Experimental evolution with *Drosophila*

Molly K. Burke and Michael R. Rose

Am J Physiol Regul Integr Comp Physiol 296:R1847-R1854, 2009. First published 1 April 2009; doi:10.1152/ajpregu.90551.2008

You might find this additional info useful...

This article cites 47 articles, 9 of which can be accessed free at:
http://ajpregu.physiology.org/content/296/6/R1847.full.html#ref-list-1

This article has been cited by 2 other HighWire hosted articles

Multiple reciprocal adaptations and rapid genetic change upon experimental coevolution of an animal host and its microbial parasite
Rebecca D. Schulte, Carsten Makus, Barbara Hasert, Nico K. Michiels and Hinrich Schulenburg
PNAS, April 20, 2010; 107 (16): 7359-7364.
[Abstract] [Full Text] [PDF]

Physiological climatic limits in *Drosophila*: patterns and implications
A. A. Hoffmann
J Exp Biol, March 15, 2010; 213 (6): 870-880.
[Abstract] [Full Text] [PDF]

Updated information and services including high resolution figures, can be found at:
http://ajpregu.physiology.org/content/296/6/R1847.full.html

Additional material and information about ***American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*** can be found at:
http://www.the-aps.org/publications/ajpregu

This infomation is current as of July 30, 2011.

American Journal of Physiology - Regulatory, Integrative and Comparative Physiology publishes original investigations that illuminate normal or abnormal regulation and integration of physiological mechanisms at all levels of biological organization, ranging from molecules to humans, including clinical investigations. It is published 12 times a year (monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 2009 by the American Physiological Society. ISSN: 0363-6119, ESSN: 1522-1490. Visit our website at http://www.the-aps.org/.

Downloaded from ajpregu.physiology.org on July 30, 2011

Am J Physiol Regul Integr Comp Physiol 296: R1847–R1854, 2009.
First published April 1, 2009; doi:10.1152/ajpregu.90551.2008.

Experimental evolution with *Drosophila*

Molly K. Burke and Michael R. Rose

Department of Ecology and Evolutionary Biology, University of California, Irvine, California

Submitted 30 June 2008; accepted in final form 25 March 2009

Burke MK, Rose MR. Experimental evolution with *Drosophila. Am J Physiol Regul Integr Comp Physiol* 296: R1847–R1854, 2009. First published April 1, 2009; doi:10.1152/ajpregu.90551.2008.—Experimental evolution is a powerful approach that can be used for the study of adaptation. Evolutionary biologists often use *Drosophila* as a model organism in experiments that test theories about the evolution of traits related to fitness. Such evolution experiments can take three forms: direct selection for a trait of interest; surveys of traits of interest in populations selected for other traits; and reverse selection. We review some of the *Drosophila* experiments that have provided insight into both the evolution of particular physiological traits and the correlations between physiological and life history traits, focusing on stress resistance. The most common artifacts that can obscure the results from evolution experiments are discussed. We also include a treatment of genomic technologies that are now available for the *Drosophila* model. The primary goal of this review is to introduce the kind of experimental evolution strategies and technologies that evolutionary physiologists might use in the future.

correlations; *Drosophila*; laboratory evolution; physiology; stress resistance

THE COMPARATIVE METHOD HAS historically provided a powerful means of inferring adaptation by correlating differences among lineages with selective pressures imposed by ecological or other factors (15). Experimental evolution with laboratory populations is an alternative tool with which to study adaptation. One of the most attractive applications of experimental evolution is to create a replicated set of populations that have been differentiated relative to replicated control populations, using a well-defined selection protocol, and then compare the allele frequencies at various loci for associations between particular types of phenotypic and genetic differentiation (37). In short, laboratory evolution allows biologists to use strong-inference tests of hypotheses concerning phenotypic and genetic responses to selection. In addition, the recent development of cost-effective genomic tools has allowed broad and systematic assays of the molecular foundations of the effects of experimental evolution.

Our main goal in this review is to demonstrate the unique advantages of the *Drosophila* model for experimental evolutionary studies. We introduce experimental selection strategies that the model allows, so that physiologists unfamiliar with such methods have the option to use them in the future. Our subsidiary interest is to highlight *Drosophila* selection experiments that have as a primary goal the dissection of the genetic architecture of complex, physiological traits.

The Drosophila Advantage

An ideal metazoan model. The genus *Drosophila* has historically served as a key model organism for experimental biology. The features that make this organism so ubiquitous and attractive for any biologist to work with, namely, a short generation time, ease of maintenance, and public genomic resources, also make the fruit fly especially suited for experimental evolution. Although *Drosophila* experimental evolution cannot use the enormous population sizes that are possible with microbial model species, it is relatively easy to maintain hundreds of fly populations, each with an effective population size on the order of 10^3. Such populations are large enough both to retain abundant genetic variation and to mitigate the confounding effects of genetic drift during selection experiments of moderate duration. And because it is easy to maintain *Drosophila* populations with abundant genetic variation, selection on them in the laboratory can produce physiological changes quite rapidly, in as few as 10 generations (reviewed in Ref. 16), less than 3 months of calendar time for some selection protocols. Fruit flies thus come with much of the speed, ease, and utility of a microbial model, yet also have a complex metazoan physiology. Evolutionary physiologists can thus produce populations of flies differentiated for their chosen physiological characters more or less at will. Furthermore, because of the greater ease of assaying a diversity of physiological characters in fruit flies, correlated responses to selection can be assayed among multiple functional characters. Indeed, the physiological complexity of adaptive responses to experimental evolution will be a major theme of this review.

