

Physiological Responses to Selection for Desiccation Resistance in *Drosophila melanogaster*¹

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SYNOPSIS. Comparative physiologists and physiological ecologists have striven to elucidate the physiological adaptations which eliminate or mitigate environmental stress. Stress tolerance is thought to influence the distribution of species as well as the fitness of individuals within various habitats. Differential stress tolerance depends in large part on physiological mechanisms which mitigate the deleterious effects of stress. Very little information is available, however, regarding the mechanisms and pathways by which such physiological adaptations arose and were modified. We point out two methods by which one can investigate the evolution of stress tolerance: phylogenetic studies and selection studies. Phylogenetic studies have the advantage that they can be used to study wild populations, with the drawback that species numbers and distribution may be limiting. In addition, for many physiologically interesting clades, the phylogenetic relationships have yet to be determined. Selection studies have the advantage that the evolution of physiological systems can be studied in response to very specific forms of stress. In addition, the phylogeny of the organisms can be experimentally manipulated and replication permits rigorous statistical analysis. The results of studies of the evolution of increased desiccation resistance in *Drosophila* are presented as an example of the methods by which insights can be obtained regarding the variables which respond to selection, the rate of evolutionary change and the process by which physiological performance changes over evolutionary time. Selection studies can be designed to provide models regarding the mechanisms, timing and directions of physiological evolution.

Stress has been defined by Koehn and Bayne (1989) as "any environmental change that acts to reduce the fitness of an organism". Stress, so defined, can encompass abiotic conditions such as meteorological conditions, biotically-induced circumstances such as parasitism or disease, or even "normal" conditions within the life history of the organism such as migration. Despite the difficulty of unequivocally quantifying its effects, ecologists and physiologists continue to employ stress as a concept because of its perceived importance for survival and reproductive success.

Comparative physiologists and physiological ecologists have striven to elucidate the physiological adaptations which elimi-

nate or mitigate environmental stress. Frequently such adaptations are thought to permit occupation of an otherwise unusable environment (see for example the many studies which have been conducted on organisms inhabiting extreme environments; Schmidt-Nielsen, 1990). Inherent in these studies is the assumption that organisms survive in stressful environments because of the action of specific physiological mechanisms which mitigate the effects of the stress on the organisms. It is clear that organisms inhabiting extreme environments often have physiological mechanisms that mitigate specific environmental stresses to a greater degree than do their close relatives from other environments. Very little information is available, however, regarding the mechanisms and pathways by which such physiological adaptations arose and were modified. Even less apparent is the process by which modifications in one system are accommodated in the mechanisms that co-

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ordinate disparate but interactive physiological systems maintaining homeostasis in the organisms. The elucidation of patterns and mechanisms by which physiological systems evolve, and by which regulatory systems accommodate changes in physiological effector systems, presents an exciting challenge for the field of evolutionary physiology.

As pointed out in the introductory article in this series, three approaches have been widely used to investigate the evolution of physiological systems. These are phylogenetic analyses, population genetics, and selection studies. In the present study, we will describe studies which have employed selection to examine the evolution of enhanced desiccation resistance in the fruit fly, *Drosophila melanogaster*.

Difficulties associated with phylogenetic analyses

The evolution of physiological systems can be studied using phylogenetic analyses of extant animal populations (Harvey and Pagel, 1991; Huey, 1987; Garland and Carter, 1994). By appropriate analysis in the context of sister species and outgroups, one can infer synapomorphies and the ancestral condition. A particularly valuable approach is provided by the simultaneous mapping on a phylogenetic tree of the physiological and anatomical traits in question with geographical and ecological data (Brooks and McLennan, 1991). Analysis of these associations can lead to substantial insights into the evolutionary history of a group and the temporal association of physiological, anatomical and ecological change.

