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SELECTION EXPERIMENTS AND EXPERIMENTAL EVOLUTION OF PERFORMANCE AND PHYSIOLOGY

John G. Swallow, Jack P. Hayes, Pawel Koteja, and Theodore Garland, Jr.

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Since a seminal paper by Arnold (1983), direct measurement of whole-organism performance has become central to functional evolutionary biology (e.g., Arnold 2003; Ghalambor et al. 2003; Kingsolver and Huey 2003). In this context, "performance" can be most easily defined by example. Assuming that individuals can be fully motivated (e.g., see Swallow et al. 1998a; Harris and Steudel 2002; Losos et al. 2002; Tobalske et al. 2004), it is relatively easy to measure maximal sprint running speed of small mammals and lizards on photocell-timed racetracks or high-speed treadmills (e.g., Calsbeek and Irschick 2007; Chappell et al. 2007). Moving down one level of biological organization to give another example, sprint speed is the product of stride length and stride frequency. In turn, stride length is affected by both limb length and gait, including possible pelvic rotation (e.g., in bipedal lizards). Limb length is the summation of the lengths of individual limb segments and is affected by how fully the limb is extended. Some gaits involve phases in which all limbs leave the ground while the animal continues to move forward, thus further lengthening the stride. Stride frequency results from neural transmission and muscular contractions that cause bones to move as force is transmitted through tendons and ligaments. And, of course, muscular contractions involve numerous structural, ultrastructural, physiological, and biochemical traits.

The example of maximal sprint speed should make it clear that measures of organismal performance constitute "complex traits" (Bennett 1989; Ghalambor et al. 2003; Swallow and Garland 2005; Miles et al. 2007; Oufiero and Garland 2007). As such, their evolution must entail changes in numerous subordinate traits (figure 12.1). If we define *behavior* as anything an animal does (or, in some cases, fails to do) and *performance* as the ability of an animal to do something when maximally motivated (see also Garland 1994), then the evolution of performance ability may have immediate impacts on behavioral repertoires, frequencies, and/or intensities in situations where the animal is maximally motivated. For example, when chased by a predator, an animal cannot choose to sprint faster than its body will allow. Indeed, several workers have emphasized that natural and sexual selection are likely to act on combinations of behavioral and performance traits via "correlational selection" (e.g., Brodie 1992; Garland 1994; Sinervo and Clobert 2003; Ohno and Miyatake 2007). Returning to the example of sprint speed, selection would seem likely to favor individuals that have *both* a behavioral propensity to sprint after prey or away from a predator *and* high physical abilities for sprinting, because such individuals would fare better than those that had either the propensity or the ability, but not both. As discussed in this chapter, depending on the genetic correlation between traits under correlational selection, their joint evolution may be either "constrained" or "facilitated."

Although organismal performance abilities clearly set boundaries within which normal behavior must occur (Bennett 1989; Sears et al. 2006), animals do not necessarily behave in ways that tax their maximal abilities (Hayes 1989a, 1989b; Hayes and O'Connor 1999; Irschick and Garland 2001; Husak 2006). Accordingly, Arnold's (1983) original morphology → performance → fitness paradigm has been extended to include the point that behavior often acts as a "filter" between performance and Darwinian fitness

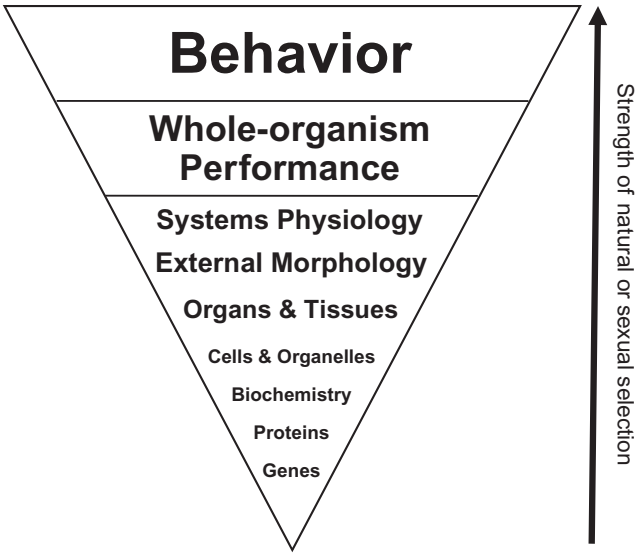

FIGURE 12.1

Complex traits, such as behavior and performance, comprise hierarchical suites of interacting subordinate traits at lower levels of biological organization. In general, selection is thought to act most strongly on phenotypic variation in traits at higher levels of biological organization, such as behavior (see also Rhodes and Kawecki this volume) and performance, as indicated by the width of the inverted triangle (and the direction of the arrow). In other words, behavior and performance have strong effects on major components of Darwinian fitness, such as survivorship and fecundity. Lower-level traits include a wide range of suborganismal morphological, physiological, and biochemical phenotypes. These lower-level traits, directly and indirectly, influence aspects of whole-organism performance ability that are crucial for survival and reproduction.

(Garland et al. 1990; Garland 1994; Garland and Carter 1994; Garland and Losos 1994; Husak 2006).

For example, when an animal detects a predator, it may or may not "decide" to flee at its maximal sprint speed. If it never runs away at top speed, then its maximal sprinting abilities become moot. Alternatively, animals with low performance abilities—either innately or because of short-term conditions, such as injury, a full stomach, or pregnancy—might modify their behavior in ways that limit their exposure to life-or-death situations where performance is crucial (e.g., Bauwens and Thoen 1981; Clobert et al. 2000). Although many workers have argued that "both natural and sexual selection are expected to operate on performance first, and secondarily on other aspects (e.g., morphology)" (Lailvaux and Irschick 2006, 264), a recent review of the limited available field data does not provide evidence supporting this proposition (Irschick et al. 2008), perhaps because behavior often intervenes between selection and performance and can often compensate

for changes in environmental conditions (Huey et al. 2003; Huey and Rosenzweig this volume). Behavior, of course, is highly plastic, but plasticity can be an important component of the response to selection at any level of biological organization (Garland and Kelly 2006). (For an interesting discussion of how performance abilities may constrain the evolution of bird song, see Podos et al. 2004.)

It is easy to conceive—and often to implement (but see Losos et al. 2002)—measures of maximal performance in the context of locomotion; indeed, most studies of performance in the sense used here do involve locomotion of some type. However, many other measures of performance, such as growth rate, thermal tolerance (Futuyma and Bennett this volume; Huey and Rosenzweig this volume), osmotic regulation, desiccation resistance (Gibbs and Gefen this volume), and rate of heat production in response to cold (Hayes and O'Connor 1999; see later sections), are ecologically and evolutionarily important. Nevertheless, to keep this contribution to a manageable size, we focus primarily on locomotor performance. This "dynamical, whole-organism" definition of performance, as recently articulated by Lailvaux and Irschick (2006), is consistent with the majority of performance measures in the literature.

The purpose of this chapter is to review selection experiments that have targeted organismal performance traits, with an emphasis on those involving locomotion or metabolism. Selection experiments are a powerful tool for investigating the linkages between biochemical, morphological, and physiological traits that influence physiological performance, as well as investigating how performance capacities may constrain or facilitate behavioral evolution (Garland and Carter 1994; Garland 1994; Lynch 1994; Rose et al. 1996; Bradley and Zamer 1999; Gibbs 1999; Feder et al. 2000; Bennett 2003; Garland 2003; Bradley and Folk 2004; Swallow and Garland 2005; Fuller et al. 2005; Gibbs and Gefen this volume; Zera and Harshman this volume). Rhodes and Kawecki (this volume) discuss selection experiments that have targeted behavior per se, several of these experiments having led to insights concerning correlated evolution of performance capacities. Both the Rhodes and Kawecki chapter and the present one provide some discussion of a selection experiment that targeted high voluntary wheel running of mice, because increases in this behavior have turned out to involve substantial components of physiology and performance (Swallow et al. 1998a; Garland 2003; Middleton et al. 2008).

Numerous selection experiments have targeted growth rates or body size, as well as such life-history traits as litter size, all of which have major "physiological" components, but these are not reviewed in the present chapter (see references in Falconer 1992; Falconer and Mackay 1996; Bunger et al. 2001; Garland 2003; Renne et al. 2003; Eisen 2005; Bell 2008). Zera and Harshman (this volume) consider the physiological underpinnings of life-history traits in insects from the perspective of selection experiments (see also Zera et al. 2007, which includes vertebrate examples). Other selection experiments have targeted physiological traits below the level of the whole organism, such as circulating corticosterone levels (Roberts et al. 2007), serum cholesterol (Dunnington et al. 1981a, 1981b), sensitivity to acute ethanol administration (Draski et al. 1992), core

body temperature (Gordon and Rezvani 2001), and hypothermic responses to specific pharmacological agents (Overstreet 2002). Again, those studies are not reviewed here.

Selection experiments have a long history and come in many types (Falconer and Mackay 1996; Garland 2003; Bell 2008; Futuyma and Bennett this volume; Irschick and Reznick this volume; Rhodes and Kawecki this volume; Rose and Garland this volume). Most of the experiments that we review have used artificial selection rather than laboratory natural selection and have involved rodents. This is for two main reasons. First, measures of whole-organism performance are physically easier with rodents than with insects, let alone microorganisms. Second, laboratory natural selection, in which an entire population must be subjected to altered environmental or husbandry conditions, is impractical or unethical with rodents. Although laboratory mice have been used most commonly, which is not surprising given the long history of genetic and breeding research (Silver 1995; Falconer and Mackay 1996; Eisen 2005), laboratory rats as well as wild rodents have also been employed. So far as we are aware, only three vertebrate studies, two selecting on body size in mice (Bunger et al. 2001; Renne et al. 2003; and Wirth-Dzięciolowska et al. 2000, 2005; see also Rosochacki et al. 2005) and the other selecting for litter size in mice (Holt et al. 2004, 2005), have gone as many as one hundred generations, hence crossing into the category of "long-term" selection experiments (Laurie et al. 2004; Travisano this volume). Surprisingly, with one short-term exception (Shikano et al. 1998), small fishes (e.g., guppies, zebra fish, mosquito fish) have yet to be the subject of a selection experiment designed to alter whole-animal performance (see also Garland 2003).

THE IMPORTANCE OF REPLICATION

Properly designing and analyzing a selection experiment is nontrivial (e.g., see Robertson 1980; Falconer and Mackay 1996; Rose et al. 1996; Roff 1997; Fuller et al. 2005; Bell 2008; Roff and Fairbairn this volume). In addition to population size, the exact details of how a phenotype will be scored, or how the environment will be altered in an attempt to induce selection, careful attention needs to be paid to the choice of base population and also to the degree of replication (Fry this volume; Huey and Rosenzweig this volume; Rauser et al. this volume; Rhodes and Kawecki this volume; Simões et al. this volume). Unfortunately, a number of interesting experiments that targeted performance or physiological traits are difficult to interpret because of the base population used, lack of replication, and/or lack of a control line (discussed later). With respect to replication, the basic problem is that any finite population will undergo genetic changes due to the stochastic processes of mutation and random genetic drift (founder effects may also be important in selection experiments if a line is begun with very few individuals; e.g., see example calculations in Garland et al., 2002; see also Fry this volume on speciation experiments). Genetic changes caused by random mutation and drift will, in turn, lead to phenotypic changes. These changes may occur for any trait, including one that is directly targeted by artificial selection. Hence, without replication of the selected lines

(and maintenance of nonselected control or oppositely selected lines), it is difficult to know if any observed change in the mean phenotype of a single selected line is really because of the selection that was intentionally imposed (for a similar discussion, but of "unreplicated" comparative studies, see Garland and Adolph 1994). The same would be true, but even more so, for any other traits that change across generations in a selected line (i.e., traits other than the one intentionally targeted for selection). So-called correlated responses to selection are notoriously more variable than the direct response to selection (Hill 1978; Robertson 1980; Falconer and Mackay 1996; Roff 1997; Bell 2008).

