

Allometric engineering: an experimental test of the causes of interpopulational differences in performance.

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Allometric Engineering: An Experimental Test of the Causes of Interspecific Differences in Performance

EVER SINCE HUXLEY [1] FIRST DREW attention to the biological significance of relative size and shape, evolutionary and functional biologists have studied the allometric scaling of diverse morphological, physiological, and ecological traits [2]. Allometric equations not only quantify the size dependence of a trait, but can also permit comparisons among individuals, populations, or species [3, 4] that differ in body size. Consequently, allometric analyses are often a key step in tests of hypotheses of trait evolution [2-5]. Nevertheless, such analyses involve statistical, not experimental, adjustments of body size [6]. Moreover, inferences about the proximate or mechanistic causes of dramatic differences in the intercept and slope of the allometry among taxa are risky, because many factors influence morphological and physiological traits [7].

Here we apply a novel method for experimentally manipulating body size, and we use this method to explore the mechanistic bases for interpopulational differences in the allometry of locomotor performance and morphology of hatchling lizards (*Sceloporus occidentalis*). Compared with hatchlings from northern populations (Oregon and Washington), hatchlings from a southern population (California) are large, have long hindlimbs, and have high burst speed and cruising stamina (Figs. 1 and 2). The high locomotor performances of southern hatchlings might be a mechanistic consequence of large body size (Fig. 1), of relatively long limbs [8] (Fig. 2A), or of other physiological or morphological differences. The involvement of these factors can potentially be tested at least three ways. First, the small northern hatchlings could be raced once they had grown to the size of (large) southern hatchlings, but this comparison would confound size and age. Second, analysis of covariance can often be used to determine if differences in a trait persist when body size is adjusted statistically, but such analyses may be misleading because causal factors responsible for trait divergence among populations may be different from the factors determining allometric scaling among individuals within a population. Third, between-population overlap in hatchling body size can be experimentally increased by removing yolk from the large southern eggs [9], thereby producing southern hatchlings that are similar in size to the small northern hatchlings (Fig. 1). If performances of size-matched hatchlings from the north and south are now

comparable, then the observed interpopulational differences in performance would appear to be a simple allometric consequence of interpopulational differences in egg size. Alternatively, if locomotor performances of southern hatchlings are still high despite size standardization, then the observed interpopulational differences should be attributable to other evolved differences, not just to size.

We obtained eggs laid in captivity by females (southern California, central Oregon, and southern Washington) during May and June 1988 [10]. To produce miniaturized hatchlings (cover and Fig. 1) from the southern population, we partially removed yolk from some of the freshly laid eggs of California females [11]. A few eggs from each clutch were unmanipulated (control), and a few eggs were poked with the syringe but had no yolk removed (sham-manipulated) [11]. Yolk removal produces miniaturized hatchlings because egg size and hatchling size scale with near isometry in both unmanipulated and manipulated eggs of *S. occidentalis* [9]. The yolk-reduced, sham-manipulated and unmanipulated eggs resulted in a graded size series of hatchlings (see cover for a comparison of the resultant range of sizes of hatchlings from a single clutch). Additionally, many clutches were unmanipulated [12] and thus provide among-population comparative data. All eggs (hatchlings) were incubated (raised) under standardized conditions [9]. When hatchlings reached 3 weeks of age, we measured their size, hindlimb span, and maximum burst speed on a laboratory racetrack [13]. One to 2 weeks later, we measured their stamina on a slowly moving treadmill [14].

Some of the interpopulation differences in stamina persisted despite experimental reduction in body size [15] (Fig. 2B). Miniaturized hatchlings from California still had much higher stamina than did those from Washington but not hatchlings from Oregon [15] (Fig. 2B). Thus, significant interpopulation differences in stamina of hatchlings, though in part an allometric consequence of differences in egg and hatchling size, are in large part due to other mechanistic causes, presumably those affecting aerobic capacity [4, 16].

