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REVIEW

LABORATORY SELECTION FOR THE COMPARATIVE PHYSIOLOGIST

ALLEN G. GIBBS*

Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA

*e-mail: agibbs@mail.arl.arizona.edu

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Summary

An increasingly popular experimental approach in comparative physiology is to study the evolution of physiological traits in the laboratory, using microbial, invertebrate and vertebrate models. Because selective conditions are well-defined, selected populations can be replicated and unselected control populations are available for direct comparison, strong conclusions regarding the adaptive value of an evolved response can be drawn. These studies have shown that physiological systems evolve rapidly in the laboratory, but not always as one would expect from comparative studies of different species. Laboratory environments are often not as simple as one thinks, so that the evolution of behavioral differences or selection acting on different life stages can lead to unanticipated results. In some cases, unexpected responses to laboratory selection may suggest new insights into physiological mechanisms, which might not be available using other experimental approaches. I outline here recent results (including success stories and *caveats* for the unwary investigator) and potential directions for selection experiments in comparative physiology.

Key words: selection, experimentation, laboratory, evolution.

Introduction

In the last decade, comparative physiologists have increasingly borrowed ideas and methods from evolutionary biology (Garland and Carter, 1994). A particularly important approach has been the use of phylogenetic information in comparative studies (Garland and Adolph, 1994). Knowledge of the evolutionary history of organisms can indicate whether variation among taxa reflects adaptation to their habitat or is perhaps an artefact of their evolutionary history. However, several potential problems arise in phylogenetic analyses (Lauder et al., 1993; Leroi et al., 1994). The current environmental conditions experienced by species may differ from their historical selection regimes, so that organisms may actually be adapted to conditions that no longer exist. Characters may be genetically correlated with each other, making it difficult to determine which one is actually the product of natural selection. In some cases, a proper phylogenetic analysis may be impossible, because rarity, extinction or political conflicts may make appropriate taxa unavailable.

Another approach is selection studies in the field (Endler, 1986). These are common in evolutionary ecology and often examine traits with a major physiological component (Reznick and Travis, 1996). The benefit is that the Darwinian fitness of variants is actually determined, rather than performance variables that contribute to fitness. Drawbacks include the labor-intensive nature of the work, which may limit sample sizes, and the complexity of the real world. Unknown variables, including historical factors, apparently insignificant habitat features or random events such as floods or droughts, may affect results in an unforseen manner.

Laboratory evolution provides a complementary approach to phylogenetic analyses and field studies. Populations of organisms are subjected to a defined stress over multiple generations, and the products of evolution are examined. Selection experiments have been used extensively by evolutionary biologists, often to study physiological problems such as stress resistance (e.g. Hoffmann and Parsons, 1989). Several comparative physiologists have begun to collaborate with evolutionary biologists and use laboratory evolution as part of their research program (e.g. Bennett et al., 1990; Huey et al., 1991; Gibbs et al., 1997) or have developed their own laboratory evolution systems (Lynch, 1980; Swallow et al., 1998a), but this is still a new approach for most physiologists. The objective here is to provide an introduction to laboratory evolution as an experimental approach and to discuss why a comparative physiologist would use it. In addition to providing several examples in which laboratory evolution has already contributed to comparative physiology, I will suggest a few ways in which selection experiments might allow insights not available using other approaches.

Types of laboratory selection

Experimental evolution is as old as the field of evolutionary

biology. Indeed, the very first chapter of the *Origin of Species* (Darwin, 1859) describes the results of selection by plant and animal breeders. Selection experiments can take many forms (for more detailed discussions, see Rose et al., 1990, 1996). Screens for mutants having a particular phenotype are routine in microbiology laboratories. In 'artificial selection', the investigator deliberately chooses those individuals that will produce the next generation. This approach is very useful for understanding the genetic basis for phenotopic variation (Hartl and Clark, 1989), but the traits studied are often not particularly interesting to physiologists (e.g. bristle number in *Drosophila melanogaster*).

Another approach, 'natural selection in the laboratory', involves subjecting populations to a specific environment, such as a high or low temperature, and allowing them to evolve by whatever means available in their new habitat. The investigator does not choose which individuals will reproduce, nor is any attempt made to specify the mechanism of adaptation. For example, a population exposed to high levels of predation in the laboratory might evolve greater speed or maneuverability to escape, crypsis, unpalatibility or self-defense. A variant of laboratory natural selection is 'natural truncation selection', or culling selection (Rose et al., 1990), in which a lethal stress is applied until a significant fraction of the population is dead, and the survivors are used to found the next generation. Studies using types of laboratory natural selection more closely reflect what happens in nature, so they will be emphasized here.

