PATERNAL CONDITION DRIVES PROGENY SEX-RATIO BIAS IN A LIZARD THAT LACKS PARENTAL CARE

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Sex-allocation theory predicts that females in good condition should preferentially produce offspring of the sex that benefits the most from an increase in maternal investment. However, it is generally assumed that the condition of the sire has little effect on progeny sex ratio, particularly in species that lack parental care. We used a controlled breeding experiment and molecular paternity analyses to examine the effects of both maternal and paternal condition on progeny sex ratio and progeny fitness in the brown anole (Anolis sagrei), a polygynous lizard that lacks parental care. Contrary to the predictions of sex-allocation theory, we found no relationship between maternal condition and progeny sex ratio. By contrast, progeny sex ratio shifted dramatically from female-biased to male-biased as paternal condition increased. This pattern was driven entirely by an increase in the production of sons as paternal condition improved. Despite strong natural selection favoring large size and high condition in both sons and daughters, we found no evidence that progeny survival was related to paternal condition. Our results emphasize the importance of considering the paternal phenotype in studies of sex allocation and highlight the need for further research into the pathways that link paternal condition to progeny fitness.

KEY WORDS: Body condition, good genes, paternity analysis, selection analysis, sex allocation, Trivers–Willard hypothesis.

In their landmark paper on adaptive sex allocation, Trivers and Willard (1973) hypothesized that mothers in a good physiological condition should bias the sex ratio of their progeny in favor of the sex that provides the greatest relative fitness return for a given increase in maternal investment. This influential idea has sparked numerous theoretical extensions and empirical tests in what has become one of the most active research areas in evolutionary biology (Burley 1982; Charnov 1982; Frank 1990; Godfray and Werren 1996; Leimar 1996; Hardy 2002; Komdeur and Pen 2002; Wapstra et al. 2007; West 2009). Indeed, Carranza (2002) speculated that “probably no other case exists in behavioral ecology where a couple of pages have sired so many studies.” Despite this substantial body of subsequent research, the generality of the Trivers–Willard hypothesis remains largely uncertain outside of mammals (Brown 2001; Cameron 2004; Sheldon and West 2004), whose high levels of maternal investment provided the original template for the theory (Trivers and Willard 1973). However, the underlying assumption that progeny sex ratio can be altered in response to environmental stimuli has been verified in a variety of organisms, many of which lack extensive parental care (Charnov 1982; Hardy 2002; West and Sheldon 2002; Wapstra et al. 2007; West 2009).

The original Trivers–Willard (1973) hypothesis was tailored to the mating system and reproductive biology of caribou (i.e., polygyny, single offspring per reproductive episode, extensive post-partum maternal investment). Under these conditions, Trivers and Willard predicted that mothers in a good condition should preferentially produce sons. This specific prediction was
based on three assumptions: mothers in a good condition produce offspring in a good condition, offspring in a good condition become adults in a good condition, and differences in adult condition have a greater impact on the reproductive success of males than of females. This final assumption is derived from the observation that, for most polygynous species, reproductive success is more skewed among males than among females (Andersson 1994). Thus, differences in relative fitness are predicted to be greater between high- and low-condition males than between high- and low-condition females, and mothers in a good condition should therefore invest preferentially in sons. These assumptions are realistic for many polygynous mammals, and recent meta-analyses have found general support for the Trivers–Willard hypothesis in this group (Cameron 2004; Sheldon and West 2004).

