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Relationship between the energetic cost of burrowing and genetic variability among populations of the pocket gopher, *T. bottae*: does physiological fitness correlate with genetic variability?

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Summary

Many studies have reported relationships between genetic variability and fitness characters in invertebrates, but there is a paucity of such studies in mammals. Here, we use a statistically powerful paired sampling design to test whether the metabolic cost of burrowing, an important physiological trait in the pocket gopher, *Thomomys bottae*, correlates with genetic variability. Three pairs of pocket gopher populations were used, with each pair selected from a different subspecies and comprising one high genetic variability and one low genetic variability population. Genetic variability was measured using average allozyme heterozygosity and two measures of DNA fingerprint band sharing. In addition, the cost of burrowing for individuals from each population was determined from the oxygen consumption per gram of body mass per unit of work performed. Our results indicate that the cost of burrowing was significantly higher in populations with lower genetic variability (3-way ANCOVA, P=0.0150); mass-adjusted cost of burrowing in the low variability populations averaged 0.57±0.24 ml O_2 g^{-1} kgm^{-1} and that in the high variability populations averaged 0.42±0.19 ml O_2 g^{-1} kgm^{-1}. The magnitude of the population differences in cost of burrowing was associated with the magnitude of difference in genetic variability. We conclude that population differences in genetic variability are reflected in physiological fitness differences for a trait that is essential to gopher survival.

Key words: metabolic efficiency, burrowing, genetic variability, fitness, inbreeding, genetic drift, *Thomomys bottae*.

Introduction

Pocket gophers excavate extensive burrow systems for foraging, shelter, food storage and reproduction. Because burrowing is a costly activity for pocket gophers (Vleck, 1979), efficient burrowing is likely to be favored by natural selection. In fact, evidence for the selective advantage of burrowing efficiency comes from the geometry of gopher burrows that are constructed to minimize cost of burrowing (Vleck, 1981).

Pocket gophers in California exist in many isolated and semi-isolated populations that, together, exhibit an extraordinary range of genetic variation. Mean heterozygosity values among populations range from near zero to almost 20% (Patton and Smith, 1990). We were therefore able to test whether genetic variability in pocket gophers is related to burrowing efficiency. We used metabolic efficiency, as determined by oxygen consumption during burrowing, as a surrogate for physiological fitness, or vigor, because burrowing is (1) an activity that is crucial to the survival of this highly fossorial species, (2) has been shown to be energetically costly (Vleck, 1979) and (3) is likely to be correlated with overall fitness in this species.

Although it is generally accepted that genetic variability in populations is required for evolutionary adaptation to changing environments, the role of heterozygosity in determining differences between individuals in physiological fitness has been dismissed by a number of researchers (Caro and Laurenson, 1994; Caughley, 1994; Dawson et al., 1987; Lande, 1988; Ouborg and Groenendael, 1996; Pimm et al., 1988, 1989; Schwartz et al., 1986). Conversely, there are many studies that demonstrate a significant relationship between genetic variability and a wide range of fitness characters (for reviews, see Britten, 1996; Mitton, 1997; Zouros, 1987; Zouros and Foltz, 1987). Few such studies, however, have been conducted on mammals, probably because of the technical and logistical difficulties of acquiring sufficiently large samples to detect correlations between levels of heterozygosity and phenotypic traits (Britten, 1996; Zouros and Foltz, 1987). In the present study, we overcame the problem of small sample size by using a statistically powerful paired design and testing whether individuals from populations with low genetic variability were less efficient burrowers (had a higher energetic cost of burrowing) than those from high variability populations

of the same subspecies. We measured genetic variation and cost of burrowing on individuals from three pairs of *T. bottae* populations; both populations in each pair were from the same subspecies but had substantially different levels of genetic variability.