In contrast, interpopulational differences in burst speed disappeared when body size was standardized and thus were causally related to interpopulational differences in egg size and thus hatchling size (Fig. 2C). Miniaturized southern hatchlings were no faster than were similarly sized northern hatchlings [17]. Moreover, because miniaturized southern hatchlings from California still had

Allometric engineering: an experimental test of the causes of interpopulational differences in performance.

longer legs [18] (Fig. 2A) but not faster speeds (Fig. 2C) relative to northern hatchlings, interpopulational differences in burst speed are unlikely to be purely a mechanistic consequence of differences in relative hindlimb length, despite presumed biomechanical links between these traits [4, 8].

Developmental manipulation of body size ("allometric engineering") adds to comparative biology a powerful new experimental dimension that can be used with diverse taxa [9, 19]. Adult size can also be manipulated by the use of genetic engineering of the hormonal control of growth rate [20]. However, this technique can currently be applied in only a few taxa. Size manipulation by either technique may allow comparisons between populations with limited overlap in body size [21], thereby permitting inferences on the proximate causes of trait evolution. Moreover, both techniques provide a direct experimental, not merely statistical, evaluation of the proximate influence of body size. For example, manipulation of hatchling size shows that interpopulational differences in sprint speed are probably an allometric consequence of interpopulational differences in egg size, but that interpopulational differences in stamina and morphology, though in part due to size, necessarily involve additional evolved factors [4, 16, 22]. A comparison of experimental with traditional analysis of covariance (ANCOVA) analyses [15, 17, 18] demonstrates that purely statistical analyses of patterns can sometimes present a misleading portrait of the role of body size in populational differentiation in locomotor performance and morphology.

Of course, size manipulation [9, 19, 20] provides insights only into proximate--not ultimate--causes of interpopulational variation in traits. For example, our results do not suggest whether contemporary interpopulational patterns reflect natural selection in southern populations for large size or fast speed or both. Nevertheless, size manipulation does show that selection on size alone is unlikely to account for all the major interpopulational differences in locomotor performance (or the converse). Moreover, if the relative fitness of size-manipulated animals is measured in natural populations, some insights into the ultimate causes of interpopulational variation can be gained [9, 23].

REFERENCES AND NOTES

- [1] J. S. Huxley, *Nature* 114, 895 (1924).
- [2] Allometric equations are power functions of the form $y = [am.sup.b]$, where m is usually body size (mass). S. J. Gould, *Biol. Rev.* 41, 587 (1966); W. A. Calder, *Size, Function and Life History* (Harvard Univ. Press, Cambridge, MA, 1984); R. H. Peters, *The Ecological Implications of Body Size* (Cambridge Univ. Press, Cambridge, 1983); K. Schmidt-Nielsen, *Scaling: Why Is Animal Size So Important?* (Cambridge Univ. Press, Cambridge, 1984).
- [3] P. H. Harvey and G. M. Mace, in *Current Problems in Sociobiology*, King's College Sociobiology Group, Ed. (Cambridge Univ. Press, Cambridge, 1982), pp. 343-361.
- [4] T. Garland, Jr., *Am. J. Physiol.* 247, R806 (1984).
- [5] R. Lande, *Evolution* 33, 402 (1979); B. Riska and W. R. Atchley, *Science* 229, 668 (1985).
- [6] Because different curve-fitting models often yield different results, any conclusions may be model dependent [M. D. Pagel and P. H. Harvey, *Science* 244, 1589 (1989)].
- [7] R. Levins and R. C. Lewontin, *The Dialectical Biologist* (Harvard Univ. Press, Cambridge, MA, 1985); R. Hilborn and S. C. Stearns, *Acta Biotheor.* 31, 145 (1982).
- [8] In general, speed and stamina are typically correlated with body size within populations. Moreover, long hindlimbs are thought to enhance speed by increasing stride length [V. B. Sukhanov, *General System of Symmetrical Locomotion of Terrestrial Vertebrates and Some Features of Movement of Lower Tetrapods* (Nauka, Leningrad, translation into English by Amerind, New Dehli, 1974); M. Hildebrand, in *Functional Vertebrate Morphology*, M. Hildebrand, D. M. Bramble, K. F. Liem, D. B. Wake, Eds. (Belknap, Cambridge, MA, 1985), pp. 27-38; T. Garland, Jr., *J. Zool. London Ser. A* 207, 425 (1985); D. B. Miles, *Am. Zool.* 27, 44A (abst.) (1987); H. L. Snell, R. D. Jennings, H. M. Snell, S. Harcourt, *Evol. Ecol.* 2, 353 (1989)]. For a counter example see J. Tsuji, R. B. Huey, T. Garland, Jr., R. G. Shaw, *ibid.* 3, 240 (1989) and [4].
- [9] B. Sinervo, *Evolution* 44, 279 (1990).
- [10] Near-term, gravid females were collected near Wrightwood, CA, on the east side of Table Mountain (altitude, 2230 m), near Terrebonne, OR (750 m), and near Lyle, WA (200 m).
- [11] Ten to 50% of total egg mass was removed with a 26[degrees] sterile syringe ($n = 13$ clutches). Incubation success of sham-manipulated controls and size-reduced eggs is similar, ~70%, as is post-hatching survival in the laboratory [9]. Size reduction does not artifactually influence growth rate [9]. Moreover, because the size-reduced lizards from California still have very high

