INVITED PERSPECTIVES IN PHYSIOLOGICAL AND BIOCHEMICAL ZOOLOGY

Analyses of Physiological Evolutionary Response

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ABSTRACT

Selection studies are useful if they can provide us with insights into the patterns and processes of evolution in populations under controlled conditions. In this context it is particularly valuable to be able to analyze the limitations of and constraints on evolutionary responses to allow predictions concerning evolutionary change. The concept of a selection pathway is presented as a means of visualizing this predictive process and the constraints that help define the population's response to selection. As pointed out by Gould and Lewontin, history and chance are confounding forces that can mask or distort the adaptive response. Students of the evolutionary responses of organisms are very interested in the effects of these confounding forces, since they play a critical role not only in the laboratory but also in natural selection in the field. In this article, we describe some methods that are a bit different from those used in most studies for examining data from laboratory selection studies. These analytical methods are intended to provide insights into the physiological mechanisms by which evolutionary responses to the environment proceed. Interestingly, selection studies often exhibit disparate responses in replicate populations. We offer methods for analyzing these disparate responses in replicate populations to better understand this very important source of variability in the evolutionary response. We review the techniques of Travisano et al. and show that these approaches can be used to investigate the relative roles of adaptation, history, and chance in the evolutionary responses of populations of *Drosophila melanogaster* to selection for enhanced desiccation resistance. We anticipate that a wider application of these techniques will provide valuable insights into the organismal, genetic, and molecular nature of the constraints, as well as the factors that serve to enhance or, conversely, to mask the effects of chance. Such studies should help to provide a more detailed understanding of the processes producing evolutionary change in populations.

Introduction

From a practical viewpoint, science should be predictive. To the extent that science can serve the needs of society, it must be able to predict outcomes, offer solutions, and foresee trends. From a theoretical viewpoint, the predictive nature of science is essential to the scientific method. Hypotheses can be formed and tested, but if the next test of the hypothesis will give a different result and thus interpretation, the validity of the hypothesis and elucidation of underlying principles are undermined.

In physiological ecology and evolutionary physiology, the concept of organismal adaptation to the physical environment has been a central and often guiding theme (Feder et al. 1987; Schmidt-Nielsen 1990; Hoffmann and Parsons 1991; Prosser 1991). Difficulties with experimental repeatability and the generality of results have also plagued these fields, since the species that are the most unique (and thus unreplicated) are often those with the most derived and, by implication, adaptive traits. As pointed out by Gould and Lewontin (1979), history and chance are confounding forces that can mask or distort the adaptive response.

In recent years, evolutionary physiologists have used selection studies as one means of examining the evolution of organismal traits under environmental selection. The results of these experiments demonstrate that an enormous number of traits can evolve in surprisingly short periods of time. In this article, we address the question of the degree to which the evolution of traits of interest to physiological ecologists can evolve in a predictable manner. We also discuss experimental designs and methods of data analysis that allow investigators to address the relative roles of adaptation, history, and chance in the evolution of organismal traits.

Selection Studies: the Pros and Cons

Selection studies have played a prominent role in the elucidation of evolutionary processes. Using selection studies of *Drosophila*, for example, the evolution of traits critical in the ecology of populations, including life-history traits (Rose 1991; Rose et al. 1996; Sgro and Partridge 2001) and traits related to

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the physiological ecology of organisms (Hoffmann and Parsons 1991, 1993; Huey and Kingsolver 1993; Djawdan et al. 1997), have been examined. It is only in the last two decades that the use of highly defined replicated procedures for selection studies has permitted a new approach to the study of evolution of physiological processes. The advantages of the use of *Drosophila* for the analysis of the evolution of physiological processes have recently been reviewed (Bradley et al. 1999; Gibbs 1999). These articles point out the advantages of selection studies, namely the capacity to know and indeed control the phylogeny of the organisms under study; the replicate nature of the populations, permitting rigorous statistical analysis of the results; the strict control of environmental parameters, permitting analysis of the effects of single discrete environmental variables on evolutionary responses; and the capacity to analyze not only the direction but also the rate of evolutionary change.

Harshman and Hoffmann (2000) point out a number of difficulties inherent in the interpretation of the data from selection studies. Selection studies in different laboratories, where they inevitably use different selection criteria, populations, and environments, produce different and often contradictory results. In addition, the responses of organisms to laboratory selection do not necessarily mimic those responses seen in wild populations, due either to the relaxation of constraints that occur in nature or to the distinct genetic makeup of the populations (e.g., inbreeding). Harshman and Hoffmann (2000) list five explanations for inconsistent outcomes in selection studies with *Drosophila.* Two of these have to do with sources of variation in responses among replicates that can obscure or even distort the selection response: genetic differences among replicate populations subjected to identical selection regimes and the occurrence of multiple mechanisms underlying the selection response. Both of these sources of error, or noise, in the observed response are actually of great interest to physiological ecologists studying the evolution of populations in response to environmental conditions.

