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Selection experiments: an under-utilized tool in biomechanics and organismal biology

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1. Introduction

Questions in 'evolutionary biomechanics' range along a continuum that is bounded by the purely evolutionary (e.g. How have morphological features changed over time?) and the purely mechanistic (e.g. How do organisms work?). In between are questions that call for rigorous analyses and deep understanding of both evolution and mechanism. For example, does efficiency tend to increase within a lineage over evolutionary time (Lauder, 1991a)? Related to this question, many biologists wish to know how well adapted are the functions of organisms. Are functional abilities very well suited to the ecological conditions in which organisms live and to support the normal behaviours that they exhibit? Are organisms generally close to optimal? Or are they merely adequate and certainly far from perfect most of the time (Gans, 1993; Garland, 1998; Garland and Huey, 1987; Ward, 1992)? Some workers hold that 'evolution by natural selection is a process of optimization' (Alexander, 1996, p. 2). George Bartholomew (1987, p. 14), on the other hand, argues that 'Natural selection increases fitness but it produces systems that function no better than they must. It yields adequacy of adaptation rather than perfection'. Or, to quote Lewontin (1987, p. 158): 'The most that can be said for organisms is that they make the best of a bad situation. But do they even do that?' The latter view is also held by Carl Gans (Gans, 1983, pp. 101–102; see also Gans, 1991), who wrote: 'In spite of occasional statements to the contrary, there can be little argument that natural selection is unlikely to be a mechanism for generating perfection in individual animals. ... that the structure of an animal allows [it] to perform particular actions, highly advantageous under a particular set of circumstances, does not require perfect matching, but only adequacy'.

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An issue related to the extent of adaptation is how often organisms show 'multiple solutions' (sensu Bartholomew, 1987), or different adaptive responses. For example, if natural selection causes the evolution of high maximal sprint running speeds in several lineages of lizards (Bonine and Garland, 1999; Snyder, 1954; Van Damme *et al.*, this volume) will some lineages accomplish this via increases in stride length (which could involve increases in leg length and/or changes in kinematics and gait [Irschick and Jayne, 1999]) while others show increases in stride frequency (which presumably would entail changes in muscle contractile characteristics, fibre-type composition [Bonine *et al.*, 2001], and possibly innervation)? Many evolutionary biologists might expect multiple solutions (e.g. see Figure 2 in Futuyma, 1986). To quote Ernst Mayr (1961, p. 1505), 'Probably nothing in biology is less predictable than the future course of evolution. ... Unpredictability also characterizes small-scale evolution. Breeders and students of natural selection have discovered again and again that independent parallel lines exposed to the same selection pressures will respond at different rates and with different effects, none of them predictable'. If multiple 'solutions' to an adaptive 'problem' are identified, then is it useful to think of some of them as being 'more optimal' than others?

Another type of hybrid question has to do with whether the way organisms are 'designed' constrains their evolutionary potential (Burt, 2001; Deban, this volume; Garland and Carter, 1994; Wagner and Schwenk, 2000). For instance, we presume that the shells of ancestral turtles evolved as an adaptation (i.e. in response to natural selection), but we also believe that they currently place rather severe constraints on the kinds of respiratory and locomotor mechanics that could potentially evolve in future turtles. Such constraints would, of course, reduce the likelihood that organisms could become optimally adapted. And this type of question is also related to the issue of multiple solutions. All constraints are ultimately genetic in origin. 'Genetic constraints' exist when alleles are absent from a population and/or when alleles affect multiple traits (pleiotropy) in ways that run counter to the prevailing selection (Schluter, 1996). Particular alleles may be present in some populations but not in others because of chance effects, such as random genetic drift. The probability that chance events will have an important influence on the response to selection depends on population size, as well as on the strength of selection. Organisms that exist in relatively small populations are more likely to experience random genetic changes that influence and interact with the genetic response to selection (i.e. adaptive evolution), and hence should be more likely to exhibit what might be viewed by biologists as 'multiple solutions'.

The foregoing brief introduction should make it clear that questions about the evolution of form and function are diverse, complicated and interrelated. Not surprisingly, such questions can (and should) be addressed in many different ways (Alexander, 1996; Feder *et al.*, 2000; Garland and Carter, 1994; Huey and Kingsolver, 1993; Rose and Lauder, 1996; Wainwright and Reilly, 1994). Interspecific comparisons have always been common in evolutionary biomechanics (Brown and West, 2000; Domenici and Blake, 2000a; Rayner and Wooten, 1991; Thomason, 1995), and advances in the last 20 years have demonstrated numerous ways in which they can be enhanced by a thorough consideration of phylogenetic information (Brooks and McLennan, 1991; Eggleton and Vane-Wright, 1994; Garland, 2001; Garland and Ives, 2000; Garland *et al.*, 1999; Harvey and Pagel, 1991; Lauder, 1991a; Martins, 1996; Rohlf, 2001). Indeed, most of the chapters in this volume involve comparisons of

species, and several of them utilize independent phylogenetic information in the choice of species to be studied and/or for data analysis. A more recent thrust in evolutionary biomechanics and related fields has been the study of individual variation within species, which provides the raw material upon which natural (or sexual) selection can act (Arnold and Bennett, 1988; Brown and Brown, 1998; Carter *et al.*, 1999; Chappell *et al.*, 1999; Clobert *et al.*, 2000; Garland and Else, 1987; Hammond *et al.*, 2000; Hayes and O'Connor, 1999; Jung, 1992; Macrini and Irschick, 1998; McKitrick, 1986; Parsons and Djatsckenko, 1983; Price, 1987; Raikow *et al.*, 1990; Reilly and Lauder, 1988; Robinson *et al.*, 1996; Van Damme *et al.*, 1997; Walsberg *et al.*, 1986; reviews in Garland and Losos, 1994; Kolok, 1999).

The traditional approach of species comparisons, conducted within the context of a well-supported hypothesis about phylogenetic relationships, can tell us much about what has happened during past evolution. In a complementary fashion, studies of individual variation within populations can inform us about present evolution in action, including what traits are currently under selection, the strength and form of any selection, and the extent of heritable variation and covariation (Grant and Grant, 1995; Kingsolver *et al.*, 2001; Reznick and Travis, 1996). The thesis of this chapter is that selection experiments form a logical bridge between these two approaches (Feder *et al.*, 2000; Garland and Carter, 1994; Gibbs, 1999; Huey and Kingsolver, 1993). They allow one to study evolution in action, but under more controlled and reproducible circumstances than are possible in the wild, and they allow one to project into the future. If extended for enough generations, they may allow one to observe fundamental changes in both phenotypic and genetic architecture, which can then be attributed to past selection that has occurred in a relatively well-defined manner (as compared with what occurs in nature). Finally, they allow one to determine whether potential constraints imposed by the initial genetic variance–covariance matrix have actually been important in determining the course of adaptive evolution.

In a typical experiment, selection occurs at the level of some whole-organism trait, such as behaviour, body size, or a major component of fitness (e.g. fecundity). The experimental unit is the line (population), which is genetically closed once the experiment begins. Because any finite population will undergo genetic changes caused by random genetic events, a selection experiment needs to involve at least two lines, one of which serves as a control. Within a given line, selection can occur for higher or lower values of a trait. An experiment that involves selection in both directions (in separate lines) is termed bidirectional, and involves at least three lines (one selected for high values, one bred randomly as a control, one selected for low values).

Once selected and control lines have diverged (Rose, 1984; Schlager, 1974), they can be compared with respect to subordinate traits that are thought to cause differences at the organismal level (Rose *et al.*, 1984; Schlager *et al.*, 1983). Thus, selection experiments allow one to test hypotheses concerning form and function, such as may be derived from interspecific comparative studies or from *a priori* models of function (see Kardong, this volume). They can also be used to test hypotheses about developmental and allometric constraints (Brown and West, 2000; Emlen, 1996; Weber, 1990), about mechanisms of adaptive radiation (Travisano and Rainey, 2000), and about the relative roles of adaptation, chance and history in evolution (Travisano *et al.*, 1995).

Accordingly, evolutionary physiologists and behavioural biologists often use selection experiments. By allowing one to alter phenotypes at higher or lower levels of

biological organization – and then determine quite precisely what other traits change in concert – they have the potential to open many difficult areas in evolutionary biomechanics to experimental study. Surprisingly, however, functional morphologists rarely use selection experiments.

In this chapter, I first present a brief overview of some of the types of studies that are conducted under the broad heading of selection experiments (or 'experimental evolution'). I then discuss our own laboratory's experiment in which we have used selective breeding to create four replicate lines of house mice that exhibit high voluntary wheel running as compared with four unselected (control) lines. We have discovered several behavioural, physiological and morphological characteristics that have evolved in concert with elevated locomotor activity. Next, I outline one possible strategy for integrating selection experiments into a larger research programme in evolutionary biomechanics. Finally, I argue that the reason selection experiments have rarely been employed in evolutionary biomechanics and functional morphology is mainly the result of historical traditions, not a fundamental difference in ease of application to physiological or behavioural vs. morphological traits.

2. Selection experiments

Although virtually non-existent in evolutionary biomechanics, selection experiments have a long history (Bell, 1997; Falconer, 1992; Falconer and Mackay, 1996; Garland and Carter, 1994; Gibbs, 1999; Hill and Caballero, 1992; Hill and Mackay, 1989; Robertson, 1980; Roff, 1997; Rose *et al.*, 1990; Travisano and Rainey, 2000). They have occurred in a non-scientific context since human beings first began developing agriculture, including the gradual process of domesticating various plants and animals (e.g. on dogs, see Morey, 1994; Trut, 1999; Vila *et al.*, 1997).