Materials and methods

General methods

Gophers (*Thomomys bottae* Eydeux and Gervais 1836) were live trapped from each site during the winter and spring between January 1995 and May 1997 and brought to the lab. Populations are described in Hildner et al. (2003). To minimize environmental contributions to their phenotypic variance, gophers were housed under controlled conditions (light 6.00–20.00 h, temperature 22°C) for an acclimation period of at least 17 days before experiments were conducted. All gophers were provided with unlimited food (Purina Rodent Pellets, St Louis, MO, USA) and water. After the acclimation period, the cost of burrowing and resting metabolic rate (see Hildner, 2000) of each gopher were measured. Following these measurements, a digestive efficiency experiment was conducted on the gophers (Hildner, 2000), after which the gophers were euthanized, and liver, kidney, heart and tail tissue were collected and stored at –80°C for genetic analyses. Sample sizes are given in Table 1.

Cost of burrowing

Cost of burrowing was determined from the oxygen consumption per gram of gopher per unit of work performed. Oxygen consumption during burrowing was measured using an open-circuit respirometry system modified from Vleck (1979), consisting of a 1 m-long Plexiglas tube filled to approximately 10 cm from the open end with a constant density (1.63 g cm^{-3}) of sand (RMC Lonestar Lapis Lustre 30 Mesh; Davenport, CA, USA). Three different diameter tubes were used; the diameter of the tube was empirically determined and was dependent on the mass of the gopher such that the gopher moved the entire volume of sand as it burrowed (58–120 g gophers were placed in a 5.72 cm tube, 120–190 g gophers in a 6.35 cm tube, and >190 g gophers in a 6.99 cm tube). The tube was connected *via* an airtight seal to a chamber where the gopher could push the excavated sand. Wire mesh prevented the gopher from entering the chamber. Airflow through the tube was kept constant at 1.4 l min^{-1} (Cole-Parmer N092-04 Flowmeter, Vernon Hills, IL, USA), and the fractional oxygen concentration of air leaving the chamber was determined using an Ametek S-3A oxygen analyzer connected to a computer for data acquisition and analysis (Sable Systems, Salt Lake City, UT, USA). Carbon dioxide and water were absorbed (baralyme and drierite, respectively) from air samples prior to oxygen analysis, and water vapor was also absorbed prior to air flow measurement (Fig. 1).

Before introducing a gopher into the tube, air was allowed to flow through the tube until the system stabilized, and the oxygen analyzer was set to the baseline value of 20.94% (the percentage of oxygen in the compressed air tank). After removal of the gopher at the end of the trial, the system was again allowed to stabilize to ensure that the baseline oxygen concentration remained constant during the experiment. In cases where the baseline shifted (<0.3%), a baseline correction was performed on the data using the Sable Systems analysis software.

Individual gophers were weighed and placed in the open end of the tube, which was then connected to the respirometry chamber with an air-tight collar. Gophers typically began burrowing shortly after being introduced to the chamber and continued to burrow until they reached the end of the tube, achieving a steady-state rate of oxygen consumption for at least a 10-min period. Gophers failing to burrow continuously were removed from the chamber and re-tested later. Only gophers that burrowed consistently for two burrowing trials were used in the analyses.

Using these criteria, 81 gophers were measured. During each burrowing trial, the distance that the gopher burrowed (*D*) and the amount of the tube filled with sand (*F*), which the gopher did not push completely out of the tube, were recorded (Fig. 1). Additionally, using two stopwatches, we recorded to the nearest second the amount of time that each gopher spent digging, as well as the amount of time spent pushing sand. These amounts were summed to calculate the total amount of time each gopher

Table 1. *Population average values for mass-adjusted cost of burrowing, fingerprint dissimilarity for 33.15 and MS1 fingerprint probes, and allozyme heterozygosity*