Cohan and Hoffmann (1989) examined in detail the effects of uniform selection on replicate populations. It is well known that separate lineages, when exposed to similar selection conditions in similar environments, often converge on similar phenotypes. The authors suggested that due to genetic differences in the populations initially, this parallelism is likely to involve disparate genetic mechanisms. For these reasons, selection of two lineages under identical conditions and resulting in similar phenotypes can actually promote genetic divergence and increase overall genetic diversity. To test these ideas, Cohan and Hoffmann (1989) examined multiple lineages, as well as replicate populations within these lineages, under conditions of uniform selection for enhanced ethanol resistance. They found that all the lines responded similarly with regard to increased ethanol resistance. On examining five other phenotypic characters, however, the authors found that two of these showed statistically significant increases in variation. These results demonstrate increased phenotypic and presumably genetic variation for several traits under conditions of uniform selection.

In this article, we examine some methods that are a bit different from those used in most studies for examining data from laboratory selection studies. These methods are intended to provide insights into the physiological mechanisms by which evolutionary responses to the environment proceed (for example, a selection pathway as discussed below) and to provide methods for analyzing disparate responses in replicate populations to better understand this very important source of variability in the evolutionary response. Just as the variable responses of individuals in populations provide insights into the phenotypic variability on which selection can operate (Bennett 1987; Chown 2001), similarly the variability in the responses of populations to seemingly identical selection pressures provides valuable insights into the pathways and progress of evolutionary adaptations to the environment.

In discussing these approaches for examining the data from selection studies, we will deal almost exclusively with data from studies in our laboratory. Our approach derives not from the inherent superiority of these data to those gathered by others but rather from our familiarity with them. We feel that these approaches can be universally used, although they do require sufficient replication of populations within selection regimes to permit the proper analyses of the variability in evolutionary response.

A Description of the Selection Procedures

Between 1980 and 1995, Dr. Michael Rose of the University of California, Irvine, and his colleagues produced a number of populations of *Drosophila melanogaster* using a variety of selection criteria (Rose 1984; Rose et al. 1996). Each selection experiment used five separate replicate populations subjected to identical selection procedures and five replicate control populations. Populations selected for postponed reproduction (O populations) exhibited postponed senescence, longer life, and increased stress resistance. M. Rose subsequently derived large outbred populations from the O populations and subjected them to a variety of selection regimes.

For the purpose of our present discussions, the important feature of the selection studies was that they provided a variety of populations exhibiting a broad spectrum of resistance to a specific ecologically important stress, namely starvation. We used these populations to examine the correlation of physiological traits with the evolved differences in stress resistance (Djawdan et al. 1997). The correlation between the lipid content of the flies and their starvation resistance was quite high, with an r^2 value of 0.95. We found that carbohydrate content was also strongly correlated with starvation resistance, although the r^2 value for this correlation was only 0.79. We were concerned that lipid and glycogen might simply be covariates, that is, flies genetically predisposed to accumulate energetic resources might

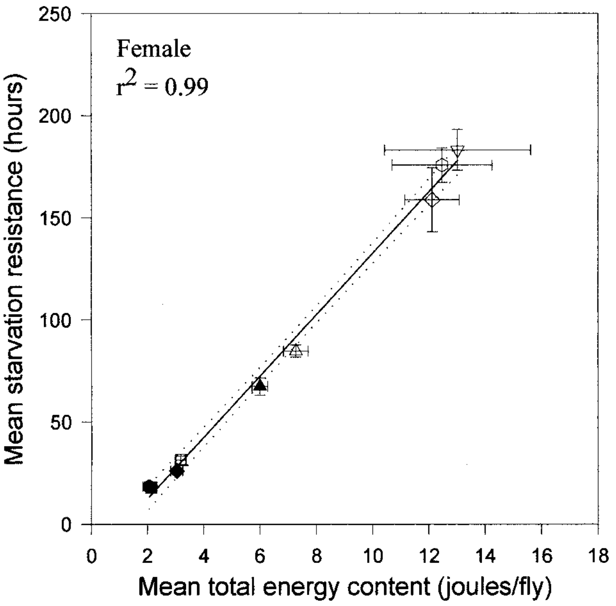

Figure 1. Relationship between mean (± SE) starvation resistance and mean (± SE) total body energy content in female *Drosophila melanogaster* from nine distinct selection regimes (Djawdan et al. 1998). Each symbol represents the mean value from four to five replicate populations within a selection regime. A least squares regression is fitted to the values as a solid line. The 95% confidence intervals around the regression are shown as dotted lines.