The more recently developed tools of 'genetic engineering' (e.g. transgenesis, knockouts) usually attempt to alter only one or perhaps a few gene loci. In the wild, however, natural and sexual selection are thought to act most directly on complex phenotypes (e.g. behaviour, life history traits) that are typically highly polygenic (affected by many genes, most of which probably have relatively small effects). Thus, allele frequencies at many loci may change in response to selection. Therefore, from the perspective of evolutionary biomechanics, selection experiments offer the major advantage of being more representative of the type of genetic changes that occur in nature.

Selection experiments come in many varieties, and four types will be mentioned below: artificial selection, laboratory culling, laboratory natural selection and field introductions. While reading about these, it is useful to keep in mind some of the general considerations that apply when designing any selection experiment. The following is not meant as an exhaustive list, and anyone planning to conduct a selection experiment would be well advised to consult others who have conducted such experiments, experts in the husbandry of the candidate organism, and both basic and advanced reference works in quantitative genetics (Bell, 1997; Falconer and Mackay, 1996; Hill and Caballero, 1992; Hill and Mackay, 1989; Robertson, 1980; Roff, 1997). Unfortunately, I do not know of an easy 'how-to' manual for selection experiments. In any case, here are some factors to consider (Rose *et al.*, 1990, 1996).

First, the organism needs to have a relatively short generation time. Secondly, it needs to be amenable to breeding in conditions imposed by the experimenter, although

the actual housing conditions might range from plastic cages inside a controlled-environment room to semi-natural enclosures (including ponds) to small natural ponds or islands (Losos *et al.*, 1997). Thirdly, it should be relatively small in body size because fairly large numbers will be required (e.g. hundreds of individual per generation). Thus, mice would generally make better subjects than would capybara, guppies would be better than carp, and *Drosophila* would be better than Monarch butterflies. Fourthly, a control (unselected) line should be maintained, and both the selected and control lines should be replicated (see Section 2.6). Fifth, one must decide whether selection will be implemented in both directions, that is, by establishing separate selected lines for both high and low values of the phenotype (see Section 3.5).

Sixth, the source of the original populations needs to be considered carefully. For many organisms (e.g. house mice, guppies, *Drosophila*) one has a choice between using wild animals and domesticated or laboratory stocks of the same species. Some biologists are averse to using domesticated or laboratory strains because they presume them to be 'degenerate' in one or more ways (see references in Dohm *et al.*, 1994; Richardson *et al.*, 1994). However, as noted by Ricker *et al.* (1987), this claim is usually made in the absence of hard evidence, such as comparisons of wild and laboratory forms. For many domesticated organisms, concerns about differences between them and their wild counterparts can be addressed empirically by raising both under common laboratory conditions, then measuring traits of interest (Geiser and Ferguson, 2001). We have done this with house mice, and found that, although the laboratory strain (Hsd:ICR) and wild population (trapped from a Wisconsin stable) that we happened to compare differed, on average, in many traits, especially behavioural ones, the former certainly did not seem to be 'degenerate' in terms of physiology or morphology (Dohm *et al.*, 1994; Garland *et al.*, 1995; Richardson *et al.*, 1994). Whether such lab–wild differences as may exist represent a problem for a selection experiment in evolutionary biomechanics will depend on the organism and trait(s) in question.

If one chooses to work with wild-stock animals, then it may be best to use a stock that has been in captivity for at least several generations and hence has had some time to adapt to laboratory conditions. If not, then this adaptation will be occurring simultaneously with any selection imposed by the investigator. Hence, some workers have argued that long-term captive stocks are the best choice. Of course, long-term captive 'wild' animals grade into 'domesticated' forms.

If one chooses to study a domesticated or laboratory form, then many options as to particular strain may be available. Obviously, one needs to use a strain with substantial genetic variability, that is, one that is randombred or outbred, not highly inbred. But for many organisms, such as mice, many such strains are available. Some of these have been created by originally crossing a number of inbred lines, then allowing several generations to occur to attain linkage equilibrium before further work is done (e.g. a selection experiment). Such a source population offers the advantage that (1) it can, in principle, be re-created if and when needed, and (2) the pre-existing inbred-strain progenitors would facilitate identification of specific genes once divergent lines had been created (see discussions in Britton and Koch, 2000, 2001).

Seventh, population size and selection intensity need to be considered. Given that all real experiments are limited in terms of the total number of animals that can be maintained, larger population sizes within lines will come at the cost of fewer replicates per treatment (see Section 2.6 below). Very small population sizes are generally

to be avoided because random genetic drift will tend to overpower selection imposed by the investigator. For house mice and Norway rats, many selection experiments have involved 8–13 pairs per line (DeFries *et al.*, 1978; Koch and Britton, 2001; Lynch, 1986, 1994; Marley *et al.*, 1998; Swallow *et al.*, 1998a). These population sizes, for which the effective population size (Ne) can be almost doubled by a within-family selection regimen (see below), are somewhat smaller that one might wish from a quantitative-genetic perspective, but on the other hand they are perhaps not wildly unrepresentative of wild populations. Work with commensal populations of house mice, from which domesticated strains were presumably derived, has shown that they often occur in relatively small populations, with further demic structure, that leads to substantial genetic differentiation (references in Boursot *et al.*, 1993; Myers, 1974).

The simplest formula used to describe phenotypic response to selection is $r = h^2 s$, where r = the response to one generation of selection, h^2 = the narrow-sense heritability (additive genetic/total phenotypic variance), and s = the selection differential (difference between the phenotypic mean of the individuals chosen as breeders and the whole population before selection). The total response over many generations of a selection experiment should be greater for larger populations and for stronger selection. Thus, for a given population size, the generation-to-generation response to selection can be increased by choosing a smaller proportion of individuals as breeders, hence increasing s, but this will reduce the effective population size, increase drift and inbreeding, and hence reduce the ultimate total response. One good compromise is to choose 50% of the individuals within a line as the breeders, which maximizes the total response while sacrificing some speed of response (Falconer and Mackay, 1996).

If a primary goal of a selection experiment is to maximize the divergence between control and selected lines, and if extremely large effective population sizes (Weber, 1996) are impractical, then it may make sense occasionally to introduce 'migrants' from the original base population, at least if it is available (e.g. if it was a large, outbred population maintained by a commercial breeder, or perhaps a population from a restricted location in the wild). This might be anathema from the pure quantitative-genetic perspective, as it would confound attempts to estimate such parameters as realized heritability, but nonetheless it might allow one to avoid or delay selection limits. For example, repeated genetic exchange between dog and wolf populations is thought to have been an important source of genetic variation for selection (Vila *et al.*, 1997).

Finally, the type of selection to be imposed can be varied. Some simple methods are as follows (Falconer and Mackay, 1996). In individual selection, breeders are chosen solely with respect to their own phenotypic values. The term 'mass selection' is often used for this procedure when the selected individuals are placed together *en masse* for mating, as with *Drosophila* in a bottle.

In family selection, whole families are chosen as breeders based on the mean phenotype of the family as compared with other families within the line. Family selection may be useful when the trait under selection has a low heritability.

In within-family selection, individuals are chosen as breeders based on how far they deviate from the mean for their family (and sex). One big advantage of this method is that, by ensuring that each family contributes equally to the next generation, effective population size is almost doubled.

Sib selection refers to a situation in which breeders are chosen not for their own phenotype but instead based on that of their siblings. This procedure may be required

if a trait can only be measured destructively (e.g. something that requires dissection or a terminal physiological test), such that the individuals measured cannot subsequently breed. Thus, one could measure half of the individuals in each family, determine which families scored highest, then use the remaining half-families as breeders. Variations on this theme would include freezing sperm and eggs from all individuals prior to destructive measurements, or allowing all individuals to produce litters before they were measured (this latter protocol can become very expensive because all individuals within a line would need to produce litters in each generation, rather than only the selected subset).

In some cases, such as milk yield in ungulates or horn size in certain beetles, the trait of interest may be expressed in only one sex, in which case selection will only be imposed on that sex (Emlen, 1996). In other cases, a trait may occur in both sexes, but whether it is actually the same trait in both sexes is unclear. One way to get at this sort of question is to select on one sex only and test for correlated response in the other sex, as has been done with 'aggression' in house mice. Ebert and Hyde (1976) selected on agonistic behaviour in female house mice, then tested for correlated response in male aggressiveness (Hyde and Ebert, 1976) (as well as maternal aggressiveness: Hyde and Sawyer, 1979).

2.1 *Artificial selection*

Artificial selection involves captive populations in which individuals in each generation are measured for a phenotypic trait (or combination of traits) that is of interest. Some top or bottom fraction of individuals is then chosen as the breeders to produce the next generation. This is called truncation selection. One variation on truncation selection is taking the highest-scoring (or lowest-scoring) male and female from within each family, then allowing them to mate with other individuals in their line (but outside of their own family). This 'within-family selection' increases the effective population size, reduces the rate of inbreeding, and helps to eliminate the possibly confounding influences of some maternal effects. (On the negative side, within-family selection reduces the possible intensity of selection as compared with 'mass' selection, which involves choosing breeders without regard to their family membership.) Within-family selection has been used in our own selection experiment, described below, and in many other studies of rodents (DeFries *et al.*, 1978; Falconer, 1973; Koch and Britton, 2001; Marley *et al.*, 1998).