Phylogenetic analyses also present a number of daunting challenges, however. Often, precise knowledge of the phylogenetic relationships within a physiologically interesting group is lacking. In addition, key species which delineate important evolutionary steps in the phylogeny may be extinct. Garland and Adolph (1994) point out that it is important to examine more than two species in order to provide statistical support tests for the association of expressed traits with phylogenetic position. This point is also critical when examining groups of organisms from different ecolog-

ical regions. For example, if one were to compare desert rodents with rodents from more mesic regions, it would be critical that the desert rodents not be from one clade and the mesic rodents from a different, single clade. In such a case, clade is confounded with environment and one cannot differentiate the effects of selection from drift (Leroi *et al.*, 1994).

Let us imagine the ideal situation in which the phylogenetic relationships of the organisms are well known, phylogeny is not confounded with environmental factors and species distributions and numbers are sufficient to permit rigorous statistical analysis. Under these circumstances, the precise selection factors which are leading to the physiological differences are not known. For example, if a set of desert species is known to have evolved from ancestors in a mesic region, did the physiological features unique to the desert species evolve as a result of selection for desiccation resistance, reduced biological productivity, cold nights, or open terrain? These uncertainties do not negate the value of knowing the evolutionary history of the group regardless of cause. They do, however, provide little insight into the precise process by which physiological mechanisms respond to and are shaped by selection. Selection studies can provide insights into these questions.

Selection studies

Selection studies have a number of features which permit rigorous experimental design and address the uncertainties associated with phylogenetic studies. Firstly, the environmental variable being manipulated can be rigorously controlled. For example, desiccation resistance can be selected for in the absence of either correlated or non-correlated differences in temperature, diet, latitude, etc. Secondly, the populations can be replicated to allow for rigorous statistical analysis. In addition, in selection studies the phylogenetic relationships of the populations can be controlled (note the design of selection studies in *Drosophila* below). Finally, and perhaps most importantly, selection studies can be repeated. If indeed, the conditions and selection criteria are fully defined, then the experiment is in principal

fully repeatable. As with all experimental procedures, it is this capacity, or failure, to repeat results and predict outcomes which is the most important aspect of the scientific method.

Naturally, drawbacks and constraints occur with selection studies as well. Some studies involving rare plant species or large animals might not easily accommodate replication of populations at will. Although species with long generation times are difficult to study, most organisms which can be cultured in the lab (*e.g.*, weevils, peas, mice, algae) can be used as the subjects of selection studies.

Selection studies are by their very nature reductionist. A well designed selection experiment seeks to eliminate all forms of selection other than the specific variable being controlled. Selection studies do not attempt to mimic nature in all its chaotic and stochastic glory. Instead, they hope to provide insights into the pace, directions and mechanisms of evolutionary change.

Studies on selection for desiccation resistance in drosophila

In 1980, one of us (MRR) derived 10 populations from a single outbred population of *Drosophila melanogaster* which had been maintained in the laboratory for five years. Five of these populations were maintained on a two-week generation cycle (the B or baseline populations), while the other five populations were used to test evolutionary theories of aging. Their generation time was extended in a step-wise fashion over several generations. Eventually, these flies were maintained on a ten-week generation time (the O or old flies).

The O flies exhibited not only postponed reproduction but also increased longevity and increased resistance to a variety of stresses including desiccation, starvation, and exposure to ethanol vapors. In order to explore the capacity of the flies for further evolved stress resistance, two further populations were derived from each of the five O populations (Fig. 1). Five of these underwent selection in each generation for resistance to desiccation resistance (D populations). Flies were reared in the usual fashion as larvae and pupae. On day four of

adult life, the flies were placed in cages without food or water and in the presence of a porous container of desiccant. The flies were left in the cage until 80% had died of desiccation. The remaining 20% were removed to a separate cage and given food. Eggs were collected to start the next generation. Breeding populations levels were always maintained above 2,000 flies, so the initial populations prior to selection were about 10,000. In each generation, at the time that the D flies are placed in the desiccation cages, the C flies were also placed in cages. These flies were given access to water in agar but no food was provided. The C and D flies were therefore subjected to identical rearing conditions and generation time; the only difference being the absence of water during desiccation selection in the D flies.