show correlated increases of both lipid and carbohydrate, causing both variables to be associated with increased starvation resistance. Yet replicate populations selected for enhanced desiccation resistance showed a greater increase in carbohydrate than lipid, whereas populations selected for starvation resistance showed the reverse trend. It is clear that at least in these populations, lipid and carbohydrate do not simply covary but that metabolic fuel can be differentially stored based on selection pressure predominantly as either lipid or carbohydrate.

We calculated the energetic value (in joules) of lipid and carbohydrate in all of the populations to examine the relationship between total stored energy and starvation resistance. We found a very strong correlation with an r^2 of 0.99 (Fig. 1). Given our understanding of the physiology of starvation resistance in animals, it might seem trivial and obvious that starvation resistance in an animal is proportional to the energy content in its nutrient stores. However, several nontrivial conclusions derive from these observations: (1) The correlation illustrated in Figure 1 does not merely demonstrate a physiological process linking an animal's energy stores and its starvation resistance but rather demonstrates an evolutionary process by which starvation resistance evolves in these populations of flies. The results provide a specific quantitative explanation for the evolution of both enhanced and decreased resistance in flies under a variety of environmental conditions. (2) Knowledge of the physiology of starvation resistance would suggest four significant parameters that might be subject to selection: lipid content, carbohydrate content, protein content, and metabolic rate. The metabolic rate of the populations did not evolve. Similarly, the remaining dry mass following the subtraction of lipid and carbohydrate content (i.e., protein content) did not change despite the capacity of organisms to use protein for energy metabolism. It is unclear why only two of the four predicted variables exhibit evolutionary changes in our populations. Certainly metabolic rate and body size have been shown to change in other populations of *Drosophila melanogaster* following stress selection (Hoffmann and Parsons 1991). It should be pointed out that our studies were carried out using ad lib. feeding. Under these conditions, it may be that accumulation of lipid and carbohydrate had fewer deleterious effects on other aspects of fitness than did changes in organ size or reductions in whole body metabolic rate. Under any circumstances, only lipid and carbohydrate content changed over multiple generations, and these two factors enable us to determine and predict very precisely the starvation resistance in all the populations.

A Selection Pathway

The results shown in Figure 1 suggest another very interesting possibility. Using 43 populations of flies that have been reared and/or selected in a variety of environmental conditions (i.e., four to five populations within nine selection regimes), we find that the populations do not deviate from a very distinct linear pattern. Might it be that this linear region represents physiological or genetic constraints on the mechanisms by which flies could evolve new levels of starvation resistance (Fig. 2)? We refer to this concept as a selection pathway, the notion that the process by which the physiological or behavioral performance of the flies evolves can be precisely defined using multiple quantitative variables. Although in the present case the relationship of the variables is linear, in other circumstances the mathematical relationships may be different.

The implications of this hypothesis are that for the character of starvation resistance, specific predictable changes in the insect's physiology lead to enhanced starvation resistance, and the same characters evolving in the opposite direction lead to reduced performance. It implies that the response involves a limited number of variables and that these are genetically determined, definable, and predictable.

Two factors should temper our enthusiasm for a wholehearted acceptance that the line in Figure 1 represents a selection pathway. First, the line represents a correlation among traits; the exact path by which the populations reached these points is not known. Arnold (1987), in his discussion of constraints in the context of population genetics, points out that evolutionary change need not be direct and can in fact for lengthy periods be nonadaptive. The advantage of selection