Allometric engineering: an experimental test of the causes of interpopulational differences in performance.

stamina, a stressful test of physiological capacity, size-reduction does not appear to hamper performance artifactually.

[12] Washington (n = 7 clutches), Oregon (n = 16 clutches), and California (n = 9 clutches). The offspring from unmanipulated clutches obtained from California females were comparable to the shammanipulated and unmanipulated controls obtained from experimentally manipulated clutches [11] with regard to hatchling size (Fig. 1), speed, and stamina (Fig. 2, B and C).

[13] Mass (in grams), hindlimb span (millimeters measured between the fourth toe on each hind leg with hind legs stretched out laterally to either side). Individuals were raced six times (with 1-hour rest between races) by chasing them along a 1-m "racetrack." As a lizard ran down the racetrack, it interrupted photoreceptors spaced at 10-cm intervals, and the interval time between light stations was electronically sampled by a computer [R. B. Huey and P. E. Hertz, *J. Exp. Biol.* 110, 113 (1984); D. B. Miles and R. G. Smith, *Funct. Ecol.* 1, 281 (1987)]. Hatchlings were raced at 34[degrees]C, the optimal temperature for sprinting and the approximate average field body temperature [A. F. Bennett, *Anim. Behav.* 28, 752 (1980); F. H. van Berkum, *Am. Nat.* 132, 327 (1988); J. S. Tsuji, *Physiol. Zool.* 61, 230 (1988)]. Maximum burst speed was the single fastest speed (centimeters per second) over any 20-cm interval from among all six trials.

[14] To determine "cruising stamina" we placed hatchlings on a rubberized belt of a treadmill moving at 0.25 km/hour. When necessary to keep them running smoothly, we tapped them lightly on the tail and hindlimbs. Stamina was measured as the elapsed time (minutes) until a hatchling was exhausted, as verified by the loss of the righting response [F. H. van Berkum, R. B. Huey, J. S. Tsuji, T. Garland, Jr., *Funct. Ecol.* 3, 97 (1989)].

[15] Because the size of Oregon hatchlings and miniaturized hatchlings from California was similar, these populations could be compared without the confounding effects of size. This ANCOVA (log-transformed variables) indicated that the difference in stamina between Oregon and California hatchlings [0.25 log (min)] was not significant [$F(1,78) = 2.53$, P [is greater than] 0.12; covariate for size not significant (ns), P [is greater than] 0.60]. ANCOVA comparing Washington hatchlings and experimentally miniaturized hatchlings from California indicated that the difference between populations (0.59) was significant [$F(1,86) = 20.98$, P [is less than] [is less than] 0.0001; covariate for size ns, P [is greater than] 0.81]. It is illuminating to compare the results obtained from this experimental analysis with a purely statistical

