Allometric Engineering: A Causal Analysis of Natural Selection on Offspring Size

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Techniques of offspring size manipulation, "allometric engineering," were used in combination with studies of natural selection to elucidate the causal relation between egg size and offspring survival of lizards. The results experimentally validate premises underlying theories of optimal egg size: fecundity selection favoring the production of large clutches of small eggs was balanced by survival selection favoring large offspring. However, large hatchlings did not always have the highest survival, contrary to most theoretical expectations. Optimizing selection on offspring size per se was the most common pattern. Moreover, matches between average and optimal egg size were qualitative, not quantitative, perhaps reflecting known functional constraints on the production of large eggs.

The effect of egg and clutch size on fitness has been a central focus in evolutionary ecology (*1*) since the work of Lack (*2*, *3*). For organisms with extended parental care (for example, birds and mammals), Lack predicted that natural selection favors the clutch (or litter) size that produces the most surviving offspring (*2*). He tested this hypothesis by manipulating clutch size, and this elegant protocol has become the standard in avian studies. For organisms without parental care (for example, most amphibians, reptiles, fish, and insects), Lack (*3*) realized that natural selection should instead favor a compromise (optimizing selection) between the quantity and quality (size) of offspring. His prediction was based on two premises: (i) egg size is inversely related to clutch size (egg number), but (ii) offspring quality and hence offspring survival is positively related to egg size. Optimizing selection results from the conflict between fecundity selection (presumably favoring many small eggs) and survival selection (presumably favoring a few large eggs). At evolutionary equilibrium the average egg size should match the optimum. The first premise is usually supported in descriptive studies (*4*) and experimental tests (*5*). However, with the exception of a few descriptive studies (*6*, *7*), the second premise has not been tested by direct manipulation of offspring size analogous to manipulations of clutch size. Therefore, we set out to test the second of Lack's premises. We combined new techniques of offspring size manipulation (*4*, *5*), "allometric engineering" (*8*), with a field study of natural selection to determine the causal relation between egg size and offspring quality as indexed by survival in natural populations of a lizard.

We collected eggs from reproductive side-blotched lizards (*Uta stansburiana*), a small iguanid lizard (3 to 10 g) that matures in 1 year, at two localities in the inner Coast Range of California, along Del Puerto Canyon Road (Stanislaus County, California) and near Los Baños Grandes along Billy Wright Road (Merced County, California) (*9*). Females typically lay small eggs (mean of 0.39 g) on the first clutch and larger eggs (0.43 g) on later clutches (*5*, *6*, *10*). Lack's hypothesis would be supported if (i) females that lay intermediate-sized eggs leave the most surviving offspring (optimizing selection); (ii) the optimum egg size is small in the first clutch of the season but larger in later clutches; and (iii) the observed seasonal shifts in average egg size in these clutches coincides with shifts in the optimum.

Optimizing selection can be difficult to detect because the narrow size range of individuals in natural populations (*11*–*13*) results in a low statistical power. Allometric engineering techniques (*4*, *5*, *8*) permitted us to decrease hatchling size by 1.3 SDs and increase hatchling size by 1.6 SDs (*14*). Moreover, by experimentally separating size from other correlated traits (*4*, *5*, *8*), we could verify the causal links between size and fitness (*11*–*13*).

Newborn hatchlings were weighed (g), marked, and released (May through August) within 3 days on the site in small groups in close proximity to female home ranges. A total of 1668 control, miniaturized, and gigantized hatchlings were released at the two study sites over two reproductive seasons (*14*). We recorded offspring survival to 1 month and to maturity (*15*). We used a cubic spline algorithm (*12*) to determine the shape of the fitness function relating offspring survival and offspring size. Significance tests for directional and optimizing survival selection (*16*) were based on jackknife estimates of *t* values (*13*). Hatchlings that were not recaptured during any of these censuses were assumed to have died (*17*). We analyzed patterns of hatchling survival for first-clutch hatchlings separately from later clutches because hatchlings from later clutches must have competed not only with other later clutch hatchlings but also with first clutch hatchlings, which were larger by virtue of their age.