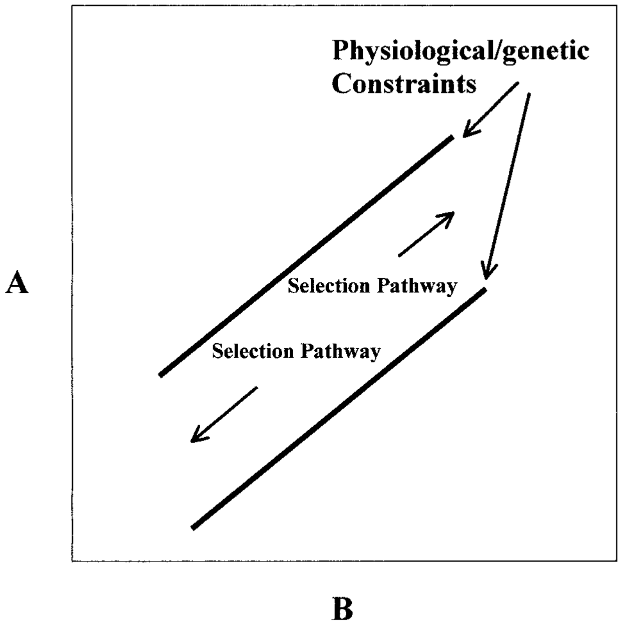

Figure 2. Diagram illustrating the correlation between a quantitative performance value *A* and a value *B* that is calculated using multiple organismal characteristics. The heavy diagonal lines delineate physiological and/or genetic constraints on the value of *B* as populations evolve to have greater or lesser values of *A*. The selection pathway implies a specific path that must be followed as the value of *A* increases or decreases over evolutionary time.

studies is that the evolutionary experiment can be repeated, and the selection pathway can be assessed generation by generation. We emphasize that the selection pathway as described in Figure 2 is a hypothesis, and as such, can be tested.

The second cause for concern in our acceptance of the selection pathway is the variance about the population means for each selection group. Although the mean responses of the populations within a selection group fall on a straight line with an r^2 value of 0.99, each selection group shows substantial variance around this mean. This variance is of considerable interest, for if a selection pathway does exist, what are the factors that contribute to variance in the evolutionary process, and what are the evolutionary implications of this variance? The following sections propose some new approaches for examining this issue.

Repeatability of Replicate Evolutionary Pathways

A further method for exploring sources and directions of variability in responses to selection uses a valuable feature of the selection studies, namely the replicate structure within treatment regimens. In the experimental design used by Dr. Michael Rose, five selected and five control populations were maintained for each type of selection protocol. Since the populations used for the selection studies were derived from five initial outbred populations that had long been maintained in the laboratory (O populations), each selected population could be paired with a control population derived from the same parent population. The selection and control regimes were therefore carried out five times on populations with different genetic backgrounds.

In the selection regime for enhanced starvation resistance, selected populations were provided with only moist agar until 80% of the flies had died. The survivors were then given food, and eggs were subsequently collected for the next generation. Eggs were collected from the control populations, which had not undergone starvation, at the same time (Chippindale et al. 1996). In the selection regime for enhanced desiccation resistance, selected populations were desiccated until 80% mortality was reached, while the controls were provided with moist agar. When selection was terminated, all D populations and the controls were provided with moist food and allowed to recover before egg collection for rearing of the subsequent generation (Gibbs et al. 1997).

It was found that the flies subjected to selection for enhanced resistance to starvation (SO populations) showed a marked increase in the total energy stored as lipid and carbohydrate but with lipid storage predominating on both a caloric and weight basis (Djawdan et al. 1997). By contrast, the flies selected for enhanced desiccation resistance (D populations) also showed a marked increase in the storage of energy; but in this case, the flies stored on average more carbohydrate than lipid, a strategy that presumably enhanced their water content as well, since glycogen is stored in conjunction with water of hydration and lipid is stored in a water-free state.

We can examine these evolutionary trends using the replicate structure of the selection studies to determine the repeatability and variability of these responses. Figure 3 shows the change in the energy stored as lipid and carbohydrate in each of the replicate SO populations (SO_1–SO_5), compared with the same parameters in the O populations from which they evolved. During strong selection for starvation resistance, each of the SO populations showed a much greater increase in energy stored as lipid than carbohydrate. The average slope of the lines is $0.12 + 0.02$ (mean + SE). All five populations responded strongly and similarly, albeit with slightly different degrees of intensity; that is, the lines are slightly different in length.

The C populations served as controls for the desiccation-selected (D) populations and had access to water (but not food) when the D flies were undergoing desiccation selection. The C flies were therefore subjected to mild starvation selection. Figure 4 shows the response of the replicate C populations (C_1–C_5). All five populations of C flies show a marked increase in their lipid energy content relative to their increase in carbohydrate energy content; the mean slope of the five lines being 0.06 ± 0.02. The C populations did not show as large an increase in lipid energy content as did the SO populations (the arrows are shorter), a result that can quite reasonably be attributed to the fact that they underwent much milder selection for enhanced

