Mechanistic Analysis of Natural Selection and a Refinement of Lack's and Williams's Principles

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ABSTRACT: Results from several physiologically based manipulations were synthesized to investigate two selective trade-offs involving offspring number versus offspring quality and costs of reproduction in an annual lizard *Uta stansburiana*. Lifetime reproductive success of experimentally size-altered progeny was studied to address the offspring number and offspring quality trade-off. Causes of natural selection on adult reproductive costs were assessed with three complementary manipulations of clutch size, egg size, and total clutch mass. Selective trade-offs between offspring size and number arose from two opposing episodes of directional selection. Fecundity selection favored female parents that laid large clutches of small offspring, but fecundity selection was balanced by survival selection that favored large offspring. Thus, the offspring number and quality trade-off had a strong stabilizing effect on mean egg size across generations. However, strength and direction of selection arising from adult reproductive costs varied among years. Because reproductive traits were heritable ($h^2 = 0.61$), selection on adult reproduction led to a large evolutionary response to natural selection. Patterns of selection detected in natural phenotypic variation were largely corroborated by phenotypic manipulations. However, maturational costs of reproduction that were detected with phenotypic manipulations were missed by traditional selection analysis of natural phenotypic variation.

Keywords: life-history trade-off, natural selection, phenotypic manipulation, physiology.

Selective constraints on life-history adaptation arise from allocation trade-offs between number versus size of offspring (Lack 1954) and current versus future reproductive success (Williams 1966; Gadgil and Bossert 1970). In both allocation paradigms, an enhancement of one fitness trait is expected to lead to changes in other traits that can have negative effects on fitness. Selective constraints are readily measured using a "selectionist's" paradigm (Arnold 1983) in which negative covariation among phenotypic traits and fitness is described in terms of the shape of the adaptive landscape or fitness surfaces (Lande and Arnold 1983; Arnold and Wade 1984). Selective constraints on life-history adaptation are also rooted in physiological mechanisms that govern alternative allocation pathways in an organism. A proximate link between physiology and reproductive allocation should arise from the endocrine system (Marler and Moore 1988; Ketterson and Nolan 1994; Sinervo and DeNardo 1996), particularly if the reproductive traits share a common mechanism of regulation. Hormonal and physiological mechanisms constrain evolutionary change if they result in trade-offs among suites of life-history traits. Physiological constraints are readily assessed using paradigms of field endocrinology (Wingfield and Moore 1987) in which exogenous hormone implants are used to induce variation in life-history traits.

Some authors have argued that phenotypic manipulations do not provide inferences regarding the genetic basis of trade-offs that should be expressed as negative genetic correlations between fitness traits (Reznick 1985, 1992; Leroi et al. 1994). This limitation applies to manipulations that perturb the correlation structure of life-history trade-offs by manipulating an environmental factor like food availability (e.g., Leroi et al. 1994). Environmental manipulations are subject to genotype-by-environment interactions that may confound assessment of genetic correlations (Reznick 1985). However, manipulation of a physiological cause that links two or more phenotypic traits in a life-history trade-off are not subject to the same criticism (Sinervo and Basolo 1996; Sinervo and DeNardo 1996; Sinervo and Doughty 1996; Sinervo and Svensson 1998).

A genetic correlation can arise from two sources: linkage and pleiotropy (Falconer 1981). The role of linkage in promoting genetic correlations is best understood with molecular tools, classical genetic crosses, and linkage studies. However, mechanisms underlying pleiotropy can be explained by manipulating a common endocrine mechanism that links traits. Life-history trade-offs should be reflected in a negative genetic correlation between fitness

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Am. Nat. 1999. Vol. 154, pp. S26–S42. 0003-0147/1999/15401S-0003$03.00.

traits. If proximate endocrine mechanisms form the basis of life-history trade-offs, then manipulations of the endocrine system should induce negative correlations between components of the life history that influence survival or reproductive success (Marler and Moore 1988; Sinervo and McEdward 1988; Sinervo 1990, 1993, 1994*a*, 1994*b*, 1998, 1999*a*, 1999*b*, 1999*c*; Sinervo and Huey 1990; Ketterson et al. 1991; Sinervo and Licht 1991*a*, 1991*b*; Sinervo et al. 1992; Ketterson and Nolan 1994; Sinervo and Basolo 1996; Sinervo and DeNardo 1996; Sinervo and Doughty 1996).

Genetic correlations are estimated from breeding studies or artificial selection experiments (Falconer 1981), and because statistical power and control over environmental variation are important concerns, genetic variation is usually measured under artificial, not natural, conditions. However, laboratory-based estimates are inadequate when the ultimate goal for estimating genetic variation is to predict response to natural selection in the wild (Lande and Arnold 1983; Sinervo and Doughty 1996; Sinervo 1999*a*). In this case, estimates of genetic variation based on parent-offspring regressions of free-ranging animals are superior to estimates from laboratory-reared animals. In the laboratory, environmental effects may be unrealistically low (the maturational environment is carefully controlled) or totally different from the environment in the wild (e.g., genotype × rearing environment). Unfortunately, estimates of heritability in nature are difficult to obtain owing to attrition during the process of natural selection. Estimation of genetic correlations requires even larger sample sizes. Only in exceptional cases where long-term studies have developed a large database (Merila et al. 1994; Sinervo 1999*b*) is it possible to estimate genetic correlations in natural populations with reasonably narrow confidence limits. It is not just for these pragmatic reasons that field biologists have resorted to endocrine manipulations as an experimental source of inference regarding life-history trade-offs. Manipulations allow for cause-and-effect relations to be documented for natural selection and for cause-and-effect relations to be documented among traits comprising the life-history trade-offs. In earlier research, I have argued for an eclectic approach that integrates genetical and manipulative studies in natural populations (Sinervo and Basolo 1996; Sinervo and DeNardo 1996; Sinervo and Doughty 1996; Sinervo 1999*a*). Manipulation of the endocrine system appears to alter allocation trade-offs by mechanisms similar to those that are initiated by genetic control (Sinervo 1999*b*).

Manipulation of a single endocrine mechanism should provide useful information regarding life-history trade-offs. However, the impact of the endocrine system is best understood by studying the regulation of several hormones that act upstream or downstream of each other in a causal chain of reproductive events (DeNardo and Sinervo 1994*a*, 1994*b*; Sinervo and DeNardo 1996; Sinervo 1999*b*). Independent verification from several manipulations confirms that pharmacological results, which might arise from a single manipulation, are not contaminating the interpretation of trade-offs. More important, most endocrine systems are based on negative and positive regulatory feedback loops that entail the interaction between two or more hormones, and such regulation undoubtedly generates many of the classic trade-offs of life-history biology.

I use the side-blotched lizard (*Uta stansburiana*) as a model system to investigate the role of hormones in regulating life-history traits and how adaptation takes place in the context of the constraints that arise from endocrine regulation of life-history processes. Classic paradigms developed by Lack (1947, 1954) and Williams (1966) provide the selective context for understanding the evolution of reproductive allocation. David Lack (1947) is best known for his hypothesis that avian clutch size is limited by the amount of care that parents provide to offspring. The selective premium accrued by investing in additional offspring should be counterbalanced by the decreased fledging success in large clutches owing to limitations on parental effort per offspring. Similarly, in organisms without parental care, Lack (1954) further suggested that production of large numbers of small offspring is balanced by the high survival of a few large offspring (Sinervo et al. 1992). Maternal effects such as egg size are thought to confer a strong fitness advantage to the offspring (Lack 1954; Smith and Fretwell 1974; McGinley et al. 1987), and because egg size can be genetically transmitted to offspring (e.g., Sinervo and Doughty 1996), egg size should also respond to the force of natural selection and undergo the process of adaptation. Likewise, a second paradigm of life-history biology, trade-offs involving costs of reproductive allocation, provides the selective context for understanding selection during adult phases of the life history. Williams (1966) refined Lack's ideas and considered the parental costs of reproduction that would arise from further investment per offspring to offset deficiencies in offspring survival. Investment in current reproduction is expected to result in a cost to future reproductive success that lowers parental survival, fecundity, or growth, which might reduce body size and fecundity.