Laboratory selection offers several advantages for studying the evolution of physiological systems, including replication of selection treatments, repeatability, well-defined selection conditions and controls. By subjecting multiple populations to selection, one can be more confident that any observed changes are actually caused by selection, not by chance. In many cases, laboratory evolution experiments are equivalent to a two-species comparison, but their replication provides greater statistical power than in similar natural systems (Garland and Adolph, 1994). Selection can be repeated, in other starting populations, other species or other laboratories. The selection regime is known, because it is imposed by the investigator. A single feature of the environment can be varied (temperature, food, water, etc.) while keeping other factors constant. Rigorous controls for the selection treatment can be developed, such as unselected control populations maintained under the same conditions as the ancestral population. Even better controls are possible in some species in the form of cryopreserved ancestral stocks.

Results from laboratory selection experiments

Comparative physiologists have traditionally sought species from habitats where performance or survival should be limited by environmental conditions (e.g. deserts, high latitudes or high altitudes). By simulating these stresses in the laboratory, selection studies have tackled several problems of great interest to environmental physiologists: energetics, locomotion, water balance and thermal tolerance. Although limited in number, these studies reveal several important things about the evolution of physiological systems.

Physiological evolution is rapid enough to study in the laboratory

Clearly, selection experiments will be limited to organisms with short generation times, so that significant physiological changes can evolve, be detected and be published before the next grant renewal. Traditional comparative studies involve organisms separated by thousands to millions of generations, whereas even a rapidly developing organism such as *D. melanogaster* undergoes fewer than 40 generations in a year. Do physiological systems evolve rapidly enough to be studied in the laboratory?

Microorganisms certainly evolve rapidly enough; *Escherichia coli* go through more than 2000 generations per year, while significant differences in fitness evolve within a few hundred generations (Travisano et al., 1995; Elena et al., 1996). Because of their asexual mode of reproduction, evolution in bacteria occurs primarily by the action of selection and drift on randomly occurring mutations. One must wait for these to appear, but with large populations and fast generation times, the wait is not very long. For example, after 5 years (10^4 generations), Lenski and Travisano (1994) estimated that each of 12 replicated *E. coli* lines had undergone approximately 7.5×10^{11} cell replications per line. Given typical mutation rates (2.5×10^{-3} mutations per replication) and a genome size of 5×10^6 base pairs, these authors calculated that every possible point mutation had occurred an average of more than 100 times in each population.

As long as genetically diverse populations are used as founders, the evolution of sexual organisms can also occur quite rapidly in the laboratory. Physiological differences appear between stress-selected and control populations of *Drosophila* in less than 10 generations (Rose et al., 1992; Blows and Hoffmann, 1993; Hoffmann and Parsons, 1993a,b; Archer, 1999) (Fig. 1A). Laboratory selection has also been applied successfully to mice, in which selected populations evolved significantly greater running performance in only 10 generations (Swallow et al., 1998a,b; Koteja et al., 1999) (Fig. 1B). Thus, even vertebrates can make good subjects for laboratory evolution.

Not all characters that should evolve do so

One advantage of laboratory evolution is that the selective regime is relatively well-defined, in comparison with nature. (As will be seen below, even in the laboratory, selective regimes are not always as simple as one would think.) In principle, any physiological process affected by selective conditions can and should evolve, so we should be able to predict the results of selection beforehand. Laboratory selection involving resource limitation (food or water deprivation) provides systems in which specific predictions regarding the evolution of physiological processes can be made. Essentially, organisms face a bookkeeping problem; they contain a certain quantity of the resource, it is consumed

at a certain rate and death occurs when that resource drops to a certain level. Recent studies using *D. melanogaster* illustrate an important point: evolution does not always proceed as expected.

In the case of desiccation selection, fruit fly populations are placed in a dry environment, and those individuals that survive longest are used to found the next generation (a type of natural truncation selection). At the organismal level, evolution has only three parameters with which to work: desiccation-selected flies should have more water entering selection, lose water less rapidly and be better able to tolerate dehydration stress. Each of these organismal traits itself is an integrated measure of multiple physiological processes (e.g. water can be lost by transpiration through the cuticle, during respiration from the spiracles or by excretion). We recently developed a complete water budget for five desiccation-selected populations (D flies) of *D. melanogaster* and their five controls (C flies) (Gibbs et al., 1997). After more than 120 generations of selection, the D flies survive desiccation 2–3 times longer than the C flies. They are able to do so because they contain more water when they enter selection and they lose water less rapidly (Fig. 2). However, C and D flies contain the same amount of water at the time of death (i.e. their dehydration tolerance is the same). Thus, only two of the three expected differences have evolved in these populations.

Starvation selection provides a similar problem to desiccation. Flies should evolve greater storage of energy, especially of energy-dense lipids. They should also evolve reduced metabolic rates, so that these reserves will last longer.