Lack's first prediction, that selection on egg size should be optimizing, was found in seven of eight cases (Fig. 1, except in first clutch Los Baños 1990). Contrary to theory, however, optimizing selection did not usually result from a simple interaction between conflicting directional-selection components. The fecundity component (m_e curve) was invariably directional and favored females that produced large clutches of small eggs, whereas the offspring survival component (l_e curve) was strictly directional and favored the largest egg size in only two of eight cases [later clutch Los Baños (LB) and Del Puerto (DP.) in 1989]. Survival selection was significantly optimizing in three cases (LB first clutch 1989, LB and DP later clutches 1990), optimizing but nonsignificant in two cases (DB first clutch 1989 and 1990; however, if data from the 2 years are pooled, significant optimizing selection is detected, $P < 0.05$), and unrelated to size in one case (LB first clutch in 1990; however, see below).

The discrepancy between the expected pattern of strictly directional survival selection that favors large egg size and observed patterns reflects a complicating effect of season and hatchling sex. At LB, for example, directional selection consistently favored large female hatchlings (Fig. 2, A and B). Nevertheless, size selection on males was complex. In three of four cases, size selection on males to 1 month of age included a significant optimizing component that favored intermediate-sized hatchlings in addition to a significant directional component that favored large hatchlings. In the fourth case, size selection was directional but favored small male hatchlings. The sex of the hatchling is important because aggression (including cannibalism) by adult males, as well as dispersal away from adult male territories, depends on hatchling sex (*16*).

Lack's hypothesis also predicts that the average egg size should track a seasonal shift (if one occurs) in the optimal egg size. The prediction always holds qualitatively. Both average and optimal egg sizes increased seasonally in three of four cases (Fig. 1), and neither shifted in the remaining case (DP 1990) (*18*). Quantitatively, however, average egg size did not shift nearly as much (~11% increase) as did optimal egg size (~50% for cases with a shifting optimum). Moreover, in these cases, the average egg

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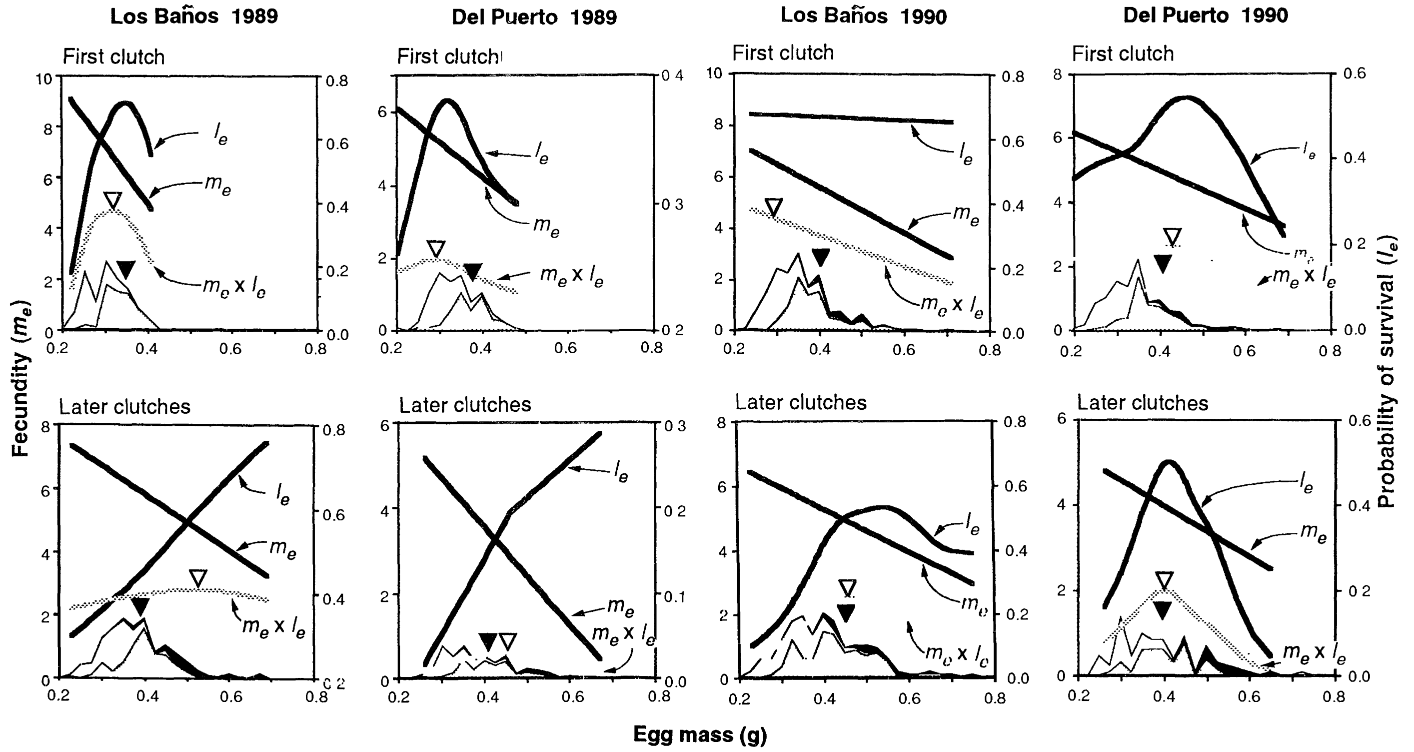