The model accommodates a range of experimental strategies. There are three major strategies for experimental evolution research (Fig. 1), and *Drosophila* studies can employ these strategies particularly well. First, we have direct selection for a particular trait, such as stress resistance, which reveals both the response of the selected character itself, as well as the response to selection of any other functional traits that might be of interest. Second, we have the application of what we term "pseudocomparative" methods to extant, selectively differentiated populations. An example of a pseudocomparative assay would be one measuring a particular trait, such as adult body size, in populations that have been selected for a range of life

Address for reprint requests and other correspondence: M. K. Burke, Dept. of Ecology and Evolutionary Biology, Univ. of California, Irvine, CA 92617-2525 (e-mail: burkem@uci.edu).

 0363-6119/09 $8.00 Copyright © 2009 the American Physiological Society

Downloaded from ajpregu.physiology.org on July 30, 2011

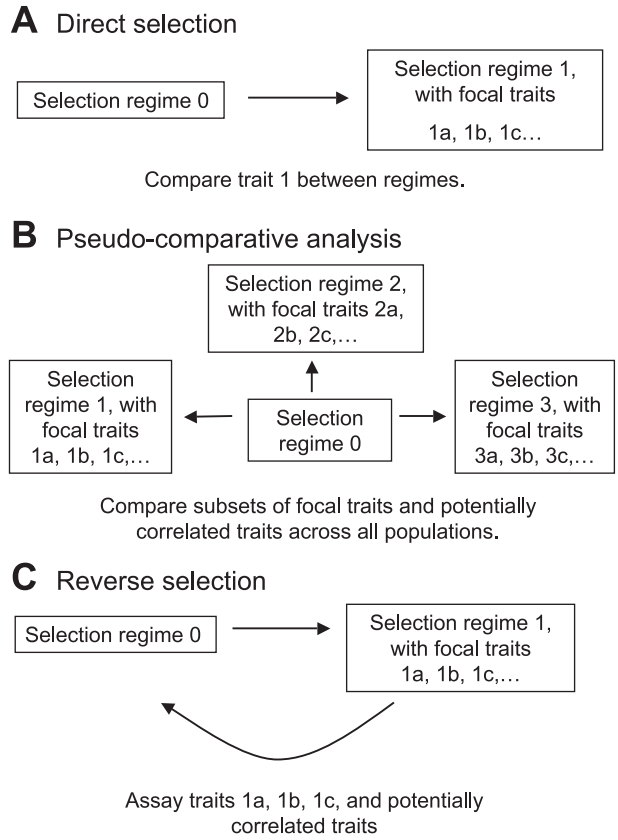


Fig. 1. An illustration of the three experimental evolution strategies described in the text. *A*: using direct selection, investigators can change the ancestral condition (selection regime 0) simply by selecting for the phenotype of interest (*focal trait 1*) in an experimental population. Subsets of focal traits (1a, 1b, 1c, etc.) could represent different directions of selection for a trait. *B*: in pseudocomparative analysis, phenotypes of interest can be assayed in experimental populations that have already been selected for a variety of conditions. For example, assaying adult body size (*focal trait 1*) in populations selected for a range of development times (*focal traits 2a, 2b, 2c . . .*) could reveal that body size is correlated with development time. *C*: in reverse selection, populations that had been previously selected for a particular phenotype of interest (*selection regime 1*) are returned to preselection conditions (*selection regime 0*).

history traits, perhaps to infer how particular life history traits affect adult body size. Third, we have reverse selection, in which populations are returned to an ancestral selection regime, and the subsequent evolution of physiological characters is monitored. These methods can be applied in varied combinations and sequences, with some laboratories having developed elaborate experimental "systems," which consist of collections of populations selected in different directions, for any number of related traits (e.g., Ref. 39). We will use the Rose laboratory system, briefly outlined in Fig. 2, as a point of discussion in the following sections.

Experimental evolution can self-correct, identifying artifacts and explaining inconsistent results. There are certainly disadvantages to the laboratory evolution approach. Laboratory selection experiments can never exactly mimic selection in the wild, and laboratory environments evolutionarily domesticate wild-caught flies in ways that are often unclear to investigators. Similarly, unintended selection can arise easily because of unanticipated effects of laboratory procedures. Physiological systems do not always evolve as one would predict, and even apparently simple selection regimes can have complicated outcomes (37). Selection on one life stage may affect other stages, and males and females may respond differently to selection. Of course, these problems potentially present confounding factors to investigators using more traditional approaches as well. The advantage of using laboratory selection is that these problems can be detected and investigated experimentally. *Drosophila* evolution experiments have thus highlighted important ambiguities, because they have the capacity to resolve such ambiguities. This topic will be treated in detail below.

The model is amenable to a variety of molecular techniques. A number of problems make the genetic dissection of physiological traits difficult. The "candidate gene" approach involves identification of candidate genes on which selection may have acted, usually based on suggestive evidence for a mechanistic link between variation in a particular phenotype and variation at the molecular level (13). Because of the enormous wealth of information in the *Drosophila* literature, such suggestive evidence is plentiful in this model species. The candidate gene tactic is therefore commonly used by investigators studying adaptation in systems of laboratory-evolved populations of *Drosophila*, and it has sometimes been successful (3–5, 9, 47). Even so, this approach is biased because it is dependent on prior knowledge of candidate genes. Examining adaptation using a whole genome approach avoids the problem of bias in selection of candidate genes. Presently, there is a variety of commercial genomic technologies available for whole genome screens of *Drosophila,* and although they are expensive, they are becoming ever more cost effective.