The accepted way to study both direct and correlated responses to selection is by comparing all of the replicate selected lines with all of the replicate control (and/or oppositely selected) lines by a "mixed-model" nested analysis of variance (ANOVA). The effect of selection is considered a "fixed effect" and is tested relative to the variation among the replicate lines, which is viewed as a "random effect" in statistical terms. Thus, if we had four replicate lines that had been selected for, say, high wheel running (discussed later) and four nonselected lines, then the degrees of freedom for testing the effect of selection would be one and six, regardless of how many individual mice might be measured for a particular trait. Other approaches are possible for analyzing selection experiments that lack replication (Henderson 1989, 1997; Konarzewski et al. 2005), but they are no substitute for proper replication (see also Eisen and Pomp 1990; Rhodes and Kawecki this volume).

Replication also allows tests for "multiple solutions" in response to a particular selective regime (Garland 2003). Selection that acts at a relatively high level of biological organization (figure 12.1)—such as voluntary behavior, whole-organism performance, or major components of life history (e.g., age at first reproduction)—is particularly likely to result in different adaptive responses both at the phenotypic level of subordinate traits and at the genetic level. At the phenotypic level, detailed studies of physiology, morphology, and biochemistry can elucidate whether a higher-level trait has evolved via changes in different subordinate traits. At the genetic level, a first-pass "black box" approach to determine whether different genes underlie the response to selection in replicate lines is to cross those lines and examine the traits of interest in the F1, F2, and/or backcross populations (see also Rhodes and Kawecki this volume). For example, if two replicate lines selected in the same direction for a given trait have different alleles at some of the loci influencing that trait, then a cross should produce an F1 whose mean trait value exceeds that of the two lines (e.g., Eisen and Pomp 1990; Bult and Lynch 1996). Moreover, crosses of replicate selected lines can be useful for renewed selection to break through selection limits (Bult and Lynch 2000).

EXPERIMENTAL EVOLUTION OF MICE IN DIFFERENT THERMAL ENVIRONMENTS

Although temperature is often the environmental factor of choice in laboratory natural selection studies with *Drosophila* and microbes (Gibbs and Gefen this volume; Huey and Rosenzweig this volume), we are aware of only one such study with rodents (see also

Garland 2003). Barnett and Dickson (1984a, 1984b, 1989, and references therein) allowed wild-captured house mice to breed for many generations in either cold (~3°C) or warm (~23°C) environments. They chronicled the changes in size, anatomy, life history, and physiology that resulted, and they performed several common-environment experiments to distinguish genetic changes that resulted from the multigenerational exposure to cold and warm environments from the short-term acclimation effects of temperature. That is, they transplanted mice from cold environments to warm ones and vice versa, so that four groups of mice could be compared. Those groups were (1) evolved at 3°C and tested at 3°C; (2) evolved at 3°C, then acclimated to and tested at 23°C; (3) evolved at 23°C and tested at 23°C; and (4) evolved at 23°C and tested at 3°C. By acclimating mice to temperatures other than the one at which they evolved, short-term acclimation effects of temperatures were controlled, so that genetically determined changes could be demonstrated. Multigenerational cold exposure resulted in increased body mass and fat content of mice. In addition, the population in the cold became more fertile, evolving an earlier age of first reproduction and shorter tails. Mice in the cold evolved faster growth rates—an important measure of physiological performance and one with clear potential links to ecological success. Likewise, to support the faster growth rates of their young, females from populations with multigenerational exposure to the cold environment produced milk with higher fat and protein content than females that were transferred to the cold environment from populations with multigenerational exposure to the warm environment (Barnett and Dickson 1984b).

WIND TUNNEL FLIGHT IN *DROSOPHILA*

Weber (1996) developed an ingenious apparatus and procedure that allowed him to select for wind tunnel flight in *Drosophila* with very large population sizes; estimated effective sizes in his experiments were $500 \leq N_e \leq 1{,}000$. The apparatus was essentially a 1.5-meter-long wind tunnel with forty equal internal compartments pierced by 4-centimeter circular holes, providing a cylindrical potential flight path. Flies were released into the downwind end and would instinctively fly toward a bright light at the upwind end. Flies moving toward the light faced wind speeds that increased in regular increments from zero in the first compartment to a maximum in the last. Walls were coated with a dry, slippery coat of Fluon such that flies could only advance by flying. Trials were ended by CO_2 anesthesia that then allowed the phenotypic distribution of the compartment reached to be scored. Up to fifteen thousand flies were fractionated in a single run, and the top few percent were selected as breeders for the next generation. According to Weber (1996, 206), "Various tests and observations show that the trait is actually a composite of phototaxis, activity level, flying speed, and aerial maneuvering skill." In other words, a mixture of behavior and performance was being positively selected.

The mean apparent flight speed in each of two replicate lines increased from about 2 to 170 centimeters/second and did not reach a plateau in one hundred generations.

Competitive fitness tests conducted at generations 50 and 85 indicated little or no loss of fitness in the selected lines as compared with two control lines (see Weber 1996 for details).

Marden et al. (1997) used a computerized system for three-dimensional tracking of large numbers of individual free-flying flies to assess performance at generation 160. The selected lines showed significant increases in mean flight velocity, decreases in angular trajectory, and a significant change in the interaction between velocity and angular trajectory. However, maximal flight velocity differed little between the selected and control lines, although individuals from the selected lines attained maximal performance levels much more frequently. The authors concluded that although the selection regimen had changed the relative effort and/or the frequency of phenotypes capable of attaining a preexisting maximal performance level, it was not able to increase maximal flight performance over entire populations. These results are in sharp contrast to an experiment that selected for high voluntary wheel running in mice (Swallow et al. 1998a; Garland 2003; see also later discussion in this chapter and Rhodes and Kawecki this volume), which found that both mean and maximal voluntary speeds increased significantly (Girard et al. 2001; Koteja and Garland 2001; view movie at www.biology.ucr.edu/faculty/Garland/ Girard01.mov). This difference is important because an increase in the maximal speed or intensity of a behavior is presumably more likely to entail changes in underlying performance capacities.

ENDURANCE RUNNING AND STRESS-INDUCED ANALGESIA IN MICE

As a performance measure, maximum endurance capacity has received considerable attention. The ability of an organism to sustain physical activity is determined by multiple biochemical and physiological factors, and by such environmental factors as temperature. The evolution of increased endurance capacity has particularly important ramifications for organisms that are wide ranging. For example, selection for higher aerobic capacity may have played an important role in the evolution of endothermy (Hayes and Garland 1995). Endurance capacity may constrain many behaviors, including foraging, mate searching or courtship, territorial defense, and migration. Endurance was important in human evolution (Bramble and Lieberman 2004), and endurance capacity strongly influences the outcome of sporting events, such as marathons. Furthermore, endurance capacity has been linked to disease susceptibility and longevity (Wisløff et al. 2005; Koch and Britton 2007; Koch et al. 2007). Until relatively recently, the heritability of endurance capacity in human populations has been unclear, with estimates of "heritability" (not narrow sense) ranging from essentially zero (Perusse et al. 1987) to over 0.9 (Klissouras 1971). Research with animal models has established that genetic factors explain a significant amount of variation in both exercise capacity in an untrained state (Koch and Britton 2001) and in the physiological responses to training regimens (Troxell et al. 2003).

Bunger et al. (1994) reported the results of sixty generations of selecting laboratory mice for an index combining high body weight and high "stress resistance," where the

latter denoted the distance to exhaustion on a treadmill. To our knowledge, this was the first attempt to select on a measure of locomotor performance in a rodent. Unfortunately, this selection experiment was conducted in the former East Germany at a time when publications in English were little supported (L. Bunger, personal communication). Although some interesting results have emerged from this work (e.g., those of Falkenberg et al. 2000 on blood enzymes and substrates), they seem to have been underappreciated in the larger scientific community.

It may seem that estimating an animal's endurance capacity during locomotor activity is a relatively straightforward task, which can be easily achieved by forcing the exercise on a treadmill. However, exposure to threatening, emergency conditions elicits a transient decrease of pain sensitivity—so-called stress-induced analgesia (SIA)—which also affects thermoregulatory mechanisms, locomotor performance, and metabolism (Sadowski and Konarzewski 1999; Lapo et al. 2003a, 2003b, 2003c). The result can be a counterintuitive depression (rather than mobilization) of metabolism in response to acute stressful stimuli, such as those applied during the measurements of performance traits (e.g., shocks from electric grid during forced running or cold exposure during the measurement of thermogenic capacity). Therefore, results obtained from such measurements may to some extent reflect an animal's propensity to develop SIA. Indeed, a comparison of mice from lines selected for high and low propensity to develop SIA (Panocka et al. 1986) showed that the maximum thermoregulatory rate of oxygen consumption (achieved during either swimming in cold water or exposure to HeO_2 atmosphere at $-2.5°C$) was lower in the high-SIA than in the low-SIA lines (Sadowski and Konarzewski 1999; Lapo et al. 2003b).

Thus, a response to selection for performance traits measured in conjunction with the application of stressful stimuli could be partly attributable to selection on propensity to develop SIA, rather than mechanisms limiting physiological performance per se. Consequently, selection for performance traits measured with protocols based on forced activity (e.g., endurance running on treadmill) may yield results different from those based on voluntary activity (e.g., voluntary wheel running—see later discussion).

ENDURANCE RUNNING IN RATS AND VOLUNTARY WHEEL RUNNING IN MICE

Treadmill endurance capacity has also been the subject of a within-family selection experiment that used rats (Koch and Britton 2001). Selection for low and high endurance capacity was based on distance run to exhaustion on an inclined motorized treadmill (with electrical stimulation grid) whose speed was gradually increased. The starting velocity was 10 meters/minute and was increased 1 meter/minute every 2 minutes. The selected lines were not replicated, and no unselected control lines were maintained, which is not an ideal design (see also later discussion of work by Konarzewski and colleagues). The selected lines diverged rapidly, and after six generations the low-capacity runners (LCR) and high-capacity

runners (HCR) differed by 2.7-fold in treadmill endurance capacity, with the majority of the change (relative to earlier measurements obtained from the starting population) occurring in the up-selected line (Koch and Britton 2001). By generation 11, the ratio between the average phenotypes of the two selected lines was 4.5 (Wisløff et al. 2005).

In such an experiment, it is possible that lines will diverge in their willingness to run, via changes in brain circuitry related to motivation, reward, or pain sensitivity (Li et al. 2004; Rhodes et al. 2005; Keeney et al. 2008). If so, then a difference in endurance measured during forced exercise might emerge even in the absence of changes in actual physiological capacities for exercise. Thus, it is important that it was found that, by generation 7, maximum oxygen consumption (VO_2max)—a key determinant of maximum aerobic speed and hence of endurance capacity per se—differed by 12 percent between the HCR and LCR lines (Henderson et al. 2002).