Major goals of life-history analysis are to uncover the heritable genetic variation that allows for life-history adaptation, heritable genetic covariation that constrains adaptation, and plasticity in reproduction that allows for short-term adaptive response to a variable environment. While experiments have great utility in elucidating mechanisms of physiological regulation that underlie life-history allocation trade-offs, we must also identify causes of natural selection. In this regard, experimental manipulations

have been extremely useful in disentangling the causes of natural selection on life-history trade-offs in the side-blotched lizard *U. stansburiana*. In addition, path analysis is ideally suited to developing cause-and-effect hypotheses for the action of selection on natural variation (Wright 1921; Kingsolver and Schemske 1991; Mitchell 1993). Moreover, path analytic hypotheses of cause and effect can be directly tested by referencing manipulations of reproductive allocation to results from path analysis of natural variation.

I synthesize results from manipulative and comparative studies that have addressed the following proximate and ultimate causes that shape juvenile and adult phases of the life history: physiological bases of the clutch size and egg size trade-off (Sinervo and Licht 1991*a*, 1991*b*); the selective trade-off that results in a balance between fecundity selection and offspring survival selection as a function of egg size (Sinervo et al. 1992; Sinervo and Doughty 1996); plasticity in reproductive allocation that leads to shifts in egg size as an adaptive response to seasonal shifts in intensity of survival selection that acts on offspring (Sinervo et al. 1992); the selective trade-off between current versus future reproduction of adult females, which reflects costs of reproduction (Sinervo and DeNardo 1996); environmental effects that obscure reproductive costs (Sinervo and DeNardo 1996); and evolutionary responses to natural selection on offspring and adults (Sinervo 1998).

Material and Methods

Side-blotched lizards (*Uta stansburiana*) were studied in a population that is located on a 250-m-long outcropping of sandstone adjacent to Billy Wright Road, Merced County, California (~2 km east of Los Baños Creek). Data are from previous studies of natural selection on offspring size (Sinervo et al. 1992; Sinervo and Doughty 1996), natal dispersal (Doughty et al. 1994; Doughty and Sinervo 1994), heritability (Sinervo and Doughty 1996), sexual selection (Sinervo and Lively 1996), proximate physiological control of clutch size and egg size (Sinervo and Licht 1991*a*, 1991*b*), and costs of reproduction (Sinervo and DeNardo 1996). I have collected data on offspring survival from 1989 to the present and data on survival of the female parent from 1990 to the present.

Heritable Variation in Egg Size, Manipulations of Yolk Volume, and Control of Maternal Effects

I individually mark all parents and follow their marked progeny through a complete life cycle to estimate heritable variation in life-history traits of free-ranging animals. However, nongenetic maternal effects remain confounded with estimates based on parent-offspring correlations. Without a half-sib analysis of maternal traits based on sires (Falconer 1981), it is difficult to estimate the magnitude of such maternal effects. Unfortunately, sire-based analyses are difficult in natural populations because matings cannot be controlled. Moreover, sires do not express traits for female reproduction and a breeding value would be needed for sires, which is likewise impractical in nature. Accordingly, we rely on dam-daughter correlations in assessing genetic variation in the wild. Laboratory incubation of all eggs under standard conditions (−200 kPa; 28°C) controls for effects that might otherwise arise from maternal oviposition site in nature. Hatchlings were released randomly with respect to the female parent's territory to eliminate potential effects of maternal territory on offspring survival, maturation, and reproduction. Finally, two key manipulations of egg size, which is a likely maternal effect, were performed.

I weighed freshly laid eggs and aspirated ~20% of the yolk from half of the eggs in a clutch using a sterile syringe. Hatchlings were miniaturized in proportion to amount of yolk removed (Sinervo 1990). Half of the eggs in a clutch were sham-manipulated controls with the syringe inserted, but no yolk withdrawn. I also gigantized hatchlings by ~20% via ablation of a subset of follicles on one or both ovaries of the female parent (Sinervo and Licht 1991*a*, 1991*b*). The complementary manipulations of egg and hatchling size inflate natural variation by approximately 2 SDs.

A total of 384 and 558 individually marked hatchlings were released in the 1989 and 1990 cohorts, respectively. Partitioning of hatchlings by treatment is described in Sinervo et al. (1992). When oviductal eggs were detected in the progeny at maturity (8 mo later), I brought them into the laboratory to lay their eggs according to methods described above for female parents. I returned female progeny to their territory immediately after laying and recaptured females on second and third clutches. On the basis of intensive mark-recapture efforts, survival of offspring during life-history episodes was assessed from hatching to maturity, from maturation to the production of the first clutch, and from oviposition of the first clutch to the end of the first season of reproduction.

Selective and Environmental Effects on Adult Female Reproduction

During 1991–1994, I studied natural selection on a group of adult female side-blotched lizards (N = 148, 178, 133, and 160, for 1991–1994, respectively). Females were individually marked at the beginning of the reproductive cycle (e.g., mid-March) and were followed until they ovulated eggs (i.e., investment in eggs complete; Packard and Packard 1988), at which point they were brought into the

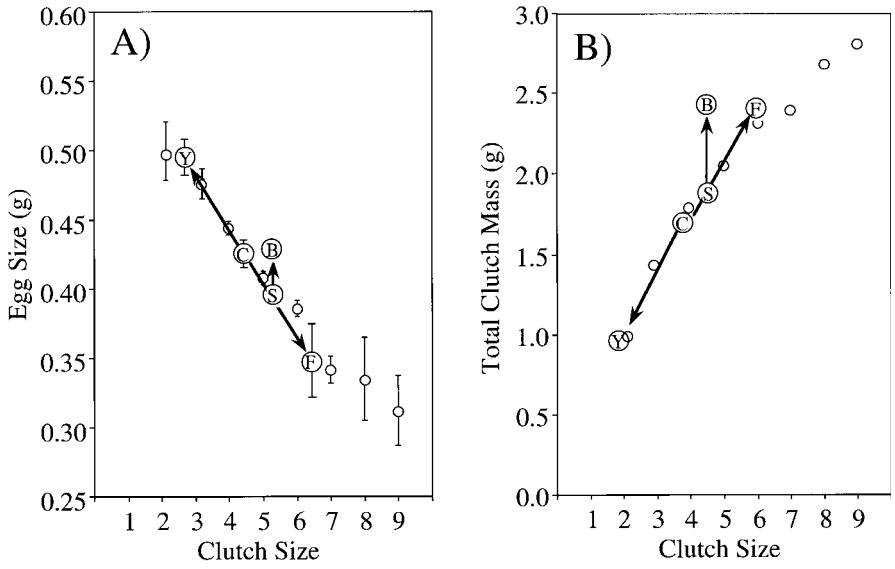

Figure 1: Natural variation (*open circles*; mean ± SE) compared to experimentally induced variation (*arrows*) between (*A*) clutch size and egg size and (*B*) clutch size and total clutch mass for female side-blotched lizards (*Uta stansburiana*) from the inner Coast Range of California. Three manipulations of the endocrine system probe the mechanistic bases of allocation trade-offs: ablation of ovarian follicles (*Y*) during early vitellogenesis reduces clutch size and total clutch mass but increases egg size; administration of exogenous FSH (*F*) during early vitellogenesis increases clutch size and total clutch mass but reduces egg size; and exogenous corticosterone implants (*B*) increase offspring size and total clutch mass, but clutch size remains unaltered. Manipulated females are compared to both unmanipulated (*C*) and sham-manipulated females (*S*).

laboratory to measure reproductive traits. Eggs in freshly laid clutches were counted and weighed to the nearest 0.01 g. Total clutch mass (g) was calculated by summing weights of individual eggs. Postlaying mass was used as an index of maternal size. Females were returned to their territories after oviposition. Survival was censused to production of the second and third clutches, which occur at approximately 1-mo intervals. The "local density" that a female experienced during vitellogenesis was estimated from the number of females observed on a female's home range (Sinervo and DeNardo 1996). A path analytic model was developed to estimate the influence of female body size (i.e., postlaying mass) and social environment (i.e., density of conspecific females that are found on an individual's home range) on total clutch mass and the effect of clutch mass on survival to future reproductive episodes (see Sinervo and DeNardo 1996).

