REPRODUCTIVE BURDEN, LOCOMOTOR PERFORMANCE, AND THE COST OF REPRODUCTION IN FREE RANGING LIZARDS

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Abstract.—A reduction in the locomotor capacity of gravid females is considered to be a cost of reproduction if it leads to an increased risk of mortality. In this study, we measured the change in endurance between gravid and postgravid female side-blotched lizards (*Uta stansburiana*) as a test of the cost of reproduction. We also altered reproductive investment in some females by direct ovarian manipulation (yolkectomy), which decreased reproductive burden by 30%. Regardless of experimental treatment, all females had lower endurance when gravid. Endurance was 28% lower in gravid females from the yolkectomy treatment and 31% lower in the unmanipulated females relative to postoviposition females. The experimental reduction in clutch mass resulted in a 21% increase in endurance of gravid yolkectomy females relative to control females. Postovipositional endurance was significantly higher in the yolkectomized females than unmanipulated females, which suggests that the cost of reproduction carries over to postoviposition performance. Unmanipulated females exhibited a significant negative association between endurance and size-specific burden. Endurance was not correlated with clutch size or size-specific burden in the yolkectomy females. Survivorship to the second clutch was higher in the yolkectomy females. The results from a logistic regression showed the probability of survival to the second clutch was significantly and positively associated with endurance after controlling for the effects of treatment. Our analyses demonstrated that the decrement in performance associated with current reproductive investment represents a cost of reproduction expressed as diminished locomotor performance and lowered survivorship to the next clutch.

Key words.—Cost of reproduction, endurance, locomotor performance, natural selection, ovarian manipulation, reproductive burden, *Uta stansburiana*, yolkectomy.

Models of life-history evolution assume the existence of tradeoffs among fitness components. For example, an increase in current reproduction by either maximizing fecundity or parental care occurs at the expense of future survivorship or fecundity or both (Williams 1966; Stearns 1976; Charlesworth 1993). Costs of reproduction are manifested as the tradeoff between current versus future allocation to reproduction (Reznick 1985, 1992; Roff 1992; Stearns 1992). Two principal mechanisms for the expression of costs of reproduction are survival costs and fecundity costs (Bell 1980; Shine 1980; Brodie 1989; Landwer 1994; Schwarzkopf 1994). Survival costs can be further partitioned into physiological costs that are mediated by metabolic tradeoffs (e.g., maintenance versus storage) and ecologically mediated costs, such as the increased risk of predation associated with foraging while acquiring energy for eggs or from the decrement in performance caused by the burden of the clutch. Distinguishing the importance of each mechanism in the expression of the costs of reproduction is a major goal for understanding the evolution of life-history patterns.

Relative clutch mass (RCM; mass of the clutch divided by total mass of the gravid female) is the dominant measure of reproductive investment in lizards and other taxa (Tinkle and Hadley 1975; Vitt and Congdon 1978; Shine 1980; Vitt and Price 1982; Dunham et al. 1988). Because estimates of mortality in natural populations are measured infrequently, most analyses assume that an increase in RCM will constrain locomotor performance. Thus, RCM and locomotor capacity

are predicted to be negatively correlated (Shine 1980; Bauwens and Thoen 1981; Cooper et al. 1990; Shaffer and Formanowicz 1996).

In general, gravid or pregnant females exhibit diminished sprint speed or endurance (Bauwens and Thoen 1981; van Damme et al. 1989; Sinervo et al. 1991; Shaffer and Formanowicz 1996). The types of comparisons vary among studies. Most have compared gravid females with other nonreproductive females, whereas other studies have compared gravid female with male performance. Regardless of the comparison, the observed difference in performance has been argued to represent a cost of reproduction imposed by the additional mass of a clutch (Shine 1980; Seigel et al. 1987; van Damme et al. 1989; Cooper et al. 1990). However, this conclusion is weakened by the fact that factors in these studies other than reproductive state may affect performance, which precludes testing causal mechanisms about the costs of reproduction.

Although many studies investigating the costs of reproduction have focused on the difference in locomotor performance between gravid and nongravid females, far fewer have investigated whether the observed decline in performance is correlated with clutch mass or egg number. This may seem to be a subtle distinction, but it is an important one. Comparing gravid versus nongravid females ignores potentially important variation in reproductive investment among individuals. Recent experimental manipulations of clutch size have documented enhanced survivorship as a consequence of

lowered reproductive investment (e.g., Landwer 1994; Sinervo and DeNardo 1996), yet the mechanism underlying the change in survivorship remains unexplored. In addition, the degree to which variation in reproductive investment among females covaries with locomotor impairment is largely unknown.

In this study, we examine how variation in reproductive investment affects the endurance and survivorship of female side-blotched lizards, *Uta stansburiana*. Rather than relying on natural variation in RCM, we experimentally reduced reproductive investment and compared the change in locomotor performance before and after oviposition. We applied the technique of allometric engineering to alter the number of eggs and total mass of a clutch by surgical removal of yolk from individual vitellogenic follicles (yolkectomy or follicle ablation; Sinervo and Licht 1991a,b). The ablation of ovarian follicles results in a multitrait manipulation, because altering clutch size causes a cascading series of changes among physiologically coupled life-history traits (Sinervo 1994, 1999). Ovarian manipulation enhances the natural variation in clutch size and therefore increases our ability to detect an effect on locomotor performance and survival. Whereas the majority of studies have used sprint speed as a measure of locomotor capacity (Seigel et al. 1987; Cooper et al. 1990), we used endurance as our measure of locomotor capacity. Endurance may be critical for those activities which require extended episodes of activity, for example, nest excavation, home range defense, foraging, and anti-predator behaviors (Garland and Losos 1994). We predicted that an experimental reduction in clutch size and clutch mass (burden) should result in an increase in endurance. Because yolkectomized females have been found to be in better condition (i.e., generally heavier and having a higher body mass per unit body length) than control females after oviposition (Sinervo and Doughty 1996), we also predicted that the yolkectomy females would maintain higher levels of endurance following egg laying.