This rat experiment forms an interesting counterpart to an experiment with mice that used within-family selection to increase voluntary wheel running in four replicate lines while also maintaining four unselected lines as controls (figure 12.2: Swallow et al. 1998a; Garland 2003; Middleton et al. 2008; Rhodes and Kawecki this volume). Mice from the high-runner (HR) lines exhibit an elevated VO_2max (Swallow et al. 1998b; Rezende et al. 2006a, 2006b), as well as increased endurance during forced treadmill

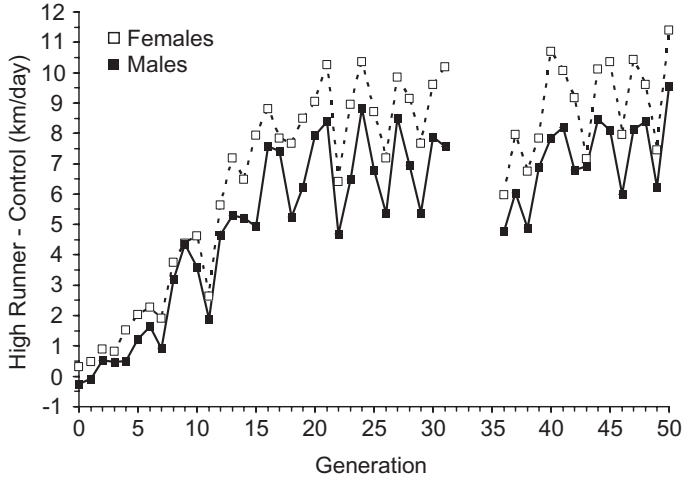

FIGURE 12.2

Average difference in voluntary wheel running on days 5 and 6 of a six-day exposure to wheels (1.12-m circumference) between four High Runner (HR) lines and their four nonselected control lines (Swallow et al. 1998a; Garland 2003; Middleton et al. 2008). After generation 31, mice were moved from the University of Wisconsin–Madison to the University of California–Riverside, and wheel running was not recorded (nor was selection applied) for four generations. For both the HR and C lines, about a third of the total revolutions (and hence distance) per day occur during "coasting" (Koteja et al. 1999; Girard et al. 2001). Although the absolute difference between the HR and control lines is somewhat greater for females, the fold difference is identical for the sexes (e.g., see Swallow et al. 1998a; Garland 2001, 2003; Rezende et al. 2006b). Note the substantial environmental variation across generations.

exercise (Meek et al. 2007). Similarly, the HCR rats also ran longer distances on wheels as compared with LCR rats (Waters et al. 2008). HCR rats ran for more time each day and ran faster on wheels. Striatal dopaminergic responses to the wheel running trial differed between HCR and LCR rats, suggesting a divergence in the central control of locomotor behaviors in these lines (Waters et al. 2008). Alterations in dopamine function have also been strongly implicated in the HR lines of mice (Rhodes et al. 2005; Rhodes and Kawecki this volume). The general consistency of results between these mouse and rat experiments suggests a fairly general relationship between physiological capacity for aerobic performance and the propensity to engage in endurance running, at least in rodents, if not mammals generally. An important area for future research in both experiments will be elucidating sex-specific differences in the lower-level traits that underlie the response to selection (e.g., see Swallow et al. 2005; Keeney et al. 2008).

Another point of consistency between the mouse wheel-running and rat treadmill endurance experiments is that in both cases, the high-selected lines became smaller in body mass. In the mouse experiment, the reduction in body mass may be partly a function of elevated circulating corticosterone levels, both at rest and during wheel running (for details, see Girard and Garland 2002; Malisch et al. 2007, 2008). However, the HCR and LCR rats do not differ in baseline corticosterone levels (Waters et al. 2008). In the mice, line variation in circulating corticosterone levels is inversely related to the ability to clear a parasitic nematode infection (Malisch et al. 2009).

Rats selected for endurance capacity diverged in control of aerobic capacity, including central mechanisms involved in the delivery of O_2 to the skeletal muscle as well as peripheral mechanisms involved in the delivery, use, and conversion of O_2 within the skeletal muscles. Interestingly, by generation 7 of selection, when maximum oxygen uptake (VO_2max) differed by 12 percent between the lines, only divergence in peripheral mechanisms were detected between the HCR and LCR rats. Higher O_2 delivery resulted from improved O_2 transport from tissue to cells, increased capillary density (Henderson et al. 2002), and increased oxidative enzyme capacity (Howlett et al. 2003)—with no significant changes in pulmonary or cardiovascular function (Henderson et al. 2002). By generation 15 of selection, when VO_2max had diverged by 50 percent between the HCR and LCR, the lines no longer differed significantly in O_2 extraction. This seeming reversal, however, is not a function of a loss of the peripheral changes (e.g., increased capillary density) observed in generation 7 but, instead, is a function of gains in cardiac output and stroke volumes, with a resultant reduction in blood transit time across the capillary beds in the HCR rats (Gonzales et al. 2006). Taken together, these results suggest that the mechanistically subordinate traits underlying a particular phenotype under selection may evolve at different rates (see also Rezende et al. 2006c; Archer et al. 2007; Guderley et al. 2008).

The rat lines selected for high or low treadmill endurance were developed to test the "metabolic syndrome" hypothesis—that many complex diseases are caused by defects in the metabolic pathways of energy acquisition, deposition, oxidation, and detoxification (Koch and Britton 2005). A comparison at generation 11 showed that the two rat lines

had diverged in a number of cardiovascular risk factors. Not only are LCR rats larger, but they have significantly higher total body fat (J. G. Swallow, unpublished data), higher visceral body fat, and higher circulating free fatty acids and triglycerides. (Similarly, mice from the HR lines have reduced body fat as compared with their control lines; see Girard et al. 2007; Vaanholt et al. 2007.) LCR rats had higher abdominal aortic blood pressure measured across multiple time periods. Furthermore, stroke volume and isolated myocyte shortening, standard measures of cardiac function, were impaired in the LCR rats as compared to HCR rats. Taken together, these data constitute evidence for the emergence of "metabolic syndrome" in the LCR line (Koch and Britton 2005; Wisløff et al. 2005). Hence, these lines are becoming an important model for biomedical research.

An unexpected result of the mouse selection for high wheel running was the discovery and increase in frequency (in two of the four HR lines) of a Mendelian recessive allele that, when present in the homozygous condition, causes a 50 percent reduction in hind limb muscle mass (Garland et al. 2002). This reduced muscle mass is related to dramatic differences in muscle fiber type composition (Guderley et al. 2008), and minimuscle individuals demonstrate that the allele has numerous other pleiotropic effects, many of which seem conducive to supporting high levels of endurance exercise (e.g., increases in mass-specific cellular aerobic capacity, heart size, and hindlimb bone lengths; review in Middleton et al. 2008). (Interestingly, however, minimuscle individuals seem to have an elevated cost of transport; Dlugosz et al. 2007.) This gene of major effect has been localized to a 2.6335-megabase interval on MMU11, a region that harbors about one hundred expressed or predicted genes, many of which have known roles in muscle development and/or function (Hartmann et al. 2008). Identification of the genetic variant that underlies minimuscle could elucidate both normal muscle function and the dysregulation of muscle physiology that leads to disease. Beyond this, QTL mapping of the HR lines, which is currently underway, has the potential to uncover "anti–couch potato" genes that positively affect voluntary activity levels, which again could have important biomedical implications.

The experiments that have increased forced treadmill endurance running in rats, while also apparently increasing voluntary wheel running; and those experiments that have increased voluntary wheel running in mice, while also increasing forced treadmill endurance, raise interesting issues concerning phenotypic plasticity (Garland and Kelly 2006). In the mouse experiment, home cage activity is also increased, as compared with control lines, when wheels are not provided (Rhodes et al. 2005; Malisch 2007; Malisch et al. 2008). Whether the same is true for the high-endurance rats is unknown. However, if activity levels are increased, then they may contribute to the physical fitness and performance abilities of the high-selected lines in question. Swallow et al. (2005) coined the term "self-induced adaptive plasticity" to refer to situations in which an organism engages in a behavior (e.g., locomotion) that in turn positively affects its ability to further engage in that behavior, notwithstanding the somewhat paradoxical use of this term to refer to a pattern of plasticity that arises as a result of selection.

A related point that has been emphasized by some exercise physiologists (e.g. Booth et al. 2002a, 2002b) is that human patterns of gene expression may be adapted to function "normally" when we engage in substantially higher levels of daily physical activity, as during the hunter-gatherer stage of our ancestors. In their view, "our current genome is maladapted, resulting in abnormal gene expression, which in turn frequently manifests itself as clinically overt disease" (Booth et al. 2002b, 399). In other words, what might be termed the ancestral physical-activity environment cannot be separated from "normal" physiological function. Or, in their words, "in sedentary cultures, daily physical activity normalizes gene expression towards patterns established to maintain the survival in the Late Palaeolithic era" (Booth et al. 2002b, 399). The rat and mouse selection experiments, or variants on them, may be well suited to address these sorts of hypotheses.

The replicate high-running selected lines of mice show a number of interesting differences that can be viewed as multiple solutions. For example, the components of wheel running (minutes/day and mean speed) differ significantly among the four replicate selected lines (Swallow et al. 1998a; T. Garland, unpublished results). And, as noted, the mini-muscle phenotype occurs in only two of the four selected lines. Pleiotropic effects of the mini-muscle allele in homozygotes (Hannon et al. 2008) also result in line differences for the affected traits. The extent to which various traits differ among the four selected lines after adjusting statistically for the effects of mini-muscle is currently under investigation.

The mouse wheel-running experiment also provides an interesting example of a possible "adaptive" genetic correlation (*sensu* Lynch 1992, 1994) between two behavioral traits. Although they do not differ from control lines in intermale or maternal aggression, mice from the HR lines show elevated predatory aggression when tested with live crickets (Gammie et al. 2003). Even when not fasted, they attack, kill, and eat crickets more rapidly as compared with the nonselected control lines (Gammie et al. 2003; T. Garland, unpublished results). The correlated response to selection by predatory aggression provides evidence that it is positively genetically correlated with locomotor activity. If this correlation is a fairly general feature of mammals, then it has implications for the evolution of an active, predatory lifestyle. Mammals such as wolverines are characterized by high activity levels (e.g., large home ranges and long daily movement distances: Garland 1983; Goszczynski 1986) and, of course, by a tendency to attack and kill suitable prey items when encountered. During the evolution of such a mammal, from an ancestor that was both less active and had lower predatory tendencies, one presumes that correlational selection would favor individuals with both relatively high activity and predatory tendency. If so, then the rate of bivariate evolution would be increased if the two traits were positively genetically correlated: the genetic architecture would "facilitate" what selection was "trying" to do (for a similar argument concerning locomotor speed and endurance in garter snakes, see Garland 1994; for a general argument that is related, see Schluter 1996). Why might locomotor activity and predatory aggression be positively related? Gammie et al. (2003) suggest that both behaviors share similar neuronal substrates, with dopamine being a likely candidate (see also Ohno and Miyatake 2007 and Rhodes and Kawecki this volume).