Fig. 1. Each panel plots empirically estimated linear regressions of fecundity (m_e) on egg mass and cubic splines (*12*) of survival probability (l_e for 1 month after release) on egg mass for hatchling lizards from two localities, for clutches one versus later clutches and for 2 years. In all cases m_e curves were significant, negative, and always linear. These relations indicate that fecundity selection was directional and favored females laying small eggs (*22*). In contrast, l_e curves varied in significance and direction, and some curves contained a significant quadratic term, indicative of optimizing selection (*22*). Also plotted are the regressions of $m_e \times l_e$ versus egg size (product of a linear regression versus a cubic spline, shaded lines), which provide an estimate of fitness (equal to the number of surviving young as a function of egg size). Detection of selection was facilitated by experimentally decreasing (white portion of histogram) and increasing (solid portion of histogram) egg size relative to natural variation in egg size (shaded portion of histogram). Mean egg size for natural variation (solid triangles) and optimum egg size (open triangles) are indicated. The optimum egg size is the reproductive strategy that leaves the most surviving offspring (*21*).

size in the first clutch was not as small as the optimum egg size, and the average egg size in later clutches was often not as large as the optimum. The population at DP, which shifted egg size in 1989 but not in 1990, suggests that seasonal changes in egg size were facultative. This observation, as well as the significant between-year variation in average egg size observed between 1989 (a nondrought year) and 1990 (a drought year), indicate that egg size showed pronounced plasticity (*18*).

Known functional and physiological constraints may explain why females do not primarily track the seasonal shifts in optimum egg size. Females with experimentally decreased clutch size, and thus with enlarged eggs (as above), often become egg-bound or produce eggs that burst at oviposition (*5*). A pronounced increase in the probability of such reproductive difficulties occurs at egg sizes >0.48 g (*5*), well below many of the later clutch (seasonal) optima detected in our studies. Females that become egg-bound would ultimately die in natural populations, and thus the production of excessively large eggs required to match the optimum egg size on later clutches would result in a cost of reproduction that decreases future reproductive success of females (*19*).

