garland, T., Jr., and Arnold, S. J. (1983) Effects of a full stomach on locomotory performance of juvenile garter snakes (*Thamnophis elegans*). *Copeia* 1983:1092–1096.

Gould, S. J., and Lewontin, R. C. (1979) The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. *Proc. R. Soc. Lond.* [*B*] 205:581–598.

Hanken, J. (1983) High incidence of limb skeletal variants in a peripheral population of the red-backed salamander, *Plethodon cinereus* (Amphibia: Plethodontidae), from Nova Scotia. *Can. J. Zool.* 61:1925–1931.

Huey, R. B., and Bennett, A. F. (1986) A comparative approach to field and laboratory studies in evolutionary ecology. In *Predator-Prey Relationships: Perspectives and Approaches from the Study of Lower Vertebrates,* ed. M. E. Feder and G. V. Lauder, pp. 82–98. Chicago: University of Chicago Press.

Huey, R. B., and Hertz, P. E. (1984) Is a jack-of-all-temperatures a master of none? *Evolution* 38:441–444.

John-Alder, H. B. (1984) Seasonal variations in activity, aerobic energetic capacities, and plasma thyroid hormones (T_3 and T_4) in an iguanid lizard. *J. Comp. Physiol.* 154:409–419.

Krebs, H. A. (1975) The August Krogh Principle: "For many problems there is an animal on which it can be most conveniently studied." *J. Exp. Zool.* 194:221–226.

Krogh, A. (1929) Progress of physiology. *Am. J. Physiol.* 90:243–251.

Lacy, F. C., and Lynch, C. B. (1979) Quantitative genetic analysis of temperature regulation in *Mus musculus.* I. Partitioning of variance. *Genetics* 91:743–753.

Lande, R., and Arnold, S. J. (1983) The measurement of selection on correlated characters. *Evolution* 37:1210–1226.

Langlois, B. (1980) Heritability of racing ability in thoroughbreds–A review. *Livestock Prod. Sci.* 7:591– 605.

Laurie-Ahlberg, C. C., Maroni, G., Bewley, G. C., Lucchesi, J. C., and Weir, B. S. (1980) Quantitative genetic variation of enzyme activities in natural populations of *Drosophila melanogaster. Proc. Natl. Acad. Sci. U.S.A.* 77:1073–1077.

Mayr, E. (1982) *The Growth of Biological Thought: Diversity, Evolution, and Inheritance.* Cambridge, Mass.: Belknap.

Pough, F. H., and Andrews, R. M. (1984) Individual and sibling-group variation in metabolism of lizards: the aerobic capacity model for the evolution of endothermy. *Comp. Biochem. Physiol.* 79A:415–419.

Putnam, R. W., and Bennett, A. F. (1981) Thermal dependence of behavioural performance of anuran amphibians. *Anim. Behav.* 29:502–509.

Ringler, N. H. (1983) Variation in foraging tactics in fishes. In *Predators and Prey in Fishes*, ed. D. L. G. Noakes, D. G. Lindquist, G. S. Helfman, and J. A. Ward, pp. 159–171. The Hague: Dr. W. Junk.

Schmidt-Nielsen, K. (1984) *Scaling: Why Is Animal Size So Important?* Cambridge University Press.

Shaffer, H. B., and Lauder, G. V. (1985a) Patterns of variation in aquatic ambystomatid salamanders: kinematics of the feeding mechanism. *Evolution* 39:83–92.

Shaffer, H. B., and Lauder, G. V. (1985b) Aquatic prey capture in ambystomatid salamanders: patterns of variation in muscle activity. *J. Morphol.* 183:273–284.

Simpson, G. G. (1953) *The Major Features of Evolution.* New York: Columbia University Press.

Smith, D., King, J. W. B., and Gilbert, N. (1962) Genetic parameters of British Large White pigs. *Anim. Prod.* 4:128–143.