EVOLUTION OF THE RATE OF ENERGY METABOLISM IN RODENTS

The performance of vital animal functions, such as food acquisition, locomotion, osmotic and thermal regulation, and reproduction, depends on a complicated network of interacting physiological processes. However, each of those processes involves—directly or indirectly—conversion of energy. Therefore, the rate of energy metabolism can serve as a unifying quantitative measure of organismal functioning (McNab 2002). In most animals, energy is eventually obtained from aerobic oxidation of organic substrates, so the rate of energy metabolism can be measured indirectly as the rate of oxygen consumption. Although describing the complicated network of physiological processes by a single value—the rate of energy metabolism or oxygen consumption—is a gross oversimplification, the question of why animals vary in metabolic rate has been a primary motivation for countless comparative and experimental studies.

Three obvious sources of variation in metabolic rate are body size, body temperature, and physical activity. Larger animals of a given kind must, on average, have higher total metabolic rates than small ones, and the exact way metabolic rate scales in relation to body size (see also Frankino et al. this volume) has important functional implications (e.g., see Garland 1983; Carbone et al. 2005). But even if we compare animals of a particular size under resting conditions and at the same body temperature, their metabolic rates may still differ by more than an order of magnitude (e.g., see Kozłowski and Weiner 1997; Kozłowski et al. 2003; McKechnie et al. 2006; White et al. 2006). Analysis of experiments in which the rate of metabolism is either directly selected or expected to change in response to selection on other traits provides a powerful tool with which to uncover underlying mechanisms (see also Hayes and Garland 1995; Garland and Carter 1994; Garland 2003; Książek et al. 2003, 2004, 2007; Konarzewski et al. 2005; Swallow and Garland 2005; Rezende et al. 2006; Brzęk et al. 2007; Kane et al. 2008; Sadowska et al. 2008).

SELECTION ON BASAL METABOLIC RATE

Basal metabolic rate (BMR) is defined as the minimum rate of metabolism measured in a normothermic, resting, postabsorptive individual under thermally neutral conditions (McNab 2002), and it is widely used as a standard for inter- and intraspecific comparisons in studies on animal energetics (Frappell and Butler 2004, and references cited herewith). We are aware of only one successful experiment in which BMR was the trait directly and purposefully selected (Książek et al. 2003, 2004; Gębczyński 2005; Konarzewski et al. 2005; Brzęk et al. 2007; figure 12.3). Konarzewski and colleagues selected for high and low mass-independent BMR (i.e., residuals from regression of BMR on body mass) in laboratory house mice (Swiss Webster outbred strain). Unfortunately, the experiment had only one line selected for each of the directions and no unselected control line, which undermined its power and forced the authors to use indirect methods to test the statistical

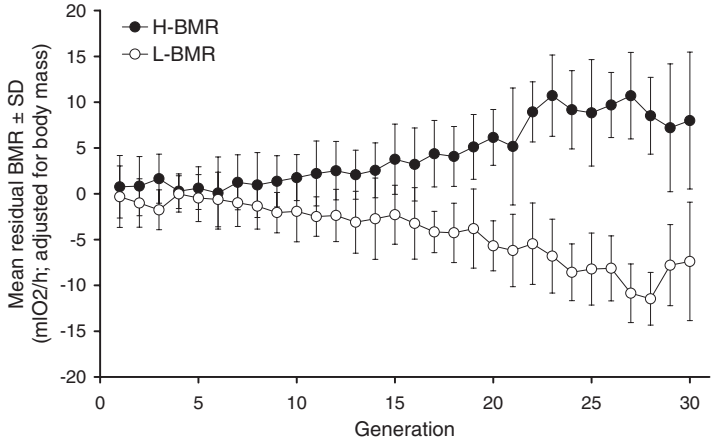

FIGURE 12.3

Changes of basal metabolic rate (BMR) in male laboratory mice selected for high (H-BMR) and low (L-BMR) values of mass-independent BMR (residuals from regression models; Książek et al. 2003, 2004; Gębczyński 2005; Konarzewski et al. 2005; Brzęk et al. 2007). The selection experiment had only one line for each direction, and therefore Henderson's (1989, 1997) approach (see text) has been used to test for the effects of selection (the means and standard deviations were calculated from family means). The data for this figure have been kindly provided by A. Gębczyński and M. Konarzewski.

significance of both the direct and correlated responses to selection (Henderson 1989, 1997; Konarzewski et al. 2005). Nevertheless, the experiment provided important results concerning variation in BMR. First, the selection was effective, and after seven generations, the lines clearly diverged (Książek et al. 2004; figure 12.3). At generation 19, the difference in BMR between the high line (59.0 ml O_2/h) and the low line (50.1 ml O_2/h) was equivalent to 2.3 phenotypic standard deviations (SD). At generation 22, the difference had increased to 10.8 ml O_2/h, or 3.3 phenotypic SD (Brzęk et al. 2007), and it appears that around generation 25 a selection limit was achieved (figure 12.3).

The results of this selection experiment were qualitatively consistent with the narrow-sense heritability ($h^2 = 0.38$, $p = 0.02$) estimated in the base population by means of parent-offspring regression (Konarzewski et al. 2005). This is an important finding, because previous studies in both laboratory mice (Lacy and Lynch 1979; Dohm et al. 2001) and a wild rodent, the leaf-eared mouse *Phyllotis darwini* (Nespolo et al. 2003; Bacigalupe et al. 2004), indicated very low or even negligible heritability of BMR. On the other hand, in a more recent study based on a much larger sample of about one thousand individuals, Sadowska et al. (2005) found a relatively high and significant heritability of mass-adjusted BMR in the bank vole *Myodes* (*Clethrionomys*) *glareolus* ($h^2 = 0.4$). Similarly, Johnson and Speakman (2007) reported high heritability ($h^2 = 0.45$, estimated from mean parent-offspring regression) of resting metabolic rate in short-tailed field voles *(Microtus agrestis)*. Thus, it seems that at least in some populations of rodents, both laboratory and

wild, there is a substantial additive genetic component to the variation of BMR. Thus, a search for genetic correlations with other traits is not hopeless.

As this selection experiment was performed on mass-independent BMR, mice from the high- and low-BMR lines did not diverge in body mass (Książek et al. 2004; Brzęk et al. 2007). Therefore, these selected lines are particularly suitable material with which to address questions concerning proximate factors underling differences in BMR (Hulbert and Else 2004, 2005). One of the plausible causes of BMR differences is a difference in the size of metabolically active organs, especially brain, heart, liver, kidneys, and the small intestine (Garland and Else 1987; Schmidt-Nielsen 1990; Hulbert and Else 2005). Indeed, the internal organs are larger in mammals than in reptiles of the same size (see Karasov and Diamond 1985; Hulbert and Else 2004, 2005), but several studies of intraspecific correlations between BMR and the internal organ size within birds or mammals have produced ambiguous results (e.g., Daan et al. 1990; Koteja 1996; Speakman and McQueenie 1996; Meerlo et al. 1996; Piersma and Lindström 1997; Chappell et al. 2007).

Results obtained from the BMR selected lines were unequivocal: mice from the high-BMR line had larger hearts, livers, kidneys, and small intestines, yet they were leaner than mice from the low-BMR line (Książek et al. 2004; Brzęk et al. 2007). Standardized differences between the organ sizes in high and low lines were 1.1–1.9 SD in generation 19, which increased to 1.4–2.2 SD in generation 22, significantly larger than a divergence that could appear as a result of random genetic drift alone, as estimated by Henderson's (1997) approach. This corroborated an earlier study, which showed a positive correlation between BMR and the mass of the internal organs across four inbred lines of laboratory mice (Konarzewski and Diamond 1995).

In addition, daily food consumption was also significantly higher in the high-BMR line than in the low-BMR line (Książek et al. 2004). These results, too, are complementary to those of another artificial selection experiment, which showed that laboratory mice from lines selected for high rate of food consumption had both larger sizes of the internal organs and a higher resting metabolic rate, compared with mice from lines selected for low rate of food consumption (Selman et al. 2001a, 2001b).

Thus, studies of these BMR-selected mice provided strong evidence that the level of BMR is associated both with the mass of the central organs involved in whole-animal metabolism and with the total rate of energy turnover in animals. This concurs with the results of studies that have shown an association between BMR and field metabolic rate (FMR)—that is, average daily metabolic rates measured in free-living animals by means of the doubly labeled water technique (Daan et al. 1990; Koteja 1991; Lindström and Kvist 1995; Ricklefs et al. 1996; Speakman et al. 2003).

Surprisingly, however, the mass of interscapular brown adipose tissue (IBAT; see also Lynch 1992, 1994)—the major site of heat production through nonshivering thermogenesis—was lower in the high- than in the low-BMR mice (Książek et al. 2004). Moreover, the maximum rate of oxygen consumption achieved during cold exposure did

not differ significantly between the lines, and mice from the high-BMR lines showed slightly deeper hypothermia after cold exposure than those from the low-BMR lines, a difference that was significant when tested with ordinary ANCOVA, but not significant when tested using Henderson's approach. Furthermore, mice from the high-BMR lines did not maintain higher body temperatures and were not able to increase body temperatures faster, after exposure to mildly cold ambient temperatures, compared with mice from the low-BMR lines (Gębczyński 2004). Thus, thermogenic capacity of the mice appeared to be genetically independent from BMR. The same conclusion emerged from a quantitative genetic analysis in the bank vole, which showed no additive genetic correlation between BMR and the maximum cold-induced rate of oxygen consumption, even though both of the traits were heritable in the narrow sense ($h^2 \approx 0.4$; Sadowska et al. 2005). These results are apparently at odds with the common assumption that evolution of high BMR in mammals and birds was driven by selection favoring better thermoregulatory capabilities (Heinrich 1977; Crompton et al. 1978; Pörtner 2004).

The "thermoregulatory" model for the evolution of endothermy had been already challenged long before any information about genetic correlations between BMR and thermogenic capacity was known (Bennett and Ruben 1979). The major concern of its critics was that the supposed benefits from a slightly elevated temperature appear to be vague when compared to immediate and severe energy costs associated with an increase in resting metabolic heat production. This problem was clearly demonstrated in an experiment on varanid lizards, which showed that doubling the rate of energy metabolism resulted in a body temperature elevation of less than 1°C (Bennett et al. 2000). Therefore, over the last three decades, alternative hypotheses have been proposed to explain how an increased level of BMR and endothermy could have evolved as a correlated response to natural selection acting on other traits (see Kemp 2006 for a recent review). Among several proposed hypotheses, the most attention has been given to the aerobic capacity model, according to which an increase BMR and endothermy evolved as a correlated response to selection for high, sustained locomotor activity, supported by aerobic metabolism (Bennett and Ruben 1979).

The crucial assumption of the aerobic capacity model is that BMR and maximal aerobic capacity (i.e., the maximum rate of oxygen consumption achieved during locomotor activity—VO_2max) are strongly connected due to shared underlying biochemical processes, physiological functions, or anatomical structures, and hence they cannot evolve independently. The model has been appealing not only because it generally fits the paleontological record (Ruben 1995), but also because the major assumption could be translated into a simple hypothesis of a generally positive genetic correlation between BMR and VO_2max and tested against readily available empirical data. Not surprisingly, several comparative and intraspecific analyses have been performed to test the hypothesis, and they provide mixed support for such a positive correlation at the comparative level (Hayes and Garland 1995; Angilletta and Sears 2003; Gomes et al. 2004; Rezende et al. 2004; Kemp 2006; Wiersma et al. 2007).