When examined after more than 100 generations, the D flies showed extraordinary increases in desiccation resistance (Fig. 2) relative both to the control (C) populations and the ancestral conditions (O flies). This is of course an essential component of any selection study, the differentiation of traits under selection.

ELUCIDATION OF THE PHYSIOLOGICAL TRAITS CONTRIBUTING TO DESICCATION RESISTANCE

Factors that could evolve

Viewed in its most fundamental form, desiccation resistance in terrestrial animals is a question of water conservation. Under conditions where ingestion is precluded, desiccation resistance is a function of, a) the amount of water available in the body, b) the amount of water that can be produced by metabolism or made available by the production of a concentrated urine, and c) the rate of water loss. Factor a) above, the amount of water in the body, could more accurately be defined as the amount of water in the body initially minus the amount of water in the body at the time of death. This represents the "expendable" water and could at least in theory be increased through increased tolerance of a loss in body water. Rates of water loss can vary due to changes in integumental permeabil-

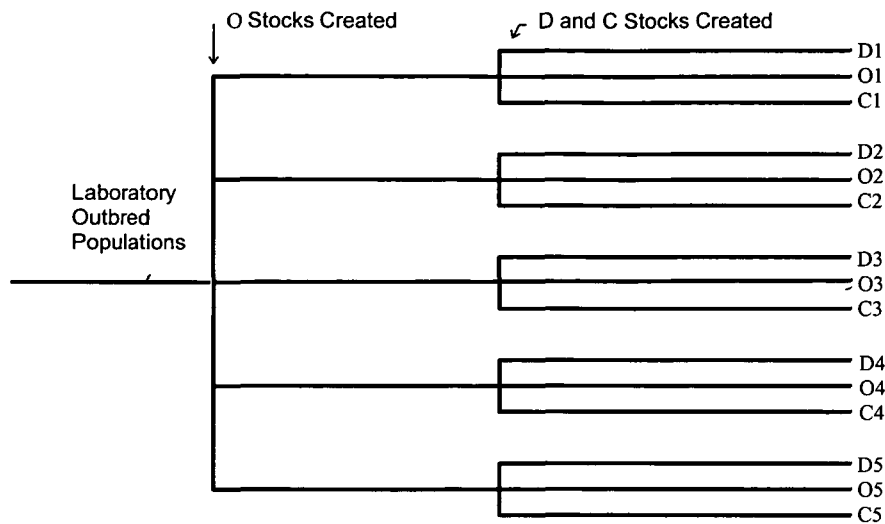


FIG. 1. A diagram illustrating the phylogenetic relationships of the laboratory populations used in studies described in this paper. Five populations (O1 through O5) were derived in 1980 from an outbred laboratory population of *Drosophila melanogaster*. In 1986, two populations were derived from each of the five O populations yielding ten new populations. For each of these pairs, the line undergoing desiccation selection was designated with a D and the control line with a C. The D and C populations were derived from the O populations and were given the same number. Thus, D1 and C1 were derived from O1. The O populations were retained for comparison. The O populations evolved separately for 66 generations prior to the production of the C and D populations. At the time the experiments described in this paper were conducted, the D and C flies had been separated from the O flies by about 170 generations.

Note that the closest relatives of the D1 population are the O1 and C1 flies. If, therefore, the D populations are found to be statistically significantly different from the C or O populations with regard to any trait, this must clearly be the result of selection and not phylogeny, since the closest relatives are all systematically different with regard to the selection regime.