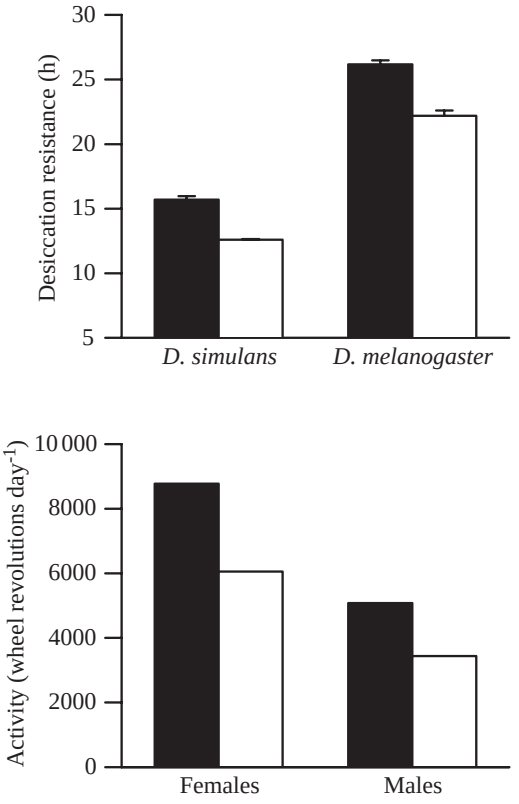

Fig. 1. Effects of short-term laboratory selection on physiological characters. (A) Desiccation resistance (as LT_{50}) of two *Drosophila* species after nine generations of selection. Values are means + S.E.M. of three selected (filled columns) and control (open column) populations. Data from Hoffmann and Parsons (1993b). (B) Voluntary wheel-running activity of mice after 10 generations of selection. Values are adjusted means for four selected (filled columns) and control (open columns) populations. Data from Swallow et al. (1998a).

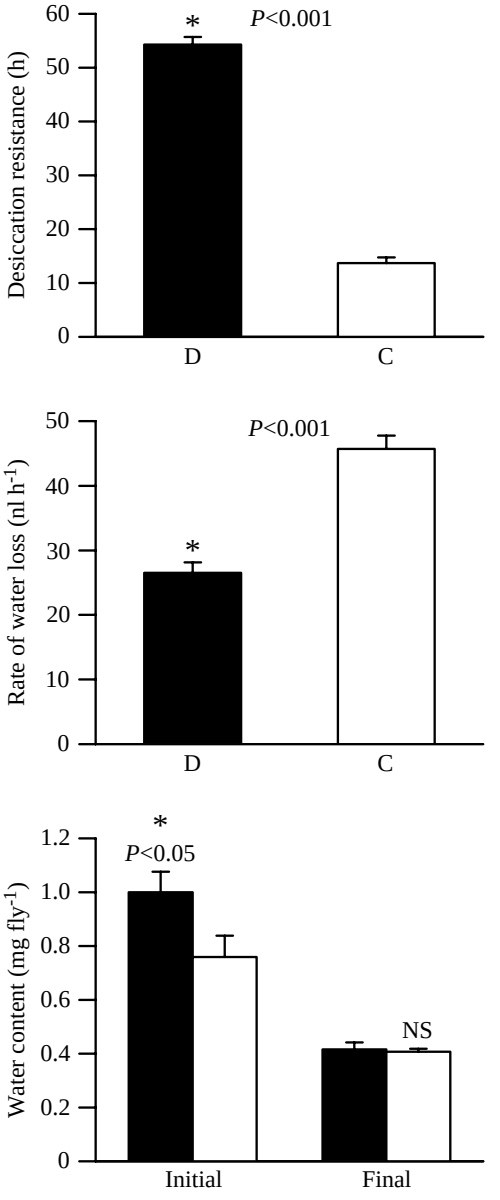

Fig. 2. Components of water balance in desiccation-selected (D, filled columns) and control (C, open columns) populations of *Drosophila melanogaster* after more than 120 generations of selection. Values are means + S.E.M. for five populations. (A) Desiccation resistance (time to death under desiccation stress). (B) Rates of water loss. (C) Amount of water per fly, entering desiccation stress (initial) and immediately after death due to dehydration (final). From Gibbs et al. (1997). Asterisks indicate significant differences between selection treatments (paired *t*-tests, $P<0.05$).

Djawdan et al. (1997) quantified energy budgets for several stress-selected *D. melanogaster* populations. As expected, starvation-selected populations accumulated very high levels of lipids and glycogen compared with their controls, enabling them to survive nearly 10 days without food (Chippindale et al., 1996). However, metabolic rates (after correction for differences in levels of energy storage compounds) did not differ among selection treatments.