However, the first determined attempts to test the hypotheses at the level of genetic variation have been made only recently. Dohm et al. (2001) found limited support for a positive genetic correlation between BMR and VO_2max in outbred laboratory house mice, whereas Sadowska et al. (2005) have shown a significant additive genetic correlation between BMR and VO_2max measured during swimming in the bank vole. The latter study also showed that the factorial aerobic scope (i.e., the ratio of VO_2max/BMR) is heritable ($h^2 \approx 0.2$), which indicates that simultaneous selection for an increased VO_2max while maintaining a low BMR should also be effective. Thus, quantitative genetic analyses with rodents to date offer partial support for the key assumption of the aerobic capacity model.

The aerobic capacity model would be ideally tested in a selection experiment in which locomotor activity or VO_2max is the directly selected trait, and BMR is tested for a correlated response. As noted, selection for high voluntary activity levels in house mice (Swallow et al. 1998a; Garland 2003) has indeed resulted in increased maximal oxygen consumption during forced exercise (Swallow et al. 1998b; Rezende et al. 2006a, 2006b), but it has not led to an increase in basal (Kane et al. 2008) or resting (Rezende et al. 2006b) metabolic rate. These results are, thus, also inconsistent with the aerobic capacity model.

However, if the assumption of a tight functional link between BMR and VO_2max holds, the latter should be lower in the low-BMR than in the high-BMR mice from Konarzewski's experiment. To test this possibility, Książek et al. (2004) and Brzęk et al. (2007) have measured the maximum rate of oxygen consumption achieved by the selected mice during swimming in cool water (25°C). Contrary to the expectation, VO_2max during swimming tended to be lower in the high-BMR than in low-BMR line, though the difference was significant when tested with normal ANCOVA, but not when tested with Henderson's approach (Książek et al. 2004; Brzęk et al. 2007). However, the mice became severely hypothermic during the test, more so than after the cold-exposure trials. Again, mice from the high-BMR line showed a significantly deeper hypothermia after the test than those from the low-BMR line. The hypothermia observed at the conclusion of the swimming trials indicated that the animals were under substantial cold stress during the trials, and a considerable part of their oxygen consumption must have been associated with heat generation rather than with locomotor activity. Moreover, individuals with lower thermogenic capability could suffer from a deeper hypothermia, and consequently a compromised capacity of the respiratory system and muscles to support locomotor activity. Thus, the VO_2max measured during the test could actually estimate thermogenic capacity (i.e., maximal oxygen consumption elicited via cold exposure) rather than maximal exercise metabolic rate (i.e., maximal oxygen consumption elicited via exercise). Therefore, it might be more appropriate to read the results as contradicting the "thermoregulatory" rather than the "aerobic capacity" model for the evolution of endothermy.

Perhaps the most intriguing result from Konarzewski's experiment comes from a comparison of cell membrane lipids (Brzęk et al. 2007). Else and Hulbert (1987; also

Hulbert and Else 1989, 1990, 2005; Else et al. 2004) found that permeability of the cell membranes to protons is higher in vertebrate endotherms than in ectotherms. Consequently, endotherms should spend more energy to maintain electric potential across membranes, should have a lower efficiency of ATP synthesis in mitochondria, and consequently should have a higher rate of heat dissipation compared with ectotherms. This difference in "leakiness" could be a cost of being an endotherm, but Else and Hulbert (1987) proposed that the "leaky membranes" could also have adaptive value as a mechanism for heat production per se. The permeability of cell membranes to protons and other ions depends on their composition of fatty acids: the more unsaturated the fatty acid chains, the higher the "leakiness" of cell membranes (e.g., Brookes et al. 1988). Based on their results, Hulbert and Else (1999, 2005) proposed the "membrane pacemaker hypothesis," according to which manipulation of membrane composition, and hence permeability, serves as a major mechanism controlling the overall level of metabolism. Farmer (2000, 2003) incorporated this hypothesis in a "parental care" model of evolution of endothermy, according to which the "leaky membranes" served as a mechanism of thermogenesis associated with incubation of eggs and young.

The lines of mice selected for high and low BMR provided a unique model for testing the assumption of the membrane pacemaker hypothesis. Contrary to the expectation derived from the membrane pacemaker hypothesis, in generation 22 of the experiment, the proportion of unsaturated bonds (unsaturation index) was higher in livers of mice from the low-BMR lines compared with the high-BMR lines, though no difference was observed in kidneys (Brzęk et al. 2007). It is easy to imagine that effective selection for high BMR could be realized through several different mechanisms, so a lack of a difference in composition of cell membranes does not necessarily falsify the "membrane pacemaker" model. However, the finding of a significant difference in the opposite direction should be treated as a serious challenge to the model, one which cannot be ignored in future research.

Finally, Konarzewski and colleagues (Książek et al. 2003, 2007) also investigated how selection for high and low BMR in mice affected their immune competence—which can also be considered an important performance trait. Immune function was measured as a response to injection of sheep red blood cells (SRBC). Mice from the high-BMR line showed a significantly lower response than those from low-BMR lines (Książek et al. 2003). This effect was even more evident when the mice were also exposed to a low ambient temperature—that is, when their energy budgets were simultaneously challenged by thermoregulation and immune response (Książek et al. 2007). As mentioned earlier, among the four control and four high-running lines of mice, variation in circulating corticosterone levels is inversely related to the ability to clear a parasitic nematode infection (Malisch et al. 2009); however, selection lines that differ in BMR have not been studied in this respect.

The scarcity of selection experiments focused on BMR is not surprising, because most of the selection experiments on vertebrates that have involved physiological traits were performed in the context of agricultural animal production. From that perspective, the important questions concern the actual energy cost of animal maintenance rather than a minimum metabolic rate measured under conditions (e.g., fasted) that do not occur under normal housing conditions. In particular, agricultural selection experiments have been designed to ascertain whether it is possible to select for low energetic costs of maintenance, because that would result in a decreased feed intake, and perhaps also would allow conversion of more of the food eaten into useful tissues—both of which would increase economic efficiency of animal production. Although the ultimate objective of animal production science (maximization of financial profit) differs from the objectives of evolutionary biology, several of those experiments have provided results that are informative in an evolutionary context.

Nielsen et al. (1997b) selected laboratory mice for high and low heat loss, measured in adult males during a fifteen-hour assay using a direct calorimetry system. Three replicate lines each were selected for high (MH) and for low (ML) heat loss, and three unselected lines were maintained as controls (MC). To control for the effect of body mass, (heat loss)/(body mass)$^{0.75}$ was used as the selection criterion (in units of kcal $\times$ kg$^{-0.75}$ $\times$ day^{-1}). Because the actual slope of the relation between heat loss and body mass of the mice may differ from 0.75, such a correction does not ensure that the trait is mass-independent (Hayes and Shonkwiler 1996). However, the results showed that, in effect, there was no unintentional selection for large or small body mass. The selection on the heat loss character was effective: at generation 15 the trait value was about 33 percent higher in the MH lines and about 20 percent lower in ML lines, compared with the control lines (Nielsen et al. 1997b). Realized heritability, calculated from divergence between MH and ML lines, was about 0.28 $\pm$ 0.01 and tended to be higher when calculated from the divergence between MH and MC lines (0.31 $\pm$ 0.01) than when calculated from divergence between ML and MC lines (0.26 $\pm$ 0.01; Nielsen et al. 1997b).

The authors intended to measure just the costs of maintenance, but, as they admitted, the difference could be related to differences in locomotor activity during the test, which could in turn have been inflated by differences in behavioral response to the calorimetric chamber, isolated from external signals (Nielsen et al. 1997b). However, other measurements showed clearly that the selection affected overall metabolism of the animals under normal housing conditions as well. Mice from the MH lines had a higher rate of food consumption, higher body temperature, and higher locomotor activity, as well as having larger metabolically active organs (liver, heart, spleen) and being leaner than mice from ML lines (mice from control lines were intermediate; Nielsen et al. 1997a; Moody et al. 1997; Mousel et al. 2001; Kgwatalala and Nielsen 2004). Kgwatalala et al. (2004) also found that corticosterone levels were higher in the MH compared to ML lines, which was

also consistent with observations that MH mice were more susceptible, and ML less susceptible, to restraint stress. Results for thyroid hormones were surprising: the level of T4 in blood serum tended to be higher in ML than in MH lines, and no clear difference was observed for T3. Thus, contrary to some expectations (Denckla and Marcum 1973; but see Hulbert and Else 2004), the differences in the rate of metabolism between the lines were apparently not mediated by an altered level of thyroid hormones.

Very interestingly, litter size at birth was higher in mice from MH than from ML lines (again, mice from MC lines had intermediate litter sizes; Nielsen et al. 1997a; McDonald and Nielsen 2006). This finding clearly shows the problem of poor exchange of information and ideas between researchers working in distinct areas. Nielsen et al. (1997a, p. 1475) noted that "why selection for higher or lower maintenance energy causes a correlated change in ovulation rate is not clear" and concluded that "the positive correlated responses in number born represent a very undesirable relationship between energy for maintenance and number born." The gloomy comment is understandable in the original context of the research, which was focused on the possibility of lowering costs of maintenance of livestock: the results showed that it indeed had been possible, but at the cost of lowering fecundity, which obviously was not good news. Apparently, the authors were not aware that their results might be a valuable contribution to the debate concerning a hypothetical link between metabolic rate and life-history traits, which has been carried on among comparative, evolutionary, and ecological physiologists.

In a seminal paper, McNab (1980) proposed that individual growth rate, reproductive rate, and the Malthusian population parameter, *r*, are positively correlated with the basal level of metabolism. These hypotheses have been tested in several comparative and within-species studies. The results of these studies have not provided unequivocal evidence for such associations, but there has been continued interest in this hypothesis nonetheless (e.g., McNab 1980, 2002; Hennemann 1983; Hayssen 1984; Padley 1985; Trevelyan et al. 1990; Harvey et al. 1991; Hayes et al. 1992; Koteja 2000; White and Seymour 2004; Johnston et at. 2007). Results from the selection experiment of Nielsen and colleagues provide important evidence that seems to support the hypothesis of a link between the metabolic rate and reproductive performance. Similarly blind were earlier evolutionary physiologists, who did not refer to these results. For example, Farmer (2000, 2003) and Koteja (2000) proposed that endothermy and high BMR in birds and mammals evolved in connection with evolution of an increased parental effort in reproduction, but they did not refer to results from this selection experiment, even though the results could be read as supporting their hypothesis.

Nielsen and collaborators subsequently put much effort into identification of the genes underlying the selected-line difference in heat loss, using two distinct methods. First, they attempted to identify quantitative trait loci (QTL) associated with the difference in heat loss (Moody et al. 1999), finding several loci influencing heat loss and other related traits such as total body mass, amount of brown adipose tissue, and gonadal fat

tissue. Though it was not possible to unambiguously link any of the QTLs to a particular gene, the authors pointed out that some of the identified regions contain genes that are potential candidates for heat loss regulators, such as genes encoding the β-subunit of the thyroid stimulating hormone, neuropeptide Y receptor Y2, or uncoupling proteins UCP2 and UCP3 (Moody et al. 1997).