Much of the significance of the Trivers–Willard hypothesis stems from the fact that its basic argument provides a very general theory for sex allocation that can be extended beyond its specific predictions for polygynous mammals (Charnov 1982; West 2009). For example, if daughters benefit more from increased maternal investment than do sons, then predictions about adaptive sex ratios are reversed. Likewise, if factors other than maternal condition differentially influence the fitness of sons and daughters, then females should bias the sex ratio in light of these additional factors. This generality is important when considering species that deviate from the original mammalian template envisioned by Trivers and Willard (1973). For example, in most lizards, maternal investment is restricted to the initial provisioning of the egg or embryo (Wapstra et al. 2007). Moreover, because many lizards produce multiple offspring per clutch or reproductive season, maternal investment strategies often involve trade-offs between offspring quality and offspring quantity (Sinervo et al. 2000). If mothers in good condition preferentially increase offspring quality rather than offspring quantity, then the original assumptions of the Trivers–Willard hypothesis may not apply. Perhaps consequently, those few studies that have tested the Trivers–Willard hypothesis in lizards have generated mixed results (Fox and McCoy 2000; Olsson and Shine 2001; Lovern and Passek 2002; Uller et al. 2006; Warner et al. 2007). It is therefore important to test not only the effect of maternal condition on progeny sex ratio, but also the underlying assumptions that maternal condition influences progeny phenotypes, and that maternal condition differentially influences the relative fitness of sons and daughters.

Although the original Trivers–Willard hypothesis focused on maternal condition, paternal condition can also influence sex allocation if fathers in good condition are better able to provision their offspring (e.g., Korpimaki et al. 2000). Paternal condition could also influence sex allocation by signaling other direct (e.g., high-quality territory) or indirect (i.e., “good genes”) benefits of mating with high-condition males, provided these benefits differentially influence the fitness of sons and daughters. Indeed, females of many species bias progeny sex allocation on the basis of phenotypic indicators of paternal quality, such as coloration, ornamentation, and body size (Ellegren et al. 1996; Svensson and Nilsson 1996; Kollikor et al. 1999; Sheldon et al. 1999; Calsbeek and Sinervo 2002; West and Sheldon 2002; Olsson et al. 2005; Roed et al. 2007). However, sex-allocation studies have generally neglected the possibility that females select for indirect genetic benefits on the basis of male condition per se (Roed et al. 2007).

In this study, we simultaneously examined the effects of both maternal and paternal condition on progeny sex ratio in the brown anole (Anolis sagrei), an oviparous lizard that lacks parental investment beyond the initial provisioning of the egg. Brown anoles are ideally suited for such a test because they are thought to possess a mechanism for biasing progeny sex ratio on the basis of sire phenotypes (Calsbeek and Bonneau 2008; Cox and Calsbeek 2010). Moreover, the polygynous mating system, female-biased adult sex ratio, and extreme male-biased sexual size dimorphism of this species indicate that reproductive success is more highly skewed among males than among females, as assumed by the Trivers–Willard hypothesis. We therefore predicted that progeny sex ratio should become increasingly male-biased as both maternal and paternal condition increase. We also tested for maternal and paternal effects on sons and daughters by examining correlations between parental condition and progeny phenotypes. Finally, we tested the actual fitness consequences of progeny sex allocation by tracking the survival of sons and daughters and examining their fitness as a function of both maternal and paternal condition. We predicted that any conditional bias in sex ratio would be accompanied by an increase in the relative fitness of that sex that was preferentially produced by dams or sires in good condition.

**Materials and Methods**

**BREEDING DESIGN**

We raised sires and dams in a laboratory common-garden to minimize nongenetic variation in body size. Sires and dams were hatched from eggs laid in captivity by an initial stock of 69 females collected from Great Exuma, The Bahamas (23°30′N, 75°45′W). A subset of dams was captured from this population as adults and maintained in captivity for over three months until they depleted any stored sperm (Calsbeek et al. 2007; Calsbeek and Bonneau 2008). Our results did not differ with respect to the inclusion or exclusion of these dams. We fed all animals an ad libitum diet of fruit flies (Drosophila, for hatchlings) and crickets (Fausta, for juveniles and adults). Food was dusted weekly with vitamin and mineral supplements (Repta-Vitamin, Fluker Farms, Port Allen, LA). Adults were housed individually in 10-gal glass terraria placed under a 40-W incandescent bulb in a reflective hood for heat (diurnal temperature range = 26–35°C) and two Repti Glo
Brown anoles store sperm for several months and repeatedly lay single eggs at ca. 11-day intervals (Calsbeek et al. 2007; Calsbeek and Bonneaud 2008). In our colony, a single bout of mating can result in the production of up to 16 successive progeny over a three-month period (Fig. S1). We provided each captive dam with two potential mates that were matched for age but paired randomly with respect to body condition. This paired mating design was implemented as a part of a separate study investigating mate choice as a function of male body size (Cox and Calsbeek 2010). However, this design also allowed us to approximate the natural reproductive biology of the species, as most wild female anoles produce offspring by more than one sire (Calsbeek and Bonneaud 2008). We allowed dams to assess both males together for two days and then permitted each male to mate exclusively with the dam for one week to minimize the influence of direct male–male interactions on mating success. We also allowed each male to mate with two dams, once as the first and once as the second male, thus controlling for potential effects of mating precedence on fertilization success.