analysis in which traditional ANCOVA of the data from the unmanipulated population samples was used. The traditional ANCOVA indicated that the difference between unmanipulated Oregon and California populations (0.41) was significant [$F(1,82) = 7.77$, P [is less than] 0.007; covariate for size ns, P [is greater than] 0.60], as was the difference between Washington hatchlings and unmanipulated hatchlings from California [difference, 0.80; $F(1,90) = 48.82$, P [is less than] [is less than] 0.0001; covariate for size ns, P [is greater than] 0.86]. However, the population comparisons with traditional ANCOVAs overestimated the level of population differentiation, because a significant allometric effect of size on stamina was not detected in these analyses in contrast to analyses using the full range of size-manipulated hatchlings [$F(1,45) = 3.82$, $P = 0.06$, $n = 47$] (Fig. 2B). Moreover, the analysis using experimentally miniaturized hatchlings is not sensitive to problems arising from ANCOVA estimates of the allometric slope [6], because the size of comparison populations is closely matched in contrast to traditional ANCOVA population comparison.

[16] E. R. Weibel and C. R. Taylor, Eds., *Respir. Physiol.* 44, 1 (1981); H. B. John-Alder, *Am. J. Physiol.* 244, R659 (1983); A. F. Bennett, R. B. Huey, H. B. John-Alder, *J. Comp. Physiol. B* 154, 113 (1984); G. E. Walsberg, M. S. Lea, S. S. Hillman, *J. Exp. Zool.* 239, 1 (1986).

[17] A comparison (ANCOVA of log-transformed data) of the burst speed of size-matched hatchlings from California with hatchlings from the two northern populations indicated that differences in each population were not significant [difference between California and Oregon, 0.018 log(m/s); $F(1,134) = 0.50$, P [is greater than] 0.84; covariate body mass ns, P [is greater than] 0.90; difference between California and Washington, -0.006 log(m/s); $F(1,151) = 0.03$, P [is greater than] 0.86; covariate for body mass ns, P [is less than] 0.02). In contrast, traditional ANCOVA using data from the unmanipulated population samples indicates that southern and northern hatchlings are different [difference between California and Oregon, 0.077; $F(1,134) = 4.663$, P [is less than] 0.03; covariate body mass, ns, P [is greater than] 0.90; difference between California and Washington, 0.063; $F(1,151) = 3.178$, P [is less than] 0.07; covariate for body mass significant, P [is less than] 0.03]. However, the population comparisons using traditional ANCOVAs overestimated the level of population differentiation because a significant allometric effect of size on burst speed was detected inconsistently in these analyses in contrast to analyses based on the full range of size-manipulated hatchlings [$F(1,56) = 6.48$, $P = 0.01$, $n = 58$] (Fig. 2C).

[18] Hindlimb span was shorter for northern hatchlings

Allometric engineering: an experimental test of the causes of interpopulational differences in performance.

[ANCOVA: factor for population $F(2,199) = 118.6$, P [is less than] [is less than] 0.01; covariate for size, $F(2,199) = 205.0$, P [is less than] [is less than] 0.01, difference in slopes, ns). However, some of the difference between the hindlimb span of northern and southern populations arises from an effect of yolk mass on morphology [ANCOVA comparing unmanipulated California hatchlings with experimentally miniaturized hatchlings and their full-sized sibs indicates that yolk volume has a significant effect on morphology, slopes were significantly different between these groups, $F(1,73) = 5.95$, P [is greater than] 0.02] (Fig. 2A).

[19] B. Sinervo and L. R. McEdward [Evolution 42, 885 (1988)] list some vertebrates and invertebrates in which larval or juvenile size can be manipulated.

[20] R. D. Palmiter, G. Norstedt, R. E. Gelinas, R. E. Hammer, R. L. Brinster, Science 222, 809 (1983).

[21] Differences in size are often implicated as mechanistic correlates of interpopulational or interspecific differences [S. J. Gould, Evolution 28, 191 (1974)], but these implications are statistical inferences only.

[22] G. F. Oster, N. Shubin, J. D. Murray, P. Allberch, Evolution 42, 862 (1988).

[23] J. A. Endler, Natural Selection in the Wild (Princeton Univ. Press, Princeton, NJ, 1986); T. Mitchell-Olds and R. G. Shaw, Evolution 41, 1149 (1987); D. Schluter, *ibid.* 42, 849 (1988).

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