In addition, we determined whether the experimental reduction in reproductive investment enhanced future survivorship. We included estimates of survivorship to the second clutch to link variation in reproductive investment and endurance with fitness costs of reproduction. Female *U. stansburiana* typically produce up to four clutches per year at approximately one month intervals. Thus, females are gravid through most of the activity season. However, mortality is high and relatively few females survive to produce three or four clutches (Sinervo and DeNardo 1996). We predicted that yolkectomized females or unmanipulated females with small clutches should have relatively higher survival because investment in current reproduction is lower, which diminishes potential physiological and ecological costs that accompany high reproductive investment (Sinervo and DeNardo 1996). Thus, yolkectomized females are predicted to have both higher levels of endurance and survival.

Materials and Methods

Our study was conducted on a 250-m long rock outcrop adjacent to Billy Wright Road, Merced County, California. Details of the study area and field methods are provided in Sinervo and Licht (1991a) and Sinervo and Doughty (1996).

Mechanistic Manipulation of Reproductive Investment

During the study we monitored the reproductive state of females by abdominal palpation. The vitellogenic cycle of female *U. stansburiana* is approximately 24 d. During the first 14 d of the cycle we determined reproductive status (i.e., number and size of vitellogenic follicles) weekly; at the end of the cycle we monitored females every two to three days. In early March, we captured a sample of early to mid-stage vitellogenic females for the follicle manipulation experiment. Surgeries were performed one day after capture. Females were injected with 0.02 ml of 0.2% lidocaine at the site of incision and placed onto a bed of crushed ice wrapped in plastic (hypothermic anesthesia). A lateral incision 10–12 mm in length was made on one side of the abdomen. All follicles from one side were pierced with an 18-gauge syringe and approximately 75–90% of the yolk was removed by carefully squeezing the follicles with blunt forceps. Because the females in this experiment had three to seven follicles prior to the ablation, we substantially reduced the clutch size (number of eggs) using this procedure. Effectively one half of the eggs were ablated by the yolkectomy. Females were sutured using 5-0 silk and placed in a terrarium for recovery from the anesthesia. We repeated the procedure on a group of sham-operated females where the follicles and ovary remained intact. We also included a group of females that were unmanipulated. Animals were released at the site of capture 12 h after surgery. These lizards were subsequently used in the analysis of endurance and survivorship as described below.

Collection and Husbandry of Gravid Females

Females retain eggs in the oviduct for ten days after ovulation, therefore we could recapture and return females to the lab with shelled eggs close to the time of oviposition. Our monitoring of females with oviducal eggs allowed us to predict oviposition within four days. We recaptured near-term, postovulatory, gravid female *U. stansburiana* during late March and early April, 1994. Our sample included the yolkectomy females and two categories of control females. We included a sample of females that underwent a sham surgery and unmanipulated females. Sample sizes for the three classes of gravid females are provided in Table 1. All lizards were brought to a field laboratory on the day of collection. Females with oviducal eggs were housed separately in a 5-liter ovipositorium (a 20-liter plastic container which was divided into four partitions). A single incandescent bulb (40W) centered over the partitions provided a thermal gradient (25–40°C) for thermoregulation. Night time temperatures averaged 25°C. The ovipositoria were kept in an environmental chamber with illumination provided by full-spectrum fluorescent lighting and incandescent bulbs. The photoperiod averaged 12L:12D during the study. Females were fed crickets daily (*Achaeta* sp., dusted with Vionate commercial vitamin supplement and calcium) and provided water ad libitum.

A square pan filled with sand was snugly placed over a 5-cm thick layer of moistened peat moss and sand. Isolated females gained access to the peat/sand soil through an 8-cm slot positioned away from the heat source. Females freely dug burrows under the pan and into the peat/sand soil. In

TABLE 1. Summary statistics for the variables for all females and each treatment. Values presented are means with standard errors given in parentheses.

	SVL (mm)	Postlay mass (g)	Clutch size (# of eggs)	Burden (g)	Prelaying endurance (s)	Postlaying endurance (s)	Difference between pre- and postovipositional endurance (s)	Percent change
Sham surgery (N = 15)	57.4 ± 0.3	4.51 ± 0.1	5.7 ± 0.2	2.08 ± 0.1	190.9 ± 10.3	259.8 ± 27.9	68.9 ± 25.6	26.5%
Unmanipulated females§ (N = 34)	57.0 ± 0.3	4.37 ± 0.1	5.4 ± 0.2	2.08 ± 0.1	190.7 ± 8.33	273.4 ± 24.7	84.1 ± 23.5	30.7%
Yolkectomy (N = 21)	56.4 ± 0.4	4.53 ± 0.14	3.2 ± 0.2	1.42 ± 0.1	240.9 ± 15.2	343.2 ± 28.2	108.2 ± 24.3	27.5%
Percent difference*			40.7	31.7	26.3	25.5		

§ $N = 33$ for postlay mass, clutch size, and burden; * Percent increase in performance of yolkectomy females relative to unmanipulated females.

most instances, females would lay their clutch within the peat/sand layer. We maintained a relatively constant soil moisture by spraying the peat/sand layer two to three times per week with water. Ovipositoria were checked a minimum of two times per day, with a maximum elapsed time of no more than 12 h between checks. Oviposition primarily occurred at night. Upon detecting a freshly laid clutch, we weighed each egg to the nearest 0.01 g and then determined total clutch mass. Each female was weighed before and after oviposition.