We begin this review with a detailed discussion of the different experimental evolution strategies, and how they complement one another; the correlation between stress resistance and life history phenotypes provides a nice example, so we focus on that as a way of illustrating our general points. We then discuss the most common types of demons to which evolution experiments fall victim. Finally, we mention molecular techniques that have informed evolution experiments over the last decade, and future directions for this kind of approach.

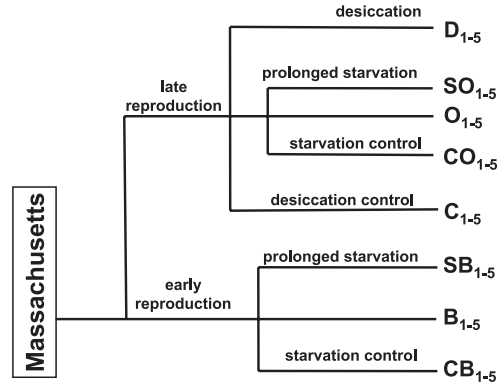


Fig. 2. An abbreviated schematic of some of the selection regimes of the Rose laboratory "system," which is made up of populations derived from a population collected from south Amherst, Massachusetts, in 1975. All selection regimes were imposed on five independently evolving replicate populations, and each population has a census size of more than 1,000 breeding adults per generation. The focal trait under direct selection is indicated above corresponding branches (branch lengths are not drawn to scale).

Downloaded from ajpregu.physiology.org on July 30, 2011

Adaptation to Environmental Stress and Its Correlated Responses

Stress-resistance characters respond to direct and reverse selection. Laboratory selection for increased resistance to starvation and desiccation produces rapid responses within populations of *D. melanogaster* (1, 6, 22, 40), and high heritability estimates have been reported for both traits (44). Direct selection for increased starvation (Rose system "SO" and SB" lines) and desiccation (Rose system "D" lines) resistance results in a correlated increase in longevity in the absence of acute starvation or desiccation, along with decreased fecundity (Ref. 40; see Fig. 3). Furthermore, some experiments suggest that lines selected for increased desiccation resistance have decreased preadult viability and slower development time than control lines (6, 7, 21). There appear to be complex trade-offs connecting resource acquisition during larval stages, adult stress resistance, and life history generally.

Results from experiments using reverse selection to study stress resistance traits reinforce some, but not all, of these correlative observations. Using a complex system of selected lines, investigators returned a variety of differentiated populations created by laboratory natural selection to their ancestral condition, including populations that had been specifically selected for increased starvation resistance (48). After 50 generations, development time, age-specific fecundity, and dry body weight measures all converged on the average values shown by populations that had been maintained under the ancestral conditions, ancestral populations that had never experienced direct selection for starvation resistance. In yet another reverse-selection study, selection was relaxed in populations that had previously been selected for starvation and desiccation resistance, and over this period of relaxed selection, starvation resistance significantly fell in reverse-selected lines, while desiccation resistance did not fall in lines reverse-selected for that trait (31).

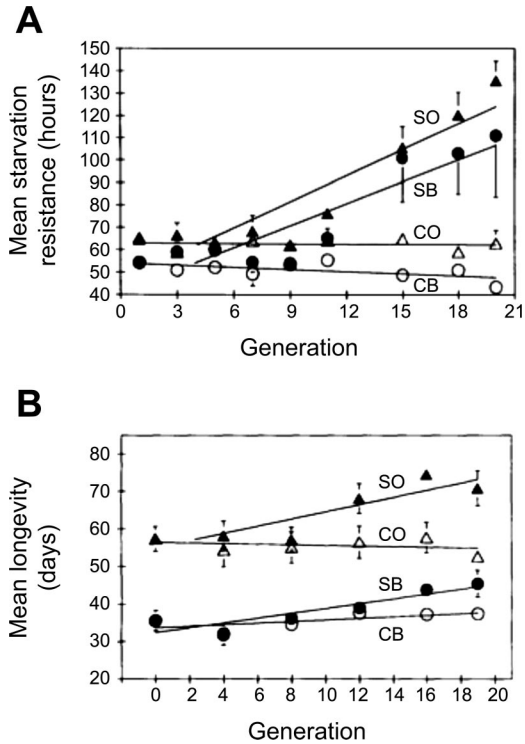


Fig. 3. *A*: direct response to selection for increased starvation resistance. The solid symbols indicate the average of the lines undergoing selection. The open symbols indicate the average of lines not undergoing additional selection. The triangles indicate the lines derived from longer-lived populations created by selection in the laboratory, and circles indicate lines derived from control populations (see Fig. 2 for details). Only the SB and SO treatments have average slopes that are significantly different from zero. *B*: indirect response of longevity to selection for increased starvation resistance. The SB and SO slopes are significantly greater than the CB and CO slopes, respectively. [From Figs. 1 and 2 of Rose et al. (40)].

Taken together, direct selection and reverse selection studies generally implicate a positive correlation between starvation and desiccation resistance and longevity; however, the evidence is more convincing in the case of starvation resistance.

Pseudocomparative surveys and reverse-selection experiments reveal correlations between stress resistance and life history characters. The mean longevity of *Drosophila* populations has been demonstrated to change as a result of manipulating the age of reproduction in a population over multiple generations (25, 30, 35, 36, 50). Furthermore, longer-lived lines (Rose system "O" lines) have generally been found to tolerate starvation and desiccation significantly better than lines with shorter average lifespans (Rose system "B" lines) (42). These pseudocomparative results were interpreted to mean that resistance to starvation and desiccation might be general physiological mechanisms necessary for maintaining health at late ages.