Why have all possible characters not evolved to increase desiccation or starvation resistance? Several possible explanations exist, each of which is subject to experimental testing. Perhaps the founding populations did not have sufficient genetic variation in dehydration tolerance or metabolic rate for differences to appear. This explanation seems unlikely for the particular examples mentioned above because the founding populations have since been shown to have substantial genetic variation for a wide range of characters (e.g. Chippindale et al., 1997; Archer, 1999). The role of initial variation can be investigated by subjecting other populations to the same form of selection or by selecting directly on metabolic rate or dehydration tolerance in the extant populations. (Ideally, this would be done by reviving cryopreserved members of the ancestral population.) If artificial selection for increased dehydration tolerance or decreased metabolic rate is successful, it would prove that genetic variation for these characters exists. In that case, factors such as negative genetic correlations with other traits that increase stress resistance may have prevented the evolution of specific traits (Lauder et al., 1993; Garland and Carter, 1994; Leroi et al., 1994). These possibilities can themselves be tested using quantitative genetic approaches (Hartl and Clark, 1989). Similar 'negative' results can occur in interspecific studies for all these reasons. An advantage of laboratory evolution is that the causes of apparent lack of adaptation are subject to experimental investigation.

Laboratory evolution can give different results from nature

As simple models for natural conditions, laboratory evolution systems can be used to test hypotheses about adaptation to different environments. The most significant environmental variable affecting the distribution and abundance of organisms is temperature. Studies of the effects of temperature on physiological systems have occupied a prominent place in comparative physiology for decades. Data are frequently presented as thermal performance curves, in which the value of a physiological trait is plotted as a function of measurement temperature (Huey and Kingsolver, 1989, 1993). A large number of comparative studies have shown that performance curves of animals from warmer habitats tend to be shifted to higher temperatures, presumably because of the action of natural selection (Huey and Kingsolver, 1989; Garland et al., 1991).

In contrast to nature, selection in the laboratory has had little effect on thermal performance curves. For example, *D. melanogaster* maintained at different temperatures for several hundred generations exhibit only minor changes in the thermal

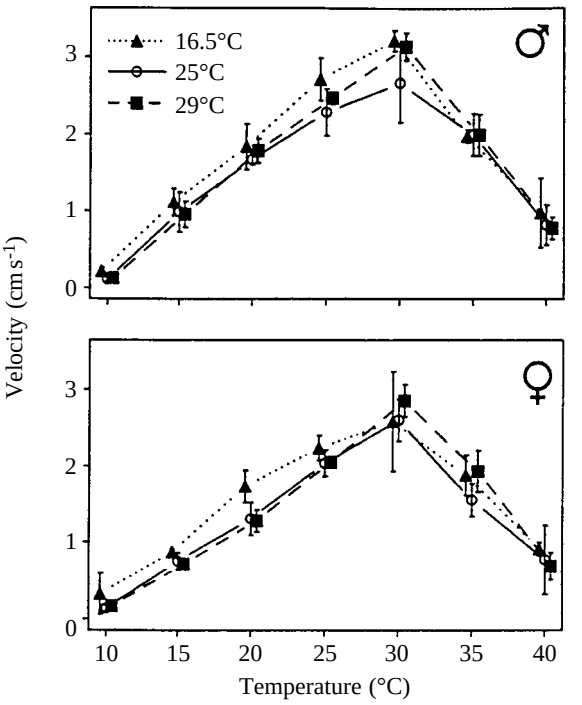

Fig. 3. Thermal dependence of walking speed in temperature-selected populations of *Drosophila melanogaster*. Flies tended to perform better at their given selection temperatures, but overall differences between selection treatments were minor. Values are means ± S.D., *N*=3 replicates. Taken from Gilchrist et al. (1997), with permission.

dependence of locomotor performance, as measured by walking speed (Gilchrist et al., 1997) (Fig. 3). After 2000 generations of selection, six lines of *E. coli* selected at 42 °C differ from their ancestral strain by less than 1 °C in lower and upper thermal limits to growth (Lenski and Bennett, 1993). Obviously, *E. coli* are not animals, and walking speed in fruit flies differs in many ways from the performance variables measured in other studies. More direct comparisons are needed to understand why and how thermal performance limits evolve in nature but not in laboratories. One possibility is that performance limits evolve more rapidly when populations are exposed to short, extreme selective bouts, rather than chronic, sublethal stress (Parsons, 1987; Mongold et al., 1999).

Desiccation selection provides another example in which the products of natural and laboratory selection can be readily compared. Numerous studies have found correlations between habitat variables and water balance in *Drosophila* spp., in both inter- and intra-specific comparisons (e.g. Eckstrand and Richardson, 1980; Stanley and Parsons, 1981; Da Lage et al., 1990; Blows and Hoffmann, 1993). As any comparative physiologist would predict, desert *Drosophila* spp. are more desiccation-resistant than mesic species. With regard to organismal components of desiccation resistance, desert flies lose water less rapidly and seem to be more tolerant of dehydration, but *Drosophila* spp. from all habitats contain similar quantities of bulk water (A. G. Gibbs and L. M.