Endurance

Endurance was measured as the elapsed time a lizard could maintain a constant speed (0.5 km/hr) on a motorized treadmill (Garland 1984; Garland and Else 1987; Huey et al. 1990). The belt surface was a triple-ply canvas cloth that provided excellent traction. Every attempt was made to minimize the propensity for females to race forward and rest on the belt by gently tapping the hind leg or tail to maintain movement within a small (25×27 cm) portion of the belt. Trials were terminated when a female failed to maintain her position on the treadmill following three successive pinches or taps. Past use of this protocol yielded repeatable measures of endurance (Garland 1988; Huey et al. 1990; Miles 1994). All measurements were made at 36°C, which is the observed field active temperature (B. Sinervo, unpubl. data; Brattstrom 1965). Females with oviducal eggs were raced over a period of two days, April 16–17, 1994, to estimate their endurance when carrying a clutch. The endurance of each female was measured again, usually within 12 h of laying their eggs. The change in endurance for each female (before vs. after oviposition) was evaluated using a repeated-measures ANOVA, with surgical treatment included as a fixed effect. Because postovipositional endurance was not normally distributed (Shaprio-Wilk test: $W = 0.86$, $P < 0.001$), we used log-transformed values for measures of both endurance and body mass in the ANOVA and subsequent analyses. Although many physiological traits covary with body size (Calder 1984; Sinervo and Huey 1990), we found no significant relationship between postoviposition body mass and endurance using an ANCOVA ($F_{1,92} = 2.41$, $P = 0.12$).

Temporal Change in Endurance

We raced an additional group of gravid females that were collected from the periphery of the main study site. We measured follicle diameter (using electronic dial calipers) and stage of reproductive cycle for each female. These data were used to determine the time of the reproductive cycle in which the decrement in locomotor abilities was manifested.

Reproductive Investment

We defined "burden" as the difference in the mass of a female before and after oviposition (Sinervo et al. 1991). We analyzed differences in burden between treatment groups with ANCOVA, with postoviposition body mass included as a covariate. We then calculated size-specific burden by retaining the residuals from the regression of burden against

postoviposition body mass, instead of the ratio of burden to body size (i.e., RCM).

Analysis of Survivorship

We extended our analysis of the costs of reproduction by estimating the link between performance and survivorship. Yolkectomy ($N = 21$) and unmanipulated ($N = 34$) females were permanently marked using unique toe clips. We released females at the site of capture after the last endurance (postoviposition) measurement and followed them through the reproductive season. Females were kept in captivity at least 24 h after the endurance trials to allow recovery. Survival patterns were determined by daily censuses of the habitat from April to June 1995 (refer to Sinervo and DeNardo 1996 for additional detail). At each census, we walked daily transects (at least ten passes) across the outcrop and recorded observations on female activity. We considered a female to have died if she was not seen in four consecutive days.

Costs of Reproduction

Our protocol is based on the paradigm that links morphology to performance and performance to fitness (Arnold 1983). In this analysis, we use survival to the second clutch as a measure of fitness. We applied path analysis to decompose the correlations between endurance, size-specific burden, and survivorship into hypothesized causal relationships among the traits (Li 1975; Mitchell 1993; Grace and Pugesek 1998). The path analysis was conducted in two parts. First, we used standard multiple regression techniques to determine the relationship between burden and performance. In this analysis, preoviposition endurance was the response variable and postoviposition body mass and burden were the predictor variables. Path coefficients were estimated from the standardized partial regression coefficients.

Next, we estimated the covariation between performance and survivorship as the path linking preoviposition endurance and survivorship to the second clutch. Survivorship was treated as a dichotomous variable (survivors vs. nonsurvivors). Because the response variable was binomial, we used logistic regression to derive a predictive model between performance and survivorship (Trexler and Travis 1993, Janzen and Stern 1998). In particular we included a term for the effects of the treatment, size-specific burden, a linear term for endurance, which estimates directional (linear) selection, and a quadratic term, which estimates variance selection. However, the coefficients from the logistic regression are not easily transformed to path coefficients (Janzen and Stern 1998). Therefore, we derived path coefficients from a multiple regression analysis. We included preoviposition endurance and body mass as the predictor variables and survival to the second clutch as the response variable. This second procedure allows us to compare the magnitude of the path coefficients with those from other studies.