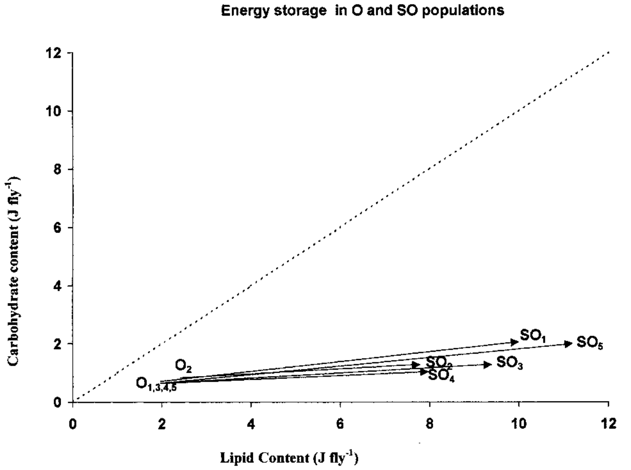

Figure 3. Relationship between mean (± SE) carbohydrate content and mean (± SE) lipid content in the five O populations (O_1–O_5) and the five SO populations (SO_1–SO_5) derived from them by selection for enhanced resistance to starvation. The arrows indicate the direction of change (but not necessarily the pathway of change) resulting from this selection. The dotted line represents the line of unity.

starvation resistance than did the SO populations. We analyzed the evolutionary responses of the C and SO populations by comparing the slopes and lengths of the response lines seen in Figures 3 and 4. Our analyses indicate that the slopes and lengths of the lines are significantly different ($P < 0.05$ and $P = 0.0001$, respectively).

The situation is markedly different when we consider the replicate populations selected for enhanced desiccation resistance (D_1–D_5). The D populations evolved from the same O populations as did the C and SO populations, yet they show a markedly different evolutionary pathway than the starvation selected flies. Figure 5 illustrates that the D populations have a much greater increase in carbohydrate energy content than the C (or SO) populations. Whereas the C populations had a mean response slope of 0.06, the D populations had a mean response slope of 2.0 ± 1.1 (Mann Whitney *U*-test, $P < 0.05$).

An obvious feature of the evolved responses of the replicate D populations, as shown in Figure 5, is the great variance in response. Although three of the populations have a response slope of <1, two of the populations show a very marked increase in carbohydrate relative to lipid with slopes of ~2 and ~6. Despite every attempt to make the selection regimes identical, we obtain markedly different responses in replicate populations.

Adaptation, History, and Chance

The previous examples regarding evolved changes in the lipid and carbohydrate energy content of selected populations illustrate the need to dissect possible causes of interreplicate variation in evolved responses. Fortunately, Travisano et al. (1995) have presented a model for experimentally approaching this problem. These authors conducted a "gedanken experiment" in which they compared the ancestral value of a quantitative trait in various ancestral populations to the value of that trait after a period of selection. Figure 6 shows a series of graphs in which the initial value of the trait in various ancestral populations (*X*-axis) is graphed versus the value of the trait in lines derived from those populations following selection (*Y*-axis). In Figure 6*A*, we can see that, for some traits, there was no initial variation among the ancestral populations and no variation in the trait for this ancestral value following selection. In Figure 6*B*, there was no variation in the trait in the ancestor, but the value of the trait diverged in various lines over the course of selection. Travisano et al. argue that since the mean value of the trait did not show a directional change in the various populations depicted, these changes reflect the random effects of chance. In Figure 6*C*, there was no variation among the ancestral or selected populations. All of the selected populations showed a directed and uniform change following selection. The authors argue that an increase in the value on the *Y*-axis is equivalent to an increase in fitness and therefore is the result of adaptation. Subsequent experimental data in the article derived from experiments with *Escherichia coli* where fitness can be measured directly. Under those circumstances, a mean increase on the *Y*-axis is equivalent to evolved adaptive change. In Figure 6*D*, the populations had no variance in the ancestral trait but showed adaptive change (an increase in the mean *Y* value). The effects of chance influenced the value of *Y* in the various populations. In Figure 6*E*, both adaptation and chance are seen to have their effects. The ancestral populations had variance in their initial trait values, as demonstrated by their distribution along the *X*-axis. The fact that the mean values of the replicates after selection (the *Y* values) were equal was taken

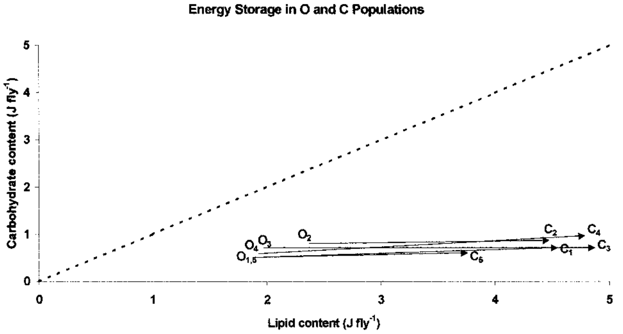

Figure 4. Relationship between mean (± SE) carbohydrate content and mean (± SE) lipid content in the five O populations (O_1–O_5) and the five C populations (C_1–C_5) derived from them. The C populations, which were mildly selected for enhanced starvation resistance, were controls for the populations selected for enhanced desiccation resistance. The arrows indicate the direction of change (but not necessarily the pathway of change) resulting from this selection. The dotted line represents the line of unity.