Offspring size manipulation has two advantages over correlational studies. Experimental manipulations verify causal links between egg size and fitness (*4, 8, 11–13*). These techniques also facilitate the detection of selection (*12*) by increasing the representation of large and small individuals in the population and thus experimentally enhancing the natural variance in offspring size by ~54% (Fig. 1). These advantages can be shown by a comparison of results from an analysis of selection on offspring size that used only natural variation in egg size with analyses that used both natural and experimentally enhanced variation. The patterns of natural selection detected with the restricted sample of natural variation agreed with analyses based on natural and experimentally enhanced variation in all cases, and there were no pathological artifacts arising from our manipulations (*20*).

The best quantitative comparison using only the data for natural variation compared to the full range of natural and experimentally enhanced variation is provided by selection on female hatchlings. For example, survival selection on female hatchlings at 1 month of age appeared to be fairly stable and directional (for example, linear), although the relative strength of selection changed on first versus later clutches (Fig. 2A). In this instance, the size dependence of survival [β coefficient, which measures the intensity of directional selection (*16*)] that was measured with both natural and experimentally enhanced variation in hatchling female survival ($\beta = 0.180$, $P < 0.0001$) was very similar to the intensity of selection that was measured with the restricted data set comprised of natural variation ($\beta = 0.196$, $P < 0.01$). Thus, the enhanced survival of large female hatchlings is causally related to greater maternal provisioning and enhanced egg size.

For male hatchlings, analyses based on experimentally enhanced variation indicated that survival was usually the best for intermediate-sized hatchlings: smallest and largest hatchlings typically had lower rates of survival at 1 month of age. Using a sample restricted to the natural variation in male hatchling size (both populations and years pooled; $n = 240$), we detected marginally significant optimizing selection on the first clutch ($P = 0.06$) that favored intermediate-sized hatchlings. Thus, the survival decrement associated with the

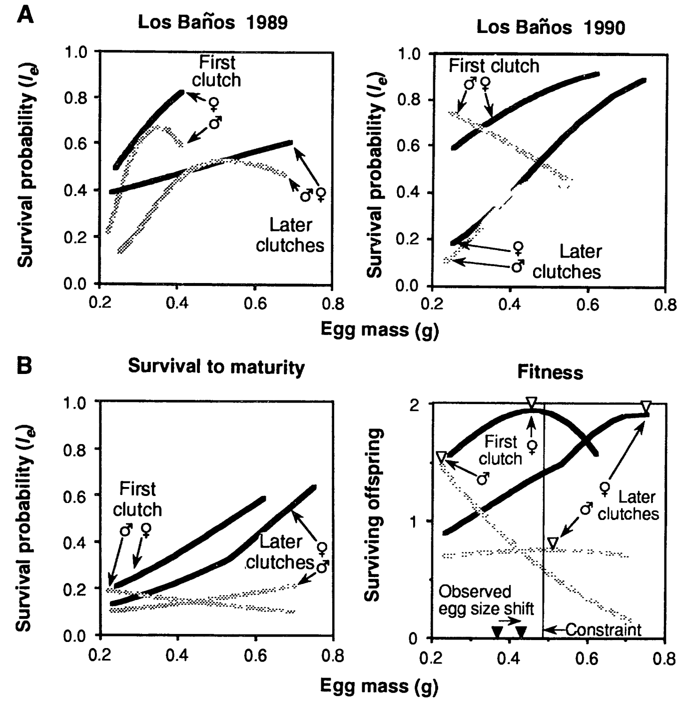

Fig. 2. (**A**) Empirically derived cubic spline (*12*) of survival probability (l_e, to 1 month) versus egg size for male and for female hatchlings from LB, for clutches one versus later clutches and for 2 years. For females, survival is generally positively (and in most cases significantly) related to egg size; for males, however, survival can be positively, negatively, or even nonlinearly related to egg size (*23*). (**B**) Empirically derived cubic spline (*12*) of survival probability (l_e) of offspring to maturity versus egg size for female (solid lines) and male (shaded lines) offspring (LB 1989 and 1990 pooled). Selection on egg mass during the first month of life persists to maturity, and selection was stronger on female offspring (*24*). Fitness (equal to the number of offspring surviving to maturity) was obtained by multiplying m_e curves (see Fig. 1) and l_e curves. Optimum egg size (open triangles) on later clutches was larger than optimum egg size on the first clutch for both female and male offspring, and thus there was selection for a seasonal shift in egg size (solid triangles). First clutch optima for both sexes bracketed observed egg size. However, later clutch optima were much higher than the observed egg size and effectively beyond functional constraints on maximum egg size (*5*).