RESULTS

Variation in Clutch Size and Clutch Mass

We detected no difference in clutch size between the sham-treated and unmanipulated females after adjusting for dif-
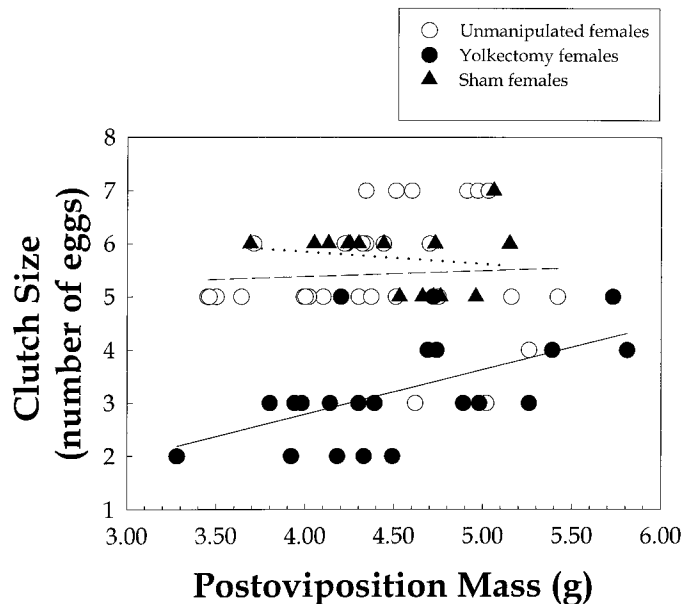

FIG. 1. Relationship between clutch size and postoviposition body mass in unmanipulated and yolkectomy female *Uta stansburiana*. Regression equations are as follows: unmanipulated females, clutch size $= 4.9 + 0.11 \times$ mass ($r^2 = 0.03$, $N = 33$, $P = 0.76$); sham females, clutch size $= 6.4 - 0.14 \times$ mass ($r^2 = 0.01$, $N = 15$, $P = 0.75$); yolkectomy females, clutch size $= -0.6 + 0.84 \times$ mass ($r^2 = 0.30$, $N = 19$, $P < 0.01$). The slopes were not statistically different (ANCOVA: $F_{2,61} = 2.37$, $P = 0.15$). Dashed line, unmanipulated females; dotted line, sham females; solid line, yolkectomy females.

ferences in body mass (Table 1; $P = 0.38$). Yolkectomy females produced significantly fewer eggs than either the sham-treated or unmanipulated females (Table 1; ANCOVA: $F_{2,65} = 46.05$, $P < 0.0001$; effect of female mass: $F_{1,65} = 3.39$, $P = 0.07$). Ablation of the vitellogenic follicles resulted in a 41% reduction in the number of eggs relative to the unmanipulated females. The slope between clutch size and body mass was similar between yolkectomy, sham, and unmanipulated females, although the correlation between body size and clutch size was always stronger for the yolkectomy females (Figure 1).

Gravid females from the yolkectomy treatment had a significantly smaller burden than the unmanipulated females, even after adjusting for differences in body mass (size-adjusted means $\pm$ SE for burden: yolkectomy, 1.45 ± 0.11 g; sham females, 2.11 ± 0.13 g; unmanipulated females, 2.05 ± 0.09 g; $F_{1,63} = 9.55$, $P < 0.001$). Even though the yolkectomy diminished the mass of the clutch by 32%, the manipulation did not alter the slope between burden and clutch size (Figure 2).

Differences between Pre- and Postoviposition Endurance

Regardless of their treatment status, females showed higher levels of endurance after oviposition compared to their performance while gravid (Table 1, Figure 3). The change in locomotor capacity was significant for all treatments (Table 2). The results from an ANOVA revealed that yolkectomy females exhibited significantly higher levels of endurance while gravid than either the sham or unmanipulated females
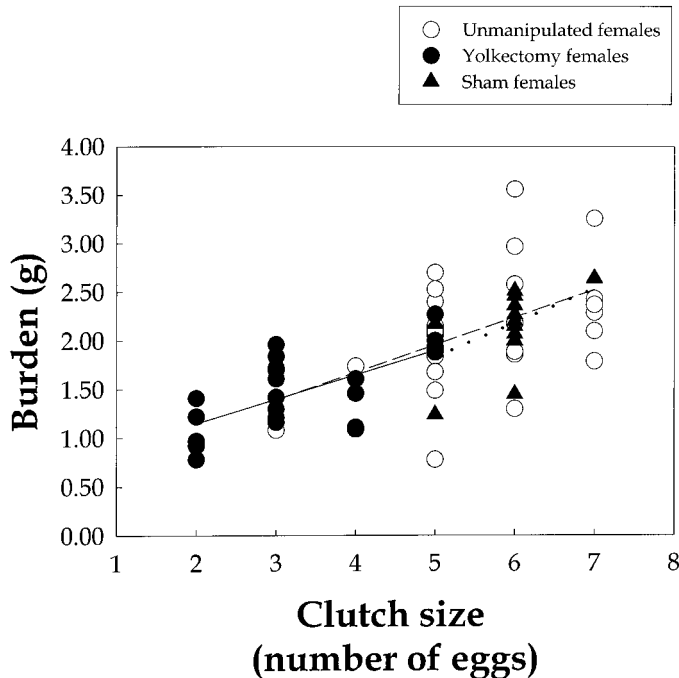
FIG. 2. The association between reproductive investment (burden) and clutch size (CS). Regression equations describing the change in burden with increasing clutch size are as follows: unmanipulated females, burden = $-0.29 + 0.28 \times$ CS ($r^2 = 0.42$, $N = 33$, $P <$ 0.05); sham females, burden = $0.07 + 0.35 \times$ CS ($r^2 = 0.32$, $N = 15$, $P < 0.05$); yolkectomy females, burden = $0.67 + 0.25 \times$ CS ($r^2 = 0.52$, $N = 19$, $P < 0.05$). Results from ANCOVA showed no differences in the slopes ($F_{2,61} = 0.22$, $P = 0.64$). Dashed line, unmanipulated females; dotted line, sham females; solid line, yolkectomy females.