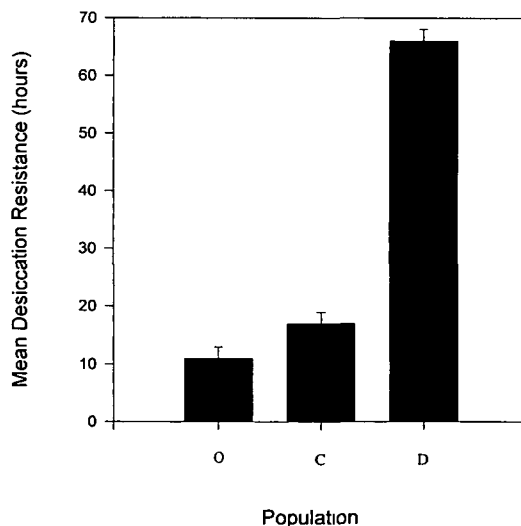


FIG. 2. Survival time in hours (mean \pm SE) of flies placed in vials without food or water and in the presence of a desiccant. Note that the D flies are highly differentiated with regard to desiccation tolerance.

ity, changes in salivation or excretion, and through changes in respiratory water loss.

The above list represents a lengthy though not exhaustive compilation of mechanisms and characters which, on the basis of other studies in insect physiology, might be expected to evolve under selection for desiccation resistance. Our challenge was to determine if these had indeed evolved, and to determine if other unexpected responses might also have occurred.

Factors that do evolve

Graves *et al.* (1992), conducted some of the first tests seeking physiological correlates to the capacity to resist desiccation in the D and C flies. They found that the D flies had increased whole body levels of glycogen. They argued that this condition might be physiologically associated with the capacity to survive in dry air. We have corroborated these measurements, and ex-

panded them by also quantifying the lipid stores of the D, C and O flies (Djawdan *et al.*, 1998). We found that D and C flies both have larger energy stores compared to their O ancestors, perhaps as result of the mild starvation selection they undergo. The C flies store most of their energy as lipid, while the D flies store significantly more as carbohydrate, principally glycogen. It can be seen then that selection for desiccation resistance has led to a shift in metabolic allocation in these flies with the desiccation-resistant flies storing more energy as glycogen than the C flies.

Hoffmann and Parsons (1989*a, b*) examined populations of *Drosophila* subjected to selection for desiccation resistance. Their studies differ from ours in a number of ways. In particular, they used only three replicate lines and these were initiated from isofemale lines. They found that the populations selected for desiccation resistance had lower metabolic rates than did the control lines. They argued that this reduction in metabolic rate might contribute to desiccation resistance by reducing respiratory exchange and therefore respiratory water loss. They argued further (Hoffmann and Parsons, 1991) that reduced metabolic rate might be a general character of stress-selected lines and might contribute to several forms of stress resistance. We therefore tested this hypothesis in our populations. We found, as did Hoffmann and Parsons (1989*a, b*), that the desiccation-selected lines (D populations) had lower metabolic rates than did the controls (C populations). As discussed above, the D populations have greater accumulations of glycogen than do the C and O populations. We reasoned that these large glycogen stores contribute to body weight but not to metabolic rate. We measured the glycogen and lipid content of flies in our D, C and O populations. When the weight of lipid and glycogen was subtracted from the dry body weight, and metabolic rate was expressed as a function of this value, no significant differences in metabolic rate were observed. This argues that changes in the metabolic rate of the flies had not occurred as a result of selection for desiccation resistance. Instead, increased accumulation of metabolic storage com-

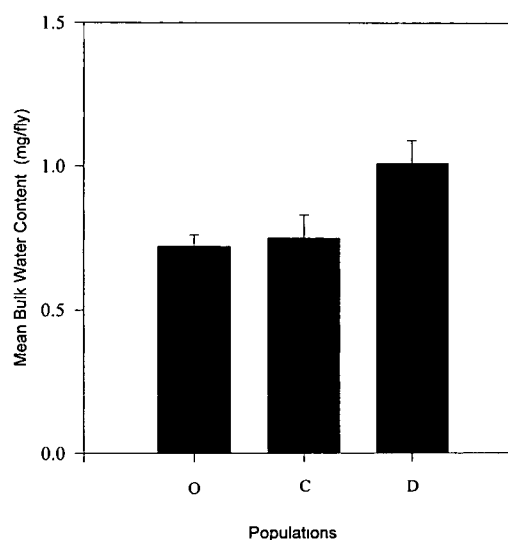


FIG. 3. Water content of flies (mean \pm SE) on day four of adult life. The D flies contain significantly more water than the C or O flies (D and C data from Gibbs *et al.*, 1997).

pounds, principally glycogen, has occurred and this leads to a lower metabolic rate per mg of total body weight.