We collected tissue samples (2-mm, tail tip) from all progeny, of captive breeding involving thousands of individual hatchlings “poor” (<0.5 SD), “average” (±0.5 SD), or “good” (>0.5 SD) relative to the population mean for each sex. Similar statistical results were obtained regardless of whether condition was treated as continuous or categorical.

We analyzed patterns of progeny sex allocation by treating each sire (n = 59) or dam (n = 67) that produced progeny as an observation and summing his or her total progeny across both potential mates. We calculated sex ratio as the proportion of sons to total progeny and tested for effects of maternal or paternal condition on sex ratio using generalized linear models (GLMs) with logit links and binomial error distributions, following procedures advocated by Wilson and Hardy (2002). The ratio of Pearson’s χ2 to residual degrees of freedom never differed significantly from 1 (P > 0.05), so we did not scale for overdispersion (Wilson and Hardy 2002). We conducted these analyses in two different ways. First, because the precision of an estimate of sex ratio increases with the number of offspring contributing to that estimate, we

PATERNITY ANALYSES
We collected tissue samples (2-mm, tail tip) from all progeny, dams, and candidate sires and then genotyped each individual at six microsatellite loci: AAGG-38, AAAG-70, AAAG-76, AAAG-91, AAAG-94, and AAAG-95 (Bardeleben et al. 2004). We performed PCR at a total volume of 10 μL with 1 μL template DNA, 1 x GeneAmp PCR Buffer II (Applied Biosystems, Carlsbad, CA), 1.5 or 2.0 mM MgCl2 (Table S1), 0.4 mM dNTPs, 0.25 μM of each primer (forward and reverse), and 0.3 U of Taq polymerase. PCR cycles consisted of an initial denaturation step at 94°C for 5 min followed by 29 or 35 cycles of 45 sec at 94°C (Table S1), 1 min at primer-specific annealing temperatures (T_a, Table S1), and 1 min at 72°C, followed by a final extension for 5 min at 72°C. All PCRs were performed on a DNAEngine Thermal Cycler (Bio Rad Laboratories, Hercules, CA). Loci were pooled for genotyping (Table S1) on an AB13100 Genetic Analyzer (Applied Biosystems) and all genotypes were scored by visual inspection using GeneMapper software against a GeneScan 500 LIZ size standard (Applied Biosystems).

We collected a total of 133 sons and 119 daughters. Because dams were housed individually, maternity was known with certainty for all of these offspring. We assigned paternity using the software package CERVUS (Marshall et al. 1998) following methods reported elsewhere (Calsbeek and Bonneaud 2008). A total of 24 tissue samples from hatchlings, four samples from dams, and 10 samples from potential sires were either lost or failed to amplify at most loci. Consequently, paternity could only be confidently assigned to 110 sons (83%) and 101 daughters (85%).