FIG. 3. Log of endurance (mean $\pm$ SE) of female *Uta stansburiana* before and after oviposition.

($F_{2,63} = 4.75$, $P < 0.01$). No statistical difference in endurance existed between the sham-surgery and unmanipulated females (Table 1, Figure 3).

We measured endurance on all females within a period of two days. However, there was some variation among females in the number of days between the performance measurements and oviposition. Some females laid eggs 12 h after racing, whereas others did not lay for another six to ten days. Therefore, we regressed our measure of preoviposition endurance against time until oviposition. No effect was evident for the unmanipulated females ($F_{1,22} = 1.83$, $P = 0.19$) or yolkectomy females ($F_{1,12} = 0.75$, $P = 0.40$).

The proportional increase in endurance after egg laying was homogeneous among treatment groups. Endurance increased 27.5% for the yolkectomy females, 27% for the sham females, and 31% for the unmanipulated females. However,
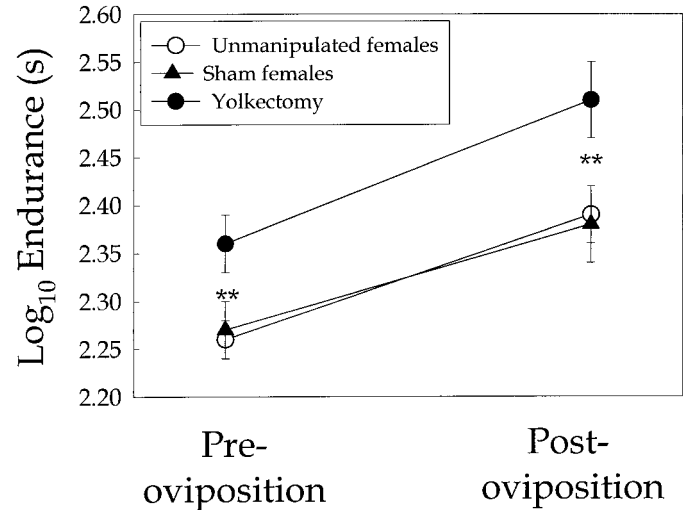
yolkectomy females exhibited higher endurance after oviposition than either the sham or unmanipulated females (Table 2, Figure 3). The mean change in endurance was 108 s for yolkectomy females, 69 s for the sham-surgery females, and 84 s for unmanipulated females (Table 1). The repeated-measures analysis showed no evidence of an interaction between treatment and the change in endurance before and after oviposition.

Temporal Change in Endurance

We used an additional sample of females to examine the change in endurance during vitellogenesis and ovulation. Endurance was negatively associated with follicle diameter ($r = -0.64$, $N = 14$, $P < 0.01$). For every 1-mm increase in diameter, endurance declined by 33 s (Figure 4). Females with follicles smaller than 8 mm averaged 234 ± 13.25 s on the treadmill, whereas females with oviducal eggs averaged 173.5 ± 13.25 s. We found no difference in endurance between those females that had just ovulated compared to females that were close to oviposition.

Relationship between Reproductive Investment and Endurance

The treatment groups differed in burden and locomotor performance while gravid, as described above. Yolkectomy females showed significantly lower burden and higher endurance, before and after oviposition, than unmanipulated

TABLE 2. Repeated-measures analysis comparing endurance of female *Uta stansburiana* lizards before and after oviposition. Treatment describes the overall effect of the follicle ablation on endurance relative to unmanipulated and sham surgery females. The time effect, or repeated measure, estimates the difference between pre- and post oviposition measure of endurance. The interaction between treatment and time tests for a difference in the change in performance specific to the different treatments.

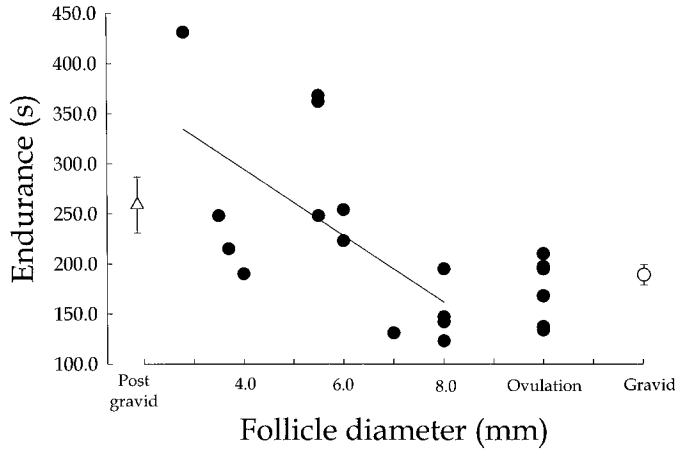| Source of variation	Numerator df	Denominator df	Wilk's Δ	Exact F	P-value
Treatment	2	62	0.88	4.29	0.01
Time	1	62	0.66	31.53	0.001
Treatment × time	2	62	0.98	0.47	0.62

FIG. 4. Changes in endurance for females that were raced prior to or after ovulation. The line describes the relationship between endurance and follicle diameter (FD). The regression equation is as follows: endurance = 427 − 33.24 × FD. Average endurance time is significantly greater for preovulatory females than for females with oviducal eggs ($N = 14$ for preovulatory females; $N = 6$ for females with oviducal eggs; $t = 7.07$, $P < 0.001$). The open circle designates endurance for unmanipulated females while gravid. The open triangle represents endurance for females after oviposition.

females. These patterns suggest a possible functional link between burden and endurance.

