Selection Experiments as a Tool in Evolutionary and Comparative Physiology: Insights into Complex Traits—an Introduction to the Symposium[1]

JOHN G. SWALLOW[2],* AND THEODORE GARLAND, JR.†

*Department of Biology, University of South Dakota, Vermillion, South Dakota 57069
†Department of Biology, University of California, Riverside, Riverside, California 92521

"The whole organism is so tied together that when slight variations in one part occur, and are accumulated through natural selection, other parts become modified. This is a very important subject, most imperfectly understood." (Darwin, 1859, *The Origin of Species*).

"Hence if man goes on selecting, and thus augmenting, any peculiarity, he will almost certainly modify unintentionally other parts of the structure, owing to the mysterious laws of correlation." (Darwin, 1859, *The Origin of Species*).

"Biological reality is so complex that we are very far from any reasonably mechanistic understanding of evolutionary processes." (Felsenstein, 1988, p. 468)

"Apparent complexity usually means a lack of understanding." (McCarthy and Roberts, 1989, p. 134)

This symposium focused on the use of selection experiments to probe complex traits. By "complex traits," we generally mean phenotypes that involve multiple subordinate traits and are polygenic. In addition, they will generally be affected by various environmental factors (*e.g.,* both developmental and immediate temperature and nutrition), as well as interactions between genetic and environmental factors (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Under this definition, complex traits tend to be those at higher levels of organization, including behavior, life history, and organismal physiology. The field of quantitative genetics developed to deal with complex traits, and artificial selection experiments, dating from the late 1800s, quickly became a cornerstone (Hill and Caballero, 1992).

We searched BIOSYS Previews to gauge how usage of complex traits, selection experiments, and some related terms have changed over the period 1969 through 2003. Figure 1 indicates some interesting trends. Complex traits were not found in the period 1969–1978. They were found in four articles during 1979–1983, and the usages there were consistent with our general definition. Relative to the other terms shown in Figure 1, usage of "complex trait" has skyrocketed in recent years. A substantial fraction of this increase is found in

the biomedically oriented literature, which has followed from the recognition that such diseases and conditions as human schizophrenia are complex traits (*e.g.,* Sullivan *et al.*, 2003; see also Koch and Britton, 2005). In addition, major research efforts are being directed at the genetic dissection of complex traits in such model organisms as house mice (*e.g.,* www.complextrait.org; www.jax.org/phenome; www.webqtl.org). The agricultural world is also adopting various approaches in genomics and bioinformatics in attempts to understand complex traits (*e.g.,* Eggen, 2003).

Classical comparative physiology sought to understand "how animals work" (Schmidt-Nielsen, 1972), and was highly successful in this enterprise (*e.g.,* see brief reviews in Mangum and Hochachka, 1998; Hochachka, 2000). An outstanding goal of modern comparative and evolutionary physiology is understanding how complex traits have evolved, including identification of factors that shape and constrain the evolution of physiological capacities (Feder *et al.*, 1987). By explicitly incorporating an evolutionary perspective and modern tools from evolutionary biology, comparative physiologists are making great strides towards understanding the evolution of animal form and function. Several publications in the last decade have identified selection experiments (of various types) as one of the most important tools in the expanding field of evolutionary physiology (*e.g.,* Garland and Carter, 1994; Rose *et al.*, 1996; Bradley and Zamer, 1999; Gibbs, 1999; Feder *et al.*, 2000; Bennett, 2003; Garland, 2003; Bradley and Folk, 2004). Our symposium attempted to illustrate the utility of selection experiments for understanding traits that are commonly studied by comparative, ecological, and evolutionary physiologists, including metabolism, locomotion, stress resistance, and aspects of life history.