smallest and largest male hatchlings that was detected in natural variation is causally related to egg size. Moreover, this result demonstrates the enhanced statistical power of experimentally enhanced variation. Whereas the deleterious effects of large and small hatchling size of male offspring were marginally significant with a pooled sample ($n = 240$) that was comprised of only natural variation, such effects were significant in most clutches and most years for the population at LB with relatively small sample sizes (as small as $n = 72$) that were comprised of natural and experimentally induced variation (Fig. 2B). Thus, allometric engineering provides a causal assessment of selection on offspring size that greatly enhances the detection of selection relative to natural variation.

Our results clearly validate Lack's first premise (*2*), which is thought to govern the evolution of egg size: egg number is inversely related to egg size in these and other lizards (*4*, *5*). However, our results question the universality of Lack's second premise (*2*), on which most evolutionary models (*1*) of offspring size are based. Large offspring do not invariably survive best: survival depends on offspring size, season, and even offspring sex. The strength of selection on offspring size also varies between years and between populations (*21*): The most common pattern was a combination of directional and optimizing survival selection for egg size during the first month of life, not directional survival selection, as is commonly believed (*22*). Because we did not detect effects of egg size on body size or fecundity of offspring at maturity, the effects of egg size on fitness were mediated largely by the effects on offspring survival at maturity (Fig. 2B) (*23*). Moreover, the match between average and optimal egg size (Figs. 1 and 2) is only qualitative, not quantitative, perhaps reflecting a known functional constraint on the maximum egg size females can produce. Our allometric engineering techniques, which can be applied to a wide range of egg-laying organisms in addition to other reptiles (*4*, *5*, *8*), could be used to extend the generality of our observations on adaptation and constraint in the evolution of lizard reproductive patterns to other organisms without parental care.