To evaluate the relationship between burden and endurance (both before and after oviposition), we first calculated residuals by regressing each variable against the log-transformed values for postoviposition body mass. These analyses resulted in size-specific measures of burden and endurance for each female. We performed the same procedure for clutch size. Only the unmanipulated females demonstrated a negative association between size-specific burden and postoviposition endurance (Table 3). Also, the unmanipulated group of females had significant negative correlations between the clutch size residuals with both postoviposition endurance and the change in performance (i.e., the difference in pre- and postoviposition endurance, Figure 5). However, we did not find any significant associations between size-specific burden or clutch size residuals with endurance for the yolkectomy females. Nevertheless, endurance of gravid females differed between treatments after adjusting for differences in burden
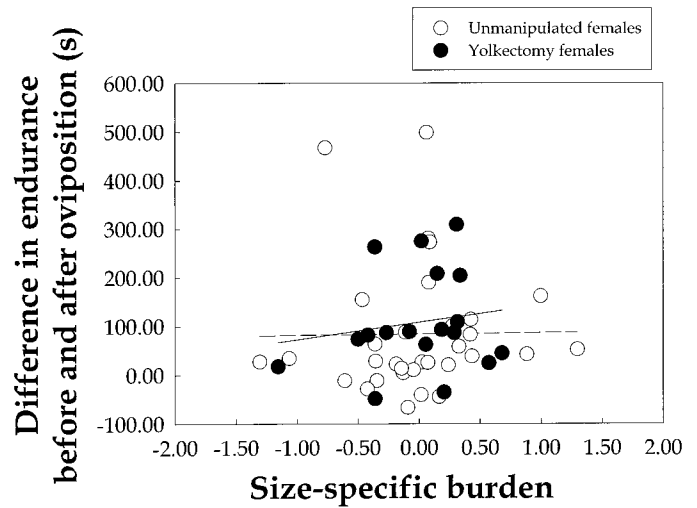

FIG. 5. Relationship between the change in endurance (difference between pre- and postoviposition endurance) and size-specific burden (burden carried per unit body length, derived from the residuals of the regression of burden on postoviposition body mass). The solid line is the regression line for unmanipulated females, and the dashed line corresponds with the regression for yolkectomy females.

(ANCOVA: $F_{1,47} = 7.50$, $P < 0.001$). Thus, females with higher values for burden exhibited lower endurance times. This provides additional evidence that the shift in performance is mediated by burden.

Costs of Reproduction, Endurance and Survivorship

A key question is whether the decrement in endurance due to reproduction is associated with an increase in mortality. Survival in yolkectomy females was higher (18 of 21, 86%) than the unmanipulated females (21 of 34, 62%; $\chi^2 = 3.87$, $P < 0.04$). We found that yolkectomy females carried a lighter clutch of fewer eggs relative to unmanipulated females and were in better condition (higher residuals from a regression of body mass vs. snout-to-vent length) after oviposition. Therefore, we hypothesized that females with lower burden and higher endurance should exhibit higher survivorship. The analysis of selection using a logistic regression resulted in a significant model ($\chi^2 = 10.03$, $P < 0.03$). The model revealed no significant effect of treatment or size-specific burden on survivorship. Instead, we detected signif-

TABLE 3. Correlations between reproductive traits and locomotor performance. All correlations were based on residuals derived by regressing log-transformed reproductive and locomotor traits on log-transformed body mass.

	Preoviposition endurance residual	Postoviposition endurance residual	Difference between pre- and postoviposition
Unmanipulated females			
Burden residual	−0.11	−0.40*	−0.35
Clutch size residual	−0.13	−0.59**	−0.59**
Yolkectomy females			
Burden residual	−0.29	0.22	0.22
Clutch size residual	0.28	0.35	0.12

*, $P < 0.05$; **, $P < 0.01$.

TABLE 4. Results from the analysis of selection using logistic regression. The linear term evaluates the magnitude of directional selection, whereas the quadratic term estimates the impact of stabilizing selection.

	Coefficient	SE	Likelihood ratio	P
Intercept	-11.52	7.04	2.67	0.10
Treatment	0.31	0.47	0.47	0.49
Endurance (linear)	0.125	0.07	3.81	0.05
Endurance^2 (quadratic)	-0.002	0.0001	4.25	0.03
Size-specific burden	0.63	0.61	1.09	0.29

icant directional selection on endurance when holding the effects of treatment and size-specific burden constant (Table 4). Thus, females with high endurance exhibited a higher probability of survival. In addition, the quadratic term was significant, suggesting stabilizing selection on endurance. The results of the logistic regression support the hypothesis that the effects of burden on survivorship occur indirectly through the effects on performance.

We used path analysis to uncover hypothesized causal relationships among the variables. Postoviposition body mass was a poor predictor of endurance (Figure 6). Rather, burden was the best predictor of preoviposition endurance (Figure 6). Yolkectomy females were characterized by smaller levels of size-specific burden and higher values for endurance than unmanipulated females ($F_{1,49} = 7.03$, $P < 0.01$). Females with high endurance tend to have a greater probability of surviving to the second clutch, which is consistent with the results from the logistic regression (Figure 6). Although there was no significant difference in endurance between treatments among the females that died, the surviving yolkectomy females had higher levels of endurance when gravid than the surviving unmanipulated females ($t = 3.07$, $P < 0.003$). We also examined the path between the predicted endurance

(based on the regression of endurance on burden) of a female and survivorship to the second clutch. We found that survival is significantly and positively associated with predicted endurance; surviving females have higher levels of endurance than the non-survivors (Figure 6). We conclude from these analyses that the covariation between performance (endurance) and survival is a consequence of burden. Therefore, an increase in burden impairs stamina, which in turn significantly elevates the risk of predation.