Sullivan, B. K., and Walsberg, G. E. (1985) Call rate and aerobic capacity in Woodhouse's toad (*Bufo woodhousei*). *Herpetologica* 41:404–407.

Taigen, T. L., and Wells, K. D. (1985) Energetics of vocalization by an anuran amphibian (*Hyla versicolor*). *J. Comp. Physiol.* 155:163–170.

Tolley, E. A., Notter, D. R., and Marlowe, T. J. (1983) Heritability and repeatability of speed for two-and three-year-old standard bred racehorses. *J. Anim. Sci.* 56:1294–1305.

Toolson, E. C. (1984) Interindividual variation in epicuticular hydrocarbon composition and water loss rates of the cicada, *Tibicen dealbatus* (Homoptera: Cicadidae). *Physiol. Zool.* 57:550–556.

van Berkum, F. H., and Tsuji, J. S. (in press) Among-family differences in sprint speed of hatchling *Sceloporus occidentalis. J. Zool. (Lond.)*

Watt, W. B. (1977) Adaptation at specific loci. I. Natural selection on phosphoglucose isomerase in *Colias* butterflies: biochemical and population aspects. *Genetics* 87:177–194.

Watt, W. B. (1983) Adaptation at specific loci. II. Demographic and biochemical elements in the maintenance of the *Colias* PGI polymorphism. *Genetics* 103:691–724.

Wells, D. K., and Taigen, T. L. (1984) Reproductive behavior and aerobic capacities of male American toads (*Bufo americanus*): is behavior constrained by physiology? *Herpetologica* 40:292–298.

Discussion

LINDSTEDT: I agree with Bennett that there are identifiable individuals which are low performers – they have a low maximum oxygen consumption, a low maximum running speed, etc. – and there are other individuals that are high performers. But on any given day a low-performing animal may outperform the high-performing animals: the ranges of their performances overlap, even if their means are repeatedly different. The likelihood of finding mechanistic differences to account for those mean differences may be rather low. We still need to have the broader overview between species, where we have a higher signal-to-noise ratio.

BENNETT: I am not advocating that we abandon all other approaches for the study of variation, nor that we should ignore the means. But we should take interindividual variations into account in addition. Sometimes the least and most able individuals overlap, but you can still find that the individual differences are statistically repeatable.

LINDSTEDT: Yes, we do try to do that. By the same token, I think we have to be cautious about throwing out simpler statistics because they are simple, especially if we risk losing some biological insight with greater statistical sophistication. For example, in using stepwise multiple regression, it can be hard to intuit what the result means. Also, we have found that as we increase the sample size, the total proportion of explained variation changes very little, yet the relative contributions of various independent variables to the total explained variation changes a great deal. Again, that leads me to gain less insight.

BENNETT: I agree that many times the simple statistics are adequate, but where they are inadequate, we should not continue using the old models and old ways of doing things. The stepwise multiple regression approach seems to me to be a tool to suggest further directions for study. If you find that no factors are correlated with performance (measured as burst speeds), that may tell you that you should be looking at other factors. When you do have significant correlations, then you have a basis for further experimental analysis. It is a first pass in looking for important variables.

SCHEID: I agree that interindividual variability is really important. Nature intends to tell us something that we have mostly neglected so far. Now, is it not true that if you want to address the interindividual variability, then you have to look at the intraindividual variability first? In fact, the only thing that remains beyond intraindividual variability is true interindividual variability.

BENNETT: That's right. You have to be able to make repeated measures on individuals. This is feasible for some factors and unfeasible for other factors. If you are looking at whole-body lactate content, for instance, you can do that only once. But there are a large number of physiological characters, such as blood flow parameters, that we can now sample nondestructively because of improved instrumentation.

SCHEID: We now have improved techniques to work on uninstrumented, nonanesthetized animals, which is mandatory if you want to ask questions about variability. I think that the techniques were not formerly available to address this variability in a meaningful way.