REFERENCES AND NOTES

1. C. C. Smith and S D. Fretwell, *Am. Nat.* **108**, 499 (1974); G. C. Williams, *ibid.* **100**, 687 (1966); *Adaptation and Natural Selection* (Princeton Univ. Press, Princeton, NJ, 1966), H. M. Wilbur, *Am. Nat.* **111**, 43 (1977), S. C Stearns, *Q. Rev. Biol.* **51**, 3 (1976); W. Y Brockelman, *Am. Nat* **109**, 677 (1975); M. A. McGinley, D. H. Temme, M. A. Geber, *ibid.* **130**, 370 (1987).
2. D. Lack, *Ibis* **89**, 302 (1947).
3. ______, *The Natural Regulation of Animal Numbers* (Clarendon, Oxford, 1954).
4. B. Sinervo, *Evolution* **44**, 279 (1990).
5. ______ and P. Licht, *Science* **252**, 1300 (1991); *J. Exp. Zool.* **257**, 252 (1991).
6. G. W. Ferguson and S. F. Fox, *Evolution* **38**, 342 (1984).
7. B. C. Jayne and A. F. Bennett, *ibid.* **44**, 1204 (1990).
8. B. Sinervo and R. B. Huey, *Science* **248**, 1106 (1990). Allometric engineering provides an experimental assessment of the size effects on offspring traits that are detected in correlational analyses. Offspring size is manipulated in lizards by altering levels of yolk utilization in the egg (*4*) or yolk provisioning of the egg by the female (*5*).
9. Near-term gravid female *Uta stansburiana* were collected from March to August 1989 and 1990. All eggs were collected and incubated under standardized conditions (28°C, −200 kPa) (*4*). Hatchling size increased with egg size (*4*, *5*).
10. R. A. Nussbaum, *Oecologia* **49**, 8 (1981).
11. J. A. Endler, *Natural Selection in the Wild* (Princeton Univ. Press, Princeton, NJ, 1986); M. J. Wade and S. Kalisz, *Evolution* **44**, 1947 (1990).
12. D. Schluter, *Evolution* **42**, 849 (1988).
13. T. Mitchell-Olds and R. G. Shaw, *ibid.* **41**, 1149 (1987).
14. To increase the frequency of small hatchlings, we collected near-term females from our study sites and aspirated ~18% of the yolk from half of their freshly laid eggs (*4*, *5*, *8*) This manipulation produced qualitatively normal hatchlings (*4*, *5*, *8*) that were miniaturized by ~18%. The remaining eggs in these clutches served as sham-manipulated (syringe inserted but no yolk removed), full-sized controls. To increase the frequency of large hatchlings, we anesthetized (with metafane) early vitellogenic females and surgically removed the yolk from all but two or three of their follicles (*4*). In this way, yolk normally allocated to the entire clutch was distributed to the two or three remaining follicles as the follicles completed growth. This approach resulted in eggs ~20% larger than those from either sham-manipulated or unmanipulated females (*5*). Survival of offspring from sham-manipulated (anesthesia and surgery but no follicles ablated) and unmanipulated females was similar. Sample sizes for each treatment are presented below by locality, year, and clutch (number of miniaturized hatchlings released, number of controls, and number of gigantized; total number of these hatchlings recaptured at 1 month): LB 1989 first clutch (65, 72, 0; 72), later clutches (98, 117, 32; 85); 1990 first clutch (167, 182, 33; 230), later clutches (64, 91, 21; 68); and DP 1989 first clutch (56, 51, 0; 40), later clutches (104, 87, 25; 38); 1990 first clutch (150, 144, 22; 142), later clutches (30, 42, 15; 31).
15. After the hatchlings were released, we staged several major recaptures on-site (LB, radius ~300 m; DP, radius ~100 m) and in a large, surrounding buffer zone (LB, extending an additional 250 m; DP, extending 200 m). We recaptured hatchlings first during the summer, at about 4 weeks (28 ± 10 days) after they were initially released. We recaptured them again at maturity the following spring (March to May).
16. R. Lande and S. J. Arnold, *Evolution* **37**, 1210 (1983).
17. This assumption was probably valid because dispersal rates into our buffer zone were very low (~5% of all recaptures) [P. E. Doughty, thesis, University of Tennessee, Knoxville (1991)] and capture efficiency during the summer was high (89%) and independent of hatchling size ($P > 0.36$).
18. Analysis of covariance (ANCOVA, with covariate for female postlaying mass significant, $P < 0.05$) of mean egg size revealed significant differences in egg size between years ($P < 0.001$), populations ($P < 0.001$), and first versus later clutches (0.0001), as well as significant population by year, clutch by year ($P < 0.01$), and population by clutch ($P < 0.05$) interaction effects. The interaction between population and clutch was not significant ($P > 0.16$). ANCOVA of egg size (popu-

lations and years treated separately) revealed that the seasonal change in egg size was significant ($P < 0.05$) for LB 1989, LB 1990, and DP 1989, but not for DP 1990 ($P > 0.05$).