In a pioneering effort in evolutionary physiology, Carol B. Lynch used within-family artificial selection to change nesting behaviour of laboratory house mice. She maintained a total of six lines, two selected for making small nests, two selected for large nests, and two bred randomly as controls. Lynch's goal has been to understand the evolution of thermoregulatory phenotypes (behavioural, morphological and physiological), viewed as an integrated suite of interacting traits (an 'adaptive syndrome'). Her selected lines have been used in many subsequent studies, and informative parallels have been drawn with clinal variation in wild populations of house mice in North America (Bult and Lynch, 1997; Lynch, 1986, 1992, 1994).

Artificial selection experiments that would be of interest from the perspective of vertebrate evolutionary biomechanics are easy to imagine. For example, one might select directly on some aspect of fish swimming performance, such as the C-start or perhaps the critical swimming speed in a step-test. Once the selected lines had

diverged from their control lines, they could be compared with respect to various subordinate traits that are thought to affect performance (see Domenici, this volume; Lauder, this volume).

Unlike laboratory natural selection and laboratory culling experiments (see next sections), artificial selection allows the investigator to make detailed choices as to what, exactly, is under selection. Very particular aspects of behaviour, performance, morphology (Emlen, 1996, Weber, 1990; Wilkinson, 1993) or physiology can be targeted. If desired, selection can easily be focused on traits at relatively low levels of biological organization, such as blood pressure (Schlager, 1974), hematocrit (Schlager and Weibust, 1976), leukocyte count (Weir and Schlager, 1962), enzyme activity (Zera and Zhang, 1995) or endocrinological function (Chai, 1970).

2.2 *Laboratory culling*

A variant of laboratory natural selection is termed laboratory culling (Rose *et al.*, 1990). Here, a population is exposed to a lethal stress until some fraction dies; the survivors are allowed to breed. This type of selection is practised commonly with non-vertebrates, such as *Drosophila* (see examples in Rose *et al.*, 1990), but rarely with vertebrates, in part because of ethical considerations.

2.3 *Laboratory natural selection*

In laboratory natural selection, individual phenotypes are not measured each generation, nor are breeders specifically chosen by the investigator. Rather, a freely breeding population is exposed to altered environmental conditions, such as different temperatures or salinities, or to altered husbandry conditions, which could favour changes in demographic schedules. Assuming that additive genetic variance exists for relevant traits, the population will adapt to the new conditions. These sorts of experiments are most common with non-vertebrates, including *Drosophila* (Gibbs, 1999; Rose, 1984; Rose *et al.*, 1996; Zera and Harshman, 2001), bacteria (Mongold *et al.*, 1996; Travisano and Rainey, 2000; Travisano *et al.*, 1995), and viruses, but have also been employed with vertebrates.

An example of laboratory natural selection with a vertebrate is Barnett and Dickson's experiments in which wild house mice were used to establish two breeding colonies, one housed at approximately room temperature and the other in the cold. They performed two such experiments, for 9–14 generations, once in Scotland (Barnett *et al.*, 1975: average room temperatures of 21 and –3° C) and then again in Australia (23 and +3° C) (Barnett and Dickson, 1984a,b). In both experiments, various changes were observed, at least some of which seemed to be adaptive to the cold (Barnett and Dickson, 1989). The results were rather complicated, however, and in both cases only a single line was kept in either the 'control' (room temperature) or 'experimental' (cold) condition. This lack of replication makes it virtually impossible to say whether apparently adaptive changes were really caused by the different temperatures, rather than being the result of founder effects, unique (and possibly non-adaptive) mutations or genetic drift. Still, the experiments show that laboratory natural selection can be implemented with vertebrates.

Examples of laboratory natural selection experiments that would shed light on biomechanical issues are also easy to envision. For example, one might establish a

breeding colony of fish in a large aquarium or an artificial stream or pond, under conditions standard for the species (e.g. guppies, mosquito fish, goldfish). After a number of generations, the colony could be split into four subcolonies. Two of these would retain the original conditions and serve as control lines. The other two would experience a strong current, such that successful spawning would require males and/or females to swim in place. Depending on how food was offered and on how feeding occurred, the current might also exert selection via foraging success. In either case, one would expect swimming performance to evolve to be higher in the lines experiencing the strong current.

Another possibility would be to house subcolonies with or without predators, such as other fish or perhaps snakes. One would, of course, need to choose experimental parameters carefully, such as, size of tank, amount of cover, number of fish, type of predator(s), number of predators, so that an appropriate number of fish were preyed upon each generation.

A logical extension from the foregoing sorts of experiments would be to study populations that have recently faced introductions of predators or competitors into their natural habitat. Many populations of fish now live with introduced fish (or other animals), but none seem to have been exploited by workers in evolutionary biomechanics. Nevertheless, such possibilities lead logically to the consideration of intentional field introductions.

2.4 *Field introductions*

The final type of selection experiment to be mentioned here is the intentional introduction of organisms to natural areas. One example of this is Losos' work with *Anolis* lizards originally introduced to small Caribbean islands by Schoener (Losos *et al.*, 1997, 2000). Another is Reznick's work with Trinidadian guppies (Reznick, 1996; Reznick *et al.*, 1997). Both of these systems would offer interesting opportunities for biomechanical analysis, and indeed locomotor performance of the guppies is currently under study by C.K. Ghalambor, J. Walker, and D.N. Reznick (pers. comm.).

2.5 *Phenotype hierarchies, targets of selection (including unintentional), and experimental designs*

Selection experiments involve whole organisms, but the actual target(s) of selection within a given experiment can range from the level of behaviour (including competitive and social behaviour, which involve interactions of multiple individuals [Hyde and Sawyer, 1979; Ruzzante and Doyle, 1993]) to organismal performance ability (e.g. maximal burst speed or endurance) and on down to organ level (e.g. heart size, leg length), physiological (e.g. heart rate, blood pressure), hormonal, biochemical (e.g. enzyme activities at the cellular level) or even molecular (e.g. targeting of particular DNA polymorphism). In general, the design details of a selection experiment will dictate much, but not all, of the details regarding the level of selection and also whether multiple levels (or components within a level) are involved.

In nature, several workers have argued that selection acts most directly on behaviour (the choices that an animal makes when foraging, seeking mates, dealing with predators) or on major components of life history, less directly on such aspects of organismal performance as locomotor speed or stamina, and even less directly on

lower-level traits that determine performance ability (e.g. leg length, maximal heart rate, muscle fibre-type composition) (Domenici and Blake, 2000b; Garland, 1994; Garland and Carter, 1994; Garland and Losos, 1994; Irschick and Garland, 2001; Stirling *et al.*, 2002). Therefore, a case can be made that selection experiments targeted at the whole-animal level are the most relevant to evolution in nature. Of course, the higher in level the target of selection, the greater the number of ways that evolutionary response might occur.

The extent to which one can impose the level of biological organization at which selection occurs will depend in a general way on the type of selection experiment employed. In an artificial selection experiment (Section 2.1), a particular phenotype (such as some specific aspect of swimming performance) must be measured on every individual in the selected lines in every generation. Hence, very particular phenotypes can be specified (see Section 3), such as wheel running on days 5 and 6 of a 6-day test, with a 12 : 12 photoperiod, on a wheel of a certain diameter, constructed of a particular mesh size.

Laboratory natural selection experiments, on the other hand, do not require measurement of individual phenotypes (except in generations in which one wishes to compare the control and selected lines), and so no particular phenotype is being selected *a priori*. Rather, the environment is altered, and nature is allowed to take its course. As a result, the way organisms respond may be relatively unpredictable (Gibbs, 1999; Rose *et al.*, 1990). Whether this is a curse or a virtue depends on one's goals.

But even artificial selection experiments often involve highly composite phenotypes (Gibbs, 1999), such that the response to selection may involve traits at multiple levels of organization. In our mice, for example, which have been selected for high total revolutions run per day, we have observed changes in both speed and, to a lesser extent, duration of wheel running per day, and in both motivation and ability for wheel running. At lower-levels of organization, the motivational changes seem to involve (at least) alterations in brain dopamine function, whereas the changes in running ability seem to involve (at least) body size, ability of muscles to uptake glucose, and muscle size (especially in some lines). In Weber's (1996) selection for *Drosophila* flying speed in a wind tunnel, he noted that 'Various tests and observations show that the trait is actually a composite of phototaxis, activity level, flying speed, and aerial maneuvering skill' (p. 206). To this list one might add both motivation and flying ability. If ability has actually changed, then some one or more of the various morphological, physiological and neurological subordinate traits that affect ability must have changed as well.

With respect to the possibility of mimicking nature, laboratory natural selection may be a better choice than artificial selection. But the greater range of possible outcomes in the former might prove frustrating from a biomechanical perspective. For example, as discussed in Section 2.3, if one attempted to alter the selective regimen on fish swimming performance by changing current or predation, then the evolution of higher swimming performance would not be a foregone outcome. Rather, it would need to be tested at various generations, as the experimental populations might instead respond through behavioural alterations that largely obviated the need to increase swimming performance ability. Hence, from a biomechanical perspective, it might be more desirable to forego most pretences of mimicking nature and instead select directly on performance, or even on a lower-level trait thought to affect performance in a key way.