Gibbs *et al.* (1997) examined the water content of the various populations of D and C flies. When combined with our measurements of the water content of the O flies (Nghiem, Rose and Bradley, unpublished) we can see that the D flies show a substantial and highly significant increase in total body water (Fig. 3). Gibbs *et al.* (1997) also examined the rate of water loss from D flies. Again, combined with our measurements of this parameter in O flies (Nghiem, Rose and Bradley, unpublished) we can see that D flies lose water at about 26 ± 2 nl per hour (mean \pm SE) while C and O flies lose water at 45 ± 2 and 36 ± 2 nl per hour, respectively. It is clear that the D flies have evolved both an increase in water content and a decrease in water loss rate. Gibbs *et al.* (1997) have argued that at least part of the increased water content in the D flies is due to the use of glycogen as opposed to lipid as an energy store. Glycogen, as a starch, is stored in association with water of hydration. This water becomes available when the glycogen is broken down and metabolized. Some of the additional water

the D flies is apparently not associated with glycogen, but its precise compartmental location is not yet clear.

A number of additional traits have evolved in the D flies in response to selection. Williams *et al.* (1997) demonstrated that selection for desiccation resistance in the D flies had led to an increase in discontinuous respiration, as measured by the discontinuity of CO₂ release by the flies. They attributed this phenomenon to an increase in spiracular closing by the flies. Subsequent analyses of water release has shown that water vapor is released in bursts simultaneously with the CO₂ release, supporting the contention that CO₂ release pattern is regulated by spiracular control in *Drosophila* (Williams *et al.*, 1998).

Discontinuous ventilation in insects has long been thought to promote water savings through a reduction in respiratory water loss (Levy and Schneiderman, 1966*a, b, c*; Edney, 1977; Hadley, 1994). It has been argued that when an insect's spiracles are closed, CO₂ builds up in the insect. When the spiracles subsequently open, the rate of CO₂ diffusion from the insect is greater than it would be if the spiracles were constantly open. By contrast, the gradient for water respiratory water loss would not change if the internal air stores remain fully hydrated at all times. The diffusion rate for water during the open phase is governed by the gradient from these saturated, hydrated tissues to the outside air. As a result, if periods of spiracular opening are relatively short, discontinuous ventilation promotes increased rates of CO₂ loss relative to rates of water loss from the respiratory system (Lighton, 1996). In *Drosophila melanogaster*, Williams *et al.* (1997) found that the D flies, which had been selected for increased desiccation resistance, also showed increased variability in the rate of CO₂ loss, suggesting increased spiracular control. Subsequent studies have demonstrated that *Drosophila* are capable of exhibiting a cyclical respiratory pattern with a closed phase (Williams and Bradley, 1998). However, neither highly discontinuous nor cyclical breathing patterns were found to be associated with concomitant decreases in rates of respiratory water loss. Williams and Bradley

TABLE 1. Responses we have observed to selection for desiccation resistance in *Drosophila melanogaster*. See text for references.

1. Increases in carbohydrate content.
2. Increases in bulk water content.
3. Decreases in cuticular water content.
4. Changes in respiratory pattern.
5. Changes in behavior.

(1998) were left with the conclusion that respiratory pattern did respond to selection for desiccation resistance, but without any demonstrable change in the rate of respiratory water loss. This conclusion is very interesting when viewed in the context of recent studies with other insects which express doubt that patterns of discontinuous ventilation in insects play a significant role in reducing respiratory water loss (Hadley and Quinlan, 1993; Lighton and Berrigan, 1995).