DISCUSSION

Multitrait Manipulations, Evolution of Reproductive Investment and the Costs of Reproduction

Our experimental manipulation of clutch size yielded three results. First, yolkectomy females produced fewer eggs and a smaller clutch mass relative to controls (Figures 1, 2). Clutch size in yolkectomy females was reduced by 41%, yet reproductive burden was reduced by only 32%. Thus, there was some compensation in the clutch size/egg size relationship. That is, clutch mass changed less than clutch size. Regardless of treatment, females with small clutches tend to have eggs that are longer and cylindrical (Sinervo and Licht
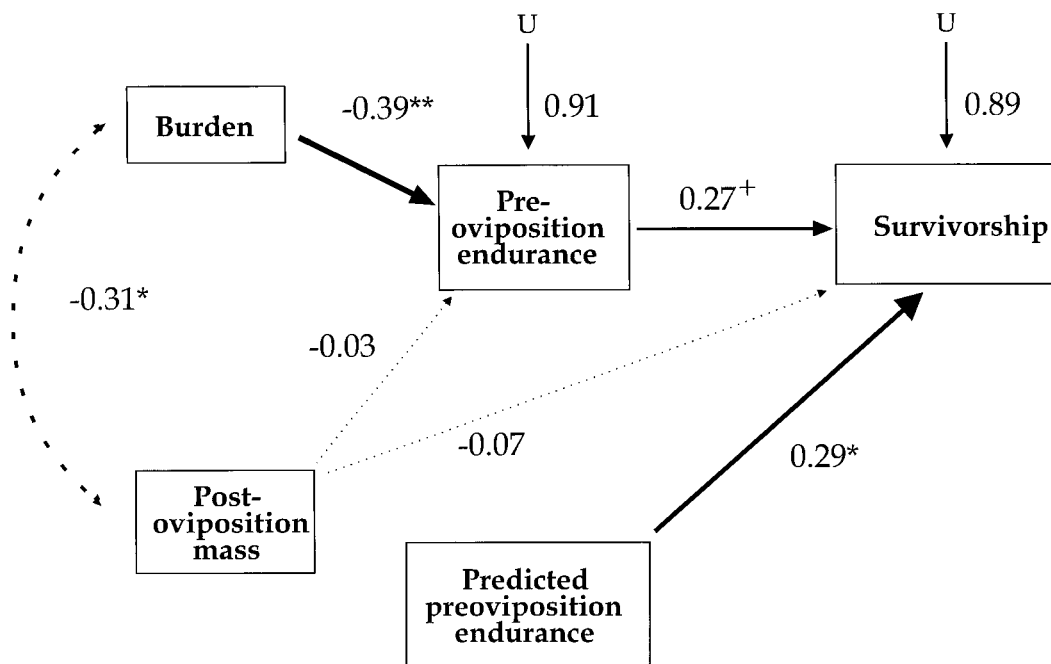

FIG. 6. Path diagram that depicts the relationships among reproduction, performance, and survivorship. Correlations are presented as double-headed arrows and single-headed arrows are the standardized partial regression coefficients. Dashed arrows signify negative coefficients. Significant paths ($P < 0.05$) are given by thick lines and bold coefficients. $^+$ $0.1 < P < 0.05$, $*$ $P < 0.05$, $**$ $P < 0.01$.

1991a,b). Yolkectomy females followed this pattern by producing fewer but larger eggs with an altered shape. Second, the decrease in size-specific burden was associated with higher values of endurance for yolkectomy females compared to controls (Figure 3). The experimental manipulation yielded a 26% increase in endurance while gravid compared to unmanipulated controls or sham-manipulated females. The difference in performance between control and yolkectomy females remained after oviposition (Table 1). Third, our analyses demonstrated that endurance was significantly correlated with survivorship after controlling for the effects of treatment and size-specific burden (Table 4, Figure 6). These results support the hypothesis that variation in reproductive burden affects locomotor performance and that variation in performance affects survivorship.

Our experimental manipulation of clutch size has several advantages for investigating costs of reproduction. First, reducing the number of yolked follicles is analogous to follicular atresia that is observed in females from natural populations (Sinervo and Licht 1991b). Second, manipulating a single phenotypic trait such as clutch size results in a multitrait cascade of changes in several coupled phenotypic characters (Sinervo and Svensson 1998; Sinervo 1999). For example, ablating developing follicles has been argued to represent a mechanistic perturbation of ovarian regulation (Sinervo 1999). Thus, reducing clutch size results in a correlated suite of changes in the resulting clutch. Not only is the number of eggs affected, but so too are egg size, egg shape, and burden (Sinervo 1994, 1999; Sinervo and Licht 1991a). Third, the variation in reproductive traits as a consequence of the yolkectomy treatment is consistent with the natural levels of variation occurring in this population of *U. stansburiana* (Sinervo 1999). Therefore, our yolkectomy experiment provides an opportunity to examine the predicted functional tradeoff between burden, performance, and survivorship.