Regardless of the intended target of selection in any particular experiment, the possibility of unintentional or 'cryptic' selection should be kept in mind (Gibbs, 1999; Rose *et al.*, 1996). This can occur in several ways, including when husbandry practices or environmental conditions unintentionally vary such that selection occurs either on the trait of interest (possibly in opposition to the intentional selection) or on one or more other traits, which may themselves affect the target trait. Alternatively, logistical considerations or the very nature of the measurement protocol may cause complications. For example, in a selection experiment to alter frequencies of wing morphs in crickets (*Gryllus firmus*), some selection for early development was unavoidable (Roff, 1990). In an experiment that attempted to 'automate' measurement of body size, Baptist and Robertson (1976) sorted flies by having them walk through a series of slits of decreasing size. Here, the trait under selection was not body size (e.g. length or mass) *per se*, but rather a combination of body size and willingness (or motivation) to walk through the device (see Roff, 1997, pp. 135–136). Weber's (1996) automated selection for flying speed (see above) would involve similar issues. In our own experiment with mice (see Section 3, below), we intended to select on a trait – wheel running – that would involve both behaviour (willingness to run, motivation) and physiology/morphology (ability), because we wanted to understand how traits at different levels of biological organization evolve in a coordinated fashion. Nevertheless, it is likely that various types of unintentional selection have occurred in our lines.

2.6 *The importance of replication*

Replication of experimental lines, and consistency of response, is crucial in order that ensuing differences can be attributed to the effects of selection rather than founder effects and/or random genetic drift, perhaps in combination with the occurrence of unique mutations. Many early selection experiments involved only a single selected line or two, one selected for high values and the other for low (Falconer, 1992). Even today, selection experiments are conducted without replication (Koch and Britton, 2001; Nakamura *et al.*, 1993). Typically, the lack of replication is attributable to the cost and logistics involved in increasing the overall size of a selection experiment. Hence, while a lack of replication may be understandable, it does have important consequences.

Even lines of organisms that are not under divergent selection may be expected to diverge genetically and hence phenotypically because of (1) chance differences in allele frequencies that occurred at founding of the lines, (2) subsequent random genetic drift, and (3) unique mutations. These same considerations will apply to lines that are under divergent selection. Therefore, if a phenotypic difference between two selected lines (one selected for high values, the other for low) is found after some number of generations, then it may not have been caused by the selection that was imposed. Divergence caused by founder effects and genetic drift (as well as limits to selection caused by the exhaustion of additive genetic variance, e.g. Weber, 1996) can be reduced by increasing the sample size within each line, but this is often impractical with vertebrates.

Replication of lines within a given treatment (e.g. selection for large body size) avoids problems of spurious correlated responses (DeFries *et al.*, 1978; Henderson, 1989, 1997; Rose *et al.*, 1996). The limitations of selection experiments that do not include replication are quite analogous to those of two-species comparisons (Garland and Adolph, 1994).

Another advantage of replication is that it may allow one to discover 'multiple solutions' (sensu Bartholomew, 1987), or different adaptive responses. The response of a population to selection is contingent on the alleles that are segregating within it, and this has the potential to change each generation, not only because of selection but also because of random mutation and genetic drift (and, at least in the wild, immigration). Thus, the course of phenotypic adaptation may differ among lines because certain favourable alleles are unavailable to certain lines. An example of this seems to come from our lines of house mice that have been selected for high levels of wheel running (see Section 3.4 below): two of the selected lines have 'used' the mini-muscle allele, whereas two others apparently lost it at founding or by subsequent drift, and have had to 'make do' without this allele. Many other empirical examples indicate that responses to selection are often unpredictable, especially with regard to traits other than the one(s) under intentional selection (see Mayr, 1961, p. 1505). As noted by Gibbs (1999, p. 2713), 'even simple selection regimes can allow evolution to proceed in complicated ways.' It is even possible that selected lines will show novel (i.e. unknown or at least unexpected, based on studies of wild organisms) mechanisms of adaptation (Gibbs, 1999; e.g. the mini-muscles in our mice, as discussed in Section 3.4).

3. Selection for increased activity levels in house mice

Since late 1993, we have been conducting an artificial selection experiment with outbred laboratory house mice. The general goal of this research programme has been to elucidate the correlated evolution of behaviour and morpho-physiological traits. More specifically, we have sought to understand how increased daily activity levels evolve, at the levels of both motivation and ability. We chose voluntary wheel running as the target of selection, and have emphasized various morphological and physiological traits studied by exercise physiologists in our search for correlated responses. Unsurprisingly, our goals and interests have changed as the experiment has progressed, and we are currently placing substantial emphasis on understanding the neuroendocrine basis of increased wheel running as well as implications for general health and longevity.

Voluntary (or 'spontaneous') wheel running was chosen for several reasons. First, it has served as a laboratory model of general activity levels, exploratory behaviour, foraging, dispersal and motivational state, and has been used to study effects of hormones, sex, age and circadian periodicity (see review in Sherwin, 1998). Such a wealth of background information is invaluable for interpreting results of a selection experiment. Secondly, as noted by Dewsbury (1980), wheel running is more sensitive than are most other standard behavioural tests to various experimental manipulations. Thirdly, mice (and other rodents, both domestic and wild [Dewsbury, 1980] will engage in this activity at levels (many kilometres per day) that seem high enough to tax physiological capacities. Fourthly, the measurement of wheel running is easy to automate with computers. Fifth, wheel running is highly repeatable on a day-to-day basis (Swallow *et al.*, 1998a), so the phenotype can be scored rather accurately for choosing breeders. Sixth, available information in the literature before we started suggested a narrow-sense heritability of about 0.2, which is high enough to allow measurable response to selection within a reasonable number of generations. Finally, wheel running may be relevant to activity levels in nature, although this is controversial (Sherwin, 1998). In any case, as our main goal was to develop a model system in

which to study the correlated evolution of activity levels and exercise capacities, the ecological relevance of the particular activity was not of primary concern.

We have tested for correlated responses to selection in a variety of other behavioural traits (e.g. activity budgets [Koteja *et al.*, 1999b], nesting [Carter *et al.*, 2000], open-field behaviour [Bronikowski *et al.*, 2001], maternal care [Girard *et al.*, 2002]) and in various morphological and physiological traits (e.g. cost of wheel running [Koteja *et al.*, 1999a], body size [Swallow *et al.*, 1999], body composition [Swallow *et al.*, 2001], litter size [Girard *et al.*, 2002], insulin-stimulated glucose uptake of isolated skeletal muscle [Dumke *et al.*, 2001], anti-oxidant enzyme gene expression and catalytic activity [Bronikowski *et al.*, 2002; Thomson *et al.*, 2002]). Below, I will highlight a few of our results, with an emphasis on some that may be of particular interest from a biomechanical or physiological perspective.

3.1 *Experimental design and methods*

Our base population was the outbred ICR strain of mice, purchased from Harlan Sprague Dawley (Swallow *et al.*, 1998a), one of several commercial breeders who maintain this strain. The ICR strain has never been intentionally inbred, and has relatively high levels of genetic variation, comparable to those found in wild populations of house mice (Carter *et al.*, 1999).

After two generations of random mating in our laboratory (designated generations –2 and –1, and hence not appearing on the following graphs), we established eight closed lines, each consisting of 10 pairs of parents each generation. Four of these were chosen randomly to experience subsequent selection. When selection and control lines are compared by nested analysis of variance (replicate lines nested within linetype), the degrees of freedom in the denominator of the F-test are based on the number of replicates. Thus, with a total of eight lines, the linetype comparisons are based on 1 and 6 d.f. The relatively few d.f. for this comparison emphasizes the importance of maintaining replicate selected and control lines.

Each generation, all offspring from the selected lines are placed individually in cages with attached rat-sized wheels (1.12-m circumference) for 6 days. Wheel running is recorded by computer in 1-min bins. The total number of revolutions on days five and six are used as the selection criterion. Individuals are sorted within family, and the highest-running male and female are chosen from within each family to become parents of the next generation. Sib-mating is disallowed, so breeders are paired with an individual from another family within their line. In each of the four control (unselected) lines, a random sample of two males and two females is chosen from each litter and tested with wheels. Of these, one male and one female are randomly chosen as breeders, with sib-mating again disallowed. (If pedigrees are followed across multiple generations, then one can avoid inbreeding to an even greater extent, e.g. by also avoiding matings of first cousins. One can also implement systematic rotational breeding schemes to minimize inbreeding [Falconer and MacKay, 1996; Koch and Britton, 2001].)

3.2 *Direct response to wheel running*

Figure 1 shows, for both sexes, that the selected lines have increased in wheel running whereas the control lines have remained largely unchanged. Males always run less than