Population	Subspecies	Genetic variability (H/L)	Adjusted cost of burrowing (ml O_2 g^{-1} kgm^{-1})	Fingerprint dissimilarity 33.15	Fingerprint dissimilarity MS1	Allozyme heterozygosity
Patrick's Point, Humbolt Co.	*laticeps*	L	0.59±0.25 (23)	0.09 (22)	0.12	0.006 (23)
Rio Dell, Humbolt Co.	*laticeps*	H	0.41±0.15 (11)	0.41 (15)	0.37	0.026 (19)
Susanville, Lassen Co.	*saxatilis*	L	0.56±0.13 (15)	0.22 (15)	0.30	0.002 (15)
Adin, Modoc Co.	*saxatilis*	H	0.32±0.13 (8)	0.51 (9)	0.57	0.020 (9)
Angels Camp, Calaveras Co.	*navus*	L	0.54±0.32 (12)	0.58 (11)	0.62	0.036 (11)
Solano Co., Solano Co.	*navus*	H	0.51±0.22 (11)	0.62 (12)	0.71	0.060 (12)

Sample sizes for each mean are given in parentheses. Sample sizes for MS1 fingerprint dissimilarity are the same as for 33.15 fingerprint dissimilarity.

spent working in minutes. The rate of soil displacement was calculated as [(g soil/cm)/1000](D min^{-1})(60) to arrive at the kg of soil moved per hour. The average distance the sand was displaced in meters was estimated as [(D/2)+S–(F/2)]/100 (for definitions of variables, see Fig. 1).

Rates of oxygen consumption were calculated according to equation 8 of Depocas and Hart (1957), as modified by Hill (1972; equation 2). The mean rate of oxygen consumption measured during the 10-min steady state of burrowing (ml O_2 g^{-1} h^{-1}) was corrected for standard temperature and pressure (STP) and then divided by the rate of the soil displacement (kg h^{-1}) and the average distance the sand was moved (m) to arrive at an estimate of cost of burrowing (ml O_2 g^{-1} kgm^{-1}). The cost of burrowing, therefore, was estimated as oxygen consumption per gram of gopher per kilogram meter of soil moved. Reported values are the means of the two burrowing trials for each gopher.

Protein electrophoresis

Liver, kidney and heart samples were surveyed for variation in 17 enzymatic and nonenzymatic proteins encoded by 28 presumptive gene loci using standard electrophoresis procedures (Patton et al., 1972; Patton and Yang, 1977; Selander et al., 1971). For details of buffer systems, see Hildner et al. (2003). The 28 loci scored were (Sdh), (Idh-1 and Idh-2), (Pgm), (Mdh-1 and Mdh-2), (Ipo-1 and Ipo-2), (Ldh-1 and Ldh-2), (Pept-1 and Pept-2), (Got-2), (Pgi), (6Pgd), (Me), (Est-2 and Est-3), (Adh-1 and Adh-2), (Xdh-1 and Xdh-2), (Ck-1 and Ck-2), (Ak-1 and Ak-2), and (Gp-2 and Gp-3), of which 12 (those underlined) were polymorphic in at least one population. Protein electrophoresis was conducted on a total of 89 gophers; estimates of heterozygosity (H) were derived from actual counts of presumed heterozygotic genotypes.

DNA fingerprints

DNA fingerprints were produced using MS1 and Jeffreys' 33.15 probes as described in detail elsewhere (Hildner et al., 2003). Briefly, for each gopher, DNA was extracted from tail tissue, purified, digested with *Hae*III, electrophoresed on 1% agarose gels in TAE buffer until bromophenol blue dye had migrated 15 cm, and transferred to Hybond N nylon membrane. Filters were hybridized with Jeffreys' 33.15 probe (Jeffreys et al., 1985) conjugated to alkaline phosphatase at 52°C for 25 min, washed according to the probe manufacturer's instructions (Lifecodes, Stamford, CT, USA) and subjected to autoradiography after applying CDP-star substrate (Tropix, Foster City, CA, USA). Filters were then stripped of old probe and hybridized with MS1 (Jeffreys et al., 1988) for 30 min at 52°C. Individuals from the same subspecies were electrophoresed on the same gel, and band sharing was only measured within gels. DNA fingerprints were successfully conducted on 84 gophers; the average level of genetic similarity in each population was measured as the mean band similarity (S). Here, we present the average band dissimilarity (D=1–S) (Soulé and Zegers, 1996), a value that is significantly correlated with average heterozygosity (Stephens et al., 1992).