HUEY: The standard statistical method of measuring repeatability is the intraclass correlation coefficient, which measures the proportion of the variation that is due to difference among individuals versus within individuals. By that measure, sometimes the types of measurements that Bennett was referring to are highly repeatable: most of the variation is among individuals and not within individuals. For example, Art Dunham and I looked at sprint speeds of lizards in natural populations over a whole year, and the repeatabilities are on the order of 0.5 to 0.6, which is higher than in thoroughbred horses. That is probably high enough that we can begin to analyze the mechanistic basis of individual variation and also look at the adaptive significance of that variation.

POWERS: One problem of reproducing the same experiment on the same individual is that some organisms become trained. In addition, we found that we cannot put more than one individual in a track at a time because of behavioral interactions between them.

BENNETT: For running speed, in about half of the species that we observe, we see what we assume is a conditioning effect, from day one to day two, but after that the means stay exactly the same, the order of the individuals stays the same. Some species show this initial effect, others don't.

POWERS: One thing we have to do with fish is to acclimate them to water that is moving at a constant speed, for thirty to sixty days. Everything from then on is very reproducible. I am sure that a lot of the variation in the literature is a function of this training phenomenon and where the organisms came from.

FLORANT: I think that developmental effects can be extremely important, and I wondered whether you were rearing these animals in the lab or being careful about the developmental processes that were going on prior to, during, and after birth.

BENNETT: All the animals that we have been dealing with are adult animals, taken directly from the field and tested within a matter of days. In the breeding studies, gravid animals are collected and young animals are born under constant conditions in the laboratory. The whole issue of developmental effects and constancy of rank-ordered performance over time has not even begun to be explored.

FUTUYMA: Suppose you are interested in very short term acclimation effects, the capacity of the individual to change its phenotype from moment to moment, which is the opposite of repeatability. How do you deal with that? There are interesting questions there as well.

BENNETT: You begin by immediately asking questions about your equipment and techniques, and get that out of the way first. Then perhaps you can begin building correlations from moment to moment by measuring the variables sequentially, to see whether you are getting tracking of one variable by the other.

ARNOLD: We can examine the capacity to change performance over short or long periods of time as traits, and that's a virtually unexplored area. But it is not the opposite of repeatability. Suppose we define a new variable that represents the capacity to change performance as a function of an elevation in temperature from 10 °C to 20 °C. We can measure its repeatability, we can ask whether it is inherited, whether it is genetically correlated with other traits, and so forth. The statistical field for dealing with such traits is sometimes called profile analysis of variance.

FEDER: I want to shift the focus of this discussion to the point that Bennett made about the prospect for performing natural experiments using natural populations. I am very excited about this prospect. Using the variation in populations as a substrate, altering an environmental variable for individuals in a population or adding individuals to a population and looking at the effects could potentially be a very powerful technique. Dennis Powers said that it may soon be possible to take individual genes and move them into or out of individual organisms, which could offer us a lot of insight.

POWERS: It is already possible for some species. In lower vertebrates, it will probably take another year.

DAWSON: There are detraining or conditioning effects that go with captivity. When we studied cold resistance in small birds, we found that the animals maintained in outdoor flight cages, given the seeds of the type that they were using naturally, abandoned their winter fattening, perhaps because they had assured meals and more complicated cues. They also had much lower cold resistance than freshly captured animals. If one is dealing with badly distorted responses, which is sometimes a risk with wild animals, that ought to be determined. A good deal of what one may be dealing with in animals long standing in captivity may not be relevant to the natural situation.

FLORANT: In keeping hibernators for many years in the lab, the hibernators begin to free-run, and it is as if certain physiological responses occur at "nonadaptive" times of year. This obscures the optimal time that the animal performs a particular response under natural circumstances.

BENNETT: These are valid concerns. One way of keeping track of them is to run appropriate controls, so that we can place boundaries on the magnitude of the captivity responses.

DAWSON: By attempting to determine repeatability, if you start early enough, you can discern if there are any effects of that type. That is not done a lot. This is a caveat about use of material from animal dealers, which may have a very fuzzy history indeed.