Experimentally Induced Variation in Reproductive Output of Adult Females

I used three complementary manipulations of clutch size, egg size, and total clutch mass of field-active females to address experimentally costs of reproduction (Sinervo and DeNardo 1996): direct ovarian manipulation (follicle ablation; Sinervo and Licht 1991*a*, 1991*b*), ovarian stimulation using exogenous gonadotropin (ovine follicle-stimulating hormone [FSH]; Sinervo and Licht 1991*a*), and increased allocation to egg size via exogenous corticosterone. Follicle ablation simulates the natural process of follicular atresia by which females can adjust clutch size in a downward direction by resorbing some follicles. Yolk from follicles on the left ovary was surgically removed (yolkectomy), which effectively terminates their growth. Follicles on the right ovary remained intact. Follicle ablation decreased clutch size and total clutch mass but increased egg size (fig. 1). FSH increases clutch size by stimulating the growth of additional follicles that might otherwise undergo atresia. Plasma levels of FSH of a second group of females were elevated using ovine FSH that was delivered in a custom-made elvax pellet (designed to match levels in previous laboratory studies; Sinervo and Licht 1991*a*). Exogenous FSH increased clutch size and total clutch mass but decreased egg size (fig. 1). Plasma levels of corticosterone were elevated in a third group of females using crystalline corticosterone (Sigma) that was delivered in saline-soaked silastic implants, which were designed to elevate corticosterone to the upper physiological plasma levels observed for field-active lizards (Wilson and Wingfield 1992; DeNardo and Licht 1993; DeNardo and Sinervo 1994*a*, 1994*b*; Sinervo and DeNardo

1996). Exogenous corticosterone increased clutch mass (fig. 1) and offspring size, but not clutch size (fig. 1).

In early March, I surgically implanted females with hormone implants (corticosterone: $N = 18$ in 1991, $N = 60$ in 1992, and $N = 42$ in 1993; FSH: $N = 30$ in 1993). A separate group of females received follicle ablations ($N = 41$ in 1992 and $N = 45$ in 1993) 10 d after implant experiments. Controls for surgery consisted of a large group of unmanipulated females (see above) and sham-manipulated females (see Sinervo and DeNardo 1996) that received a saline implant ($N = 19$ in 1991, $N = 61$ in 1992, and $N = 55$ in 1993). Survival of females was assessed from surgical manipulation to production of first, second, and third clutches.

Results

Heritable Variation in Egg Size and Covariation between Egg Size and Clutch Size

Egg size of the female parent was correlated with egg size of her daughter at maturity (fig. 2, *top*); however, experimental removal of yolk from freshly laid eggs had no effect on eggs produced by daughters when they matured (fig. 2, *bottom*). Thus, yolk volume per se did not act as a maternal effect to alter the egg size that daughters produced at maturity. The slope of the mother-daughter regression of egg size is equal to 0.304, and thus, $h^2 = 0.61 \pm 0.29$. Evidence of a genetic correlation should be reflected in significant covariation between a parental trait such as egg size and a second progeny trait such as the daughter's clutch size (and vice versa). Female parents that laid large eggs produced daughters that laid large eggs (fig. 2, *top*), and daughters also laid small clutches (fig. 3). A negative correlation between a dam's clutch size and daughter's egg size is suggestive of a genetically based trade-off (fig. 3). Mechanistic sources for the genetic correlation were elucidated with experiments.

In an earlier study (Sinervo and Licht 1991*a*), I investigated a model of follicular regulation that Jones (1978) has termed "follicular selection." Presumably, developing follicles compete for circulating gonadotropin (FSH) to maintain growth rate during the earliest stages of vitellogenesis. Follicles with greater vascularization may have higher uptake of FSH and yolk and this may lead to even greater vascularization at the expense of neighboring follicles (Jones et al. 1975, 1976; Jones 1978). If neighboring follicles do not receive sufficient FSH to sustain growth, they may undergo follicular atresia and be resorbed. Models of follicular selection are consistent with results of experimental manipulations of clutch size conducted on side-blotched lizards. Female *Uta stansburiana* that were injected with exogenous gonadotropin during the earliest

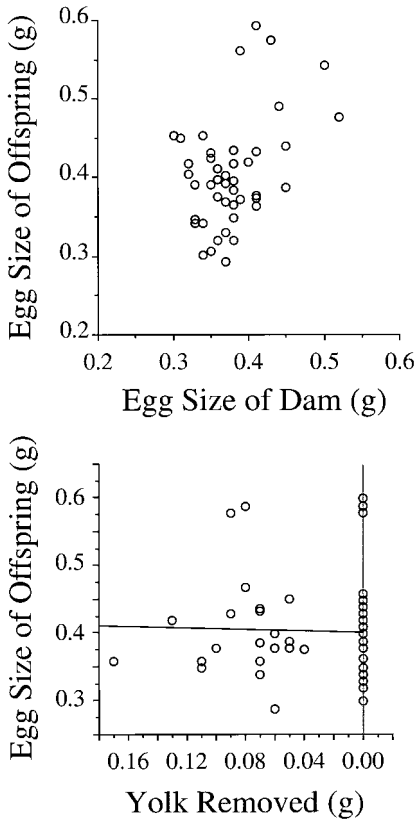

Figure 2: *Top*, Heritable variation in egg size ($h^2 = 0.61$) of free-ranging dams and their daughters. *Bottom*, Removing yolk from freshly laid eggs had no effect on egg size of progeny when they matured; thus, yolk-volume maternal effects were unlikely to confound estimates of heritability in egg size.

stages of vitellogenesis produced larger clutches (average clutch size is 6.0 eggs) relative to controls (4.3 eggs; Sinervo and Licht 1991*a*). Administration of exogenous FSH apparently may have made FSH less limiting, which promoted the growth of additional follicles on the ovary that might have otherwise undergone atresia. Manipulation of clutch size by exogenous gonadotropin also had a correlated effect on average egg size. Females that received FSH also produced smaller eggs (0.35 g) relative to controls (0.43 g in mass; fig. 3). Ablation of ovarian follicles is a surgical manipulation that simulates the converse process, in which sufficient FSH is not available for growth of all follicles and some undergo follicular atresia (Jones 1978), which in the case of follicle ablation occurs by experimental intervention. Follicle ablation also corroborated links between clutch and egg size regulation (Sinervo and Licht 1991*a*, 1991*b*). If a subset of follicles were ablated during midvitellogenesis, females produced a smaller clutch (2.3 eggs) that consisted of larger eggs (0.50 g; fig.

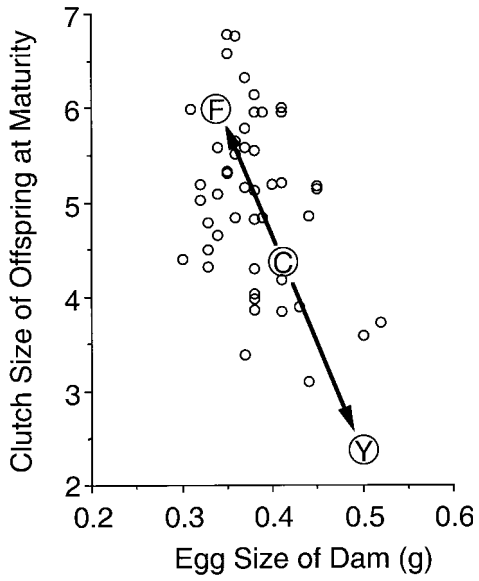

Figure 3: Covariation between a dam's egg size and a daughter's clutch size is indicative of an underlying genetic correlation. Experimentally induced covariation between clutch size and egg size (Y = ovarian follicle ablation, F = clutch size stimulation with FSH) and genetic covariation are remarkably similar, suggesting that experimentally induced trade-offs are likely to reflect genetically based trade-offs between clutch size and egg size. The genetic correlation is calculated as $r = -0.92$ (see Sinervo 1999*a*).