Matzkin, unpublished data). Recall that selection in the artificial desert of the laboratory has resulted in flies that carry 30 % more water than their controls, but that selected and control flies have similar dehydration tolerances (Fig. 2). Thus, the only component of the water budget showing similar differentiation in the laboratory and in nature is reduced rates of water loss in xeric-adapted flies.

These examples demonstrate that laboratory and natural systems may not yield similar results. Several factors may contribute to these differences. In addition to limited genetic variation or genetic correlations between characters, it is likely that selective conditions in the laboratory and in nature are not as straightforward as we think. This is obviously true in nature, but even in the laboratory, behavior and life history evolution can play important roles in the evolution of physiological characters. The hidden complexities of laboratory habitats are discussed in the next two sections; integration of field and laboratory studies is discussed later.

Even simple selection regimes can give rise to complex patterns of adaptation

Desiccation selection in the C and D populations of *D. melanogaster* appears to be a very simple system. Adult flies face a rather straightforward problem in resource management, including acquisition of water reserves. Because selection is imposed on adults only, physiological studies have concentrated on this life stage. However, recent work reveals that most of the water accumulated by the D flies is actually acquired during the larval phase and that this difference has come at the cost of reduced larval viability, relative to controls (Chippindale et al., 1998). Thus, selection on adults has yielded physiological changes throughout the life cycle of the selected populations. The situation becomes even more complicated when one considers differences between the sexes and examines the control populations in more detail.

Female flies in the D populations survive desiccation stress for much longer than males (Gibbs et al., 1997). In fact, few or no males survive the selection bout each generation (A. K. Chippindale, personal communication). For males to have non-zero fitness, they must mate successfully before selection is imposed. It appears that males are actually selected for rapid development and early-adult mating success, whereas females are selected for slower development and greater water acquisition during the larval phase (Chippindale et al., 1998). This sex-specific selection may have inhibited the evolution of even greater desiccation resistance in D females (A. K. Chippindale, personal communication).

A detailed analysis of the control (C) populations for the D flies reveals additional complexity. Because desiccation selection involves removal of both food and water, the C flies are provided with water only (Graves et al., 1992; Rose et al., 1992). Thus, the C populations undergo mild starvation selection each generation, which most males survive. Because mating can occur after selection is lifted, there has been no apparent antagonism between the sexes. Both sexes accumulate relatively large lipid reserves, although not as much as populations subjected to strong starvation selection (Chippindale et al., 1996; Djawdan et al., 1997). In hindsight, it seems that the C populations were not the ideal controls for the D populations. Better control treatments might have involved dividing the sexes before selection and applying selection separately or even creating controls for the controls: populations given water and food, instead of just water.

The D and C populations of *D. melanogaster* demonstrate that even simple selection regimes can allow evolution to proceed in complicated ways. Unintended, cryptic selection is a potentially important problem, even in control populations, and populations may use any available means to survive (Rose et al., 1996). As in nature, one needs to consider physiological processes occurring over the entire life history, not just during a selection bout.

Behavior also evolves

Behavior is an important link between potential physiological performance and actual organismal fitness in nature (Huey, 1991; Garland and Losos, 1994). Anyone who has tried to get a recalcitrant animal to run on a treadmill realizes that behavior is also an important experimental concern in the laboratory. At first glance, it would seem that behavioral changes are not generally an option in laboratory selection. Thermally selected bacteria and fruit flies cannot move to warmer or cooler parts of the laboratory, nor can desiccation-selected *D. melanogaster* seek wetter environments. In spite of these limitations, evolutionary changes in behavior appear frequently.

Examples of behavioral evolution include selection on locomotor performance in *D. melanogaster* and mice. Flies selected in a wind tunnel did not evolve higher maximal velocities, as one might expect (Marden et al., 1997). Instead, selected populations had a greater tendency to fly horizontally, with fewer changes in direction. These behavioral differences would increase their chances of making significant headway against the wind. Despite the similarity in maximal performance, a greater fraction of the selected lines had high flight speeds. A physiological explanation is that selection favored flies with higher routine flight speeds, but this change could have also been behaviorally based. Perhaps selection favored flies that ‘liked’ to fly fast, rather than those that could.

Selection for voluntary running in mice has had similar effects. Swallow et al. (1998a,b) have selected for increased voluntary locomotory activity in mice using revolutions per day on an exercise wheel as their selection character. Mice could ‘choose’ to spend more time running each day or to run faster, or both. Within 10 generations, average running speed had increased significantly in selected lines, whereas running time per day had not (Swallow et al., 1998a; Koteja et al., 1999). In contrast to the *D. melanogaster* example, maximal performance also increased (Swallow et al., 1998b).