19. D. Reznick, *Oikos* **44**, 257 (1985).
20. The lower survival of enlarged hatchling males in most years would suggest that surgical effects on the female parent might cause a drop in offspring quality even though size remains enlarged. This is unlikely for the following reasons. First, we did not detect any effects of sham surgery ($P > 0.56$), follicle ablation ($P > 0.94$) (5), or yolk removal from eggs ($P < 0.47$) (4) except for the comparable effects that manipulations and natural variation have on egg size and offspring survival ($P < 0.01$) (ANCOVA with pooled data set). Second, the pathology would have to be sex-dependent (Fig. 2): the largest female hatchlings always showed high survival at the same time that the largest male hatchlings showed low survival. Third, on the first clutch in 1989 for LB, we did not release experimentally gigantized hatchlings, and thus the decreased survival of large hatchlings was a result of natural variation. Moreover, if we pool all results from both years and populations to estimate selection on the first-clutch male hatchlings with only the natural variation, we detected marginally significant optimizing selection ($P = 0.06$, $n = 240$). Fourth, in the most extreme case we observed directional selection that favored experimentally miniaturized male hatchlings (first clutch for LB 1990, Fig. 2A), and in this case significant directional selection favoring small males was also detected ($P < 0.01$) with only the natural range of variation.
21. We tested (ANCOVA) for differences in 1-month offspring survival as a function of egg size (covariate) across clutches (first versus later clutches), between populations, and between years (as well as higher order interaction terms among these three factors). We partitioned this analysis by sex because female survival curves were linear and male survival curves included a significant optimizing component. For the female offspring, we found that most of the higher order interaction terms involving clutch (for example, first versus later clutches) and the covariate egg size were significant or marginally significant, including clutch by year ($P < 0.05$), clutch by population ($P < 0.05$), clutch by year by egg size ($P = 0.06$), clutch by population by egg size ($P = 0.05$), clutch by population by year ($P < 0.01$), clutch by population by year by egg size ($P < 0.05$), as well as the term for the covariate egg size ($P < 0.05$). Thus, the intensity of directional selection on egg mass for female offspring varies across first versus later clutches, between populations, and between years. For the male offspring, a significant (egg mass)^2 term (optimizing selection) was used as a second covariate along with the covariate egg mass (directional selection). We found significant clutch ($P < 0.01$), egg size ($P < 0.01$), and (egg size)^2 ($P < 0.001$) effects. The only interaction term that was significant was clutch by egg size ($P < 0.01$). Population ($P > 0.05$) and year effects ($P > 0.05$) were not significant, nor were any other higher order interaction terms.
22. Postlaying body mass was used as a second covariate for the calculations of the directional selection coefficients on female fecundity, m_e. Significance levels based on jackknife estimates of t values (13) for the directional (β) and stabilizing (γ) coefficients on fecundity (m_e) and offspring survival (l_e) (15) [where suggested by cubic spline analyses (12)] are as follows: LB 1989, first clutch: l_e ($\beta = 2.686$, $P < 0.01$; $\gamma = -2.495$, $P < 0.05$), m_e ($\beta = -0.618$, $P < 0.0001$); later clutches: l_e ($\beta = 0.162$, $P = 0.05$), m_e ($\beta = -0.397$, $P < 0.001$); 1990, first clutch: l_e [$\beta = -0.007$, $P > 0.05$, not significant (NS)], m_e ($\beta = -0.729$, $P < 0.0001$); later clutches: l_e ($\beta = 1.175$, $P < 0.05$; $\gamma = -0.968$, $P < 0.06$), m_e ($\beta = -0.556$, $P < 0.05$); and DP 1989, first clutch: l_e ($\beta = 0.878$, $P > 0.05$, NS; $\gamma = -1.024$, $P > 0.05$, NS), m_e ($\beta = -0.544$, $P < 0.0001$); later clutches: l_e ($\beta = 0.177$, $P < 0.01$), m_e ($\beta = -0.745$, $P < 0.001$); 1990, first clutch: l_e ($\beta = 0.510$, $P > 0.05$, NS; $\gamma = -0.444$, $P > 0.05$, NS), m_e ($\beta = -0.383$, $P < 0.05$); later clutches: l_e ($\beta = 2.161$, $P < 0.05$; $\gamma = -2.191$, $P < 0.05$), m_e ($\beta = -0.404$, $P < 0.0001$). The balance between fecundity and survival selection ($m_e \times l_e$) yielded an optimum egg size in seven of eight comparisons. The optimum egg size is the reproductive strategy that left the most surviving offspring. In one case (LB 1990, first clutch) there was no optimum per se, and the female that laid the smallest eggs in the population produced the most surviving offspring (arbitrarily defined as the optimum).
23. Significance levels based on jackknife estimates of t values (13) for the directional (β) and stabilizing (γ) coefficients for survival selection (15) [where suggested by cubic spline analyses (12)] are as follows: LB 1989, first clutch (females: $\beta = 0.186$, $P = 0.05$; males: $\beta = 3.536$, $P < 0.02$; $\gamma = -3.361$, $P < 0.03$); second clutch (females: $\beta = 0.079$, $P > 0.05$, NS; males: $\beta = 1.196$, $P = 0.06$; $\gamma = -1.051$, $P = 0.09$); and 1990, first clutch (females: $\beta = 0.150$, $P < 0.02$; males: $\beta = -0.136$, $P = 0.06$); second clutch (females: $\beta = 0.307$, $P < 0.01$; males: $\beta = 1.353$, $P < 0.01$, $\gamma = -1.201$, $P < 0.05$). When both responses were linear (for example, first clutch of 1990), we could test for significant differences between the sexes In this case, selection on egg size differed significantly depending on offspring sex (ANCOVA, $P < 0.01$), and the probability of survival was highest for largest females but smallest males.
24. The curves relating survival probability to maturity are linear, which permits a test for differences in survival between the sexes by ANCOVA. Selection on egg size to maturity was significant (covariate egg mass was significant, $P < 0.01$), and there were significant sex differences in the strength and direction of selection on the survival of male and female offspring (sex by egg mass interaction term was significant, $P < 0.05$).