This interpretation prompted a series of follow-up experiments. Longer-lived lines were reverse-selected by returning them to their ancestral reproductive schedule; after 22 generations, decreased starvation resistance was observed in reverse-selected lines, but not decreased desiccation resistance (43). After more than 100 generations of reverse selection, the reverse-selected lines had starvation resistance intermediate between those of longer-lived and shorter-lived lines, while desiccation resistance that had fallen back to the levels of the short-lived lines maintained under ancestral conditions (19). When the reverse evolution experiment was repeated and sustained for over 50 generations, longevity fell dramatically as a response to this reverse selection, as did starvation resistance, but desiccation resistance did not respond significantly (32).

These pseudocomparative and reverse selection experiments have several implications for the correlation between stress resistance and longevity phenotypes. Generally, pseudocomparison suggests a positive correlation between both starvation and desiccation resistance and longevity, while reverse selection experiments indicate a more complicated trade-off relationship. The observation that desiccation does not immediately respond to reverse selection for shorter lifespan in "O" lines suggests that increased desiccation resistance evolves in lines selected for postponed aging, but at little expense for early reproduction. On the other hand, since starvation resistance falls rapidly in reverse-selected lines in these same experiments, this trait appears to trade-off with benefits associated with early fecundity, a finding that is in keeping with estimates of a negative additive genetic correlation between these two characters (44).

Lower-level physiological mechanisms implicated by direct selection for stress resistance appear nearly identical to those implicated by pseudocomparative study. Good evidence suggests that an increase in the lipid content of adult flies underlies

Downloaded from ajpregu.physiology.org on July 30, 2011

increased resistance to starvation. Lines directly selected for starvation resistance show that lipid content accounts for almost all the variation in starvation resistance (6, 21). Pseudocomparative analysis of starvation resistance in the Rose system of lines selected for life history traits has provided additional, corroborative data characterizing the starvation-resistance response. Longer-lived "O" populations have larger lipid stores than shorter-lived "B" populations (7, 41). Further, pseudocomparative analysis of selected lines also found that the total amount of stored calories in the fly body predicted starvation resistance very accurately (11).

While the physiological basis of starvation resistance appears tied to the accumulation of energy in the form of stored lipid and carbohydrates, the physiology of desiccation resistance is more complex. A pseudocomparative study showed that neither lipid content nor total energy was significantly correlated with desiccation resistance across the spectrum of selected lines (28). That study also found that flies from short-lived "B" lines lost water weight significantly faster than flies from long-lived "O" lines, but did not find substantially different water content levels between these populations. Yet another pseudocomparative analysis of selected lines showed that carbohydrate storage was significantly correlated with desiccation resistance in female but not male flies (9). Both direct selection (17) and pseudocomparative work (9) have suggested that flies selected for desiccation resistance accumulate the carbohydrate glycogen, apparently to sequester water. Detailed analysis of lines directly selected for desiccation resistance then showed that characters regulating water balance, including reduced rates of respiratory water loss and overall water content, were the best predictors of desiccation resistance (14, 17, 51).

Moderately enhanced storage of lipid, carbohydrate, and water apparently can increase the stress resistance of flies and promote longevity. At very high levels of stress resistance, this pattern of correlation breaks down. This will be discussed in more detail in the next section.

Thus, for both starvation and desiccation resistance, direct selection experiments and pseudocomparative experiments provide complementary results. Direct selection for these stress resistance phenotypes implicates the same lower-level mechanisms that underlie starvation and desiccation resistance in lines selected for life history traits, such as later fecundity.

Drosophila Studies Have Identified Limitations Affecting Evolution Experiments

We would be remiss to outline all of the reasons why physiologists might find *Drosophila* evolution experiments useful, without also pointing out the ways in which the results of such experiments can mislead investigators. Direct selection tends to yield consistent effects on the phenotypic focal trait, in different experiments and in different laboratory systems. But the studies discussed above suggest that correlations between traits ascertained by pseudocomparative and reverse selection methods can be ambiguous. In the worst-case scenarios, selection experiments may fail to exhibit the same correlation between traits from experiment to experiment, even within the same laboratory system and employing similar selection tools. This problem of robustness can plausibly result from the action of any combination of a number of complications, or "demons," that can lurk in evolutionary experiments.

Genotype-by-environment interactions can cause changes in correlations between traits among selected populations that are handled in different ways. Within our own laboratory system, such interactions have confounded relationships between life history characters. The negative correlation between longevity and early-life fecundity that had been established by previous experiments selecting for delayed reproduction (e.g., Refs. 25 and 35) was not repeated in a later set of assays (8), after more generations of selection. In fact, the selected lines eventually exhibited increased early-life fecundity, compared with controls, in these later assays. Subsequently, several potential types of genotype-by-environment interactions that could have changed the direction of this correlation were considered, and ultimately, it was found that specific culture and assay methods had generated a misleading genotype-by-environment interaction (23, 24). A flawed fecundity assay method, rather than a real evolutionary phenomenon, caused the reversal of the correlation between longevity and fecundity. The expected correlation was recovered upon the implementation of a more appropriate assay. The take-home lesson of this Cheshire Cat pattern of appearing and disappearing correlated response to selection is not that such problems are specific to experimental evolution. Rather, such problems no doubt arise in many evolution experiments. But with experimental evolution systems, it is possible to unravel the recondite causes of such inconsistencies.