3) than controls. The genetic correlation between clutch size and egg size is negative and nearly one ($r = -0.92$; see Sinervo 1999*a*). Concordance between the genetic correlation and manipulations of clutch size and egg size regulation (fig. 3) suggests that the genetically based covariation arises from variation in production of gonadotropin releasing hormone (GnRH), production of gonadotropin (FSH) by the pituitary along with concomitant changes in plasma levels of FSH, or sensitivity of ovarian receptors to FSH. Lack of a radioimmunoassay for reptilian FSH (Licht 1970; Licht et al. 1977) and GnRH does not allow us to address these predictions at this time (Phillips et al. 1985, 1987).

Fitness Consequences of the Clutch Size and Egg Size Trade-Off for Offspring

Manipulations of clutch size and egg size elucidate mechanistic bases by which the endocrine system constrains the trade-off between offspring size and offspring number. The curve relating clutch size and egg size defines the first selective episode that acts on reproductive allocation, fecundity selection. These experiments also serve to verify the selective link between egg size and fecundity. Females that lay large clutches should also lay small eggs (fig. 4*A*), and small egg size should have cascading effects on offspring survival. However, survival of small offspring might arise from a number of causes that are merely correlated with egg size. For example, females in poor health might lay small eggs that are deficient in other micronutrients.

Offspring size manipulation or "allometric engineering" not only allows for the cause of survival selection to be ascribed to yolk volume, but allometric engineering has the added benefit of increasing the variance in offspring size. The combination of experimentally miniaturized and gigantized offspring inflated variance in egg size by 2 SDs (fig. 4*B*), which provides statistical leverage necessary to detect the shape of natural selection using fitness regression (Lande and Arnold 1983; Mitchell-Olds and Shaw 1987; Schluter 1988). I have used several controls for the effects of surgical manipulation of the female parent and for the miniaturization and gigantization procedures (Sinervo 1990; Sinervo and Huey 1990; Sinervo and Licht 1991*a*, 1991*b*; Sinervo et al. 1992). Sham surgical manipulation of the female parent per se did not affect her egg size, her clutch size, her own survival in the wild (Sinervo and DeNardo 1996), or the survival of her progeny (Sinervo et al. 1992). Moreover, eggs that were gigantized by follicle ablation in the female parent and then miniaturized back to "normal size" by removing yolk at oviposition had the same viability to hatching and offspring survival in the wild as sham-manipulated eggs. The "double manipulations" (miniaturized giant eggs) and the sham manipulations indicate that manipulations of offspring size mimicked the effects of natural differences in yolk volume among females. Selection gradients for natural variation in egg size were also comparable in magnitude to the selection gradients for experimentally reduced eggs (Sinervo et al. 1992).

During the first clutch of 1989 and 1991, the number of female offspring that survived to maturity was under strong stabilizing selection owing to two episodes of selection. Fecundity selection favored females that laid large clutches of small offspring (fig. 4*A*), and fecundity selection was balanced by survival selection that favored large female offspring (fig. 4*C*). During the course of the season, the fecundity selection differential relaxed in intensity and survival selection differential increased in intensity on later clutch progeny (see ANCOVA results discussed in Sinervo et al. 1992, Notes). These changes in fecundity and survival selection resulted in an upward shift in the optimal egg size from first to later clutches. Furthermore, survival selection continued to act differently on first versus later clutch progeny during adult life, which served to reinforce an adaptive plastic change in egg size across the season (fig. 4*C*). Mature progeny from the first clutch had high survival during the production of their own first clutch if

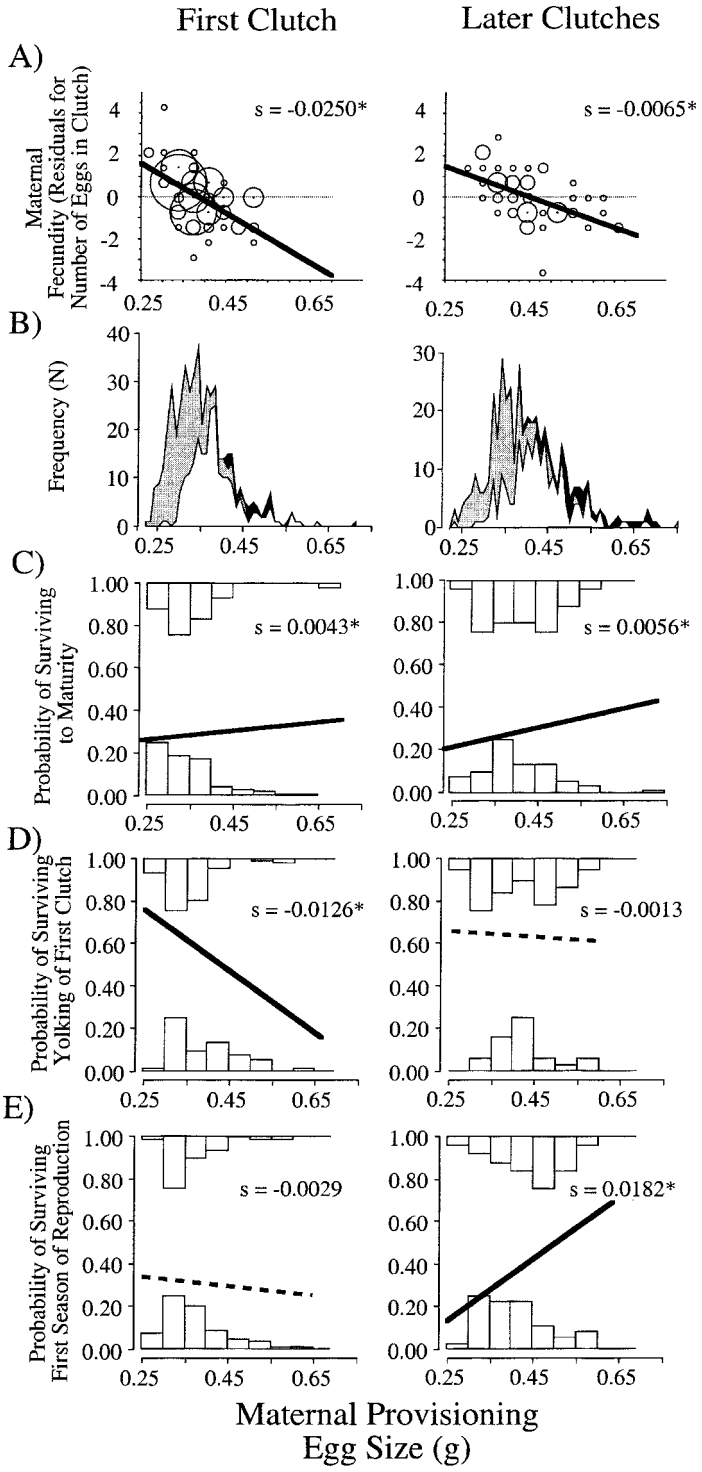

Figure 4: Natural selection on egg size during the life cycle of free-ranging female *Uta stansburiana* (data are pooled from 1989 to 1991; see Sinervo and Doughty 1996). Results are partitioned by the first clutch that females laid during the reproductive season and later clutches. Solid regression lines describe significant fitness regression surfaces (dashed lines are not significant). *A*, Fecundity selection on the first and later clutches favors the production of large clutches of small eggs (residuals for clutch size have effects of postlaying mass removed; data points that coincide are indicated by the size of the point). *B*, Frequency distributions describing natural variation (*white histograms*), experimentally decreased egg size (*gray histograms*; miniaturized by yolk removal), and experimentally increased egg size (*black histograms*; gigantized eggs by ovarian follicle ablation). Survival selection of natural and size-altered progeny during three phases of the life history: *C*, from hatching to initiation of reproduction (i.e., maturation); *D*, from initiation of maturation to production of the first clutch; *E*, from oviposition of the first clutch to the end of the reproductive season. Distribution of female offspring that survived (survival = 1) or died (survival = 0) during an episode of selection are found at the top and bottom of each panel. The fitness regressions for female offspring survival are based on experimental and natural variation (but see Sinervo et al. 1992 for a comparison of natural-selection-based natural and experimental variation).

they came from small eggs (fig. 4*D*), whereas mature progeny from later clutches had high survival during their own egg production on later clutches if they came from large eggs (fig. 4*E*). In summary, patterns of selection on first versus later clutch offspring favored female parents that change egg size during the season. Indeed, individual females showed a significant tendency to increase egg size during a single season (0.401 ± 0.009 vs. 0.437 ± 0.012 on first vs. second clutch, $P < .01$), tracking the shift in optimum egg size. Even though females plastically increased egg size, individual differences among females are significantly repeatable from first to second clutches (see Sinervo and Doughty 1996 for a repeated-measures ANOVA in heritable variation in egg size).