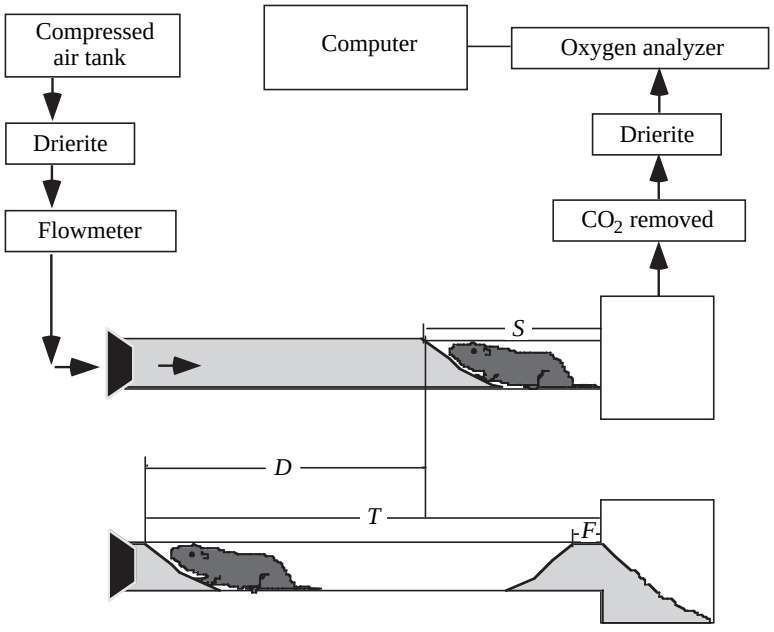

Fig. 1. Schematic of burrowing apparatus with distance measurements. Distance from the starting end of the tube was recorded to the nearest tenth of a centimeter at the commencement of burrowing (S) and when the gopher reached the end of the tube or stopped burrowing consistently (T). The total distance burrowed (D) was then calculated as D=T–S. In cases where the gopher did not push all of the sand out of the tube and into the chamber, the amount of the tube that was filled with sand (F) was also recorded to the nearest tenth of a centimeter.

Analysis

To test whether populations with low genetic variability have relatively high burrowing costs, 3-way ANCOVAs were conducted using the program JMP (SAS, Cary, NC, USA) with subspecies, genetic variability class (high/low) and sex as main effects, and log(mass) as a covariate. All main effects were treated as fixed. The dependent variables were cost of burrowing (log ml O_2 g^{-1} kgm^{-1}) and oxygen consumption during burrowing (log ml O_2 g^{-1} h^{-1}). Using the methodology described in Quinn and Keough (2002), the full model was run to test for heterogeneity of slopes, and the interactions between the covariate and the main effects were removed because no evidence of heterogeneity was found. Non-significant interactions among the main effects were also removed using a conservative criterion of P>0.25. Only results of the reduced model are presented here.

In the ANCOVA for cost of burrowing, error variances were heteroscedastic (Levene's test P=0.04). For this reason, we also ran the analysis using a reciprocal transformation of cost of burrowing (Y=cost^{-1}) as the dependent variable, which resulted in homoscedastic error variances.

Results of this ANCOVA were qualitatively the same as those for the ANCOVA with log-transformed cost of burrowing as the dependent variable, but the *P*-values for genetic variability and the interaction between genetic variability and subspecies were smaller (P=0.004 and P=0.003, respectively). Because our results and interpretation are unaffected, we only present the results of the original analysis.