In a separate but related experimental context, laboratory domestication can be considered as the result of a particularly important type of genotype-by-environment interaction. The dependence of any genetic correlation on the environment to which a population is exposed will be massively affected by that population's introduction to a laboratory. Flies that have been recently sampled from nature generally undergo a period of rapid adaptation to laboratory conditions during which several life history characters improve (27, 45). Assays of life history characteristics in newly domesticated fly populations could thus indicate deceptive positive correlations between traits. This idea was experimentally demonstrated by comparing genetic correlations in laboratory-adapted flies under normal conditions and in a novel environment (44). As expected, the correlations were more positive in the novel environment, compared with the conditions to which the flies had initially adapted.

Inbreeding depression may also contribute to the disappearance or reversal of correlations. This has been observed for correlations between life history characteristics, particularly between characteristics for which life history theory predicts an evolutionary trade-off, for example, a negative correlation between early-life fecundity and longevity (52). This negative correlation has been repeatedly shown in some laboratories (25, 35), but other studies reveal positive correlation between these two particular traits (e.g., Ref. 18). This contradiction was examined by observing patterns of fecundity and longevity in inbred lines derived from an outbred stock that had previously been shown to exhibit a trade-off between these two characters (34). Indeed, in lines created by three generations of full-sib mating, these same genetic correlations became generally positive. Inbreeding depression presumably lowered the value of these two characters in the inbred lines, with varying

Downloaded from ajpregu.physiology.org on July 30, 2011

levels of inbreeding depression among inbred lines, thereby generating positive correlations among life history characters to such an extent that the original, negative correlation between these characters was obscured (34). Such correlations are not robust under inbreeding, raising significant questions concerning the use of inbred lines to make generalizations about normally outbred species, which most *Drosophila* species are. Although inbred lines remain those most commonly used by *Drosophila* biologists in general, certainly, the best evolution experiments make use of larger, more outbred lines. It bears repeating how easy and cost-effective maintaining such lines can be.

Finally, correlations among physiological and life history characters sometimes just break down over the course of long-term selection experiments. As discussed previously, a positive correlation between selection for stress resistance and longevity has often been observed (37). One study provides evidence for the disappearance and even reversal of this evolutionary correlation, in the same system of populations (33). These results suggest that the long course of sustained selection between two experiments led to this breakdown, which the authors argue did not result from inbreeding, changes in linkage disequilibrium, or genotype-by-environment interactions. A second set of related experiments showed that more direct laboratory selection for extreme values of stress resistance can also lead to a breakdown in the correlation between stress resistance and longevity (2). These findings suggest that when long-term selection produces substantial enhancements in functional characters, correlations between these characters may collapse (38). We doubt that such findings, which required assays of lines that had been continuously selected for nearly 20 years and therefore hundreds of generations, could have been discovered in any other model. So, although particular demons can haunt evolution experiments, we argue that yet another attractive aspect of the *Drosophila* model lies in its history of identifying these demons.

What Can Drosophila Evolution Experiments Reveal About the Genetic Architecture of Physiological Traits?

The sequencing of entire genomes and advances in microarray technologies have made it feasible to interrogate entire genome sequences. Several platforms of whole genome arrays can be used in a number of ways to identify and characterize the genetic changes that result from selection in the laboratory. Such experiments can compare transcripts and DNA sequences of populations evolved in the laboratory to the ancestors from which they were derived. They can also make comparisons directly between different selected lines.

Arrays can detect changes in gene expression that result from laboratory selection. One attractive application of array technology is the comparison of global gene expression patterns between selected and unselected lines. This method allows the investigator to observe the magnitude and direction of any expression change, and the use of replicate lines allows an assessment of the level of parallel evolutionary response within selection treatments.

Sørensen et al. (46) compared transcript levels between populations subjected to seven different selection regimes and one control regime using whole genome gene expression arrays. They were able to detect consistent differences in gene expression between replicate lines selected for stress resistance and control lines and obtained a clear signal after 10 generations of selection. The changes in gene expression in lines selected for increased longevity, desiccation resistance, and starvation resistance showed marked similarities. This is perhaps unsurprising, given the results described in the previous section but still a very useful genomic window on the genetic foundations shared by these three characters. They also identified functional groups of genes affected by selection for stress resistance, which were diverse (46).

There are a number of studies that assess expression changes among *Drosophila* lines selected for different behaviors. Although perhaps not viewed as a traditionally physiological trait, behavior is an important link between potential physiological performance and actual organismal fitness in nature (16). Genes with different expression levels have been identified in lines selected for aggressive (10, 12) and mating behavior (26). Interestingly, selection experiments for the same focal trait, when carried out in different laboratories, tend to implicate different genes. Of course, many of the demons mentioned in the previous section could cause this phenomenon. Edwards et al. (12) and Dierick and Greenspan (10) both found that direct selection produced more aggressive populations of flies, but did not find that the same genes were differentially expressed in their experimental populations (Table 1 provides a complete description of these genes; see Ref. 20). Both studies were carefully designed and employed such exemplary features as minimal inbreeding depression and replication of selected lines. Of course, the two studies differed in some significant ways, including their specific selection protocols, the founder populations for selected populations, and methods of preparing flies for genomic analysis. But it is troubling that two studies with such similar goals did not implicate the same genetic mechanisms underlying selection for aggressive behavior. The number of genomic analyses of selected lines is relatively small so far, though increasing, and it may prove an interesting challenge to determine the robustness of direct selection regimes in terms of their reproducibility at the level of global gene expression.