Endurance of Gravid Females

Preoviposition endurance of unmanipulated and yolkectomy females was lower compared to their performance after oviposition. The difference in endurance in *U. stansburiana* is not as great as reported in other species (e.g., Garland and Else 1987; Cooper et al. 1990). The difference may be attributable to higher clutch masses of the other species. Despite the difference in magnitude of the decline in performance, our results are consistent with earlier studies.

A key question is what contributes to the decline in performance. Our experiment suggests both biomechanical and physiological effects of reproductive investment on endurance. The change in performance may result from the mass of the clutch, a broad physiological diminishment due to reproduction (Brodie 1989), or both. Two results of the yolkectomy experiment support a biomechanical explanation. First, gravid yolkectomized females exhibited significantly greater endurance relative to control females (Figure 3). If the decrement in performance was due to gravidity alone and not the added burden imposed by the mass of the clutch, then there would not be a difference between the treatment groups. Because endurance was affected by the reduction in clutch

size, burden, or both, our results contradict the physiological cost hypothesis. The significant negative correlation between follicle size and endurance (Figure 4) provides additional support for a biomechanical cause of lowered performance due to reproduction. Second, endurance increased soon after oviposition, in most instances no later than 12 h after oviposition. However, the elevated levels of endurance in the yolkectomy females after oviposition supports a broader physiological effect during reproduction. The yolkectomy females were in better condition (based on regressions of body mass on snout-to-vent length) and maintained higher levels of endurance than the control females. Thus, the yolkectomy females may have had greater energetic reserves compared to the controls, which enhanced performance.

A Mechanistic Explanation for the Decline in Endurance

Endurance is likely to be affected by the distance moved per stride and the energetic cost of each stride (Snyder 1952; White and Anderson 1994; Farley and Ko 1997). The effects of reproduction on performance may have two components. First, the additional mass of the clutch increases the costs of locomotion (i.e., a pure mass effect). Second, the physical presence of the eggs impairs speed or endurance, because the distention of the abdomen from carrying the clutch hinders axial bending and hind limb kinematics (Sinervo et al. 1991). Consequences of abdominal distension are reduction in pelvic rotation, flexion of the vertebral column, and small stride length. Thus, reproduction has a twofold impact on locomotion. First, the added mass of the clutch increases the energetic cost of transport, limiting or reducing the distance an animal can move. Second, the constrained lateral flexion and reduced stride length means a female must take more strides per unit distance, which exacerbates the mass effect and further lowers endurance. Therefore, the effects of reproduction on performance are attributable to the interactions among clutch mass, cost of transport, and limb kinematics (Shine 1988).

Relationship between Burden and Locomotor Performance

The unmanipulated females exhibited a significant negative correlation between size-specific burden and either endurance after oviposition or the difference between pre- and postoviposition endurance. Furthermore, these females also exhibited a significant negative correlation between the number of eggs in a clutch, corrected for body size (clutch size residuals), and the difference in pre- and postoviposition performance (Table 3). Unmanipulated females with high values for burden or clutch size had lower levels of endurance, and they exhibited lower recovery values. Thus, the unmanipulated females showed a smaller change in endurance following oviposition. This pattern supports the hypothesis that an increase in burden results in a decline in performance and represents a cost of reproduction, which is consistent with other studies (Shine 1980; Seigel et al. 1987; Van Damme 1989; Sinervo et al. 1991; Shaffer and Formanowicz 1996).

Reproductive Investment, Performance, and Fitness

If performance affects fitness (Arnold 1983), then the lower endurance exhibited by the unmanipulated females should

result in higher rates of mortality compared to the yolkectomy females. Indeed, the field data support this prediction. In general, a reduction in endurance among gravid females may be critical for three reasons. First, escape from predators may involve an interaction between sprint speed and endurance. Initial velocity and maximum speed may be complemented by flight distance and the ability to continue sustained activity. Field observations revealed a fourfold increase in snake activity, which coincided with a rise in mortality among adult *U. stansburiana* (Sinervo and DeNardo 1996). The observed difference in survivorship between yolkectomy and unmanipulated females (high endurance and low burden vs. low endurance and high burden, respectively) in the wild may be due to predation. Second, lowered endurance may affect the ability of a female to monitor her home range and defend potential nest sites. Third, endurance may be critical for the period of sustained activity during nest excavation.

Yolkectomy females had significantly lower rates of mortality than unmanipulated females. Furthermore, the survival to the second clutch was significantly associated with endurance, which in turn was affected by the manipulation of burden via yolkectomy (Table 4). Based on the results from the logistic regression and path analyses (Figure 6), we suggest the following scenario to explain the observed patterns of reproductive investment, performance, and survivorship. Females with high burden relative to body size experience diminished endurance. Furthermore, females with higher values of burden tend to be in poorer condition compared to females with lower burden after oviposition. Survival to the second clutch (a measure of future reproductive success) is higher in females with lower burdens, as shown by the yolkectomy experiment. Survival was also associated with preoviposition endurance. Yolkectomy females have significantly higher levels of endurance after adjusting for burden. Consequently, the difference in endurance is a reflection of differences in size-specific burden between the treatments. This suggests that the difference in survivorship is a consequence of burden, which is mediated by performance.

Our results provide evidence that an increase in current reproduction has a detrimental effect on survivorship and specifically on survivorship to the second clutch. The mechanistic cause of the decline in performance should be investigated by biomechanical analyses to compare limb kinematics among yolkectomy and control females. These studies can be complemented by analyses to compare the cost of transport among gravid and postoviposition females. Such integrative studies are necessary to reveal the causal bases for the reduction in locomotor performance associated with reproduction.

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