Survival selection as a function of egg size also depended on offspring sex (not shown, but see Sinervo et al. 1992). Whereas survival selection on female progeny is purely directional, survival selection on male progeny was usually stabilizing and at times favored male progeny from small eggs (Sinervo et al. 1992). Large egg size enhanced survival of female progeny, but large egg size was usually a liability for male progeny. The exact source of differential effects on female versus male progeny is beyond the scope of this article but appears to be related to striking frequency-dependent selection that acts on three alternative male strategies before and during maturation (Sinervo and Lively 1996; B. Sinervo, unpublished data).

Fitness Consequences of the Clutch Size and Egg Size Trade-Off for Adult Females

Manipulations of endocrine mechanism demonstrate that egg size, clutch size, and total clutch mass are a tightly regulated complex of traits (fig. 3) that promote a selective trade-off between fecundity and egg size (fig. 4*A*). The trade-off between offspring number and offspring quality is further influenced by progeny survival selection as a function of egg size during early life (fig. 4*C*) and during their adult life (fig. 4*D*, *E*). Selection on progeny during late life could arise from two causes. A long-lasting maternal effect arising from the mother's egg size might continue to affect progeny survival. Alternatively, as progeny initiate egg production, they turn on their own genes for clutch size and egg size that might lead to a cost of reproduction that reduces survival.

Allometric engineering of offspring size does not allow us to resolve the selective impact of reproductive costs during adult life, and I devised a number of ovarian manipulations to address these issues. Measures of experimentally induced costs must also be referenced to natural variation. Accordingly, I measured the number of clutches that a female produced during the reproductive season as a function of natural variation in clutch mass. The pattern of phenotypic selection on total clutch mass was obscured by environmental effects that were correlated with female aggregations. Path analysis was used to remove effects of the local density environment on a female's clutch mass and number of clutches produced during the reproductive season (fig. 5). Presumably, density-free residuals should reveal selective costs that are not obscured by density-induced reproductive plasticity.

In path analysis, any two paths that are linked to a common cause can be used to derive the correlation between traits (Li 1975). For example, a positive path between density and clutch mass in 1991, but negative path between density and number of clutches, yields a density-induced correlation between clutch mass and number of clutches $= 2 \times -0.33 \times 0.36 = -0.24$ (fig. 5). In 1992, a positive path between density and clutch mass and a positive path between density and number of clutches yield a density-induced correlation between clutch mass and number of clutches $= 2 \times 0.50 \times 0.27 = 0.27$ (fig. 5). In 1993, density had a positive effect on number of clutches but a nonsignificant negative effect on clutch mass, and the environmentally induced correlation between clutch mass and number of clutches $= 2 \times 0.36 \times -0.15 = -0.11$ was not significant (fig. 5). Finally, in 1994, density had a negative effect on number of clutches, but no effect on total clutch mass and the environmentally induced correlation between clutch mass and number of clutches $= 2 \times -0.27 \times 0.00 = 0.00$ was not significant (fig. 5).

I compared natural correlations between density-free residuals for total clutch mass and number of clutches with causal relations (fig. 6) that were experimentally induced by follicle ablation, FSH implants, and corticosterone implants. I measured cascading effects of each manipulation on number of clutches produced during the reproductive season. In 1991, groups of females with experimentally enhanced total clutch mass (corticosterone implants) had high survival (Sinervo and DeNardo 1996) and produced significantly more clutches during the reproductive season than controls. This is contrary to expectations that there was a cost to reproduction. Effects are not merely a pharmacological effect of the hormone corticosterone on survival because similar patterns are seen in natural variation (fig. 6*A*). Females that laid a large natural total clutch mass in 1991 also enjoyed higher survival than females that laid a small total clutch mass (fig. 6*A*). This result highlights the importance of measuring natural variation to rule out the possibility that experimentally induced causes do not lead to a spurious pharmacological effect.

In 1992, natural variation in total clutch mass residuals yielded the expected costs of reproduction, in that females with naturally high clutch mass residuals survived to pro-

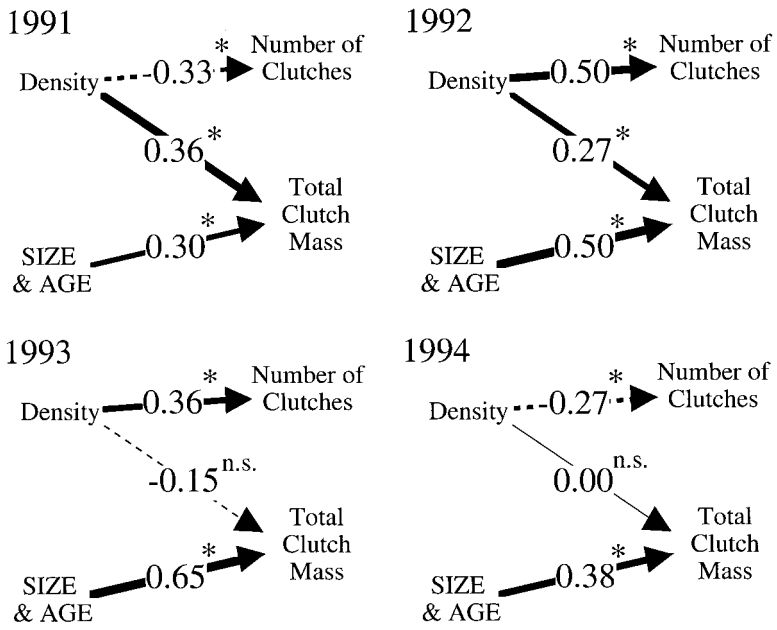

Figure 5: Path analysis of environmentally induced correlations between total clutch mass and number of clutches produced during the reproductive season. Investment in current reproduction can become correlated with future reproductive success because the environmental cause density affects both traits (see text). In addition, female size or age can also affect total clutch mass. Removing environmental influences of density and female size on reproduction yields residuals (see fig. 6) that describe the selective trade-off between current investment and future reproductive success. Thickness of arrows is proportional to the magnitude of the path coefficient. Positive paths are denoted by solid lines, and negative paths are denoted by dashed lines.

duce fewer clutches during the season (fig. 6*B*). In addition, experimentally reducing total clutch mass by ablating ovarian follicles enhanced survival and increased the number of clutches produced during the season relative to controls (fig. 6). The complementary manipulation of enhanced clutch mass via corticosterone implants did not affect survival in 1992. The difference between the two experimental manipulations may be due to the timing of each relative to the female reproductive cycle. Corticosterone affects early follicle development and energy acquisition but has no obvious phenotypic effects on females after laying (Sinervo and DeNardo 1996). In contrast, follicle ablation simulates later-acting processes of follicular atresia. Follicle ablation causes females to be in better condition after laying, and this may be the route by which postlaying survival is enhanced. The difference between the two manipulations suggests selection in 1992 acted on postlaying costs of reproduction.