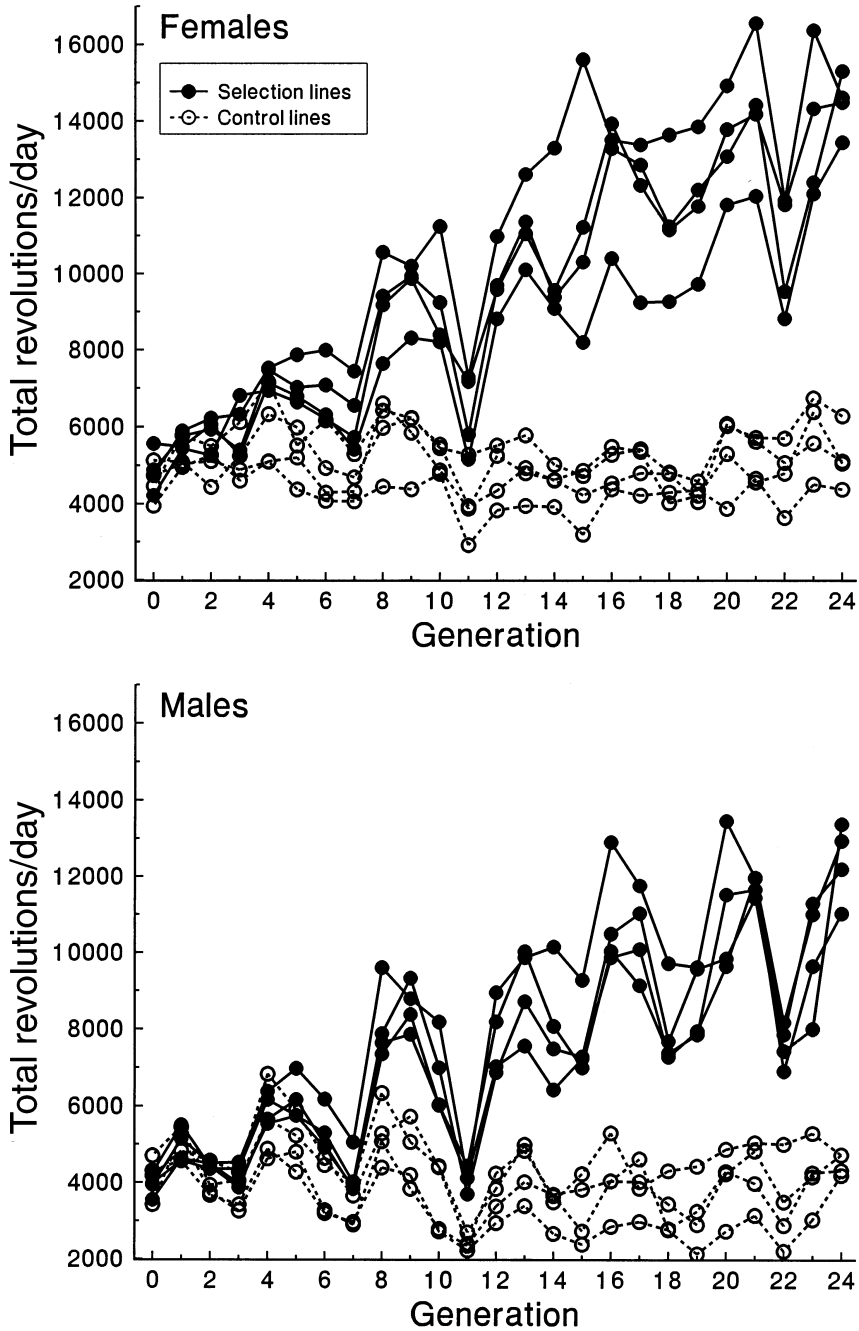

Figure 1. *Wheel running (line means) of eight lines of house mice either selected for high wheel running or bred randomly as controls (Swallow* et al., *1998a). Dips in wheel running that occur approximately every four generations (especially in males) correspond to summer generations, during which elevated humidity (and sometimes temperature) may reduce activity. Note that females always run more than males, but that the response to selection, relative to control lines, is similar in the two sexes (*Figure 2*).*

females, but the fold-difference between the average of the selected and control lines is virtually identical (*Figure 2*). Another way to look at the divergence between selected and control lines is a simple histogram that pools all of the replicates (*Figure 3*). Note that low-running individuals have been eliminated by the process of selection, and that the selected lines contain many individuals that far exceed the maximum values observed in control lines. Moreover, the difference between our selected and control lines almost spans the range of variation that has been reported among species of wild rodents (*Figure 4*).

Over the first 10 generations of the experiment (Swallow *et al.*, 1998a), the selection differential averaged 0.94 phenotypic standard deviations per generation (this is also termed the selection intensity). This value can be compared with those reported in studies of natural populations of a wide range of organisms (Endler, 1986; Kingsolver *et al.*, 2001). A value of 0.94 is approximately in the middle of the range of values reported by Endler (his Figure 7.2), but the distribution is highly right-skewed, and the vast majority of values are lower. The values reported by Kingsolver and colleagues are, on average, lower than those reported by Endler, which may be for a variety of reasons (J.G. Kingsolver, pers. comm.). In any case, it is clear that the intensity of selection imposed in our experiment has been considerably stronger than is routinely observed in natural populations.

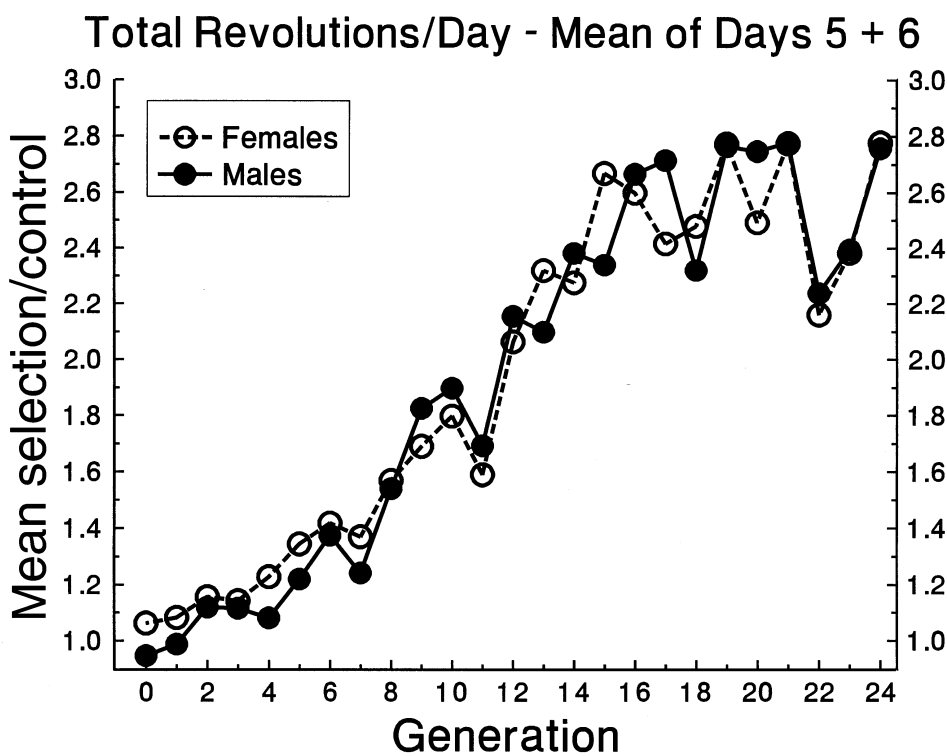

Figure 2. *Ratio of mean wheel running for the selected compared with the control lines. Note that wild female house mice from a Wisconsin population ran 68% more than female Hsd:ICR mice (days 5 and 6 of wheel exposure; data recalculated from Dohm* et al., *1994), a differential that was matched in the selection experiment by generation nine.*

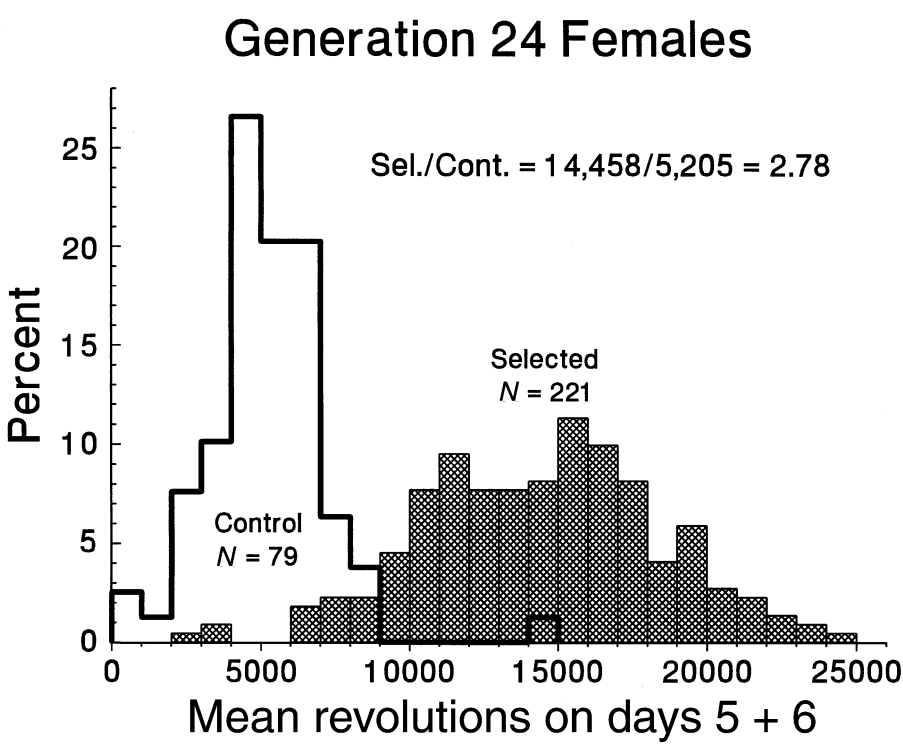

Figure 3. *Histogram for wheel running of generation 24 females, showing all selection versus all control-line individuals pooled.*

Typically higher selection intensities are, of course, just one of many ways in which selection experiments differ from nature. Other obvious differences are the smaller population sizes and lack of migration in experimental situations. This leads to the topic of selection limits or plateaus. As can be seen in *Figure 2*, the difference between our selected and control lines (about 2.7-fold) has been relatively stable since about generation 16. Such limits are routine in selection experiments, especially those that have been performed with vertebrates (Bell, 1997; DeFries *et al.*, 1978; Falconer, 1973, 1992; Falconer and Mackay, 1996; Hill and Caballero, 1992; Hill and Mackay, 1989; Lynch, 1986, 1994; Robertson, 1980; Roff, 1997).

For instance, Goodale (reported in Bell, 1997, pp. 154–156) selected for increased body mass in house mice for more than 80 generations, with the goal of producing rat-sized animals. Population size varied between about 100 and 1000 individuals. Body mass increased from approximately 25 to 43 g, for a total increase of about seven phenotypic standard deviations (initially about 2.5 g), over the first 35 generations (average selection intensity was about one). After generation 35, no further increase in body mass occurred, although wide fluctuations occurred, similar to those apparent in our experiment (*Figure 2*).