Williams *et al.* (unpublished) have also conducted preliminary studies of the behavior of the D, C and O flies. Using computer-based digital analysis of video records of the walking behavior of the flies, we find that the D flies are more quiescent than the C and O when subjected to desiccation in dry air, a behavioral shift which is genetic in origin and was evolved under selection for desiccation resistance. This behavior may play an important role in the ability of these flies to survive long periods in dry air.

Where do we go from here?

Table 1 lists the physiological changes observed to date in the D flies relative to the C and O flies. These differences are observed in experimental and control populations after two generations of identical rearing. These are therefore not the result of environmental or maternal effects, but are genetic in origin. We cannot be sure that each of these traits actually influences desiccation resistance since they may be associated with other such traits through genetic pleiotropy. How then can we further examine the physiological role of these traits and the process by which evolutionary change occurs in physiological systems?

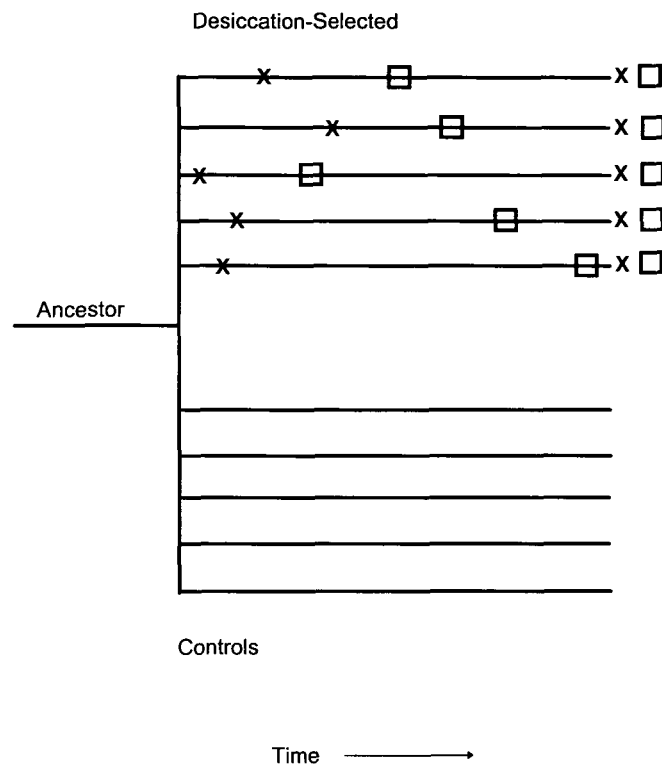


FIG. 4. A diagram illustrating the phylogeny of ten fly lines derived from an ancestral population. The five upper lines represent desiccation-selected populations, while the five lower lines represent five control populations. The selected lines are shown to become differentiated with regard to two traits illustrated by an X or an open box. The passage of time is shown from left to right. The location of the X or box symbol indicates the time at which the traits in the selected lines become statistically significantly different from those in the control populations. Note that the time at which traits become differentiated varies between the five selected lines.

The pattern of evolutionary change

The studies described above involved examination of the D, C and O flies after more than 100 generations of selection for desiccation resistance. A great strength of selection studies is the fact that they can be repeated. We are now selecting five new pairs of D and C populations from the five O populations and reexamining the patterns of evolutionary change leading to increased desiccation resistance. We are finding that the final results of selection are similar to those obtained before. Some of the traits shown in Table 1 change within 10 generations of the initiation of selection. These studies allow us to determine the timetable and order by which important traits vary during selection.

The process by which such experiments can be analyzed is illustrated in cartoon

form in Figure 4. Consider 10 replicate lines derived from an ancestral population. Selection is initiated at the branch point and the passage of time is shown going from left to right. After a considerable period of time (the right edge of the figure) the desiccation-selected lines can be seen to have two distinct traits (illustrated by the Xs and Open Boxes) which are not found in the controls. The point at which the symbols have been placed on the population's time line represents the point at which, a) a plus/minus trait converts in sign or b) the mean of a quantitative trait in the selected population becomes significantly different from the mean value in the control line. If we are aware of these traits from the beginning the experiment and follow the traits over time as selection is proceeding, we can determine the time, and variance in time, over

which these traits appear. We can also determine the order in which evolved changes in traits occur. In Figure 4, the traits marked by Xs appear early in selection and with low temporal variance, while the traits marked by an Open Box always appear later than the X transition, with a time interval which is quite variable. The rate at which a trait changes under selection can be assumed to be due to the genetic variance for the trait within the population, the heritability of the trait, and the degree intensity of selection of the trait. By careful examination of the timing and order of transition of traits in our experimental populations, we can obtain detailed insights into evolutionary processes by which desiccation resistance can increase.