While results from experimental and natural variation during 1991 and 1992 were largely consistent, there was a striking discrepancy between experimental and natural variation during the spring of 1993 (fig. 6*C*). Natural phenotypic variation in clutch mass during 1993 did not have a significant impact on survival of subsequent clutches. In contrast, two manipulations that enhanced clutch mass (corticosterone implants and follicle-stimulating hormone implants) exacerbated costs of reproduction and lowered survival to subsequent clutches. Conversely, reducing total clutch mass via follicle ablation ameliorated costs of reproduction and enhanced survival to subsequent clutches. Reasons for the discrepancy between natural and experimentally induced covariation during 1993 are discussed below and relate to the difference between maturational versus postlaying costs of reproduction.

Discussion

Evolutionary Response to Natural Selection and Inferences from Experimental Manipulations

Given that reproductive traits in side-blotched lizards are heritable and genetically correlated, the traits should respond to the force of natural selection. A comprehensive estimate of natural selection entails measurement of lifetime reproductive success of females (Clutton-Brock 1988) as a function of the maternal effects of reproductive traits on offspring survival and the costs of reproduction that arise when offspring turn on their own endocrine machinery at maturity. A number of experimental manipulations must be used to dissect the causes of selection

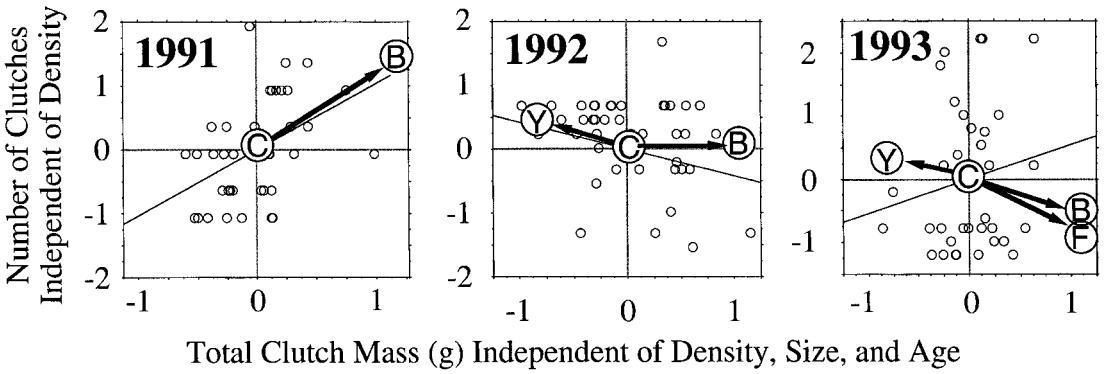

Figure 6: Natural covariation between total clutch mass residuals and number of clutches produced during the season is significant and positively correlated in 1991, significant and negatively correlated in 1992, but not significantly correlated in 1993. Natural covariation is represented by open circles and regression lines. (N.B.: Residuals for natural variation have environmental effects of density, size, and age removed by path analysis; see fig. 5 and text). In 1991, enhancing clutch mass with corticosterone implants (*arrows*, *B*) enhanced survival, and females produced more clutches compared to controls (*C*). This experiment corroborates the natural positive correlation between residuals for clutch mass and number of clutches. In 1992, reducing clutch mass (*Y*) enhanced survival, and females produced more clutches compared to controls (*C*), which provided experimental corroboration of the natural negative correlation between residuals for clutch mass and number of clutches. In 1993, all three manipulations of clutch mass had significant and consistent effects on female survival and clutch production. Inducing large clutch mass with FSH (*F*) or corticosterone (*B*) reduced survival while reducing clutch mass (*Y*) enhanced survival. Despite strong "costs of reproduction" detected in the experiments, no significant costs were found in natural phenotypic variation.

across juvenile and adult phases of the life history. Moreover, using a variety of complementary manipulations during each phase of the life history ensures that selection is not a spurious consequence of one particular manipulation. Finally, referencing the manipulations to controls (e.g., sham manipulations) and natural variation corroborates the validity of the experimentally induced effects as they pertain to inferences about evolution of the life history and rules out additional spurious experimental artifacts.

Fecundity selection favored females that produced large clutches of small eggs (figs. 1, 3, 4*A*), but fecundity selection was balanced by survival selection that favored the survival of progeny from large eggs. The net result of these counterbalancing forces resulted in stabilizing selection or an optimal egg size; however, the optimum shifted during the season. The difference in optimum egg size on first versus later clutches favors reproductive plasticity on the part of the female parent. Moreover, there was a tendency for selection to favor an even larger seasonal shift in egg size (Sinervo 1998) because the observed shift in egg size is small compared to the shift in optimal egg size (Sinervo et al. 1992). In particular, females should produce much larger later clutch eggs since the optimum is much higher than observed egg size on later clutches. However, functional constraints arising from the pelvic girdle of vertebrates (Congdon and Gibbons 1987; Sinervo and Licht 1991*a*, 1991*b*) may limit further increases in egg size on later clutches. Females with naturally large eggs and experimentally enlarged eggs have a higher frequency of burst eggs at laying or become oviductally egg bound at a higher frequency (Sinervo and Licht 1991*b*). Such proximate constraints reflect a functionally based cost of reproduction associated with the production of large offspring (Sinervo 1994*a*, 1994*b*). While it may be possible to evolve a larger pelvic girdle in females to accommodate larger eggs, the pelvic girdle is central to lizard locomotion (Snyder 1952, 1954) and may be subject to additional biomechanical constraints.

Additional costs of reproduction arise from allocation per se. Survival selection as a function of reproductive output by mature offspring was quite strong and directional but does not always favor adult females that lay a small total clutch mass. For example, natural and experimentally induced phenotypic selection during 1991 favored females that laid a large total clutch mass (TCM). Selection differentials (s) based on an analysis of natural and experimentally induced variation were also consistent with the magnitude and direction of an evolutionary response ($R = h^2 \times s$; Falconer 1981) to natural selection that resulted in a significant increase in total clutch mass from 1991 to 1992 (fig. 6). For example, the observed response to selection is given by

$$R_{\text{observed}} = \text{TCM}_{1992} - \text{TCM}_{1991} = 0.24,$$

and the response to selection calculated from the experimental selection differential (see Sinervo 1998 for com-

putation of selection differentials from experimental variation) is given by

$$R_{\text{calculated}} = h^2 \times s = 0.61 \times 0.422 = 0.26$$

(see fig. 6*A*).

Natural and experimentally induced phenotypic selection during 1992 favored females that laid a small total clutch mass. The direction of natural and experimentally induced selection differentials during 1992 was consistent with an evolutionary response to selection that resulted in a significant decrease in clutch mass from 1992 to 1993:

$$R_{\text{observed}} = \text{TCM}_{1993} - \text{TCM}_{1992} = -0.50.$$

However, the magnitude of this observed response to selection is far too large to be explained by the response calculated from heritability and the experimentally induced selection differential:

$$R_{\text{calculated}} = h^2 \times s = 0.61 \times -0.217 = -0.13$$

(see fig. 6*B*).

Cryptic Natural Selection as Detected by Phenotypic Manipulations

The discrepancy between observed and calculated response to selection is a red flag that indicates additional episodes of selection might have been missed in analysis of phenotypic variation. Alternatively, the between-year change in egg size and clutch mass could easily be explained in terms of an environmentally induced change across years. For example, experimentally watering plots of desert habitat demonstrate that additional rainfall triggers an increased clutch size in female side-blotched lizards compared to females on control plots (James and Whitford 1994). Both 1992 and 1993 were wet years, and it is unlikely that a change in clutch or egg size was induced by a change in rainfall between years. In the absence of any other abiotic differences between years, we are left with the first hypothesis—undetected natural selection.

Natural history observations indicate that maturing progeny experienced unusually high mortality (>50%) as they began to yolk their first clutch during early March 1993. This mortality occurred before they had a chance to lay their eggs and before I could index their reproductive investment. Moreover, Sinervo and DeNardo (1996) observed a four- to fivefold increase in the number of predatory snakes in the spring of 1993. Large numbers of predatory juvenile snakes hatched in the fall of 1992, which were not present before 1992. The alleviation of a 7-yr drought came about with heavy El Niño rains in March 1992. I hypothesize that increased predation risk in the spring of 1993 induced a high maturational cost of reproduction. Reproductive burden is known to decrease sprint performance of gravid female lizards (van Damme et al. 1989; Sinervo et al. 1991), which enhances the risk of predation by snakes (Shine 1980).