The second method used by this group was to investigate differences in gene expression between the lines selected for high and low heat loss. Allan et al. (2000) performed a genome-wide scan of genes differently expressed in hypothalamus and brown adipose tissue (BAT) in mice from inbred lines derived from the MH and ML lines, followed by Northern blot analysis that enabled identification of those genes that showed different expression. These analyses revealed that ribosomal protein L3 (RPL3) mRNA was expressed at a higher level in the ML than MH mice. This protein forms a central channel through which newly synthesized peptides emerge, and, therefore, its function potentially affects all aspects of metabolism. In an extension of the project, Wesolowski et al. (2003) applied microarray analyses followed by real-time PCR and Northern blotting to investigate differences in the expression of several plausible genes in the hypothalamus. They found two genes that had expression significantly different between the inbred mice derived from MH and ML lines: oxytocin and a tissue inhibitor of metalloproteinase 2 (Timp-2), both of which had a higher expression in mice with high heat loss. The role of the latter in regulating heat loss is unclear, but an increased expression of the oxytocin gene is consistent with a higher level of locomotor activity and a higher body temperature of the mice from MH lines (Wesolowski et al. 2003).

In the next study, McDaneld et al. (2002) applied opposite tactics: instead of scanning the entire genome for candidate genes, the authors specifically examined whether the differences between MH and ML lines could be attributed to altered expression or functionality of uncoupling protein UCP1 in BAT (the protein which plays a central role in heat generation in BAT). They compared UCP1 expression in MH and ML lines and also compared heat loss in two new lines, derived from inbred MH and ML lines, but which also had a knockout UCP1 gene. The expression of UCP1 was higher in the mice from ML than MH lines, and heat loss did not differ between mice with functional and knockout UCP1 gene. Thus, the difference in energy expenditure between the lines is not related to UCP1-mediated thermogenesis in BAT (McDaneld et al. 2002). We should also add that the lower expression of UCP-1 in the MH lines is consistent with a lower mass of BAT and a lower thermogenic capacity observed in mice selected for high BMR in Konarzewski's experiment described earlier.

RATE OF METABOLISM AS A HYPOTHETICAL CORRELATED RESPONSE

In the two selection projects described so far, the basal or average rate of energy metabolism were targets of selection and the selected lines were used in attempts to identify proximate morphophysiological and genetic factors underlying the observed differences.

Although success in identifying the mechanisms involved is, so far, limited, the rapid progress of molecular techniques warrants continued effort. However, evolutionary physiologists would also like to know whether selection for some traits possibly related to Darwinian fitness of free-living animals could trigger evolution of the rate of metabolism. In other words, the question is whether an increased or decreased rate of metabolism is likely to arise as a correlated response to selection for ecologically relevant traits.

Although animals from any selection experiment could reasonably be used to test for a correlated response in the basal or standard metabolic rate, few experiments have been designed specifically for that purpose. Recently, one of us (P.K.) has launched a large-scale selection experiment on a nonlaboratory rodent, the bank vole *Myodes (Clethrionomys) glareolus*. The voles are selected for three characters that are thought to have played an important role in the mammalian adaptive radiation (Eisenberg 1981; Kemp 2007): increased aerobic capacity, the ability to grow on a low-quality herbivorous diet (Karasov and Martinez del Rio 2007), and the intensity of predatory behavior. All three traits are adjusted for body mass during selection (figure 12.4). (For a recent discussion of adaptive radiation in the wild, see Seehausen 2006. For an example of experimental adaptive radiation in a bacterium, see MacLean and Bell 2002.) To produce satisfactory power for statistical tests, four replicated lines are maintained in each of the three selection directions, and four unselected, control lines (Sadowska et al. 2008).

After three generations of within-family selection, the maximum rate of oxygen consumption achieved during voluntary swimming was 15 percent higher in lines selected for high aerobic capacity than in the control lines (252 vs. 219 ml O_2/h, $p = 0.0001$). When fed a low-quality diet made of dried grass, voles from lines selected for the ability to cope with an herbivorous diet lost about 0.7 gram less mass than voles from the control lines (-2.44 vs. -3.16 g/4 days, $p = 0.008$). In lines selected for predatory behavior toward crickets, the proportion of "predatory" individuals was higher than in the selected lines (43.6 percent vs. 24.9 percent, $p = 0.045$), but "time to capture" calculated for the successful trials did not differ between the lines (Sadowska et al. 2008).

In the next generation, basal metabolic rate was significantly higher in the lines selected for high aerobic capacity than in the control lines (unpublished results), as expected from the positive additive genetic correlation between the traits estimated in the base population (Sadowska et al. 2005). This result fits the hypothesis that selection favoring high locomotor activity powered by aerobic metabolism could have been an important factor in the evolution of high BMR in mammals. BMRs of the other selection lines ("herbivorous" and "predatory") did not differ from those of their control lines, but the direct effect of selection was also less profound in these lines than in the high-aerobic lines. The experiment is continuing, and the selected lines of voles will provide a useful case for testing hypotheses concerning the correlated evolution of complex traits that may relate to adaptive radiation of rodents.

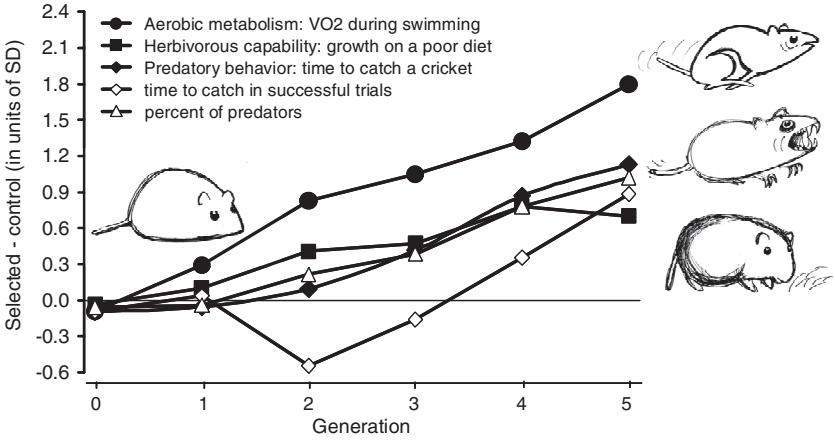

FIGURE 12.4

Multidirectional selection experiment on bank voles *(Myodes glareolus)* intended to provide a laboratory model of adaptive radiation (Sadowska et al. 2008): a "generic," omnivorous rodent species (generation 0), is selected to create highly aerobic athletes (right, top), aggressive predators (middle), and efficient herbivores, capable of growing on low-quality diet (bottom). Four replicate lines are maintained in each of the four line types (three selected plus unselected control). The effects of five generations of selection are shown as differences between mean values of the selected traits, expressed in units of phenotypic standard deviation (filled symbols). For the "predatory" lines, changes of proportion of predatory individuals and their performance in successful trials are also shown (open symbols). The SD is a mean of standard deviations in pooled control and pooled selected lines, and it has been calculated separately for each generation. See text for further explanation. The drawings of voles have been kindly provided by January Weiner.

SELECTION ON VO_2Max AND THE CORRELATION BETWEEN BMR AND VO_2Max

Another selection experiment (J. P. Hayes, B. Wone, M. K. Labocha, E. R. Donovan, and M. W. Sears) is taking a two-pronged approach to testing the aerobic capacity model. This experiment has used Ibg/HS (i.e., heterogeneous stock) laboratory mice as the base population. Four replicate lines are being selected for each of three treatments. The first treatment is no selection (i.e., control lines). The second treatment is selection to increase mass-independent VO_2max elicited during forced treadmill exercise. The third treatment is selection to achieve a negative relationship between mass-independent VO_2max and mass-independent BMR. More specifically, the third treatment chooses mice with the most negative cross-product of mass-independent VO_2max and mass-independent BMR, with the additional restriction that selected mice have positive VO_2max residuals (see also Roff and Fairbairn this volume, and Rhodes and Kawecki this volume, on index selection). Because a correlation is calculated as the covariance

of two traits divided by the product of their standard deviations, selecting for the most negative cross-product should also select for negative correlations between mass-independent VO_2max and mass-independent BMR.

This selection experiment is currently in its sixth generation, and initial results will soon be forthcoming. If selection for increased mass-independent VO_2max is achieved, then a correlated increase in BMR in those lines would support the assumption of the aerobic capacity model. An increase in mass-independent VO_2max with no increase in mass-independent BMR would argue against the assumption. However, responses to the third treatment may be the most revealing. If it is possible to select for lines of mice with a negative correlation between BMR and VO_2max, then that result would suggest that the physiological machinery of terrestrial vertebrates does not produce an inescapable link between these two metabolic traits. Such a result would argue against the aerobic capacity model. However, the vitality of such laboratory populations would also have to be assessed, because although selection for populations with a negative correlation between BMR and VO_2max might be possible in a benign laboratory environment, those populations might suffer fitness losses in comparison with wild populations in natural environments. Ultimately, it will be of considerable interest to compare the results of this experiments with the results of other research groups selecting for voluntary running, endurance capacity, BMR, and VO_2max (see earlier discussion).

This selection experiment by Hayes and colleagues attempts to address a specific "macroevolutionary" event, the evolution of mammalian endothermy, using a microevolutionary test. It is certainly possible that this type of microevolutionary study will be conducted on a scale (e.g., too few generations) that is insufficient to test such a macroevolutionary hypothesis. However, some macroevolutionary hypotheses make very specific and testable predictions. In the case of the aerobic capacity model, the specific prediction is that resting metabolism and aerobic capacity are inescapably positively related, at least in the vertebrate lineages that led to birds and mammals (Bennett and Ruben 1989; Hayes and Garland 1995). If this prediction is correct, then it can be argued that resting metabolism and aerobic capacity should be positively related in all extant birds and mammals, except perhaps for species that are extremely specialized. More specifically, these two characters should exhibit a strongly positive additive genetic correlation (for more on the importance of genetic correlations, see Rauser et al. this volume; Roff and Fairbairn this volume). Falsification of this prediction for any species of bird or mammal would undermine the model, although it could always be argued post hoc that the ancestral forms in which endothermy evolved were somehow fundamentally different from modern birds and mammals. Thus, at least in some circumstances, selection experiments may be useful for attempts to falsify a macroevolutionary hypothesis. Indeed, this example suggests that whenever a macroevolutionary hypothesis incorporates a broad prediction about necessitous features of physiological machinery, then selection experiments may be one appropriate way to test this type of hypothesis. Ultimately,

understanding the evolution of any "macroevolutionary" feature of physiology or performance will likely benefit from a variety of approaches (Garland and Carter 1994; Feder et al. 2000; Garland 2001; Kemp 2006; Futuyma and Bennett this volume; Kerr this volume).

SEXUAL SELECTION: EFFECTS OF ORNAMENTS ON PERFORMANCE

Morphology can affect whole-organism performance, and selection on performance can affect morphology. Sexual selection can lead to the very rapid evolution of exaggerated morphological and behavioral characters when individuals of a given sex, usually males, experience a mating advantage. Two processes are thought to drive the elaboration of secondary sexual characters: intrasexual selection (typically male-male competition) and intersexual selection (typically female choice). Thus, secondary sexual characters function as weapons in the context of intrasexual conflict, as attractive advertisements in the context of intersexual choice, or as both if the character serves dual purposes (Andersson 1994). The consequences of sexual selection, and rapid changes in morphology, on whole-organism performance are inadequately addressed in the sexual selection literature.