SEX RATIO AND PARENTAL CONDITION
Prior to mating trials, we measured snout-vent length (SVL, nearest millimeter) and body mass (nearest 0.01 g) for all dams and sires. We measured dams prior to the onset of reproduction to avoid confounding their reproductive state (i.e., gravid or not) with their estimated body condition. Although it would be ideal to have a separate measure of maternal condition at the time each subsequent egg was fertilized, we deemed it impractical and potentially stressful to continually palpate females to document their frequent transitions in reproductive condition. We calculated body condition using residuals from the regression of log10 body mass on log10 SVL for all dams and potential sires included in the study, with separate regressions for each sex. We confirmed that this measure of body condition is correlated with the wet mass of abdominal fat bodies (i.e., stored energy) in a separate study of dissected females (r² = 0.35; n = 28; P < 0.001). For analysis, we treated body condition as a continuous variable. However, for graphical convenience, we also categorized body condition as “poor” (<0.5 SD), “average” (±0.5 SD), or “good” (>0.5 SD) relative to the population mean for each sex. Similar statistical results were obtained regardless of whether body condition was treated as continuous or categorical.

We analyzed patterns of progeny sex allocation by treating each sire (n = 59) or dam (n = 67) that produced progeny as an observation and summing his or her total progeny across both potential mates. We calculated sex ratio as the proportion of sons to total progeny and tested for effects of maternal or paternal condition on sex ratio using generalized linear models (GLMs) with logit links and binomial error distributions, following procedures advocated by Wilson and Hardy (2002). The ratio of Pearson’s χ² to residual degrees of freedom never differed significantly from 1 (P > 0.05), so we did not scale for overdispersion (Wilson and Hardy 2002). We conducted these analyses in two different ways. First, because the precision of an estimate of sex ratio increases with the number of offspring contributing to that estimate, we
weighted sex ratios by the number of progeny contributing to
each estimate. This is particularly useful in situations such as
ours, where many sires and dams produced only a few progeny
and were thus prone to extreme sex ratios (Fig. S1). Second, we
conducted similar analyses using unweighted estimates of sex ra-
tio. These two weighting schemes always yielded similar results
(see Results).

To determine whether observed patterns of sex-ratio bias
were driven by the differential production of sons versus daugh-
ters, we analyzed the total number of sons or daughters, rather
than the proportion of sons, as a response variable for each dam
or sire. We did this by regressing the number of sons or daughters
against maternal or paternal condition using GLMs with log links
and Poisson error distributions. We also tested for interactions
between maternal and paternal condition by constructing similar
GLMs using each sire–dam pair \((n = 81)\) as an observation. In-
teractions between maternal and paternal condition were never
significant \((P > 0.38\) for all analyses) and main effects of ma-
ternal and paternal condition were qualitatively identical to those
observed when treating each sire or dam as an observation. Thus,
we focus on results obtained using each individual sire or dam as
an observation.

Our calculation of a single sex ratio based on all of the se-
quential progeny produced by a dam assumes that these individual
fertilization decisions are all related to her condition at the time
of mating. As an alternative, we also tested whether the sex of the
first offspring that each dam produced was related to her condi-
tion at the beginning of the reproductive season. We did this using
GLM with a logit link and binomial error distribution. This analy-
sis is robust to any error due to changes in maternal condition that
could influence subsequent allocation decisions.

**PROGENY PHENOTYPES AND PARENTAL CONDITION**

We tested for effects of parental condition on progeny phenotypes
by regressing SVL, body mass, and body condition (residuals
of log\(_{10}\) body mass on log\(_{10}\) SVL) against maternal or paternal
condition. These measurements were collected for all progeny at
hatching. To avoid statistical nonindependence of datapoints, we
calculated a single mean value for all of the sons or daughters of
a given sire or dam and weighted these means by the number of
progeny contributing to the estimate.

**NATURAL SELECTION ON PROGENY PHENOTYPES**

We released 62 sons and 54 daughters from our breeding
experiment onto a small island of natural habitat in The Bahamas
\((23°30’N, 75°45’W)\) in September of 2008. Prior to release, we
permanently marked each hatching with a unique toe clip. We
recaptured surviving progeny eight months later (May 2009), at
which point they were approaching sexual maturity. The use of an
offshore island prevented dispersal, and the small size \((431 \, \text{m}^2)\)
of the island allowed us to search it thoroughly and capture nearly
every surviving lizard (Calsbeek and Smith 2007). We have pre-
viously reported analyses of natural selection on these progeny
(Cox and Calsbeek 2010), but we now expand upon this original
study by including new analyses in the context of parental condi-
tion, new multivariate selection gradients, new quadratic selection
gradients, and new measures of selection on hatching viability in
captivity prior to release.