Selection that has been imposed by human beings beyond the confines of a single laboratory has sometimes produced much larger changes. For example, breeds of domestic dogs actually exceed the range of body masses exhibited by extant members of the entire family Canidae (Bell, 1997, p. 197). The three main reasons that

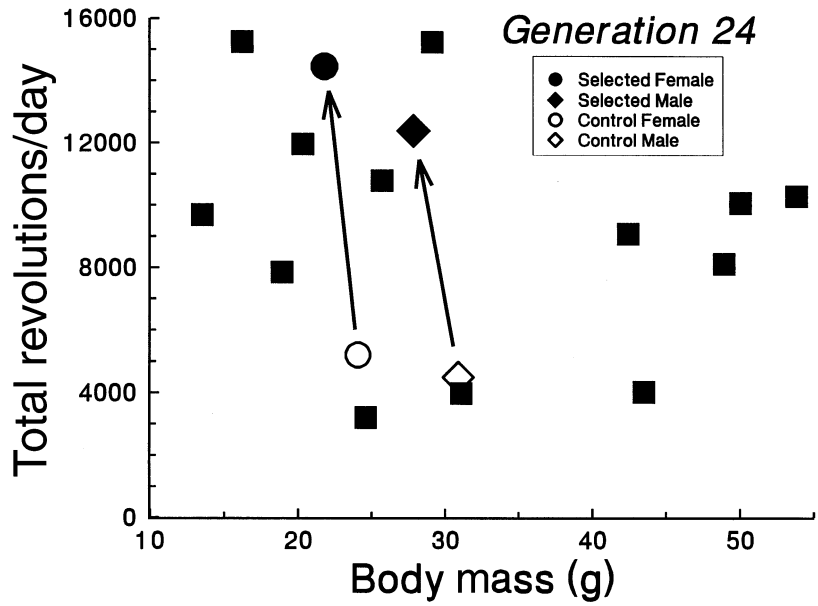

Figure 4. *Comparison of divergence in wheel running at generation 24 with that observed by Dewsbury (1980) in a comparison of 13 species of murid rodent (solid squares; only males were studied). His wheels were the same diameter as ours (1.12 m circumference), but animals were given four weeks of access rather than six days, so numbers are not directly comparable (see Swallow* et al., *1999, 2001 for examples of longer-term wheel running in our mice). In any case, divergence between our selected and unselected (control) lines almost spans the range that he observed among species.*

controlled selection experiments usually experience much narrower limits are smaller population sizes, intense selection and lack of migration, which would help to replenish genetic variance into experimental populations.

3.3 *Speed versus duration of wheel running*

An increase in total distance run per day might be accomplished by increasing the duration of running, the average speed of running, or both. In our lines of mice, the number of 1-min intervals recorded as having any revolutions has increased relatively little, so the average speed of running is the main cause of the higher total distance run (Koteja and Garland, 2001; Rhodes *et al.*, 2000; Swallow *et al.*, 1998a). This is somewhat more true for females than for males. Analysis of the single highest minute of running over a 24-h period also show an increase in the selected lines (1.9- and 1.7-fold for females and males, respectively; Koteja and Garland, 2001).

However, speeds recorded via 1-min bins may not adequately represent actual instantaneous running speeds (Eikelboom, 2001). Therefore, we performed a videotape analysis of mice running at night. After subtracting out any periods of coasting (Koteja *et al.*, 1999b) or when the mouse had exited the wheel but it was still rotating, we found that instantaneous running speeds were approximately two-fold higher in females (males have not yet been studied) from the selected lines (Girard *et al.*, 2001).

A priori, we had no reason to expect that speed would increase far more than duration of activity, but it has been gratifying from the standpoint of our interest in exercise physiology and morphology. We imagine that increasing speed of running would more quickly (across generations) tax exercise capacities than would an increase in duration of activity.

3.4 *Morphological and physiological changes*

The video-tape analyses by Girard *et al.* (2001) suggest that the apparent selection limit corresponds to a situation in which, for at least some minutes per night, mice from the selected lines are running at or near their maximal aerobic speed, which was estimated in the base population by Koteja *et al.* (1999a). Consistent with this interpretation, neither maximal oxygen consumption (VO_2max), when measured a week prior to wheel testing, nor basal metabolic rate (BMR) has responded to selection by generation 22 (unpublished results). (Maximal oxygen consumption may show differences between the selected and control lines at other ages and/or under different housing conditions [Swallow *et al.*, 1998b].) This lack of response in maximal-aerobic and resting metabolic rates is also consistent with a quantitative-genetic analysis of the base population, which suggested that additive genetic variance may not have existed for either VO_2max or BMR, although this conclusion depended on the particulars of the genetic model that was fitted to the data (Dohm *et al.*, 2001).

Various morph-physiological differences between selected and control lines exist, some of which may represent genetic adaptations for sustained exercise (Garland *et al.*, 2000). For example, mice from selected lines have more symmetrical hindlimb bone lengths (T. Garland and P.A. Freeman, unpublished results) and higher insulin-stimulated glucose uptake in some hindlimb muscles (Dumke *et al.*, 2001). Mice from the selected lines are smaller in body mass (Swallow *et al.*, 1999) and have less body fat than controls, at least under some conditions (Dumke *et al.*, 2001; Swallow *et al.*, 2001). When housed with wheels that are either free to rotate or locked for eight weeks, suborganismal training responses (e.g. increases in citrate synthase activity of hindlimb muscle, haematocrit) are often greater in selected-line animals (which constitutes a genotype-by-environment interaction), presumably because they run more (Houle-Leroy *et al.*, 2000, unpublished data). We are now studying motivation (McAleer *et al.*, 2000), and pharmacological experiments suggest altered dopaminergic function in the brains of selected-line mice (Rhodes *et al.*, 2001).

The free superoxide and hydroxyl radicals produced during aerobic metabolism react to create various toxic reactive oxygen metabolites, which can damage cell components. Moreover, generation of free radicals is elevated during strenuous exercise. Thus, we hypothesized that anti-oxidant enzymes might have increased in our selected lines, as a protective measure. Instead, we found that mice from the selected lines (especially females) exhibit reduced activity of superoxide dismutase-2 (Sod-2), a free-radical scavenger (anti-oxidant enzyme), in the liver (Thomson *et al.*, 2002). Such a difference could have negative consequences for lifespan (i.e. cause a trade-off between early-age locomotor activity and lifespan), a hypothesis that we are currently testing (Bronikowski *et al.*, 2002).

The four replicate selected lines show statistically significant differences in a number of traits. Of particular interest from a biomechanical perspective, two of the

four selected lines now contain a high frequency (approximately 50%) of individuals with small muscles (Garland *et al.*, 2000, 2002), in which the triceps surae exhibits an almost 50% reduction in mass (*Figure 5)*, along with an approximate doubling of mass-specific oxidative capacity (Houle-Leroy *et al.*, unpublished). Comparisons of offspring with their parents suggest that this phenotype is inherited as a single autosomal recessive (Garland *et al*., 2002). The phenotype has only been recorded in two of the selected lines and (rarely) in one of the control lines, and population-genetic model fitting provides evidence that the allele must have been under positive selection in the two selected lines. (Presumably, the other selected lines lost the allele, which was rare in the base population, by chance either at founding or thereafter by genetic drift.) Our working hypothesis is that these 'mighty mini-muscles' are adaptive for sustained, relatively high-speed running, perhaps because of shorter diffusion distances. In collaboration with Helga Guderley and Philippe Houle-Leroy, we are now testing this possibility. (Interestingly, recent work in evolutionary biology suggests that such genes of major effect may be more important for adaptation in nature than has been emphasized by neo-Darwinian thinking [Orr and Coyne, 1992; Smith and Girman, 2000].)

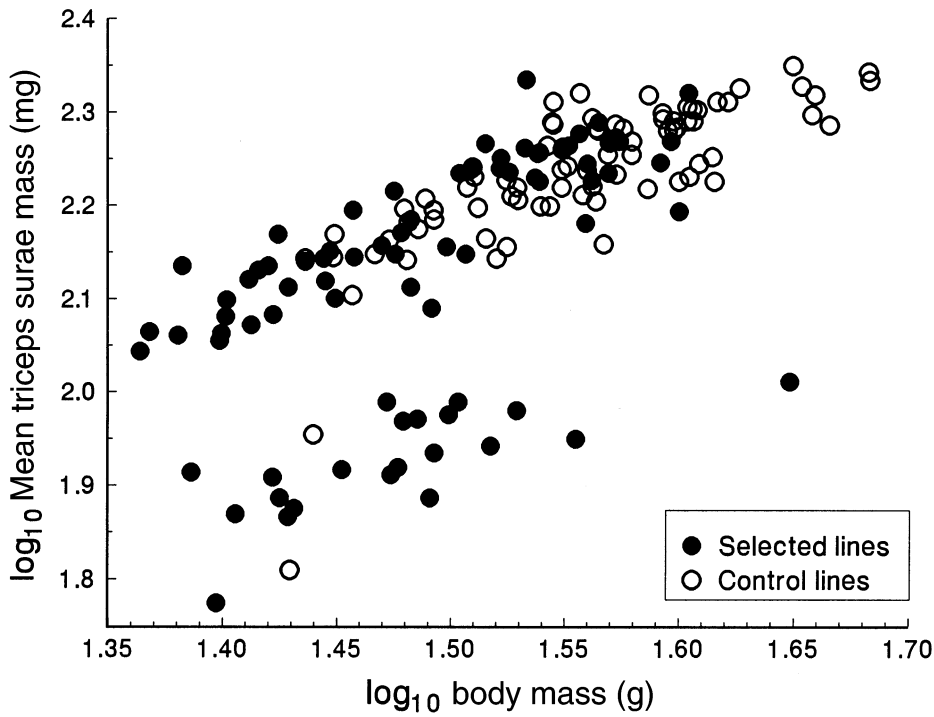

Figure 5. *'Mini-muscle' phenotype (see Section 3.4) at generation 22 of the selection experiment for increased voluntary wheel-running behaviour in house mice. Mass of the triceps surae is reduced by approximately 50% in some individuals, most of which occur in two of the selected lines. Note also that mice from selected lines tend to be smaller in body mass (Swallow* et al., *1999). Sample size is ≤20 mice in each of eight lines.*