Extravagant sexually selected structures are notable for their tremendous variation in size and form. Moreover, some structures, such as the long necks of giraffe and of sauropod dinosaurs, traditionally thought to have been elaborated by natural selection, are now thought to have been shaped by sexual selection to a significant extent (Senter 2007, references therein). The assumption that elaborate secondary sexual characteristics are costly to produce and to maintain, and thus will be opposed by natural selection, is central to all models of sexual selection (Kotiaho 2001). Direct and indirect costs as well as trade-offs associated with producing and maintaining elaborate secondary structures are becoming increasingly well documented (Andersson and Simmons 2006).

As a result, the functional or performance consequences of trait elaboration are just beginning to receive wider attention among comparative and evolutionary physiologists (Lailvaux and Irschick 2006; Oufiero and Garland 2007). For example, Emlen (2001) has suggested that developmental trade-offs associated with ornament production may entail performance costs and thus have major, but largely overlooked, implications for evolutionary diversification (see also Frankino et al. this volume). Even in the absence of developmental trade-offs, elaborated sexual ornaments may generate physiological or biomechanical constraints and impair locomotor performance, as has been suggested for stalk-eyed flies (Swallow et al. 2000; Ribak and Swallow 2007).

If sexually selected traits result in evolutionarily significant performance and physiological trade-offs, then patterns of correlated evolution should be reflected in the phylogenetic history during which the ornament evolved and diversified. Such patterns could and

should be explored in a comparative context (Garland et al. 2005; Lavin et al. 2008; Frankino et al. this volume). It also follows that if a performance decrement generated by the ornament is selectively important, then compensatory morphological and behavioral changes should arise to mitigate performance trade-offs (Oufiero and Garland 2007; see also Huey and Rosenzweig this volume). To date, however, locomotor trade-offs with secondary sexual characters have yet to be rigorously tested or demonstrated (see also Roff and Fairbairn this volume, on trade-offs between flight capability and reproductive investment in crickets).

One definitive means of determining the mechanistic and adaptive significance of sexually selected traits is to experimentally alter them via artificial selection and then compare the relative performance or fitness attributes of selected and unselected individuals (Garland and Carter 1994; Feder et al. 2000). Relatively few artificial selection studies have targeted sexually selected ornaments per se (e.g., Wilkinson 1993; Houde 1994; Emlen 1996; Frankino et al. this volume). In this section, we discuss two model systems to illustrate how selection on secondary sexual traits overlaps with, and should be informed by, the performance literature and by selection experiments.

GUPPIES

Guppies have been a widely used model organism for studies of natural and sexual selection both because they are amenable to field observations and because they can be reared with relative ease in the laboratory (Houde 1997). Their short generation time also makes them attractive as a genetic model, permitting analysis of the genetic underpinnings of many of the traits of interest (e.g., Brooks and Endler 2001a, 2001b). In guppies, measures of male ornamentation and behavioral displays that influence attractiveness show substantial additive genetic variation (Brooks and Endler 2001a). Similarly, female mating preference is heritable and is genetically correlated with male color patterns (Houde 1988, 1997; Houde and Endler 1990). Although the pattern of mate preference is more nuanced than we will delineate here, studies of individual and population-level variation indicate that female guppies generally prefer male ornaments with bright orange and black spots and a high degree of contrast (Houde 1997; Brooks and Endler 2001b).

In natural populations where predation pressure is low, males tend to be brightly colored. In populations where predation pressure is high, males are generally dull. Brightly colored males experienced a predation cost, because they are more conspicuous (Houde 1997; see also Irschick and Reznick this volume). Natural selection in high-predation areas had a variety of other effects in addition to changes in coloration, including effects on a range of life-history traits (Reznick et al. 1997) and behavioral antipredator traits (Magurran et al. 1993), including escape performance ability (O'Steen et al. 2002).

In this case, sexual selection clearly has an indirect effect on performance. These indirect effects are manifest in high-predation environments, and they have far-ranging

consequences on the phenotype. However, in the absence of predation, there is no inherent biomechanical reason why reduced locomotor performance has to be associated with bright coloration. Unless coloration is condition-dependent and linked with other systems, then selection on coloration alone should not result in any change in swimming performance. However, the latter possibility is not without merit. If the ability to bear and express ornaments are indicators of increased viability, as suggested by "good genes" models of sexual selection (Anderson 1994), then individuals that bear larger or brighter ornaments may actually display greater performance (see Lailvaux and Irschick 2006 for a review). Artificial selection could be used to directly test the connection between ornaments and performance, free of the confounding selection for antipredation abilities. Although Houde (1994) created lines of guppies divergent in coloration to test a variety of hypotheses about mate choice and sensory drive, to our knowledge no one has tested these lines for differences in locomotor performance.

STALK-EYED FLIES

Stalk-eyed flies are a particularly good model for investigating trade-offs between sexually selected traits and locomotor performance. Stalk-eyed flies (Diopsidae) have eyes placed laterally away from the head on elongated peduncles, and the degree of hypercephaly and sexual dimorphism varies dramatically species (Wilkinson and Dodson 1997; Warren and Smith 2007; see also Frankino et al. this volume). In sexually dimorphic species, exaggerated eye span is driven by intense directional intrasexual and intersexual selection on male eye span. Males with longer eye stalks usually win contests of aggression over resources (Burkhardt et al. 1994), and females prefer to mate with males bearing longer eye stalks (Burkhardt and de la Motte 1988; Wilkinson and Reillo 1994; Wilkinson et al. 1998). Furthermore, both eye stalk length and female preference are genetically heritable and thus capable of responding to selection (Wilkinson 1993; Wilkinson and Reillo 1994).

Because laterally protruding eyes seem like an inefficient arrangement for flight, trade-offs between these secondary sexual traits and aerial performance might be expected. Currently, however, comparative data are insufficient to fully address the relationship between eye span and flight performance. A comparative analysis of the wing beat frequency of thirteen species of stalk-eyed flies in relation to eye span elongation illustrates some of the compensatory mechanisms (see also Oufiero and Garland 2007) that may mitigate the costs of bearing this ornament (Swallow unpublished). As predicted by scaling laws, variation in the wing beat frequency scales negatively with body mass. Large insects oscillate their wings more slowly. Eye stalk elongation, indexed as the ratio of eye span to body length, was correlated with a reduction in wing beat frequency. Furthermore, within the dimorphic species in which males have longer eye span, wing beat frequency was lower in males than females. Mechanistically, these

results can largely be explained by correlated compensatory increases in the length and area of the wing, which serve to increase the length of the oscillating arm, and in the thorax of males of the dimorphic species. How these compensatory changes translate into differences in flight behavior and performance is currently under investigation. For example, in comparisons between species and sexes that bear longer eyestalks, there is some evidence for reduced aerial performance and maneuverability (Swallow et al. 2000). However, the reduction in performance is smaller in magnitude than a biomechanical analysis of the consequences of eye span elongation for moment of inertia would imply, providing further evidence of correlated compensatory evolution among other traits (Ribak and Swallow 2007).

One definitive means of determining the mechanistic and adaptive significance of eye stalks is to experimentally alter them via artificial selection and then compare the relative performance or fitness differences between individuals from selected and unselected lines. After more than sixty generations of bidirectional artificial selection on male *Cyrtodiopsis dalmanni*, Wilkinson and colleagues have created replicate lines of stalk-eyed flies that differ dramatically (~2 mm, 20 percent) in relative eye span (Wilkinson 1993; Wilkinson et al. 2005). Male fighting performance and likelihood of winning contests of aggression evolved with eye span (Panhuis and Wilkinson 1999). These selected lines are currently being used to investigate the consequences of eye stalk elaboration outside of the context of mate competition.

"EXPERIMENTS" WITHOUT PRECISELY DEFINED SELECTION CRITERIA

Besides the formal selection experiments described in this volume, there are many interesting examples of artificial selection and experimental evolution that are less formal but still informative. For example, the process of domestication no doubt was associated with strong selection for a wide range of functional characters (Hemmer 1990; Bradley and Cunningham 1999; Price 2002; see also Simões et al. this volume). Related to the process of domestication is selection on the physiological performance of already-domesticated animals. An excellent example of this is selection on racing performance.

Humans race camels, greyhounds, horses, ostriches, and pigeons. Horse racing has a long history, dating back at least to chariot racing by the Greeks and Romans, and a wide range of racing events for horses are still practiced throughout the world. Many people enjoy betting on racing events, and owners of racing animals are interested in increasing the earnings of their animals. Consequently, informal selection on racing performance is widespread, at least for horses and greyhounds. It is interesting to compare the informal selection on horses and greyhounds, and ask what the prospects are for improving racing performance via further selection.

HORSE RACING

Horse racing is a diverse enterprise. Races vary by distance, nature of the running surface (dirt or turf), whether there are jumps (steeplechase), and whether the jockey is pulled on a cart (trotters) or rides on the horse's back. Racehorse populations are separated by breed (e.g., standard-bred trotters, thoroughbreds, and Arabians), and they also are segregated into somewhat distinct populations (e.g., British, Brazilian, Japanese, North American, Swedish, Polish, Spanish, Turkish, Tunisian, and other populations). For those of us who study the evolution of performance, selection for racing in these populations constitutes an interesting informal experiment.

Among the most interesting aspects of selection on racing performance are (1) the rate of improvement in racing speeds and performance, (2) the rate of increase in breeding values for those traits, and (3) the amount of genetic variation that remains after many years of selection on performance. An analysis of the winning time for three major thoroughbred races over several decades in Britain suggested that winning time did not appear to be improving (Gaffney and Cunningham 1988). In contrast, winning and average race times got faster in Swedish standard-bred trotters (Árnason 2001). A possible explanation for this discrepancy is that inbreeding and a long history of selection led to the exhaustion of genetic variation for performance in thoroughbreds. Overall, it appears that heritabilities for racing performance are low (usually ~0.2 or lower), but sufficient genetic variation is present for continued genetic progress to be made (Villela et al. 2002; Bokor et al. 2005, 2007; Chico 1994; Ekiz and Kocak 2005; Oki 1995; Sobczynska 2006). The data of horse pedigrees and racing performance have led to investigations of (1) the distribution of allelic effects and scale (additive vs. multiplicative) effects, (2) the possible effects of assortative mating, and (3) the distinctions between speed and other measures of performance. To date, these studies suggest that while fastest race times show little improvement, average racing speeds do appear to be getting faster. Why this is the case deserves further study, and the results could be useful to those interested in the evolution of performance. Another interesting finding is that the informal breeding schemes used by horse breeders result in the selection of sires and dams that do not produce the most rapid genetic progress for performance (Hill 1988; Williamson and Beilharz 1999).

GREYHOUND RACING

In contrast to the long history of horse racing, the racing of dogs (specifically greyhounds) is relatively recent. The racing performance and genetics of greyhounds have not been studied as carefully as for horses, so this description is based on a single recent report on greyhounds in Ireland (Taubert et al. 2007). From 2000 to 2003, average race time over 480 meters has declined by roughly 0.5 second (30.3 to 29.8 seconds). Analysis of the greyhound data shows that heritable variation exists and that selection is increasing performance (i.e., race times are getting shorter, and the dogs are getting

faster). As is the case for horses, breeding values (with higher breeding values indicating faster race time) are increasing, but the increase is less than could be achieved by the use of optimal selection criteria.