In order to extrapolate the results of the analyses of covariance to subspecies not included in our analyses, subspecies would need to be treated as a random effect. We considered subspecies as a fixed effect here, which should be accounted for in extrapolating from our results. Treating subspecies as a random effect drastically reduces the denominator degrees of freedom (for cost of burrowing from 67 to 2). Therefore, a random effect model would reduce the power so much that it would be impossible to detect an effect of genetic variability without much larger sample sizes (J. Estes, personal communication).

Although cost of burrowing was measured at the individual level, we characterized genetic variability at the population level. Ideally, individual-level statistical analyses would be performed, while including terms for common population-level factors other than genetics. The small sample sizes, however, combined with the strong differences between populations in genetic variability (with some populations having almost no genetic variability), precluded this approach. To address the concern that our results may have arisen from population level effects unrelated to genetics, we used the Akaike Information Criterion (corrected for small sample size=AIC_c) and Akaike weights to compare the above ANCOVA model with one in which population (nested within subspecies) replaces genetic variability class (population model) (Burnham and Anderson, 1998). A total of four models were compared: (1) genetic model (ANCOVA described above) with no interaction terms, (2) genetic model including interaction terms between genetics and other factors, (3) population model with no interaction terms and (4) population model with interaction terms. If the differences among populations are due to factors other than genetic variability, the population models should provide a better prediction of patterns in the dependent variables than the models ascribing effects to genetic differences.

Mass-adjusted values for cost of burrowing were calculated using the following equation:

$$Y_{Adj} = Y\,(\text{mean } m/m)^b ,$$

where Y_{Adj} is the mass-adjusted cost of burrowing, Y is cost of burrowing (mean from the two trials), m is the mass of the animal (mean from the two trials; change in mass between the trials was not statistically significant, paired *t*-test P=0.33) and b is the slope of the regression of log of cost of burrowing on log of mass [$\log(Y)$=2.374–1.279 $\log(m)$; P<0.0001 r^2=0.44]. Mass range for the burrowing experiments was 58.2–230.7 g with a mean of 120.0±36.3 g.

Descriptive statistics, *t*-tests and correlations were performed using Statview 4.51 (Abacus Concepts, Inc., Berkeley, CA, USA). Means are reported as mean ± 1 S.D., unless otherwise noted.

Results

Oxygen consumption and burrowing costs

We measured burrowing oxygen consumption for two trials for each of 81 gophers; there were sufficient data to calculate the cost of burrowing for 80 of those individuals. Among individuals, mean oxygen consumption during burrowing for the two runs ranged from 2.05 to 5.99 ml O_2 g^{-1} h^{-1} with a mean of 3.97±0.73 ml O_2 g^{-1} h^{-1} (N=81 gophers). Values of burrowing oxygen consumption in this study agree well with those measured by Vleck (1979; 2.8–7.1 ml O_2 g^{-1} h^{-1}). Mass-adjusted oxygen consumption during burrowing ranged from 2.22 to 5.20 ml O_2 g^{-1} h^{-1} with a mean of 3.90±0.59 ml O_2 g^{-1} h^{-1}. Mean cost of burrowing for the two runs ranged from 0.17 to 2.44 ml O_2 g^{-1} kgm^{-1} with a mean of 0.66±0.43 ml O_2 g^{-1} kgm^{-1} (N=80 gophers). Mass-adjusted cost of burrowing ranged from 0.17 to 1.52 ml O_2 g^{-1} kgm^{-1} with a mean of 0.57±0.25 ml O_2 g^{-1} kgm^{-1}. The two burrowing trials for each gopher were significantly correlated for cost of burrowing (log ml O_2 g^{-1} kgm^{-1}; correlation Z test: r=0.75, P<0.0001) and burrowing oxygen consumption (log ml O_2 g^{-1} h^{-1}; correlation Z test: r=0.84, P<0.0001).