Selection on behaviors can, in turn, affect organismal physiology. An excellent example is a series of experiments in which replicate lines of mice were selected for building either large or small nests (Lynch, 1980; Bult and Lynch, 1997).

Nest-building is a thermoregulatory behavior, and high- and low-selected lines differed in their fitness at low temperatures. Pups from the high-selected lines gained weight more rapidly and had greater survival at 4 °C than low-selected pups, probably because their mothers built larger nests and rebuilt them more rapidly after cage changes (Bult and Lynch, 1997). Physiological changes associated with thermoregulation also evolved under behavioral selection. High-selected mice have higher body temperatures (Schneider and Lynch, 1984), and neuroanatomical differences have been described in the suprachiasmatic nucleus of the hypothalamus (Bult et al., 1992). The latter result is particularly interesting, because it supports a previous hypothesis regarding the function of this region in thermal regulation.

Comparative physiologists have long recognized the importance of behavior in stressful environments (e.g. behavioral thermoregulation). The studies outlined above show that behavior is no less important in the laboratory than in the field, because selection will favor individuals that behave in an adaptive manner. Behavior is a critical component of physiological performance, and attempts to select on performance may affect only how members of a population behave, rather than their ability to perform. Differences in behavior can, in effect, substitute for evolution of physiological traits, so careful observation of selected populations is necessary.

Future questions in laboratory selection

The examples provided so far illustrate the use of selection experiments in the context of traditional physiological problems: temperature, water balance, energetics and locomotion. But what can laboratory selection do for comparative physiology that is new? In this section, I suggest a few possible ways in which selection studies can provide new insights into questions relevant to physiologists.

Natural selection in the laboratory and in the field

An inescapable problem in understanding the action of selection in nature is that nature is complex. It is difficult, if not impossible, to demonstrate that any trait evolved as it did because of any particular aspect of the environment (Leroi et al., 1994). Well-controlled field studies of selection are rare and difficult to perform (Endler, 1986; Reznick and Travis, 1996). However, individual environmental variables can be independently manipulated in the laboratory so that their effects can be distinguished from others. (But do not forget the potential pitfalls discussed above!) The direct comparison of laboratory and natural systems provides the opportunity to identify and to test hypotheses regarding natural selection in the field. If laboratory and comparative studies provide similar results, this is corroborative evidence that selection is acting as we thought in nature (Lynch, 1992). When different results are obtained, then something may be missing in our understanding of one or both environments.

We have already seen that laboratory-selected and desert *D. melanogaster* exhibit different mechanisms of increased desiccation resistance. Desiccation-selected flies in the laboratory accumulate high levels of bulk water, but desert flies do not. A possible explanation is that the habitat of desert species, rotting cacti, is an ephemeral and patchily distributed resource (Breitmeyer and Markow, 1997). Thus, desert flies may be selected for the ability to fly long distances to new habitats, but their performance could be reduced by carrying a large amount of water. The D flies have no opportunity for dispersal and are less active than their controls, especially under desiccating conditions (Williams, 1998). They appear to follow a ‘hunker down’ strategy that allows them to carry extra water at little cost.

These ideas can be tested in several ways. Field studies can indicate how long favorable cactus rots last and how far individuals must fly to disperse to new habitats (Coyne et al., 1987; Breitmeyer and Markow, 1997). Laboratory studies can indicate how far flies can fly under specific thermal and humidity conditions. The flight performance of flies loaded with extra mass can be quantified to determine the effects of water load on dispersal capability. One could also devise a laboratory selection regime in which desiccated flies must seek and find a new water source. The important point here is that incongruity between laboratory and natural systems does not imply that either type of study is inappropriate. We can use each system to develop and test hypotheses in the other system.

Evolution as a process

An under-utilized advantage of laboratory selection experiments is the fact that evolution can be observed as it occurs. Thus, one can study the process as well as the outcome of evolution. However, very few studies of the time course of physiological evolution have been performed (e.g. Swallow et al., 1998a; Archer, 1999) (Fig. 4). For which problems would a fine-grained analysis of the evolution of a physiological system be useful? I suggest one example (symmorphosis) here; many others are possible.