One of the flaws in the informal breeding design used for Irish greyhounds is that the dogs chosen as sires do not have the highest breeding values (Taubert et al. 2007). Breeding values for race time were predicted for 51,332 dogs. Only 793 of the dogs were used as sires over the time period studied, and 18 of those sires produced 43.7 percent of all offspring. Sires were not optimally chosen from the perspective of improving racing time because the sires that produced the largest number of offspring were not always those with the highest breeding values. For instance, one sire that produced 991 offspring had a slightly negative breeding value. As has been suggested for horses, breeders of racing dogs would likely benefit from working more closely with animal geneticists (Hill 1988; Taubert et al. 2007).

PHYSIOLOGICAL DIFFERENCES AMONG STRAINS OF MICE AND BREEDS OF DOG

Another rich source of information for students of experimental evolution is the examination of strains or breeds of animals. These populations are fertile ground for study because it is straightforward to identify phenotypic differences that are genetically based (Silver 1995; Crawley et al. 1997; Eisen 2005; Fuller et al. 2005). The genetic differences among strains or breeds of mice, rats, dogs, and other animals may have resulted from deliberate selection for the traits of interest (e.g., blood pH, sensitivity or resistance to drugs, infection, tumor formation, estrus cycle timing, or stress response: Webster 1933a, 1933b; Weir and Clark 1955; Nobunaga 1973; Nagasawa 1976; Liang and Blizard 1978; Caslet et al. 1997; Kemp et al. 2005). Alternatively, physiological differences may be the accidental by-product of selection for other characters, or they may be attributable to founder effects or random genetic drift, with subsequent inbreeding (see also Simões et al. this volume). Thanks to Professor Michael Festing and the Jackson Laboratory, information on the origins of standard mouse strains is easily available online. Phenotypic and genetic differences among mouse strains have been extensively characterized, particularly by biomedical researchers (e.g., Barbato et al. 1998; Biesiadecki et al. 1999; Svenson et al. 2007), but we think that the genetic resources represented by these many strains have been underutilized by evolutionary biologists. The literature on strain difference is vast, and here we report only a few examples to illustrate how the outcomes of the informal or formal selection used to produce laboratory strains may be of interest to comparative, ecological, or evolutionary physiologists.

The ability to cope with hypoxia is physiologically, ecologically, and evolutionarily important (Monge and Leonvelarde 1991; Dudley 1998; Hochachka et al. 1998; Iyer et al. 1998; Hayes and O'Connor 1999; Semenza 1999). Mouse strains vary in their ventilatory response to hypoxia, with some strains coping primarily by altering tidal volume

and others having greater changes in breathing frequency (Tankersley et al. 1994). Moderate hypoxia engenders physiological responses to cope with the changed environment, but extreme hypoxia can be immediately life threatening. Inbred C57BL/6J mice survived extreme hypoxia (4.5 percent O_2) better than outbred CD-1 mice (Zwemer et al. 2007). In addition, more detailed physiological study suggested that differences in ketone metabolism are one factor that contributes to the differences in hypoxic tolerance. Another factor that may be involved in hypoxic tolerance is angiogenesis. Angiogenesis and the up-regulation of proteins involved in stimulating angiogenesis varied markedly across four strains of mice subjected to severe hypoxia (10 percent O_2; Ward et al. 2007). Likewise, the effects of severe hypoxia (10 percent O_2) on arterial blood pressure and heart rate depend in large measure on the strain studied (Campen et al. 2004). Lastly, physiological differences in how mice respond to hypoxia might also be affected by their underlying morphology, and strains of mice have genetically based differences in alveolar anatomy (Soutiere et al. 2004). The point here is that the informal or formal selection programs that lead to the production of genetically distinct strains and breeds can be helpful to evolutionary physiologists seeking to understand how integrated physiological phenotypes evolve.

Locomotor performance is one of the themes of this chapter, and several studies have measured mouse running duration during incremental step tests on a treadmill or some other measure of aerobic capacity during exercise. Aerobic capacity is higher in DBA/2 and BALB than in C57BL mice (Lerman et al. 2002), and significant differences were found in aerobic capacity among ten inbred strains that included A, AKR, Balb, C57BL, and DBA/2, among others (Lightfoot et al. 2001). In the latter study, Balb mice had the greatest endurance of any of the strains, and A mice had the lowest endurance. Related to exercise endurance is the ability of an animal to respond to training, because training can substantially increase exercise endurance. Not only does aerobic capacity vary across strains of mice, but strains also differ in the magnitude of their response to exercise training, in how far they run voluntarily, how fast they run voluntarily, and in how much time they run each day (Lightfoot et al. 2004; Massett and Berk 2005). Strains also differ in the critical speed at which lactate production reaches the point that long-term endurance is limited (Billat et al. 2005). As some of the preceding studies demonstrate, differences among strains are fertile ground for probing the morphological, biochemical, genetic, and genomic bases of variation in performance. Such studies have much to offer evolutionary physiologists.

Another underutilized resource for evolutionary physiologists is the genetic variation present among and within breeds of dogs (Parker et al. 2004). To anyone familiar with greyhounds and pit bulls, it is no surprise that these dogs have been selected for very different measures of physiological performance. Pit bulls were bred to fight, and greyhounds were bred to run. The strength of their bones reflects those specializations (Kemp et al. 2005). Likewise, the distribution of muscle and the ability of the musculoskeletal system differ markedly between pit bulls and greyhounds (Pasi and Carrier 2003).

CONCLUSION

"Survival of the fittest" is the fundamental public metaphor for symbolizing evolution by natural selection. Unfortunately, this public perception conflates physical fitness with Darwinian fitness (Wassersug and Wassersug 1986), which, although complicated, roughly equates with net lifetime reproductive success (McGraw and Caswell 1996; Mueller this volume). Nonetheless, the terminological ambiguity carries more than a grain of truth, because organismal performance traits that involve strength, speed, or stamina may often have strong effects on such major components of Darwinian fitness as survivorship and fecundity. Measures of locomotor performance are now routinely studied in comparative biology, and it has been claimed that "[l]ocomotion, movement through the environment, is the behavior that most dictates the morphology and physiology of animals" (Dickinson et al. 2000, 100).

Overall, the present volume shows that selection experiments of various types are key tools for disentangling the mechanistic basis of evolutionary diversification in all sorts of complex phenotypes (see also Swallow and Garland 2005), and this chapter reveals that performance and physiology are no exception. Selection allows researchers to create populations that explore the limits of physiological performance, while simultaneously facilitating the elucidation of genetic architecture and genes of major effect (e.g., Garland et al. 2002; Hartmann et al. 2008). By studying responses to selection on complex performance traits, biologists can determine both how such traits are integrated phenotypically and the precise mechanisms by which performance evolves (see also Zera and Harshman this volume). These responses also can show whether complex traits (e.g., locomotion, oxygen transport, and delivery: Swallow et al. 1998b, 2005; Rezende et al. 2006c) evolve by correlated progression of all component subordinate traits simultaneously (e.g., stroke volume, heart rate, hematocrit, Bohr shift, mitochondrial density), as might be posited by proponents of symmorphosis (Garland 1998 and references therein), or whether responses reflect mosaic evolution of just one or a few key traits (Garland and Carter 1994; Kemp 2006; Archer et al. 2007). Similarly, correlated responses to selection may reveal both genetic architecture and functional constraints associated with the focal trait being selected. In addition, even when inbreeding effects are minimized, selection that is intense and drastically alters traits potentially may lead to correlated responses that reduce individual Darwinian fitness and hence population viability, at least in some environments. Selected populations that evolve correlated responses rendering them inviable in nature may also reveal ecological constraints. As a hypothetical example, animals selected for high metabolic rate might also evolve as a correlated response immune systems that are too weak to allow survival in nature (see also Malisch et al. 2009).

One additional point to keep in mind regarding the design of selection experiments that target organismal performance traits (including some aspects of behavior [Rhodes and Kawecki this volume] and of life history [Nunney 1996; Zera and Harshman this volume]) is that selection for low values of performance may tend to increase the

frequency of any deleterious recessive alleles that are segregating in the population. This may result in "sickly" organisms, and the observed response to selection for low performance may have little to do with the evolutionary potential of the species in nature (Nunney 1996). Indeed, selection for low treadmill endurance in rats has led to animals that are unhealthy, as compared with their high-selected counterparts (Koch and Britton 2005; Wisløff et al. 2005). In addition, selection for low values of performance may not result in a trait that is "the same" but at the opposite end of a continuum as compared with selection for high values. For example, selection for low values of voluntary wheel running (Swallow et al. 1998a) might lead to an increase in fear of entering the wheels, rather than reduced aerobic capacity (see discussion in Garland 2003).

As concern about global warming intensifies, experimental evolution of animals in warm or cold environments could be used to test how populations evolve when faced with climate change. These mesocosm or laboratory studies would not fully reflect ecologically reality, but they would offer a great starting point (see also Huey and Rosenzweig this volume). For example, it would be interesting to know whether mammals, lizards, and birds tend to respond primarily physiologically or behaviorally when housed for multiple generations at different temperatures (see also Gibbs and Gefen this volume; Huey and Rosenzweig this volume). One intriguing possibility for experimental evolution studies would be to consider using different species for replication, an approach that has already been used with *Drosophila* species experimentally evolved with later reproduction (Deckert-Cruz et al. 2004). Similarly, in terms of response to global changes in the ozone layer, one could study the evolutionary responses to increased ultraviolet radiation in multiple species of amphibians.

In conclusion, biologists interested in physiology and performance should consider the unique knowledge that can be gained by selection experiments. In particular, these studies are highly appropriate for understanding (1) the mechanistic bases of adaptation, (2) the extent to which organismal "design" may constrain evolutionary outcomes, and (3) phenotypic integration. We encourage biologists to apply these approaches to a broader range of animal species.

SUMMARY

In the last twenty-five years, direct measurement of whole-organism performance has become central to those fields of biology that explicitly focus on those physiological, biomechanical, or molecular mechanisms that underlie variation in whole-organism traits. Measures of performance, such as maximal sprint running speed, are presumed to be more direct targets of selection in nature relative to traits below the organismal level. Moreover, voluntary behavior occurs within an envelope of possibilities circumscribed by performance limits; and, conversely, behavioral choices made by animals can shield performance capacities from the direct effects of selection. Both behavioral and performance traits are complex, consisting of numerous subordinate traits and affected by

many genes and environmental factors. In spite of the increased attention paid to whole-animal performance, relatively few experiments have intentionally imposed selection at this level, and most that have involve locomotion or metabolism of rodents. Such experiments with rodents have successfully targeted treadmill endurance-running capacities, voluntary locomotion, and metabolic rates. Some of these experiments have found interesting correlated changes in the behavior of the selected animals, as well as identifying mechanistic underpinnings of divergence at the organismal level. Experiments involving sexual selection have altered guppies and stalk-eyed flies in ways that should impinge on performance abilities, but this has yet to be tested. Finally, besides the planned selection experiments emphasized in this volume, there are many interesting examples of animal breeding that are less formal but still informative, including breeds of horse, dog, and mouse. Potentially useful model organisms for selection on target performance have yet to be exploited (e.g., small fishes). Finally, only three vertebrate studies (two selecting for increased body size in mice, the other for increased litter size) have gone as many as one hundred generations, hence crossing into the category of "long-term" selection experiments.

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