Relationship between cost of burrowing and genetic variability

For each of the three subspecies, the three measures of genetic variability consistently ranked one population as having low genetic variability relative to its paired population. Genetic variability results are summarized in Table 1 and are described in Hildner et al. (2003). Cost of burrowing was significantly greater in the populations with low genetic variability than in those with high variability (ANCOVA P=0.015; Table 2; Fig. 2). Mass-adjusted cost of burrowing in the low variability populations averaged 0.57±0.24 ml O_2 g^{-1} kgm^{-1} and that in the high variability populations averaged 0.42±0.19 ml O_2 g^{-1} kgm^{-1}. 6.1% of the total sums of squares was explained by genetic variability. As expected, there was a significant negative relationship between log-transformed cost

Table 2. *Effect of genetic variability class (high/low), sex, subspecies and log(mass) (covariate) on the log-transformed cost of burrowing (r^2=0.61)*

	ANCOVA			
Source of variation	d.f.	SS	*F*-ratio	*P*
Genetic variability (A)	1	0.179	6.221	0.015
Sex (B)	1	0.057	1.989	0.163
Subspecies (C)	2	0.039	0.683	0.509
AB	1	0.059	2.044	0.157
AC	2	0.173	3.017	0.056
ABC	2	0.098	1.701	0.190
Log(mass)	1	0.339	11.792	0.001
Residual	69	1.984		

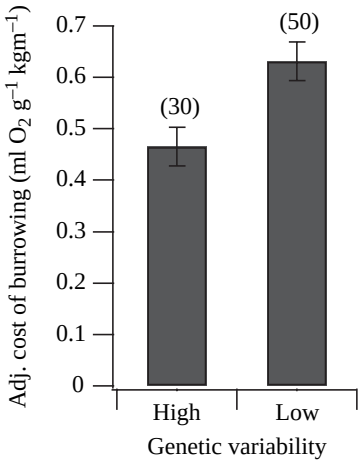

Fig. 2. The mass-adjusted cost of burrowing for individuals from high and low genetic variability populations (see Table 2). Error bars represent ±1 S.E.M. Sample sizes are in parentheses.

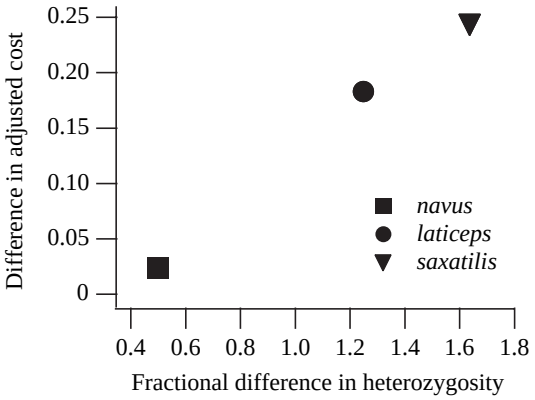

Fig. 3. Difference in adjusted cost *versus* fractional difference in allozyme heterozygosity. Each point represents one pair of populations. The ordinate is the difference in mass-adjusted cost of burrowing for the two populations, and the abscissa is the difference in allozyme heterozygosity for the two populations divided by the mean heterozygosity for that pair.

of burrowing and log(mass) (ANCOVA P=0.001). There was no effect attributable to the sex or subspecies of the gophers. The interaction between subspecies and genetic variability class, however, approached significance (P=0.056) because the magnitude of the effect of genetic variability on cost of burrowing differed for different subspecies.

Individuals from populations with lower genetic variability had a higher cost of burrowing in all three subspecies, but the difference in mass-adjusted cost of burrowing (C_{Adj}) was greatest in the two subspecies with the greatest difference in genetic variability, *laticeps* (t-test P=0.0265) and *saxatilis* (t-test P=0.0001), and was not significant in subspecies *navus* (t-test P=0.797). The C_{Adj} of gophers from the low genetic variability population was 47% higher in *laticeps*, 79% higher in *saxatilis* and 6% higher in *navus* than that of gophers from the corresponding high variability population. Although not statistically significant, there was a trend among the three subspecies for the difference in C_{Adj} to be positively correlated with the difference in genetic variability as a fraction of the pair's average allozyme heterozygosity (Kendall's Tau=1.0, P=0.12; Fig. 3). In other words, subspecies with greater difference in allozyme heterozygosity also had greater difference in cost of burrowing.