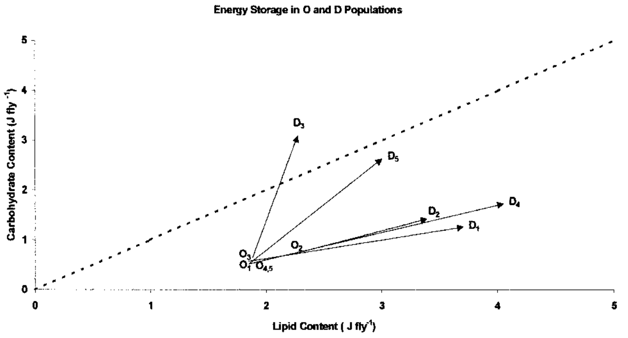

Figure 5. Relationship between mean (±SE) carbohydrate content and mean (±SE) lipid content in the five O populations (O_1–O_5) and the five D populations (D_1–D_5) derived from them by selection for enhanced resistance to desiccation. The arrows indicate the direction of change (but not necessarily the pathway of change) resulting from this selection. The dotted line represents the line of unity.

to show that adaptation had the predominant effect on the final character values, with chance playing a noticeable but minor role. Finally, in Figure 6*F*, it can be seen that the lines all showed signs of adaptation (they moved above the line of unity), but the effects of chance also caused the replicates to diverge slightly. A strong effect of history can be discerned in Figure 6*F*, however, in that the final values of the trait in the selected populations show a correlation with the initial state.

In this manner, Travisano et al. argue that through replication of lines with different ancestral conditions and the examination of evolved values, one can discern the effects of adaptation, history, and chance in the evolved responses. In fact, subsequently in the same article, Travisano et al. conducted such analyses on data from experiments using various clonal lines of *E. coli.* They demonstrated that evolved changes in the use of a novel sugar source were driven largely by adaptation, whereas the values of a second trait (cell size) showed an almost equal contribution of adaptation, history, and chance.

To our knowledge, no one to date has conducted experiments with *Drosophila* similar to those carried out by Travisano et al. There are several reasons for this. First, many selection studies are carried out with outbred populations, making it impossible to have replicate populations that are absolutely genetically identical before selection. Second, rarely are the numbers of replicates large enough to provide a rigorous test at any level other than the effects of selection.

The methods of Travisano et al. can, however, provide a useful template for further studies of the effects of history and chance on evolutionary outcomes during selection in eukaryotes. For the time being, some experimental data can be analyzed with these questions in mind, if replicate numbers are sufficient.

The Issue of an Ancestral Value

In the experiments discussed in this article, the O populations were measured at the same time as the D and C populations, thereby eliminating time-dependent block effects. For this reason, however, the O population values are not strictly ancestral values but rather are the current values for the populations from which the selected populations were derived. Three lines of evidence, however, argue that the O values represent a good estimate of the ancestral values. First, the O populations have undergone unchanging stable selection (for survival for 10 wk