DNA "tiling" arrays can detect changes in allele frequency that result from laboratory selection. A relatively new application of array technology is the use of DNA arrays on which are printed probes from the reference sequence of model organisms such as *Drosophila melanogaster*. Investigators can hybridize samples of genomic DNA from their laboratory populations of interest to these arrays, and the resulting hybridization signals will reflect levels of genome-wide gene frequency differentiation. Such assays go beyond expression analyses, which point to molecular phenotypes, in ascertaining genotypes of interest.

In a study that does not employ laboratory-selected populations of *Drosophila*, differentiated genes and chromosomal regions that result from adaptation along a latitudinal cline were assayed using this tiling array technology (49). A technique called "single-feature polymorphism mapping", in which total genomic DNA from pooled samples obtained from "northern" or "southern" populations were hybridized to a *Drosophila* tiling array, revealed "single feature polymorphisms" (SFPs) differentiating the samples. As the tiling arrays harbor features at a density of approximately one every 40 base pairs, a large number of such queries can be effectively carried

Downloaded from ajpregu.physiology.org on July 30, 2011

Table 1. *Genes identified by two different studies that report differential expression of genes as a result of direct selection for aggressive behavior*

Gene*	Biological Process*	Gene†	Biological Process†
arginase	Amino acid catabolism	CG11899	Amino acid metabolism
frizzled	Anatomical structure development	Mub	Apoptosis
Darkener of apricot	Anatomical structure development	TpnC41C	Calcium-mediated signaling
Btk family kinase at 29A	Courtship behavior	CG31475	Calcium-mediated signaling
CG14478	DNA methylation	CG10444	Channels and transporters
kismet	Embryonic development	CG3397	Channels and transporters
CG5966	Lipid metabolism	CG9295	Cuticle proteins
CG12292	Nervous system development	CG2555	Cuticle proteins
muscleblind	Nervous system development	Drs	Immune response
CG17154	Nervous system development	GNBP1	Immune response
CG1623	Nervous system development	CG4825	Lipid metabolism
couch potato	Nervous system development	Mlc1	Muscle contraction
longitudinals lacking	Nervous system development	CG5195	Neuronal function
scribbler	Nervous system development	Snap	Neuronal function
		Mfas	Neuronal function
		Dh	Neuronal function
		CG7900	Nitrogen compound metabolism
		Obp56a	Pheromone signaling
		Cyp6a20	Pheromone signaling
		CG7378	Protein phosphorylation
		kek4	Signaling
		CG8942	Signaling
		CG5955	Sugar metabolism
		Treh	Sugar metabolism
		CG32444	Sugar metabolism

Only genes with known biological processes are listed. The genes listed for the Edwards et al. (12) study are those with reportedly functional consequences. The genes listed for Dierick and Greenspan (10) are those with expression differences >25% between lines; the authors only report one gene in this set to have functional consequences. Boldface represents genes for which authors report functional consequences as a result of mutant tests. *Data from Edwards et al. (12). †Data from Dierick and Greenspan (10).

out. An interesting property of this experiment, from the point of view of experimentally evolved populations, is that SFPs are due to DNA polymorphism arising from different allele frequencies in two groups. Thus, the method potentially allows one to identify alleles across the entire genome, which have differentiated in response to experimental evolution. The resulting portrait of genomic differentiation can add to existing knowledge of candidate loci previously implicated in selection, and it also generates new candidates for follow-up studies.

Presently, we are aware of only one study that combines laboratory selection with tiling array technology, a study that identified blocks of linked markers that apparently changed in allele frequency following selection for acute starvation resistance (29). Although their primary goal was the identification of quantitative trait loci involved in the general stress response, their approach allowed them to pinpoint a number of candidate genes involved in the response to selection for starvation resistance. These included genes involved in various metabolic processes and infection resistance, heat-shock protein genes, and genes encoding insulin-like growth factors. We feel that the DNA tiling array approach is a highly complementary new tool for studies of *Drosophila* laboratory selection. There is great potential for this tool to inform investigators studying the evolution of physiological traits.

The combination of experimental evolution with genomic surveys is an incredibly useful strategy for exploring the genetic basis of physiological processes. As the costs of genomic surveys become more competitive, we expect that the number of studies that employ this combination will increase. As more of the resulting data become available, we will approach a clearer picture of the genetic architecture underlying physiological traits.

Summary and Conclusions

Drosophila have proven to be exceptionally useful as a system for experimental evolutionary studies, and *D. melanogaster* specifically so. Flies provide a complex metazoan model with which to study physiological processes, particularly because it is practical to maintain a system of moderately large, replicate populations subject to a variety of selection regimes. Although ancestral populations unfortunately cannot be preserved in the same way microbial populations can, at least not readily given the present low level of success with *Drosophila* cryopreservation and resuscitation, it is easy to maintain stable environments for control populations, and the aforementioned large population sizes minimize the confounding effects of genetic drift. Although there are certainly some problems with using the *Drosophila* model, there is a helpful body of literature explaining how and why selection experiments occasionally yield ambiguous results. In any case, these problems are far outweighed by the excellent *Drosophila* genomic resources, with technologies that are constantly being improved on, and becoming more affordable. We envision a future for evolutionary physiology that is infused with experimental evolution and genomic tools, to connect physiological performance, life history, and gene expression on the phenotypic level with polymorphism at the genotypic level. And this future is well on its way to becoming a reality.