Results of the AIC_c analysis (see Materials and methods) indicated that the genetic model with no interactions had the lowest AIC_c value and hence provided the best fit to the data. Akaike weights estimate a relative likelihood of 0.70 that the genetic model with no interactions is the best explanation of the data. The population model with no interactions provided the second best explanation of the data, with an Akaike weight of 0.26.

As with cost of burrowing, oxygen consumption during burrowing was significantly dependent on genetic variability. Individuals from low genetic variability populations had significantly higher oxygen consumption during burrowing than those from the paired high variability populations (ANCOVA P=0.02, N=81). As expected, there was a significant negative relationship between log-transformed burrowing oxygen consumption and log(body mass) (ANCOVA P=0.0009). None of the other factors or interactions had a significant effect on burrowing oxygen consumption.

Discussion

Gophers from genetically less variable populations appear to have a higher cost of burrowing than those from populations with high genetic variability. Based on the Akaike weights, the genetic model with no interactions provided a much better explanation of the data than the population model with no interactions (Akaike weights=0.70 and 0.26, respectively), indicating that differences in level of genetic variability provide a more effective explanation of burrowing efficiency than do other population differences. In addition, the two subspecies (*laticeps* and *saxatilis*) with significantly different levels of allozyme heterozygosity between the populations (for statistical tests, see Hildner et al., 2003) also had the greatest difference in cost of burrowing between the populations. *Navus*, the subspecies with the least difference in allozyme heterozygosity among populations, showed comparatively similar burrowing costs between the populations. Among all six populations, the two with exceptionally low genetic variability, found at Patrick's Point and Susanville, had the highest overall cost of burrowing.

Our results are consistent with those in a number of previous studies that demonstrate fitness consequences of low genetic variability (for a review, see Mitton, 1997). For example, Mitton and co-workers have shown that within populations of the tiger salamander (*Ambystoma tigrinum*), both growth rate and scope for activity increase with allozyme heterozygosity (Mitton et al., 1986; Pierce and Mitton, 1982). Also, in one of the few studies on a mammal, Teska et al. (1990) found that, on a low quality diet, more heterozygous individuals of the old field mouse, *Peromyscus polionotus*, had higher digestive

efficiencies and maintained body mass better than individuals with lower heterozygosity.

It should be noted that because we measure genetic variability at the population level, there are two possible causes for the association between genetic variability and burrowing efficiency found in the present study. One possibility is that the differences in fitness are caused by individual-level heterozygosity effects such as overdominance (heterozygote advantage) at individual loci or associative overdominance effects (see, for example, Pogson and Zouros, 1994). A more probable explanation is that the low genetic variability of some gopher populations is the result of inbreeding coupled with genetic drift in small, isolated populations, and therefore the lower burrowing efficiency in these populations is a reflection of homozygosity for deleterious alleles, causing inbreeding depression (for a recent meta-analysis, see Reed and Frankham, 2003). The present study does not provide sufficient information to distinguish between these alternatives. Regardless of the specific mechanism, however, our results demonstrate a significant association between genetic variability and physiological efficiency among populations.

Although correlation does not prove causation, our results suggest that differences in genetic variability influence burrowing efficiency. If the high cost of burrowing of individuals in the low genetic variability populations is indeed caused by their low genetic variability, one would expect to observe effects in other characters as well. In fact, we do see such effects; gophers from the low variability populations have both lower digestive efficiencies on a low quality diet (Hildner, 2000) and lower growth rates (Hildner et al., 2003) than gophers from the genetically more variable populations.