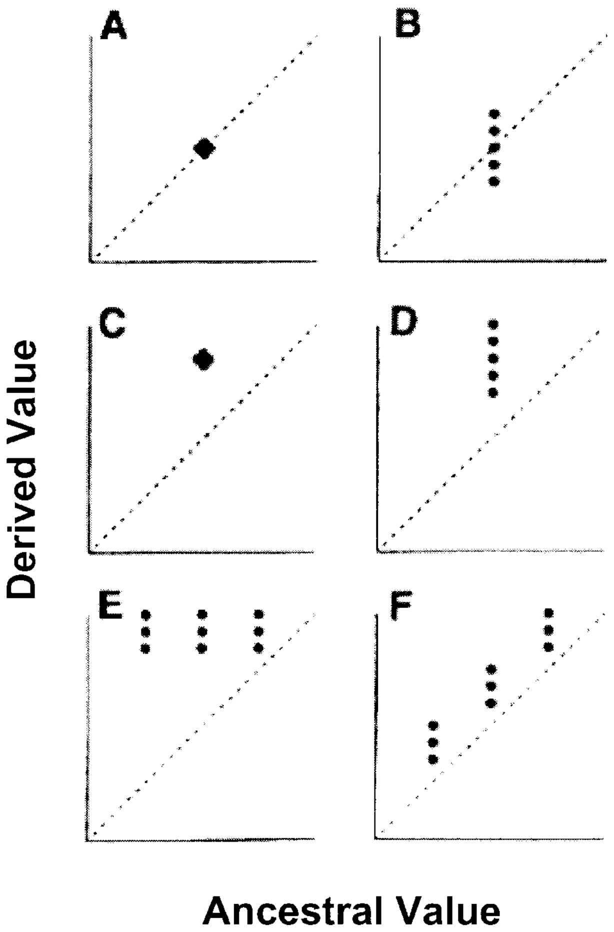

Figure 6. Diagrammatic representation taken from Travisano et al. (1995) showing the effects of adaptation, history, and chance on populations that have undergone multiple generations of selection. *A*, Populations showed no initial variation and no change over evolutionary time. *B*, No initial variation; all changes are due to the effects of chance. *C*, No initial variation; effects are due to adaptation. *D*, No initial variation; effects are due to the combined effects of adaptation and chance. *E*, Variation in the ancestral value is largely eliminated by the effects of adaptive change (an increase in the derived value). The effects of chance are observed as the variation within each of the replicate lineages. *F*, An initial variation due to history is maintained despite the effects of adaptation and chance.

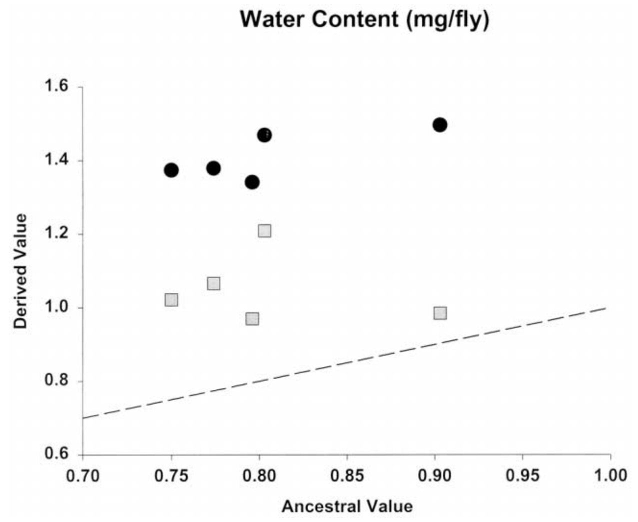

Figure 7. Mean water content on day 4 of adult life of unstressed female *Drosophila melanogaster* from the five populations selected for enhanced desiccation resistance (D_1–D_5) and their five control populations (C_1–C_5). The derived value is the water content of the D and C populations; the ancestral value is that of the O population from which both were derived. D_1–D_5 populations are represented by filled circles; C_1–C_5 populations are represented by gray squares. The dotted line represents the line of unity.

before egg laying) for more than 20 yr, and mortality during selection is very low. Second, the O populations are maintained on a long generation time at high population sizes (>1,000/generation). These conditions serve to minimize genetic drift, since neutral genetic drift is proportional to the number of generations that a population has been in isolation and inversely proportional to the population size. Third, water content in the O populations was measured in 1985 (Service et al. 1985), providing values not statistically and significantly different ($P > 0.3$) from the values we found in the present study. With regard to the characters we are examining, therefore, we are certain that the O populations have changed very little since the D and C populations diverged from them.

It follows, therefore, that although direct comparisons between the ancestral and derived populations may not be possible with many higher multicellular animal systems (it can be achieved with prokaryotes, yeast, and *Caenorhabditis elegans*), the Rose lines come as close as any to providing an experimental system for examining changes due to selection from the ancestral state given the constraint of working with many “higher” eukaryotes.

Evolution of Whole-Body Water Content

A number of evolved responses to selection have been observed in the populations subjected to selection for enhanced desiccation resistance (Bradley et al. 1999), including an increase in body water content (Gibbs et al. 1997). Using the methods of Travisano et al., we can graph the whole-body water content of each ancestral O population against the evolved whole-body water content of the C and D populations derived from them (Fig. 7; Folk et al. 2001).