Performance evaluation

A tremendous advantage in examining a well-defined physiological process is the capacity to quantitatively assess performance, and to relate this to evolutionary changes. In the case of the populations in Figure 3, we can measure not only the occurrence of traits at each point in time in each populations, but also the influence of these traits on overall organismal performance. We might find, for example, that when a population converts with regard to trait X, the mean desiccation resistance of the individuals goes up 30%, while conversion of trait Open Squares is associated with an increase of 200%. In such a scenario, the desiccation-selected populations would differ markedly in the time at which they showed large increases in desiccation resistance.

Such measurements permit us to identify traits that are associated with selection for desiccation resistance but which have no measurable effect on performance for this parameter. Additionally, we can sum the net effect of each of the traits to determine what percentage of the total effect has been accounted for by the traits we have identified. In this way we can ascertain if all the major traits have been identified. Alternatively, if during the course of selection a large improvement in desiccation resistance occurs which is not associated with change in an

TABLE 2. *A spectrum of experiments.*

1. Selection provides insights into the breadth of possible genetic responses.
2. Repetition of selection studies provides insights for ranking of these responses both temporally and physiologically.
3. Selection studies in organisms with other genetic backgrounds will reveal the universality of the responses.
4. Field studies will reveal the predictive strength of selection of studies.

identified trait, we will know that a major factor remains unidentified.

Expanding our horizons

Table 2 illustrates a spectrum of experiments which can be used for examining any selectable set of physiological. Initially, selection studies can be employed to obtain a list of candidate traits which may, but need not in all cases, respond to selection. Experiments which examine the traits, as well as organismal performance, with time can provide insights into the process, time course and pattern of physiological evolution.

Such studies have the capacity to provide insights into physiological mechanisms which have not been identified using comparative physiological methods. For example, the use of glycogen in place of lipid as an energy store is a logical mechanism for increasing body water content. This character has not, however, been one which students of insect osmoregulation have routinely examined.

Selection studies can be expanded to examine other populations or even species. Such experiments would examine evolutionary responses in populations possessing genetic backgrounds distinct from those previously used. One of us (MRR) has initiated such studies using other species of *Drosophila*.

Selection studies provide observations on the occurrence and distribution of traits influencing organismal physiological performance. They reveal the pattern of change of these traits, their quantitative role in performance and their distribution in other species. These studies can be designed with the further goal of providing testable hypothe-

ses about the process of physiological evolution in the wild.

Finally, the use of *Drosophila* for experiments of this type raises the possibility of examining the genetic basis of evolved physiological change. The success of such approaches remains an open yet exciting question.