Downloaded from ajpregu.physiology.org on July 30, 2011

ACKNOWLEDGMENTS

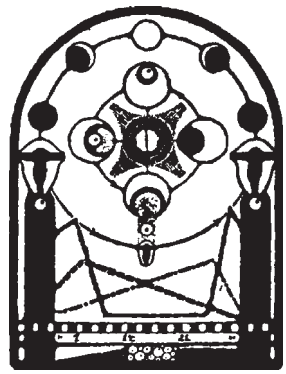
This article was invited following the symposium, "*Experimental Evolution as a Tool of Physiological Analysis*". We thank J. Hicks for suggesting and organizing the symposium, and the American Physiological Society for supporting the symposium. We also thank two anonymous reviewers for helpful suggestions that substantially changed the focus of the article. The University of California has provided research support to M. K. Burke and M. R. Rose.

REFERENCES

1. **Archer MA, Bradley TJ, Mueller LD, Rose MR.** Using experimental evolution to study the physiological mechanisms of desiccation resistance in *Drosophila melanogaster*. *Physiol Biochem Zool* 80: 386–398, 2007.
2. **Archer MA, Phelan JP, Beckman KA, Rose MR.** Breakdown in correlations during laboratory evolution. II. Selection on stress resistance in *Drosophila* populations. *Evolution* 57: 536–543, 2003.
3. **Bettencourt BR, Kim I, Hoffmann AA, Feder ME.** Response to natural and laboratory selection at the *Drosophila* hsp70 genes. *Evolution* 56: 1796–1801, 2002.
4. **Cavener D, Clegg M.** Multigenic response to ethanol in *Drosophila melanogaster*. *Evolution* 35: 1–10, 1981.
5. **Chen B, Walser JC, Rodgers TH, Sobota RS, Burke MK, Rose MR, Feder ME.** Abundant, diverse, and consequential P elements segregate in promoters of small heat shock genes in *Drosophila* populations. *J Evol Biol* 20: 2056–2066, 2007.
6. **Chippindale AK, Chu TJF, Rose MR.** Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. *Evolution* 50: 753–766, 1996.
7. **Chippindale AK, Gibbs AG, Sheik M, Yee KJ, Djawdan M, Bradley TJ, Rose MR.** Resource acquisition and the evolution of stress resistance in *Drosophila melanogaster*. *Evolution* 52: 1342–1352, 1998.
8. **Chippindale AK, Leroi AM, Kim SB, Rose MR.** Phenotypic plasticity and selection in *Drosophila* life history evolution. 1. Nutrition and the cost of reproduction. *J Evol Biol* 6: 171–193, 1993.
9. **DeckertCruz DJ, Tyler RH, Landmesser JE, Rose MR.** Allozymic differentiation in response to laboratory demographic selection of *Drosophila melanogaster*. *Evolution* 51: 865–872, 1997.
10. **Dierick HA, Greenspan RJ.** Molecular analysis of flies selected for aggressive behavior. *Nat Genet* 38: 1023–1031, 2006.
11. **Djawdan M, Chippindale AK, Rose MR, Bradley TJ.** Metabolic reserves and evolved stress resistance in *Drosophila melanogaster*. *Physiol Zool* 71: 584–594, 1998.
12. **Edwards AC, Rollmann SM, Morgan TJ, Mackay TFC.** Quantitative genomics of aggressive behavior in *Drosophila melanogaster*. *PloS Genet* 2: 1386–1395, 2006.
13. **Feder ME.** Engineering candidate genes in studies of adaptation: the heat-shock protein Hsp70 in *Drosophila melanogaster*. *Am Nat* 154: S55–S66, 1999.
14. **Folk DG, Han C, Bradley TJ.** Water acquisition and partitioning in *Drosophila melanogaster*: effects of selection for desiccation-resistance. *J Exp Biol* 204: 3323–3331, 2001.
15. **Futuyma D.** *Evolution.* Sunderland, MA: Sinauer Associates, 2005.
16. **Gibbs AG.** Laboratory selection for the comparative physiologist. *J Exp Biol* 202: 2709–2718, 1999.
17. **Gibbs AG, Chippindale AK, Rose MR.** Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *J Exp Biol* 200: 1821–1832, 1997.
18. **Giesel JT, Murphy PA, Manlove MN.** The influence of temperature on genetic interrelationships of life history traits in a population of *Drosophila melanogaster*.What tangled data sets we weave. *Am Nat* 119: 464–479, 1982.
19. **Graves JL, Toolson EC, Jeong C, Vu LN, Rose MR.** Desiccation, flight, glycogen, and postponed senescence in *Drosophila melanogaster*. *Physiol Zool* 65: 268–286, 1992.
20. **Grumbling G, Strelets V, The Flybase Consortium.** Flybase: anatomical data, images and queries. *Nucleic Acids Res* 34: D484–D488, 2006.
21. **Harshman LG, Hoffmann AA, Clark AG.** Selection for starvation resistance in *Drosophila melanogaster*: physiological correlates, enzyme activities and multiple stress responses. *J Evol Biol* 12: 370–379, 1999.
22. **Harshman LG, Schmid JL.** Evolution of starvation resistance in *Drosophila melanogaster*: Aspects of metabolism and counter-impact selection. *Evolution* 52: 1679–1685, 1998.
23. **Leroi AM, Chen WR, Rose MR.** Long-term laboratory evolution of a genetic life history trade off in *Drosophila melanogaster*. 2. Stability of genetic correlations. *Evolution* 48: 1258–1268, 1994.
24. **Leroi AM, Chippindale AK, Rose MR.** Long-term laboratory evolution of a genetic life history trade off in *Drosophila melanogaster*. 1. The role of genotype-by-environment interaction. *Evolution* 48: 1244–1257, 1994.