3.5 *The decision not to select for reduced wheel running*

Although bidirectional selection may have intuitive appeal and also offers the advantage of yielding rather large differences (as compared with what one gets by selecting in only one direction and then comparing with unselected control lines), it has at least two potential problems. First, selection for high versus low values may affect what are actually different traits. For example, selection for high locomotor performance in some sort of forced test (e.g. on a racetrack or a motorized treadmill) might bring about changes in limb lengths or proportions, muscle masses, or muscle fibre-type composition, that is, traits that directly affect performance ability. Selection for low performance might affect the same subordinate traits, but in the opposite direction. Alternatively, selection for low performance might cause changes in other traits, ones not directly related to performance ability *per se*. Thus, selection for low performance might just produce animals that are pain tolerant, for example, resistant to prodding or shocking when tested on a treadmill. Pain-tolerant animals might be of interest from some perspectives, but comparison of them with high-selected lines whose increased performance was based on changes in muscle biology would involve apples and oranges, and this complication might not become apparent to the investigator until substantial mechanistic studies had been completed. In our own experiment, we were concerned that one-way wheel running might decrease owing to an increase in neophobia (fear of new objects), such that mice simply would not enter the wheels.

A second possible problem with selecting for reduced organismal performance is that it may occur by increases in the frequency of alleles that make mice generally unhealthy. Therefore, we decided not to implement selection for low wheel running, which allowed us to increase the number of high-selected and control lines.

4. A strategy for integrating selection experiments into a research programme

As noted in the Introduction, many approaches can and should be used in evolutionary biomechanics. In this section, I outline one strategy for incorporating selection experiments into a research programme that also involves the more traditional approach of comparing species. At present, I know of no cases that have actually done all of the proposed steps.

4.1 *Generate adaptive or mechanistic hypotheses by comparing species in a phylogenetic context*

As an example of a mechanistic hypothesis, a comparison of species of rodents might suggest that leg length is positively related to maximal sprint-running ability. Such a comparison has not yet been made, but rodent species do vary widely in maximal sprint speed (Djawdan and Garland, 1988), and Garland and Janis (1993) found that sprint speed and leg length were positively correlated with (relative) leg length across 49 species of Carnivora and ungulates. Alternative hypotheses, which are not mutually exclusive, would include that speed should correlate positively with (1) metatarsal/femur ratio (review in Garland and Janis, 1993), (2) the mass (or cross-sectional) area of muscles involved in limb propulsion, and (3) the percentage of fast-twitch muscle fibres in important hindlimb muscles.

Even if none of these three traits were found to correlate significantly with speed among species of rodents, as functional biologists we would presume that one or more morphological or physiological determinants of sprinting ability must exist. Thus, it would still be of interest to conduct a selection experiment, as described below. However, such an experiment would be more of a 'fishing expedition' than if one or more putative mechanisms had been identified *a priori* in a comparative study. A similar point has been made with respect to interspecific comparative studies, even when analysed in a phylogenetic context. Given that one does identify statistical associations between a performance trait and one or more lower-level traits that may be causally related to the higher-level trait, an external model or criterion (a 'covering law'), based on detailed biomechanical, physiological or biochemical understanding, is essential for teasing apart which associations are truly causal versus only correlational (Garland and Adolph, 1994, p. 823; Lauder, 1990, 1991b). Hence, biomechanics and physiology have much to offer evolutionary analyses (Feder *et al.*, 2000; Garland and Carter, 1994).

Aside from comparative studies, hypotheses about mechanisms can come from detailed biomechanical studies of single species or from first-principles models. These, too, would suggest that, all else being equal, speed should be positively affected by limb length, muscle mass and the percentage (or amount) of fast-twitch muscle fibres (the probable effect of limb proportions is perhaps a bit less clear).

4.2 *Select on an organismal phenotype (behaviour or performance)*

Continuing with the hypothetical speed example, we might develop an experiment to select for high sprint-running speed in a convenient rodent. Such an experiment has yet to be conducted, but we have selected for high voluntary wheel running in laboratory house mice (as discussed above), and laboratory rats have been bidirectionally selected for treadmill running performance (Koch and Britton, 2001; Koch *et al.*, 1998).

4.3 *Test for correlated responses*

Once the organismal trait under selection (e.g. sprint speed) has shown divergence between the selected and control lines (we will assume that each treatment is replicated), or between lines selected for high versus low performance, then they could be compared with respect to the traits hypothesized to affect performance. Such a comparison will be a type of nested ANOVA (or perhaps a more sophisticated analysis in which pedigree information within lines is incorporated), in which replicate lines are nested within linetype (e.g. selected vs. control). In such an analysis, the degrees of freedom associated with the F statistic for testing for a linetype effect are the number of treatments minus one (numerator) and the total number of lines minus the number of treatments (denominator). For example, in an experiment with four high-selected lines and four control lines (as in our mouse experiment described above), the d.f. for testing a linetype effect are 1 and 6. Hence, it pays to have as many replicates as possible (Rose *et al.*, 1996). In such a nested ANOVA, one can also test for differences among lines within linetypes, and post hoc comparisons can be made among lines.

Thus, in a nested ANOVA, one may find consistent differences between the selected and control lines, as well as differences among lines within the selected or control treatments. The former can be termed correlated responses to selection, and

reflect (usually) the existence of pleiotropic gene action (one gene affects more than one trait, i.e. the trait under selection and the one[s] that show consistent differences between the set of selected and control lines). The latter reflect chance events, including founder effects, random genetic drift, mutations unique to particular lines, and interactions between these factors and selection. If all selected lines show a difference from all of the control lines, but the selected lines also differ among themselves, then the differences among selection lines may be viewed, cautiously, as 'multiple solutions', although that phrase may imply more effects of selection *per se* (adaptation) than are actually warranted.

4.4 *Select on the putative mechanism(s) of adaptation*

Once correlated responses (consistent differences between selected and control lines) have been identified from within the suite of subordinate traits (e.g. limb-bone lengths or proportions) that can reasonably be expected to affect the organismal trait, then it may be possible to initiate a new selection experiment in which those traits are themselves directly selected. For example, one could select directly on limb-bone proportions by X-raying individual mice prior to choosing breeders. If this trait evolves in response to selection, then we would expect the organismal trait to change as well (and in a particular direction) if the subordinate really does exert a causal influence on the organismal trait. Thus, this approach allows one to test experimentally hypotheses about the phenotypic mechanism underlying the original response to selection.

4.5 *Caveats and alternatives*

An ideal experimental outcome, as described in Section 4.4, might seem to offer very solid evidence about mechanism, but we must be cautious (Zera and Harshman, 2001). For instance, it would still be possible that both the organismal trait and the putative mechanism might change because they both depended on some third, unmeasured trait. With respect to speed and leg length, we could imagine that testosterone might affect bone growth (and hence leg length) as well as muscle mass or fibre-type composition, and that the latter might be the actual traits that affected sprint speed (leg length would not necessarily have an effect on speed if animals with different leg length altered their running styles in such a way as to 'compensate' for variation in leg length, thus allowing stride length to be invariant).

Alternatively, some of the loci that affect both leg length and speed might exist in close linkage on particular chromosomes, such that changes in the allele frequencies at one locus, which affected leg length, would occur in concert with changes in the frequency of alleles at another, which affected speed (e.g. via effects on muscle mass or fibre-type composition). These sorts of potential genetic complications could, in principle, be dissected via various genetic engineering approaches (see Feder *et al.*, 2000, and references therein), which would be feasible with such vertebrates as house mice or zebra fish.

The overall strategy outlined above begins with selection at the whole-organism level, such as performance or behaviour. This is because selection in nature is thought to act most directly at these higher levels of biological organization, rather than directly on such subordinate traits as leg length (see Section 2.5). From a

biomechanical perspective, however, it might be of more interest to alter the sequence such that one first selected on the lower-level trait and then later selected on the organismal trait.

5. Why have selection experiments not been applied in evolutionary biomechanics?

I believe that selection experiments have much to offer in evolutionary biomechanics. Indeed, I am surprised that they have not been used in this field. In thinking about why that may be, I have come up with several possibilities.

First, many morphologists may not believe that the traits they study show sufficient individual variability or narrow-sense heritability to make a selection experiment feasible. This belief is generally unfounded, as virtually all morphological and behavioural traits that have ever been studied show significant narrow-sense heritability (Mousseau *et al.*, 2000; Roff, 1997; Stirling *et al.*, 2002), and those that have been subject to selection have indeed responded (Bell, 1997). Nevertheless, it may be prudent to conduct a preliminary study, either by estimating heritability from offspring-on-parent regression, or by actually conducting a small-scale selection experiment for a few generations (Koch *et al.*, 1998), before undertaking a large-scale selection experiment. Preliminary experiments can sometimes reveal problems with trait measurement, such as low reproducibility (Dohm, 2002) or low heritability (see Dohm *et al.* [1996] on sprint speeds of laboratory house mice), which could limit the effectiveness of artificial selection.