There is no way to measure selection differentials from the phenotype of females developing a large total clutch mass because they could have been depredated before laying their eggs. Indeed, no selection was detected on natural variation in total clutch mass during the spring of 1993 (fig. 6*C*). However, experimentally altering clutch mass allowed us reliably to assess mortality effects on "high-risk" and "low-risk" phenotypes relative to control females that lay an average-sized clutch mass. Experimentally elevating clutch mass via either exogenous FSH or exogenous corticosterone induced maturational costs of reproduction (fig. 6*C*). Conversely, experimentally reducing clutch mass via follicle ablation reduced costs (fig. 6*C*). The selection differential induced during maturational phases of the life cycle was nearly sufficient in magnitude to explain the observed response to selection between 1992 and 1993 (-0.50; see above). The response to selection calculated from experimentally induced postlaying selection in 1992 (e.g., follicle ablation) and maturational selection in 1993 (e.g., FSH-induced costs) is given by

$$\begin{aligned} R_{\text{calculated}} &= h^2 \times (s_{1992} + s_{1993}) \\ &= 0.61 \times (-0.217 - 0.496) = -0.43. \end{aligned}$$

Three complementary manipulations of clutch mass (fig. 1) allowed us to detect "cryptic natural selection" that would be impossible to resolve from an analysis of "phenotypic selection" on reproductive output. The phenomenon of cryptic natural selection adds to the problems associated with phenotypic selection that have already been discussed, including statistical issues (Mitchell-Olds and Shaw 1987), issues related to environmental causes of natural selection (Wade and Kalisz 1990), unmeasured traits that are the true focus of selection (Lande and Arnold 1983; Mitchell-Olds and Shaw 1987), and detection of the shape of the fitness surface (Schluter 1988). Our results demonstrate that even in cases where the entire life cycle is available for analysis, phenotypic selection may remain undetected. This is not usually a problem with selection analyses of static morphological traits that govern shape (Grant 1986), but it is a very serious problem with life-history traits that change over time, such as growth, maturation, and reproduction. Total clutch mass was under selection in 1993, but selection operated on females during the early ontogeny of trait expression. Expression of phe-

notypic variation in life-history traits entails physiological and behavioral changes that place an organism at risk of selective mortality that can go undetected unless the physiological traits that give rise to the life-history variation can be measured. A quantitative genetic analysis of the negative genetic correlation between clutch production and maturational survival circumvents many of the problems of traditional phenotypic selection (Sinervo 1999*a*; Rausher 1992).

Environmental Effects and Detection of Costs of Reproduction

Path analysis of reproductive plasticity and the social environment provides another cautionary note regarding the detection of costs of reproduction in the absence of adequate experimental protocols. I found that density had very different effects on traits between the years 1991 and 1994 because of dramatic changes in March rainfall, density, and the predation regime. In our studies, females may have aggregated at high density because of a local concentration of resources. In these rich areas they both produced large clutches and survived to lay many clutches. In 1992, conditions at aggregation sites were good from the point of view of current effort as well as future reproductive success (e.g., number of clutches). However, as the overall population density increased from 1991 to 1994 (see Sinervo and DeNardo 1996), the sites that supported females producing larger-than-average clutch sizes became crowded. As overall population density increased, conditions at these good sites appears to have worsened with overcrowding, as was the case in 1993 and 1994, and effects of density on reproductive output became negative (fig. 7*C*, *D*). Compounding the change in density of lizards between 1991 and 1994 is an increase in the number of predatory snakes, which may cue into the high-density aggregations of females.

Path analysis of these environmental effects in side-blotched lizards reinforces the view that detection of costs is problematic in the face of reproductive plasticity that is typical in laboratory experiments (Chippendale et al. 1993; Leroi et al. 1994) and natural populations (Hogstedt 1980; Tuomi et al. 1983; van Noordwijk and de Jong 1986; Sinervo and DeNardo 1996). However, in the case of manipulations of reproductive output, effects of treatment are randomized across the varied social and abiotic environments, and environmental covariance between traits and fitness (Rausher 1992) does not confound experimental assessment of costs of reproduction.

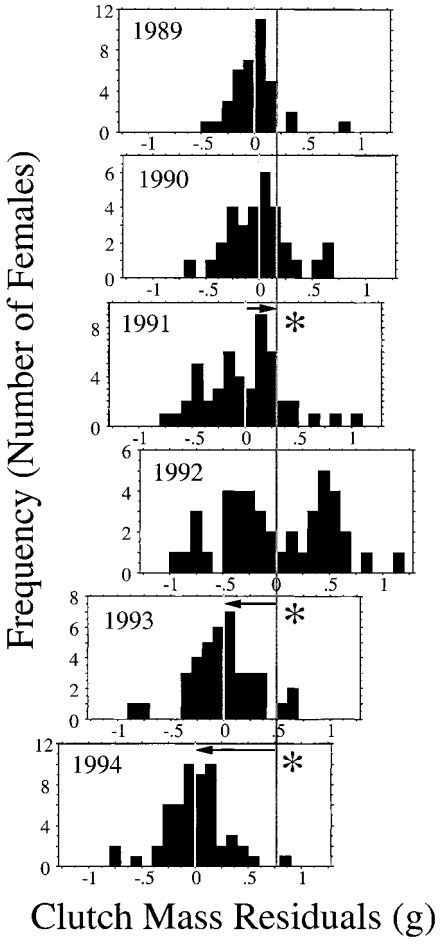

Figure 7: Changes in residuals for total clutch mass from 1989 to 1994 (positive effects of postlaying mass on clutch mass were removed by regression analysis). The means for all years (*light gray bar*) are referenced relative to 1992 (*heavy gray bar*) because 1992 was the pivotal year in which heavy March rainfall ended a 7-yr drought in California, which allowed predatory snakes to increase in density. Changes in total clutch mass were also mirrored by changes in egg size (not shown, but see Sinervo and DeNardo 1996) and clutch size (not shown, but see Sinervo 1998). Because egg size (fig. 2) and clutch size are heritable (Sinervo 1999*b*), the significant increase in clutch mass between 1991 and 1992 and decrease between 1992 and 1993 would be consistent with natural selection on reproductive allocation (fig. 6) that leads to evolved changes between generations.

Single-Trait Manipulations versus Multitrait Mechanisms and Inferences on Life-History Trade-Offs

The interpretation of heritable variation in egg size could be likewise confounded by potential environmental effect. Egg size is heritable ($h^2 = 0.61$); however, egg size is also influenced by the "maternal environment" in a way that can influence later life-history traits. The maternal effect neonate size could influence growth (Riska et al. 1985),

size at maturity (Falconer 1965), offspring performance (Sinervo 1990; Sinervo and Huey 1990), and litter size (Falconer 1965). I tested for maternal effects of egg size by removing yolk. Yolk removal had no effect on the egg size produced by offspring at maturity (fig. 2). Thus environmental maternal effects do not have manifold effects on the life history. While I have eliminated the possibility that yolk volume acts as a confounding maternal effect, I have not ruled out the possibility that maternal effects of yolk composition (Congdon et al. 1983) might influence daughter's egg size at maturity. "Allometric engineering," in which yolk is removed from freshly laid eggs, is conceptually similar to approaches that others have used to evaluate maternal effects on the expression of life-history variation. Cowley et al. (1989) used embryo transfer between different genotypes of mice to experimentally assess the magnitude of preparturition maternal effects on growth rate. Schluter and Gustafsson (1993) used clutch size manipulation on female pied flycatchers to detect the effect of clutch size on the daughter's condition.