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REFERENCES

- Brooks, D. R. and D. H. McLennan. 1991. *Phylogeny, ecology and behavior*. University of Chicago Press, Chicago.
- Djawdan, M., A. K. Chippindale, M. R. Rose, and T. J. Bradley. 1998. Metabolic reserves and evolved stress resistance in *Drosophila melanogaster*. *Physiol. Zool.* 71(5):584–594.
- Edney, E. B. 1977. *Water balance in land arthropods*. Springer-Verlag, Berlin.
- Garland, T. and S. Adolph. 1994. Why not to do two-species comparative studies: Limitations on inferring adaptation. *Physiol. Zool.* 67:797–828.
- Garland, T. and P. A. Carter. 1994. Evolutionary physiology. *Ann. Rev. Physiol.* 56:579–621.
- Graves, J. L., E. C. Toolson, C. Jeong, L. N. Vu, and M. R. Rose. 1992. Desiccation, flight, glycogen and postponed senescence in *Drosophila melanogaster*. *Physiol. Zool.* 65:268–286.
- Gibbs, A. G., A. K. Chippindale, and M. R. Rose. 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *J. exp. Biol.* 200:1821–1832.
- Hadley, N. F. 1994. *Water relations of terrestrial arthropods*. Academic Press, San Diego.
- Hadley, N. F. and M. Quinlan. 1993. Discontinuous carbon dioxide release in the eastern lubber grasshopper *Romalea guttata* and its effect on respiratory transpiration. *J. exp. Biol.* 177:169–180.
- Harvey, P. H. and M. D. Pagel. 1991. *The comparative method in evolutionary biology*. Oxford University Press, Oxford, UK.
- Hoffmann, A. A. and P. A. Parsons. 1989a. An integrated approach to environmental stress tolerance and life history variation. Desiccation tolerance in *Drosophila*. *Biological Journal of the Linnean Society* 37:117–136.
- Hoffmann, A. A. and P. A. Parsons. 1989b. Selection for increased desiccation resistance in *Drosophila melanogaster*: Additive genetic control and correlated responses for other stresses. *Genetics* 122:837–845.
- Hoffmann, A. A. and P. A. Parsons. 1991. *Evolutionary genetics and environmental stress*. Oxford University Press, New York.
- Huey, R. B. 1987. Phylogeny, history, and the comparative method. In M. E. Feder, A. F. Bennett, W. W. Burggren, and R. B. Huey (eds.), *New directions in ecological physiology*, pp. 76–101. Cambridge University Press, Cambridge.
- Koehn, R. K. and B. L. Bayne. 1989. Towards a physiological and genetical understanding of the energetics of stress response. *Biol. J. Linnean Soc.* 37:157–171.
- Leroi, A. M., M. R. Rose, and G. V. Lauder. 1994. What does the comparative method reveal about adaptation? *Amer. Nat.* 143:381–402.
- Levy, R. I. and H. A. Schneiderman. 1966a. Discontinuous respiration in insects. II. The direct measurement and significance of changes in tracheal gas composition during the respiratory cycle of silkworm pupae. *J. Insect Physiol.* 12:83–104.
- Levy, R. I. and H. A. Schneiderman. 1966b. Discontinuous respiration in insects. III. The effect of temperature and ambient oxygen tension on the gaseous composition of the tracheal system of silkworm pupae. *J. Insect Physiol.* 12, 105–121.
- Levy, R. I. and H. A. Schneiderman. 1966c. Discontinuous respiration in insects. IV. Changes in intratracheal pressure during the respiratory cycle of silkworm pupae. *J. Insect Physiol.* 12:465–492.
- Lighton, J. R. B. 1996. Discontinuous gas exchange in insects. *Ann. Rev. Ent.* 41:309–324.
- Lighton, J. R. B. and D. Berrigan. 1995. Questioning paradigms: Caste-specific ventilation in harvester ants, *Messor pergandei* and *M. julianus* (Hymenoptera: Formicidae). *J. exp. Biol.* 198:521–530.
- Schmidt-Nielsen, K. 1990. *Animal physiology: Adaptation and environment*. 4th ed. Cambridge University Press, Cambridge.
- Williams, A. E., T. J. Bradley, and M. R. Rose. 1997. CO₂ release patterns in *Drosophila melanogaster*: The effect of selection for desiccation resistance. *J. exp. Biol.* 200:615–624.
- Williams, A. E., M. R. Rose, and T. J. Bradley. 1998. Using laboratory selection for desiccation resistance to examine the relationship between respiratory pattern and water loss in insects. *J. exp. Biol.* 201:2945–2952.
- Williams, A. E. and T. J. Bradley. 1998. The effect of respiratory pattern on water loss in desiccation-resistant *Drosophila melanogaster*. *J. exp. Biol.* 201:2953–2959.

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