We quantified viability selection by regressing relative sur-
vival \((0\) or \(1\) divided by mean survival) on trait values standardized
to the population mean in unit variance (Arnold and Wade 1984).
We calculated relative survival and standardized trait values sep-
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erately within each sex. We used SVL at release, mass at release,
and condition at release as traits for these analyses. Because SVL
and mass were highly correlated, we conducted separate selec-
tion analyses for each morphological trait to avoid problems of
multicollinearity (Lande and Arnold 1983; Fairbairn and Reeve
2001). We included hatch date as a trait in each analysis so that
we could estimate selection acting directly on morphological varia-
tion, rather than age. We calculated directional selection gradients
from the partial regression coefficients of ordinary least squares
regressions including a single morphological trait and hatch date
(see Appendix S1). We estimated nonlinear selection gradients
\((i.e., \text{stabilizing or disruptive selection})\) from separate regres-
sions that included quadratic terms for the morphological trait
and hatch date, as well as morphology \(\times\) hatch date interactions.
We calculated directional selection gradients by doubling the re-
gression coefficients and their standard errors for quadratic terms
(Stinchcombe et al. 2008). We assessed statistical significance
\((P < 0.05)\) using logistic regression to account for the binomial
distribution of survival (Janzén and Stern 1998). We inferred sex
differences in selection from significant sex \(\times\) trait interactions
in these analyses. We used cubic splines (Schluter 1988) with
binomial error distributions \((\text{live, die})\) to visualize fitness surfaces
for each trait.

In addition to measuring natural selection in the wild, we
also measured survival in captivity from hatching until release to
the wild \((n = 90\) sons, 75 daughters). We estimated linear and
quadratic selection gradients for SVL, body mass, and condition
at hatching using methods identical to those described above,
but substituting survival in captivity as the measure of fitness.
The progeny that we released were maintained in captivity for a
variable period \((\text{range: 7–90 days})\) prior to release. We calculated nonlinear selection gradients by doubling the re-
gression coefficients and their standard errors for quadratic terms
\((P < 0.05)\) using logistic regression to account for the binomial
distribution of survival \((\text{Janzen and Stern 1998})\). We inferred sex
differences in selection from significant sex \(\times\) trait interactions
in these analyses. We used cubic splines \((\text{Schluter 1988})\) with
binomial error distributions \((\text{live, die})\) to visualize fitness surfaces
for each trait.

**PROGENY FITNESS AND PARENTAL CONDITION**

To assess progeny fitness as a function of maternal and paternal
condition, we calculated a single mean probability of survival
for all of the sons or daughters produced by each individual sire or dam. We calculated these survival probabilities separately for hatchlings in captivity (n = 165 progeny, 48 dams, 32 sires) and for a subset of these progeny following their release to the wild (n = 116 progeny, 46 dams, 32 sires). Differences between these sample sizes and the larger samples reported for analyses of sex ratio (n = 252 progeny, 67 dams, 59 sires) occur because breeding trials were conducted in two rounds. Survival was not measured for progeny resulting from the second round of breeding trials.

We calculated relative fitness for each sire or dam by dividing the mean probabilities of survival for all their sons and all their daughters by the population mean measures of fitness for all sires or dams. We calculated relative fitness separately for sons and daughters so that we could directly compare the overall fitness payoffs of producing offspring of either sex. We then regressed the relative fitness of sons and daughters against parental condition, which was standardized in unit variance to the separate means for dams and sires. We tested for an adaptive function of sex-ratio bias by examining how relative fitness was influenced by the interaction between progeny sex and parental condition using GLMs with Poisson error distributions and log links. We weighted each sire or dam’s estimate of relative fitness by the number of progeny contributing to that estimate and also conducted similar analyses using unweighted estimates. These two weighting schemes generally gave similar results (see Results).