A growing body of evidence indicates that many traits of traditional interest to comparative/evolutionary physiologists as measured in standard ways (*e.g.,* locomotor performance, aerobic capacity, thermal tolerance) are heritable in the narrow (additive-genetic) sense and thus capable of responding to selection. As a consequence, selection experiments are increasingly being recognized as a powerful tool for understanding the genetic and mechanistic basis of complex physiological and morphological traits (see also Scheiner, 2002). Selection experiments with behavioral traits have a longer history and are more numerous, but have often been undertaken from a more psychological or biomedical perspective (but see Lynch [1980] for what
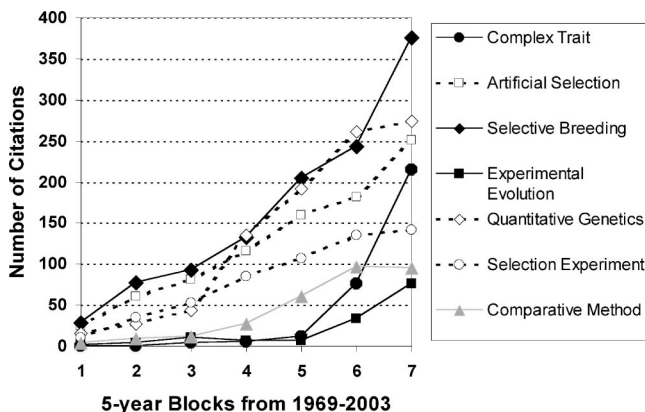
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[2] E-mail: jswallow@usd.edu

FIG. 1.   Search results from BIOSYS Previews to gauge how usage of complex trait, selection experiment, and some related terms has changed over the period 1969 through 2003. Values on X axis indicate successive 5-year periods, e.g., "1" indicates 1969–1973 and "7" indicates 1999–2003.

may be considered one of the first selection experiments in ecological/evolutionary physiology).

Selection experiments allow physiologists to effect cross-generational changes and directly observe microevolutionary processes. Thus, they allow one to test hypotheses concerning general principles of physiological evolution. For example, selection for a single trait often results in correlated changes in other traits. Such correlated responses are commonly determined by pleiotropic gene action in which one gene affects more than one trait. Shared biochemical, physiological or developmental pathways are the caused by pleiotropic gene action; mechanistic inferences can thus be derived from monitoring correlated changes in key physiological characters. Another question that can be approached is whether adaptive evolutionary changes (i.e., cross-generational changes in allele frequencies of populations in response to selection) tend to be in the same direction as plastic changes (i.e., changes that occur during development within individual organisms, such as during acclimation, acclimatization or physical conditioning) in response to a given "stress" (see Callahan, 2005; Swallow et al., 2005).

The goal of selective breeding is usually to change the mean trait value of a defined population (line) as compared to a control (non-selected) population and/ or as compared to a line selected in the opposite direction. Usually, selection is imposed at a fairly high level of biological organization. For example, in the present symposium, we have papers about mice artificially selected for high voluntary wheel-running behavior (Rhodes et al., 2005; Swallow et al., 2005) and for basal metabolic rate (Konarzewski et al., 2005), rats selected for high and low treadmill running performance (Koch and Britton, 2005), crickets selected for high or low wing morph frequency (Zera, 2005), and stalk-eyed flies selected for male eye span (Wilkinson et al., 2005). Quantitative-genetic theory predicts that the response to selection should be symmetrical in the "up" and "down" directions, but this is

often not the case, and a deep understanding of physiological mechanisms holds great promise for understanding the causes of asymmetric responses, the evolution of trade-offs, and so forth (Davidowitz et al., 2005; see also Zera et al., 1998 see also Folk and Bradley, 2005; Zera, 2005). Moreover, it is possible to select simultaneously on several traits or on combinations of traits, as must always occur in nature. Selection experiments have even targeted the degree of phenotypic plasticity of particular traits (Scheiner, 2002; Callahan, 2005).

In any case, once the populations have diverged in mean values for the trait(s) under intentional selection, they may be compared with respect to other traits that are hypothesized to share common causes, such as behaviors that may share neural or hormonal pathways (e.g., Rhodes et al., 2005) or life-history traits that may be impacted (e.g., Wilkinson et al., 2005; Zera, 2005). They may also be compared for lower-level traits that are thought to constitute the mechanisms underlying any differences in higher-level traits (e.g., Folk and Bradley, 2005; Koch and Britton, 2005; Overli et al., 2005; Swallow et al., 2005; Zera, 2005). Sometimes selection is imposed directly on a lower-level trait, such as an enzyme activity (e.g., Zera et al., 1998) or blood pressure (references in Rapp, 2000), an example in this symposium being trout selected for plasma cortisol concentration following a stress test (Overli et al., 2005).