Clutch size manipulation in birds (Lack 1947) or yolk removal from freshly laid eggs (Sinervo 1990; Sinervo and Huey 1990) are "single-trait manipulations" akin to "cut-and-paste" experiments that are common in studies of sexually selected traits (see Sinervo and Basalo 1996). The primary benefit from such "cut-and-paste" experiments is that correlations arising from confounding traits are broken by a controlled experimental manipulation (Sinervo and Basalo 1996). For example, Andersson (1982) manipulated tail feathers in widowbirds to assess the selective impact of tail length on female choice. Such protocols are particularly useful in analysis of sexually selected traits that might be indicators of good genes (Petrie 1994) since they allow one to distinguish between female choice based on ornament size versus the male's overall health. Manipulating the ornament isolates its impact on female choice. However, a single manipulation does not necessarily identify all of the relevant trade-offs (Sinervo and Basalo 1996), such as those involved in the growth and development of the structures, or in the rearing of progeny. Several manipulations (e.g., tail length, parasite extirpation) could be simultaneously applied in a factorial fashion to address more complex life-history trade-offs (Møller 1988, 1990).

In contrast, "mechanistic" experiments of ovarian regulation (e.g., follicle ablation, FSH, and corticosterone implants; Sinervo and Licht 1991*a*, 1991*b*) maintain the essence of genetically based correlations that arise from pleiotropy because they act on a mechanism that regulates two or more traits (e.g., clutch size, egg size, total clutch mass, and future reproductive success). Because mechanistic manipulations maintain the essence of genetically based correlations, they provide a powerful avenue for investigating life-history trade-offs and the process of adaptation. I have used manipulations of ovarian regulation to address the trade-off between offspring number and offspring quality as well as the costs of reproduction for female parents. Such "proximate constraints" of ovarian regulation parallel the genetic correlation between clutch size and egg size (fig. 3). The link between endocrine manipulations and additive genetic variation critically depends on the amount of genetic variation that is present along a given endocrine axis. A challenge for future studies will be to develop measures of genetic variation in the regulation of endocrine systems. In a similar vein, male hormones might be under sexual selection and may influence other life-history traits (e.g., parental care, survival). Manipulation of testosterone (Marler and Moore 1988; Sinervo 1999*c*) or other hormones that interact with testosterone such as the stress hormone corticosterone (DeNardo and Sinervo 1994*a*, 1994*b*) would capture the essence of physiological trade-offs involved in male reproduction (see Ketterson and Nolan 1999, in this issue). In addition, manipulation of GnRH or gonadotropin in males might alter the expression of secondary sexual characters (Bass 1996) and stimulate production of hormones such as testosterone that have cascading effects on male physiology. The more upstream the manipulation occurs in the regulatory network, the more life-history trade-offs are captured in the manipulation.

A Mechanistic Refinement of Lack's and Williams's Principles of Life-History Allocation

David Lack (1947, 1954) considered the evolutionary consequences of the offspring quality and offspring number trade-off, and George C. Williams (1966) refined Lack's ideas by pointing out that costs of reproduction can also limit the amount of energy parents invest in reproduction. Subsequent theoretical analyses often model each of the trade-offs in a piecemeal fashion, where only a theory for optimal egg size (Smith and Fretwell 1974; Brockelman 1975; McGinley et al. 1987) or only a theory for costs of reproduction (Shine and Schwarzkopf 1992) is considered. In light of the endocrine mechanisms that underlie allocation trade-offs in female lizards, we must develop a theory that simultaneously synthesizes a number of competing life-history allocation trade-offs. If energy is limiting in an organism, then there will be competition for resources among the different allocation pathways. Why are trade-offs so difficult to detect if they represent a syllogism similar in spirit to Liebniz's Law of the Minimum?

Analysis of regulatory networks, as presented here for side-blotched lizards, illustrates the multifarious physiological routes by which such competition and regulation arise. Life-history trade-offs do not arise from mystical black boxes that are manifest as a genetic correlation. Egg

size, clutch size, and total clutch mass are regulated by GnRH, which stimulates the production of FSH. Presumably, high plasma titers of GnRH or high plasma titers of FSH could have been present in those female utas that died during maturational phases of reproduction in 1993, which may have increased behaviors that elevate predation risk or perhaps decreased escape speed through the burdening effect of the developing clutch (Shine 1980; Bauwens and Thoen 1981; Sinervo et al. 1991).

To be sure, regulatory feedback loops of the hypothalamic-pituitary-gonadal axis or the hypothalamic-pituitary-adrenal axis make a physiological analysis of life-history traits a bit more complicated, particularly in a natural history context. Life-history trade-offs might arise from genetic variation at a number of regulatory events. For example, FSH stimulates the growth of follicles on the ovary, and the growing follicles begin producing estrogen. Estrogen stimulates the cells in the liver to produce vitelloproteins (Gerstle and Callard 1972; Yaron and Widzer 1978; Ho et al. 1982; Gavaud 1986) through estrogen receptor binding sites on the DNA that promote translation of vitelloprotein. Fat bodies (Hahn and Tinkle 1965) and vitelloproteins constitute a major energy cost during reproduction (Hahn 1967). However, follicular production of estrogen is linked to a negative feedback loop that shuts down pituitary production of gonadotropin and inhibits the further stimulation of additional follicles. This may even lead to a reduction in clutch size by the process of follicular atresia if sufficient gonadotropin is unavailable to support the growth of all ovarian follicles (Jones 1978).

Finally, the regulatory cycle is also governed by the hypothalamic-pituitary-adrenal axis. Gonadotropin stimulates adrenal secretion of the stress hormone corticosterone in reptiles (Callard and Callard 1978) and levels of plasma corticosterone (i.e., B) are highest immediately before ovulation when female side-blotched lizards have very large ovaries (Wilson and Wingfield 1992). One of the functions of corticosterone in female *Uta stansburiana* may involve energy mobilization during reproduction (Wilson and Wingfield 1992). In this fashion, clutch size, egg size, and total clutch mass are regulated by positive and negative feedback and the trade-off between these three traits is formed (Sinervo and Licht 1991*a*, 1991*b*). Because corticosterone is also the primary stress hormone in vertebrates (Johnson et al. 1992), corticosterone may play an important role in generating life-history plasticity. It is not surprising that life-history plasticity confounds an analysis of life-history trade-offs. They share common mechanisms of regulation. Mechanisms of life-history trade-offs are inextricably linked to mechanisms of life-history plasticity. The major costs of reproduction may also lie in the costs of allocation plasticity.

Assessment of the selective consequences of such a feedback loop would require that I measure changes in hormone titers across ovarian development, which is a daunting task even with this simple cascade of four hormones (GnRH, FSH, E, and B). In this regard, endocrine and surgical perturbations of regulatory feedback loops will always be useful to assess the role of individual hormones (Marler and Moore 1988, 1991; Ketterson et al. 1991) or hormone interactions (DeNardo and Sinervo 1994*a*, 1994*b*; Sinervo and DeNardo 1996) in promoting selection on reproductive allocation. Moreover, manipulations allow the phenotype to be altered in a predictable and controlled fashion, allowing detection of the elusive episodes of selective mortality during maturation. Experimental analysis allows one to detect such selection because the induced selection can be reliably assigned to treated versus control groups. Experimental assessment of natural selection on juvenile and adult life-history phases of the side-blotched lizard further argues that selection on the offspring traits must be treated in tandem with selection on adult physiology. Despite the problems entailed with analysis of phenotypic selection and the potential solution afforded by experimental manipulations, we must still endeavor to link experiments back to genetic variation that governs evolutionary change (Reznick 1985, 1992). This remains a challenge for future studies. A promising approach may lie in quantitative genetic analysis of negative genetic correlations between traits and fitness in the wild (Rausher 1992) that is coupled with analysis of the mechanistic assessment of the resulting life-history trade-offs (Sinervo 1999*b*).

Acknowledgments

I wish to thank R. Schrimp and P. Stadler for allowing us to work on their land; D. Reznick, J. Schmitt, and E. Svensson for critically reading the manuscript; and K. Allred, L. Carroll, D. DeNardo, P. Doughty, T. Frankino, J. Graff, and K. Zamudio for assistance with data collection. Research was supported by National Science Foundation grants BSR 89-19600, DEB 93-07999, and IBN 96-31757.

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