We also assessed the adaptive significance of sex-ratio bias by measuring the growth of all surviving progeny from release (shortly after hatching) to recapture (near sexual maturity). We expressed growth as change in SVL and body mass and tested for effects of sex, maternal (or paternal) condition, and the interaction of sex × maternal (or paternal) condition using ordinary least squares regression.

Results
SEX RATIO AND PARENTAL CONDITION
At the population level, dams produced an approximately balanced sex ratio (133 sons, 119 daughters; χ² = 0.78; P = 0.38). The total sex ratio produced by each dam was not related to her initial condition (weighted by total number of offspring: χ² = 0.27; P = 0.60; unweighted: χ² = 0.01; P = 0.94; Fig. 1), nor were dams in good condition more likely to produce a son as their first offspring (χ² = 0.20; P = 0.65). By contrast, progeny sex ratio shifted markedly from female-biased to male-biased as paternal condition increased (weighted by total number of offspring: χ² = 8.82; P = 0.003; unweighted: χ² = 6.04; P = 0.014; Fig. 1). Analyzing the total number of sons sired by each male as the response variable revealed that this effect on progeny sex ratio was driven entirely by an increase in the production of sons as paternal condition improved (χ² = 8.19; P = 0.004; Fig. 2). By contrast, the total number of daughters produced by each sire was unrelated to his condition (χ² = 0.02; P = 0.89; Fig. 2). Maternal condition was unrelated to either the total number of sons (χ² = 0.22; P = 0.64) or the total number of daughters (χ² = 0.80; P = 0.37) produced by each dam (Fig. 2).

PARENTAL CONDITION AND PROGENY PHENOTYPES
We did not detect any associations between maternal condition and the SVL, mass, or condition of progeny at hatching (Table 1). Nor did we find any significant associations between paternal condition and most aspects of progeny phenotype (Table 1), with the exception of a very weak positive relationship between the body condition of sires and that of their daughters (r² = 0.099; F̅₁,₃₀ = 3.28; P = 0.080).

NATURAL SELECTION ON PROGENY PHENOTYPES
Hatching survival in captivity was identical for sons and daughters (79%) and was positively correlated with mass and condition...
Production of sons and daughters was unrelated to maternal condition (left), but the production of sons increased with paternal condition (right). Top panels report mean numbers of progeny (±1SE) as a function of body condition, categorized as “poor” (<0.5 SD), “average” (±0.5 SD), or “good” (>0.5 SD) relative to the means for each sex. Bottom panels report the numbers of sons and daughters for each individual dam or sire as a function of continuous variation in condition, expressed in units of SD relative to the population means for each sex. For clarity, symbols for sons and daughters are vertically offset at each increment on the y-axis. Ordinary least-squares regressions are shown for illustrative purposes only. Significance tests were based on generalized linear models with log links and Poisson error distributions (see text).

We did not detect significant quadratic selection on any morphological traits (Table 2) and patterns of selection in captivity did not differ between sons and daughters ($\chi^2 < 0.78; P > 0.37$ for all interactions with progeny sex).

Juvenile survival in the wild did not differ significantly between sons (16%) and daughters (24%) ($\chi^2 = 1.14; P = 0.28$). Directional selection strongly favored large SVL at release, large body mass at release, and high body condition at release (Table 2, Fig. 3). However, selection was relatively stronger in sons than in daughters with respect to SVL (sex $\times$ SVL at release: $\chi^2 = 8.82; P = 0.003$) and body mass (sex $\times$ mass at release: $\chi^2 = 6.65; P = 0.009$) (Table 2). Selection on body condition did not differ between sexes (sex $\times$ condition at release: $\chi^2 = 0.58; P = 0.45$).

In addition to these strong directional components, selection on SVL and mass was characterized by negative (i.e., stabilizing) quadratic gradients (Table 2), although this nonlinear component of selection was only significant in the case of body mass of daughters ($\chi^2 = 3.92; P = 0.048$) and only when hatch date was included in our multivariate analyses (Table 2).