The concept of symmorphosis has played an important role in recent comparative physiology (Weibel et al., 1998). An important issue is ‘excess’ physiological capacity: why do some organs or tissues appear to be over-designed for their functions? Animal athletes are frequently used as models, including Thoroughbred horses and greyhounds, themselves products of selection by humans. In some cases (e.g. desiccation resistance in *D. melanogaster*), laboratory-selected ‘athletes’ can even outperform natural populations. Thus, laboratory selection provides a new source of models for symmorphosis in which one can test specific predictions about the evolution of physiological systems. For example, under symmorphosis, entire systems should evolve simultaneously (e.g. all steps of gas exchange and transport), rather than sequentially (as might happen as each step became rate-limiting). Another important prediction of symmorphosis is that selection acts to reduce excess capacity. Thus, physiological traits that have responded to laboratory selection should rapidly return to their original conditions once selection is relaxed.

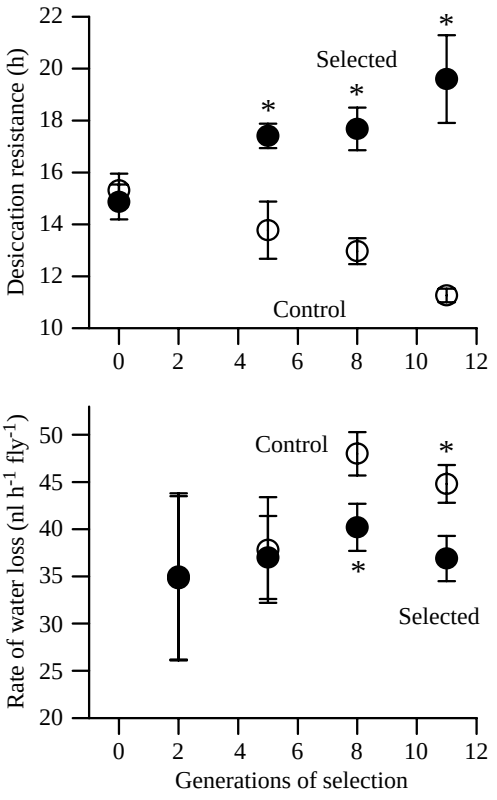

Fig. 4. Time course of evolution of desiccation resistance and water loss in *Drosophila melanogaster*. Five populations were subjected to desiccation stress each generation, until 80–90 % mortality. Control populations were simultaneously provided with water, but not food. Values are means ± S.E.M. for five populations. (A) Desiccation resistance (as mean time to death under desiccation stress) for female flies. (B) Rates of water loss, measured using groups of 20 females. Data are taken from Archer (1999). Asterisks indicate significant differences between selection treatments (paired *t*-tests, $P<0.05$).

Selection experiments and mechanistic physiology

Most laboratory evolution studies to date have concerned questions in evolutionary physiology. How do physiological systems evolve, do performance variables trade off with each other, does specialization for one habitat (e.g. a given temperature) reduce performance under other conditions? Comparative physiologists also study how organisms work. Can laboratory selection be used to study basic physiological mechanisms? In principle, the answer is yes. Comparative studies have often assumed that species are well adapted (even optimized) for their environment and, therefore, that differences between species are functionally significant. This assumption may be invalid for a variety of reasons (Lauder et al., 1993; Garland and Adolph, 1994; Garland and Carter, 1994; Leroi et al., 1994). In contrast, continuous directional selection in the laboratory will result in populations that truly are highly adapted to their specific environment. Optimality may not be achievable, even after 10^4 generations (Lenski and Travisano, 1994), but long-term selection may provide useful models for mechanistic questions. For example, several models for thermal adaptation of cell membranes have been developed from comparative studies, but none is universally accepted (Hazel, 1995). Populations of *D. melanogaster* and *E. coli* are available that have been selected at different temperatures for more than 500 generations (Lenski and Bennett, 1993; Gilchrist et al., 1997). These lines may be ideal models for membrane physiologists.

Another use of laboratory selection is to disprove hypotheses based on comparative studies. For example, the importance of epicuticular lipids in reducing water loss from insects is well known. Variation in cuticular permeability has been thought to reflect differences in either the amount or the physical properties of these lipids (Gibbs, 1998). The D flies have greatly reduced rates of water loss relative to their controls, yet their surface lipids do not differ (Gibbs et al., 1997). The unexpected failure of surface lipids to evolve suggests that some other fundamental aspect of cuticular structure or physiology has been affected. Further research on these populations could reveal factors affecting cuticular permeability that may not be readily apparent in comparative studies.

The D flies also exhibit cyclic patterns in CO_2 release that resemble the discontinuous gas exchange cycles found in many insects (Lighton, 1994; Williams et al., 1997). This pattern was originally hypothesized to reduce respiratory water loss, so its appearance in desiccation-selected flies is intriguing. However, individual flies can switch between discontinuous and continuous CO_2 release, with no effect on water loss (Williams and Bradley, 1998). In this case, selection experiments serve to discredit the original hypothesis for the function of discontinuous gas exchange. This pattern instead may simply be a side-effect of the relatively quiescent behavior of the D flies (Williams, 1998).