In the strict sense, fitness is defined as "*the average ability of organisms with a given genotype to survive and reproduce*" (Snyder et al., 1985). In practice, however, many surrogates of fitness have been used in studying the relationship between genetic variability and fitness, including such characters as developmental stability (Mitton, 1997) and growth rate (Hildner et al., 2003; Mitton, 1997). The relevance of a particular physiological trait to an individual's fitness is not always apparent, but burrowing efficiency is clearly important to the survival and reproduction of pocket gophers. Pocket gophers spend most of their time underground and they rarely venture more than a few body lengths from their burrow openings (Howard and Childs, 1959). In addition, survivorship appears related to burrowing efficiency. In a study by Sanjayan (1997) using *T. bottae* from a single population, individuals with lower cost of burrowing were more likely to survive between their release in the spring and the following winter.

Gophers are a favored prey of many avian and mammalian carnivores and, because they rarely venture far from their burrow openings, the extensiveness of the burrow system is correlated with a gopher's access to food. Indirect evidence that gophers try to limit their exposure to above-ground predators comes from a study in which the above-ground movements of gophers (*T. talpoides*) in an alfalfa field appeared to be tied to the height of the surrounding vegetation (Proulx et al., 1995); in addition, above-ground movements were less frequent and less extensive when vegetation was shorter, possibly because of the increased risk of predation. We have shown that gophers from low variability populations have higher metabolic costs of burrowing than gophers from populations with higher genetic variability and will therefore need to spend more time digging, on average, in order to obtain the same net energy gain as gophers from high variability populations or will need to spend more time foraging above ground, increasing their risk of predation.

Burrowing is an energetically costly activity for gophers. It has been estimated that the energy expended while burrowing is 360–3400 times that of moving the same distance over the surface (Vleck, 1979). The amount of burrowing necessary to meet a gopher's energy demand on a particular day varies with habitat, season and forage quantity and quality (Andersen and MacMahon, 1981; Loeb, 1987). For *T. talpoides*, a Rocky Mountain species, the average daily energy needs are between 2 and 10 h of burrowing per day, and the most common cause of death is thought to be lack of food caused by stochastic weather events that affect the rate of burrowing, thus altering energy acquisition rates (Andersen and MacMahon, 1981). Thus, everything else being equal, a reduced cost of burrowing translates into more energy available for growth and reproduction.

Previous studies have shown that gopher fitness is associated with the ability to acquire adequate forage. For example, Loeb (1981) showed that *T. bottae* in an irrigated alfalfa field had significantly larger body sizes and nearly twice the reproductive rates of those in a non-irrigated field. These differences in size and reproductive rates were probably due to year-round availability of high quality forage in the irrigated habitat (Loeb, 1981).

Based on our results, gophers from high variability populations are likely to have a foraging advantage over those from low variability populations. The cost of burrowing of gophers from populations with low genetic variability was 6–76% higher than that of gophers from high variability populations, and the two subspecies with the largest difference in genetic variability, *laticeps* and *saxatilis*, also had the largest difference in cost when comparing high and low variability populations (47 and 79%, respectively). These values, however, are almost certainly an underestimate of the energetic advantage of gophers from the more heterozygous populations because they do not take into account that gophers from high variability populations also have significantly higher digestive efficiencies (Hildner, 2000).

Finally, are such differences in vigor likely to translate into differences in population persistence? Other studies suggest that genetically less variable populations do indeed have a lower probability of persistence (Frankham, 1995; Saccheri et al., 1998; Westemeier et al., 1998), especially during periods of environmental stress (Bijlsma et al., 2000). Any loss of genetic variability that results in decreased physiological vigor or efficiency can hasten extinction because it can decrease survival and reproduction and lead to further decreases in population size and hence to more severe inbreeding and

genetic drift – the extinction vortex (Gilpin and Soulé, 1986). We predict, therefore, that low variability gopher populations will have a significantly higher extinction risk than their more genetically variable counterparts. Further studies are needed to test this prediction and the generality of our results to other populations and species.

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