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Components of Sterol Biosynthesis Assembled on the Oxygen-Avid Hemoglobin of *Ascaris*

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The parasitic nematode *Ascaris* infests a billion people worldwide. Much of its proliferative success is due to prodigious egg production, up to 10^6 sterol-replete eggs per day. Sterol synthesis requires molecular oxygen for squalene epoxidation, yet oxygen is scarce in the intestinal folds the worms inhabit. *Ascaris* has an oxygen-avid hemoglobin in the perienteric fluid that bathes its reproductive organs. Purified hemoglobin contained tightly bound squalene and functioned as an NADPH-dependent, ferrihemoprotein reductase. All components of the squalene epoxidation reaction—squalene, oxygen, NADPH, and NADPH-dependent reductase—are assembled on the hemoglobin. This molecule may thus function in sterol biosynthesis.

Ascariasis is an infection that pervades the world. The World Health Organization estimates that one billion people are infested with the etiologic parasitic helminth, *Ascaris lumbricoides* (1). Mortality rates for the disease are estimated at 20,000 per year, due mostly to biliary and intestinal obstructions. Morbidity is somewhat higher, with about one million cases per year exhibiting overt clinical manifestations (2, 3). Perhaps most devastating, ascariasis results in decreased growth and development in millions of affected children (4, 5). Little is known about the molecular metabolism of *Ascaris*. *Ascaris* appears to be microaerophilic, consistent with its location in the low-oxygen environment of the intestinal folds (6). Carbohydrate metabolism is anaerobic in *Ascaris*, but a terminal cytochrome chain exists that uses oxygen as available (7).

How oxygen is delivered to the cells remains unknown. *Ascaris* muscle has a myoglobin with high oxygen affinity (8–11), and the perienteric fluid has an abundant hemoglobin that binds oxygen 25,000 times more tightly than its mammalian homolog. (The partial pressure of oxygen at which the hemoglobin is half saturated is about 0.001 mm of mercury for *Ascaris* hemoglobin, and 25 mm of mercury for human hemoglobin.) (8, 10, 12, 13). The perienteric hemoglobin molecule is comprised of eight 40-kD subunits (14), but the structural features that promote its robust oxygen affinity are unknown. The function of this protein, which was detected spectroscopically by Keilin in 1925 (19) and characterized with respect to oxygen affinity by Davenport in 1949 (8), has also been a mystery. It binds oxygen too tightly to be

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