Novel mechanisms of adaptation

Selection experiments may provide novel insights into mechanistic physiology in two ways. First, organisms exposed to a novel environment may evolve in an unexpected manner. An example of this approach is selection for urea resistance in *D. melanogaster* larvae (Joshi et al., 1996). Numerous comparative studies have investigated mechanisms by which animals tolerate high urea levels, particularly the role of solutes that counteract the denaturing effects of urea on proteins (Somero and Yancey, 1997). *D. melanogaster* are not exposed to high levels of urea in nature, yet laboratory selection has yielded populations whose larvae develop in and consume media containing more than 300 mmol l^{-1} urea (Borash et al., 1999), levels approaching those found in elasmobranch fishes and mammalian kidneys. The mechanisms whereby these larvae tolerate such high urea levels are unknown, but they do not appear to include accumulation of counteracting solutes (Pierce et al., 1999). Instead, urea resistance in *D. melanogaster* may involve some novel mechanism(s). These mechanisms may operate in other urea-tolerant organisms, but may have been overlooked. Thus, selection in novel environments may indicate fruitful avenues for comparative studies.

Molecular analyses of laboratory-selected populations can also reveal novel mechanisms of adaptation. For example, a recent allozyme survey of the D, C and other *D. melanogaster* populations revealed that different selection treatments have affected the allele frequencies of several genes. Most striking is a dramatic increase in the frequency of the *S* allele of superoxide dismutase (SOD) in D flies, relative to all other stress-selected populations (Deckert-Cruz, 1996). This difference far exceeds the increase observed in populations selected for postponed senescence (O flies) relative to their control (B) populations (Tyler et al., 1993; Deckert-Cruz et al., 1997). The latter result is one of many that has generated intense interest in the role of free radicals in aging (Martin et al., 1996). The D and C result indicates that SOD is very important in desiccation resistance, a possibility that has not been suggested by comparative studies of water balance. Its exact role remains to be determined, and the SOD locus may simply be linked to a key gene affecting desiccation resistance. (However, a database search of nearby genes reveals no obvious candidates for a role in desiccation tolerance, either.) Until further work is done, such as physiological analyses of genetically engineered lines, we can only speculate on how SOD might affect desiccation resistance, but this result demonstrates that laboratory selection can generate novel hypotheses about physiological mechanisms.

A limitation of allozyme surveys, such as the SOD study, is that one can only study those proteins that have alleles differing in electrophoretic mobility and that can be detected easily. Changes in gene expression are sure to play an important role in evolution in the laboratory and in nature. These have been detected in the B and O populations of *D. melanogaster* using two-dimensional gel electrophoresis (Fleming et al., 1993). Assays of mRNA expression, such as subtractive hybridization or differential display, provide another means to detect differences in gene expression, with no *a priori* expectations about the outcome required. Genes whose expression increases or decreases as a result of laboratory selection are good candidates for a role in stress resistance. These methods have been applied to comparative systems, but are prone to false positives and other artefacts. The replication of laboratory selection systems should reduce these problems. If one obtains similar results for several pairs of selected and control populations, the probability of an artefact is greatly diminished. Thus, by combining molecular methods and laboratory evolution, researchers may be able to gain novel understanding of basic physiological problems.

Conclusions

Laboratory evolution is a new source of interesting variation in physiological characters. Because this approach was developed by evolutionary biologists, it is not surprising that most physiologists who use it are interested in evolutionary physiology. Laboratory selection studies have shown that physiological systems do not always evolve as one would predict, and even apparently simple selection regimes can have complicated outcomes. Unintended, cryptic selection can arise easily because of the details of laboratory treatments (Rose et al., 1996). Behavioral differences evolve rapidly and can 'substitute' for physiological evolution. Selection on one life stage may affect other stages, and males and females may respond differently to selection. These factors provide pitfalls for the unwary investigator, both in the laboratory and in the field. The advantage of laboratory selection is that these problems can be detected and investigated experimentally.

An important conclusion is that physiological systems do not necessarily evolve in the laboratory in the same way as they do in nature. This is not surprising, because the laboratory is only a simplified model for nature. Long-term selection under specific laboratory conditions is very different from the variability of the real world, and behavioral options are more limited in the laboratory. However, the contrasts between predictions, nature and the laboratory can be used to develop and test hypotheses in both environments. Laboratory selection can also reveal novel mechanisms of adaptation to the environment, which may not be detected using other approaches. The significance of these mechanisms can then be investigated in natural systems. In combination with traditional physiological methods, comparative analyses and molecular techniques, laboratory selection is becoming part of the experimental tool kit available to the comparative physiologist. To paraphrase Carl Gans (1978), 'all animals are interesting' (even *E. coli*).

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