Secondly, it may be believed that even if selection is successful it will not produce a very large difference between selected and control lines (or between high- and low-selected lines). That is, the resulting 'signal' will not be sufficient to study at least some phenomena that have inherently high 'noise'. This concern often can be rejected, as in our selected lines of mice, which almost span the range of variation that has been reported among species of wild rodents (*Figure 4*).

Thirdly, for many workers, evolutionary biology is largely viewed as an historical science, whose main (or only) goal is to explain past events. Indeed, the earlier volume entitled *Biomechanics in Evolution* (Rayner and Wooton, 1991) strongly emphasized fossils. But modern evolutionary biology is much more diverse in its temporal perspective, and desires to become a predictive science as well (Dudley, 1991; Freeman and Herron, 2001; Futuyma, 1998; Huey and Kingsolver, 1993). Selection experiments are a key component of this more expansive evolutionary biology, which views evolution as a process that can often be studied in real time.

Fourthly, selection experiments may be viewed as unnatural and hence outside of the domain of 'proper' evolutionary biology. Granted, selection experiments can never be a perfect model of the natural world, but the same is true of any model of any complex phenomenon. The important criterion is whether a given model captures enough key elements of the real phenomenon to allow insight. Selection experiments have surely done this (Bell, 1997; Roff, 1997; Travisano and Rainey, 2000). Granted, too, that selection experiments do not necessarily yield outcomes that are the same as has occurred in nature (see Gibbs [1999] for examples with *Drosophila*). Rather, '... 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' (Gibbs, 1999, p. 2714).

Fifth, the costs of selection experiments, in terms of money, personnel and time, may be perceived as too high. This excessively high cost might be in either absolute or relative terms. Admittedly, the absolute amount of time that a selection experiment requires is often rather long. For vertebrates, it would be measured in years, which is often too long to allow fruition within the length of a typical graduate career, grant period, or amount of time allotted before tenure decisions are made. On a relative scale, however, selection experiments involve relatively long periods of time during which little work is involved, for example, after pairs of mice have been formed and one is waiting for births to occur. Hence, they can sometimes be conducted simultaneously with other research projects. The monetary cost of selection experiments can be high, but it is not clear that this cost is higher than, say, a multi-species comparative study that requires collecting trips to exotic locations and probably several field seasons to obtain all of the target species (let alone possible problems in obtaining permits or in getting animals back to the laboratory in good health). And, if one adds in the need to raise each species under common conditions prior to measurement (Garland and Adolph, 1994), the effort involved becomes even greater. Thus, one should not assume that a selection experiment will be more 'expensive' than some of the more traditional approaches in evolutionary biomechanics.

A logical way to reduce the cost of a selection experiment is to avoid doing one by instead using selected lines that already exist (Jackson and Diamond, 1996; Konarzewski *et al.*, 1997, 2000; Lilja *et al.*, 2001; Lynch and Roberts, 1984; van der Ziel and Visser, 2001). This was the approach taken by Marden *et al.* (1997), who studied pre-existing lines of *Drosophila* that had been selected for voluntary (motivated by positive phototaxis) wind-tunnel flight (Weber, 1996). Although the available lines may not have been selected for the exact trait that a biomechanicist would have preferred, they may nonetheless be of interest for study. Indeed, Weber (1996) notes that 'The choice of this trait was dictated by the desire for a system that could score massive numbers' rather than by any particular interest in the functional significance or possible fitness consequences of wind-tunnel flight. Another possibility is to study multiple breeds of a domestic species (e.g. dog, horse, chicken), some of which have been bred for particular characteristics and hence may show 'extreme' morphological, physiological or behavioural characteristics, as is the case for greyhounds and thoroughbred horses (Fuller, 1951; Snow and Harris, 1985; Taylor, 1988).

As an example of existing lines that have yet to be studied from a biomechanical perspective, Wilkinson (1993) produced lines of stalk-eyed flies with altered eye-stalk size and shape. It would be of considerable interest to test the flight behaviour and performance of these animals, given that Swallow *et al.* (2000) have already shown differences in aerial performance among species of stalk-eyed flies. In the selected lines, differences in flight performance might be expected for several reasons, two of the simplest being (1) the altered centre of mass and (presumably) aerodynamics of flies with altered body proportions and (2) possible compensatory changes in the sizes of other organs that might affect flight ability (e.g. wing size or mass of flight muscles [Emlen, 1996; Nijhout and Emlen, 1998]). With respect to vertebrates, many experiments have selected for body size in both mice and rats, and these could provide nice opportunities to study the effects of variation in body size on locomotor or feeding performance (or within-species allometry in general). Most recently, Koch and Britton (2001) have selected directly on treadmill running performance in rats.

A sixth reason that selection experiments do not occur in evolutionary biomechanics is simply because the field has no tradition of using them. Scientific disciplines sometimes develop in ways that are rather non-scientific. That is, rather than adopting approaches and techniques that are the most suitable for the questions at hand, they may be constrained by sociological factors, including strong investigative traditions and dominant personalities. During their graduate and postdoctoral training, most scientists tend to adopt approaches and techniques that their mentors use. At the same time, some disciplines benefit by serendipitous interactions with scientists from other disciplines, thus allowing the incorporation of 'novel' perspectives and techniques. Thus, once a few selection experiments enter the realm of a particular field, it may be that they will experience increasingly wider use. I hope that occurs within evolutionary biomechanics.

Finally, selection experiments may be viewed as 'old fashioned' in comparison with some of the more 'modern' approaches of genetic engineering. True as this may be, this view ignores the many compensating advantages of selection experiments, especially with respect to polygenic traits (see section 2 above; Rhodes *et al.*, 2001; Rose, 1991). Although funding agencies may be more favourably disposed to experiments that use the latest technology, they can often be persuaded that selection experiments are an important approach.

6. Conclusions and prospects for the future

Replicated selection experiments should be implemented in functional morphology and evolutionary biomechanics. They are an effective way to test many simple but profound hypotheses, such as whether a trait shows potential for response to natural (or sexual) selection (and in so doing to estimate narrow-sense heritability), or whether evolutionary changes in one trait necessarily cause changes in another. Beyond this, they can be used to address many classic topics in evolutionary biomechanics, including the magnitude and nature of constraints and trade-offs (Zera and Harshman, 2001). For example, selection on the size of horns in beetles (Emlen, 1996) has been used to elucidate the dynamics of developmental processes that cause correlations among body parts (Nijhout and Emlen, 1998), a topic that has been of interest at least since discussed by Charles Darwin (1859): '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'. With respect to possible correlated responses, it should be recognized that they may occur in other organs (Nijhout and Emlen, 1998), and even at other levels of biological organization, that would not have been easy to predict. For example, it is possible that selection on, say, eye-stalk length of *Drosophila* (Wilkinson, 1993) or on horn length of beetles (Emlen, 1996) would result in changes in courtship and fighting behaviour, respectively, or perhaps in anti-predator behaviour.

Ideally, selection experiments can be integrated into an overall research programme that employs other approaches, such as comparisons of multiple species, detailed studies of single species, and mathematical or physical modelling. As in other areas of science, the greatest insights into topics in evolutionary biomechanics are likely to come from intersections of approaches.

One interesting avenue for exploration will be comparative selection experiments. That is, if different species are subjected to similar selection protocols, do they

respond in similar ways? For example, one might select zebra fish, guppies and goldfish for swimming performance then determine whether they 'use' the same mechanisms (e.g. alterations in fin size and shape, changes in muscle mass or composition) to achieve higher level of performance. This type of experiment would allow unique insights into the phenomenon of parallel evolution.

A subsidiary on this theme would be to select on performance in species that differed greatly in performance before selection. For example, one might predict that selection for increased endurance in a species with relatively low endurance would be successful, whereas selection in a species that already had very high endurance (as identified by an initial interspecific comparative study) would not be successful because that species was already at the physiological or biomechanical limits that past natural (or sexual) selection could have produced. In this case, the 'failed' selection experiment would actually yield much useful information.

A final reason for doing selection experiments is that they can also yield 'products' that are useful for biomedical research. For example, our 'hyperactive' mice may prove to be an important model for human attention deficit hyperactivity disorder (ADHD; Rhodes *et al.*, 2001). In addition (Girard and Garland, 2002; Houle-Leroy *et al.*, 2000), they provide a model in which the effects of chronic exercise (at high levels) can be studied (Eikelboom, 1999) without the need to use forced-exercise protocols, which have the disadvantage of generally causing psychological stress, which may confound effects of physiological (exercise-induced) stress. As the selected lines differ in body mass, body composition and mass-corrected food consumption (Koteja *et al.*, 1999a; Swallow *et al.*, 1999, 2001), they may prove useful for studying the regulation of energy balance. Finally, they may prove useful for studying the effects of high voluntary activity on general health and aging (Thomson *et al.*, 2002; Bronikowski *et al.*, 2002).

Although selection experiments are a non-trivial undertaking, and hence will not be feasible for all potentially interesting questions in evolutionary biomechanics, in many cases it may be possible to use pre-existing selected lines to get at interesting topics. For example, a large number of selection experiments have altered growth rate, body size or body composition (including muscle characteristics) of rodents and poultry (Falconer, 1973; Holder *et al.*, 1999; Lilja *et al.*, 2001; Moura *et al.*, 1997; Notter *et al.*, 1976). Many of these would be interesting to examine for allometric effects on various biomechanical traits, and could also be used to test for consistency of correlated responses among species.

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