The water content evolved in all C and D populations, regardless of treatment, since the whole-body water content for each population is above the line of unity. The D populations have whole-body water contents that are statistically and significantly higher than the controls ($P < 0.05$). Interestingly, the regression line (not shown) described by the five points for the D populations and that line (not shown) defined by the five points for the C populations both have slopes not significantly different from 0 ($P = 0.14$ and $P = 0.41$, respectively). Using the reasoning of Travisano et al., if the variation in the body water content in the evolved populations had been guided largely by history, we would have expected the slopes of these lines to match that of the line of unity and to be significantly different from 0. One can conclude that the historical influence of the ancestral condition contributed little to the evolution of whole-body water volume in these populations.

Water Loss Rates

In addition to increased water content, the populations selected for enhanced desiccation resistance also have a reduced rate of water loss during desiccation (Gibbs et al. 1997). If we again

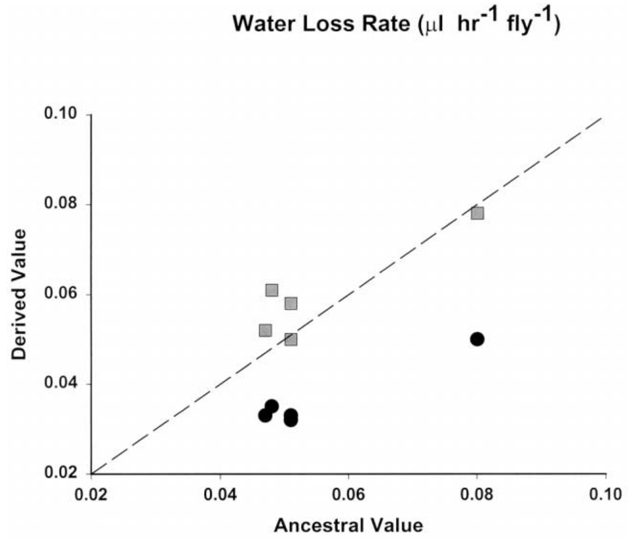

Figure 8. Mean rate of water loss on day 4 of adult life of female *Drosophila melanogaster* from the five populations selected for enhanced desiccation resistance (D_1–D_5) and their five control populations (C_1–C_5). The derived value is the rate of water loss in the D and C populations; the ancestral value is that of the O population from which both were derived. D_1–D_5 populations are represented by filled circles; C_1–C_5 populations are represented by gray squares. The dotted line represents the line of unity.

plot the evolved values for this parameter versus the ancestral values (data from Williams et al. 1998), we see that the D populations have lower water loss rates than those in the ancestral populations ($P < 0.05$) or control populations ($P < 0.05$; Fig. 8). For this parameter, a lower water loss rate corresponds with improved performance in a dry environment; hence an evolved increase in resistance corresponds to a lower value on this graph. The rate of water loss in the C populations has not significantly diverged from the ancestral values ($P = 0.60$).

If the variation among the D populations were entirely due to historical influences, the slope of the regression line described by the five points for the D populations would be indistinguishable from 1. If the variations were determined entirely by adaptation, the regression line would have a slope of 0. The regression line described by the D populations in Figure 8 has a slope of 0.53, a slope significantly different from 1 ($P < 0.001$) and from 0 ($P < 0.006$). We can conclude that both adaptation and history have influenced the patterns of evolved changes in water loss rate in the D populations.

Because their experimental design included replicates of each of the ancestral lineages, Travisano et al. (1995) were able to partition the variation due to chance, history, and selection. Using ANOVA, they partitioned the variance obtained due to measurement, replication within lineages, and selection, respectively. Cohan and Hoffmann (1989) also measured variance in replicates within lineages in their study of genetic variance in several lineages. We are proposing here that by comparing ancestral conditions to derived ones and by using regression analysis to examine the slopes of the resultant lines, information can be obtained regarding the relative strength of historical constraints and selection in the evolutionary responses to selection. As further selection experiments are carried out that incorporate replicates within lineages, both ANOVA and regression analysis, as we have used it, can provide insights into the responses of organisms to selection.

We recognize the time-sensitive nature of such analyses, for as selection studies proceed, the traits of interest may continue to evolve, and historical effects must inevitably diminish. In fact, the strength of this analysis is that the dynamics of the limits and constraints on evolution, namely history and chance, may be studied as selection proceeds. Furthermore, we appreciate that the five replicate SO, C, and D populations derive from five closely related ancestral populations (O populations); thus the historical influence may have been more limited than if the five replicate populations were derived from five ancestral populations with highly variable genetic backgrounds. It would be of interest to perform this type of analysis on replicate populations that are more genetically distinct to examine the effects of history, chance, and adaptation on the evolution of traits of interest.

Acknowledgments

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