Most of the studies mentioned in the previous paragraph fall under the general term "artificial selection" (see review of early history in Hill and Caballero, 1992) or "selective breeding." The investigators measured individual animals with respect to some phenotype of interest and then chose the soon-to-be parents for the next generation accordingly. But selection experiments come in several other varieties (Rose et al., 1996; Gibbs, 1999; Garland, 2003; Fuller et al., 2005; Riehle et al., 2005).

At one end of the continuum, the study of evolutionary responses to naturally occurring events (e.g., epidemics, droughts, invasions) constitutes a kind of fortuitous experiment, especially if these events occur repeatedly and predictably enough that the study can be replicated (Lee, 2002). Because of space constraints, we did not include any examples of this in the present symposium. As reviewed by Reznick and Ghalambor (2005), intentional field introductions involve placing a population in a new habitat in the wild, or altering its habitat by adding a new predator, a new prey species, fertilizer, a pesticide, etc. The manipulated population is then monitored across generations and compared with a control population.

In laboratory natural selection, the environment of a laboratory-maintained population is altered as compared with an unaltered control population (e.g., Folk and Bradley, 2005; Riehle et al., 2005). Laboratory culling exposes a population to a stress that is lethal (or sublethal) and then allows the survivors (or hardiest) to produce the next generation (e.g., some of the

studies cited in Rose *et al.*, 2005). Domestication generally involves some amount of intentional selective breeding and, once domesticated, organisms may be the subject of additional selective breeding programs, with varying degrees of control and replication, leading to multiple breeds or lines. The unintentional effects of various actions by human beings can also be studied from the perspective that they constitute selective factors whose consequences may be predictable. Examples include changes in commercial fisheries (*e.g.,* Koskinen *et al.*, 2002; Heath *et al.*, 2003) and in various ungulates that are hunted (*e.g.,* Coltman *et al.*, 2003).

As shown in Figure 1, usage of ''experimental evolution'' has grown in recent years (*e.g.,* see www.biology.ucr.edu/ucirpee/) and is receiving coverage in the popular press (*e.g.,* www.sanluisobispo.com/mld/sanluisobispo/news/nation/9766974.htm). This term encompasses much of what we term selection experiments, but would often exclude cases that have design features, such as lack of replication, that can seriously compromise inferences about line differences (but see Callahan *et al.*, 2005; Koch and Britton, 2005; Konarzewski *et al.*, 2005). The term experimental evolution is often favored by those who work with *Drosophila* or microorganisms (*e.g.,* Folk and Bradley, 2005; Riehle *et al.*, 2005; Rose *et al.*, 2005), which have a long history of study by population and evolutionary biologists, as well as geneticists, and which are amenable to large population sizes, a high degree of replication, and long-term (many generations) studies. Long-term selection studies may uncover evolutionary results that are different from those seen in earlier generations (Rose *et al.*, 2005). Folk and Bradley (2005) point out that the multiple solutions seen among replicate lines and among selection experiments have been somewhat frustrating for evolutionary biologists interested in repeatable outcomes. However, such solutions have been a boon for evolutionary physiologists interested in probing the mechanisms underlying the adaptations (see also Garland, 2003).

We hope that the following symposium articles will help to introduce selection experiments and experimental evolution as powerful but underutilized tools for comparative physiology, but also for other related fields, such as functional morphology (Garland, 2003). The papers cover a substantial range of physiological systems and numerous types of organisms, including ectotherms and endotherms, vertebrates, insects, plants, and bacteria. They demonstrate encouragingly rapid responses to selection in many systems, and highlight how unanticipated results (*e.g.,* not matching predictions from interspecific comparative studies) can provide important insights into physiological mechanisms of adaptation (Gibbs, 1999). They also point out how phenotypically differentiated lines can become a valuable resource not only for understanding the physiological differences among lines but also for genetic dissection of complex traits using modern approaches in genomics and bioinformatics (*e.g.,* Riehle *et al.*,

2005). Finally, they hint at an important area for future research: comparative selection experiments designed to elucidate how ''repeatable'' evolution may be across species (J. F. Harrison, personal communication; Garland, 2003). One might hypothesize, at least naively (Rose *et al.*, 2005), that comparisons of fairly closely related organisms, such as mice and rats, would reveal similar evolutionary responses, whereas comparisons of distantly related organisms (or those that differ greatly in complexity, such as mice *vs.* bacteria) would not.

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