25. **Luckinbill LS, Arking R, Clare MJ, Cirocco WC, Buck SA.** Selection for delayed senescence in *Drosophila melanogaster*. *Evolution* 38: 996–1003, 1984.
26. **Mackay TFC, Heinsohn SL, Lyman RF, Moehring AJ, Morgan TJ, Rollmann SM.** Genetics and genomics of *Drosophila* mating behavior. *Proc Natl Acad Sci USA* 102: 6622–6629, 2005.
27. **Matos M, Rose MR, Pite MTR, Rego C, Avelar T.** Adaptation to the laboratory environment in *Drosophila subobscura*. *J Evol Biol* 13: 9–19, 2000.
28. **Nghiem D, Gibbs AG, Rose MR, Bradley TJ.** Postponed aging and desiccation resistance in *Drosophila melanogaster*. *Exp Gerontol* 35: 957–969, 2000.
29. **Nuzhdin SV, Harshman LG, Zhou M, Harmon K.** Genome-enabled hitchhiking mapping identifies QTLs for stress resistance in natural *Drosophila*. *Heredity* 99: 313–321, 2007.
30. **Partridge L, Fowler K.** Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Evolution* 46: 76–91, 1992.
31. **Passananti HB, Beckman KA, Rose MR.** Relaxed stress selection in *Drosophila melanogaster*. In: *Methuselah Flies: A Case Study in the Evolution of Aging*, edited by Rose MR, Passananti HB, and Matos M. Singapore: World Scientific Publishing, 2004, p. 323–352.
32. **Passananti HB, Deckert-Cruz DJ, Chippindale AK, Le BH, Rose MR.** Reverse evolution of aging in *Drosophila melanogaster*. In: *Methuselah Flies: A Case Study in the Evolution of Aging*, edited by Rose MR, Passananti, H. B., Matos, M. Singapore: World Scientific Publishing 2004, p. 296–322.
33. **Phelan JP, Archer MA, Beckman KA, Chippindale AK, Nusbaum TJ, Rose MR.** Breakdown in correlations during laboratory evolution. I Comparative analyses of *Drosophila* populations. *Evolution* 57: 527–535, 2003.
34. **Rose MR.** Genetic covariation in *Drosophila* life history—untangling the data. *Am Nat* 123: 565–569, 1984.
35. **Rose MR.** Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution* 38: 1004–1010, 1984.
36. **Rose MR, Charlesworth B.** Genetics of life history in *Drosophila melanogaster*. 2. Exploratory selection experiments. *Genetics* 97: 187–196, 1981.
37. **Rose MR, Nusbaum TJ, Chippindale AK.** Laboratory evolution: the experimental wonderland and the Cheshire cat syndrome. In: *Adaptation*, edited by Rose MR and Lauder GV. San Diego, CA: Academic, 1996, p. 221–241.
38. **Rose MR, Passananti HB, Chippindale AK, Phelan JP, Matos M, Teotonio H, Mueller LD.** The effects of evolution are local: Evidence from experimental evolution in *Drosophila*. *Integr Comp Biol* 45: 486–491, 2005.
39. **Rose MR, Passananti HB, Matos M.** *Methuselah Flies.* Singapore: World Scientific Publishing, 2004.
40. **Rose MR, Vu LN, Park SU, Graves JL.** Selection on stress resistance increases longevity in *Drosophila melanogaster*. *Exp Gerontol* 27: 241–250, 1992.
41. **Service PM.** Physiological mechanisms of increased stress resistance in *Drosophila melanogaster* selected for postponed senescence. *Physiol Zool* 60: 321–326, 1987.
42. **Service PM, Hutchinson EW, Mackinley MD, Rose MR.** Resistance to environmental stress in *Drosophila melanogaster* selected for postponed senescence. *Physiol Zool* 58: 380–389, 1985.
43. **Service PM, Hutchinson EW, Rose MR.** Multiple genetic mechanisms for the evolution of senescence in *Drosophila melanogaster*. *Evolution* 42: 708–716, 1988.
44. **Service PM, Rose MR.** Genetic covariation among life history components—the effect of novel environments. *Evolution* 39: 943–945, 1985.
45. **Simoes P, Rose MR, Duarte A, Goncalves R, Matos M.** Evolutionary domestication in *Drosophila subobscura*. *J Evol Biol* 20: 758–766, 2007.
46. **Sørensen JG, Nielsen MM, Loeschcke V.** Gene expression profile analysis of *Drosophila melanogaster* selected for resistance to environmental stressors. *J Evol Biol* 20: 1624–1636, 2007.

Downloaded from ajpregu.physiology.org on July 30, 2011

47. **Teotonio H, Chelo IM, Bradic M, Rose MR, Long AD.** Experimental evolution reveals natural selection on standing genetic variation. *Nat Genet* 41: 251–257, 2009.
48. **Teotonio H, Rose MR.** Variation in the reversibility of evolution. *Nature* 408: 463–466, 2000.
49. **Turner TT, Levine MT, Eckert ML, Begun DJ.** Genomic analysis of adaptive differentiation in *Drosophila melanogaster*. *Genetics* 179: 455–473, 2008.
50. **Wattiaux JM.** Parental age effects in *Drosophila pseudoobscura*. *Exp Gerontol* 3: 55–61, 1968.
51. **Williams AE, Bradley TJ.** The effect of respiratory pattern on water loss in desiccation-resistant *Drosophila melanogaster*. *J Exp Biol* 201: 2953–2959, 1998.
52. **Williams GC.** Pleiotropy, natural selection, and the evolution of senescence. *Evolution* 11: 398–411, 1957.



Downloaded from ajpregu.physiology.org on July 30, 2011