**Table 1.** Progeny phenotypes at hatching were not correlated with either maternal or paternal condition. Statistics are reported for regressions using the weighted means for all sons or daughters of a given sire or dam.

<table>
<thead>
<tr>
<th>Progeny sex</th>
<th>Phenotypic trait</th>
<th>Maternal condition df</th>
<th>$r^2$</th>
<th>$P$</th>
<th>Paternal condition df</th>
<th>$r^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daughters</td>
<td>Snout-vent length</td>
<td>47</td>
<td>-0.003</td>
<td>0.695</td>
<td>31</td>
<td>-0.021</td>
<td>0.425</td>
</tr>
<tr>
<td></td>
<td>Body mass</td>
<td>47</td>
<td>0.000</td>
<td>0.991</td>
<td>31</td>
<td>0.043</td>
<td>0.225</td>
</tr>
<tr>
<td></td>
<td>Condition</td>
<td>47</td>
<td>0.001</td>
<td>0.853</td>
<td>31</td>
<td>0.099</td>
<td>0.080</td>
</tr>
<tr>
<td>Sons</td>
<td>Snout-vent length</td>
<td>57</td>
<td>0.007</td>
<td>0.537</td>
<td>37</td>
<td>0.013</td>
<td>0.493</td>
</tr>
<tr>
<td></td>
<td>Body mass</td>
<td>57</td>
<td>0.016</td>
<td>0.333</td>
<td>37</td>
<td>-0.007</td>
<td>0.621</td>
</tr>
<tr>
<td></td>
<td>Condition</td>
<td>57</td>
<td>0.012</td>
<td>0.406</td>
<td>37</td>
<td>-0.002</td>
<td>0.797</td>
</tr>
</tbody>
</table>
Table 2. Selection gradients for linear (β) and quadratic (γ) selection on hatching females (F) and males (M). Gradients are partial regression coefficients from models including hatch date and a single morphological trait (see Appendix S1 for equations). Separate regressions were performed for each morphological trait to avoid problems of multicollinearity. Quadratic selection gradients were calculated by doubling regression coefficients and their standard errors. Selection on morphology at hatching was estimated from variation in survival over a two-month period in captivity. Selection on morphology at release was estimated from variation in survival over an eight-month period following release to the wild. Asterisks indicate statistical significance (P<0.05), as determined by logistic regression.

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Linear selection β±1SE</th>
<th>Quadratic selection γ±1SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Hatching viability</td>
<td></td>
<td></td>
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<tr>
<td>SVL at hatching</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>75</td>
<td>0.11±0.07</td>
<td>−0.15±0.10</td>
</tr>
<tr>
<td>M</td>
<td>90</td>
<td>0.05±0.05</td>
<td>0.04±0.06</td>
</tr>
<tr>
<td>Both</td>
<td>165</td>
<td>0.07±0.04</td>
<td>−0.01±0.05</td>
</tr>
<tr>
<td>Mass at hatching</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>75</td>
<td>0.17±0.07*</td>
<td>0.10±0.12</td>
</tr>
<tr>
<td>M</td>
<td>90</td>
<td>0.11±0.05*</td>
<td>0.06±0.07</td>
</tr>
<tr>
<td>Both</td>
<td>165</td>
<td>0.12±0.05*</td>
<td>0.01±0.05</td>
</tr>
<tr>
<td>Condition at hatching</td>
<td></td>
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<td></td>
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<tr>
<td>F</td>
<td>75</td>
<td>0.13±0.07</td>
<td>0.09±0.15</td>
</tr>
<tr>
<td>M</td>
<td>90</td>
<td>0.12±0.05*</td>
<td>−0.13±0.14</td>
</tr>
<tr>
<td>Both</td>
<td>165</td>
<td>0.12±0.04*</td>
<td>0.04±0.06</td>
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<tr>
<td>Juvenile viability</td>
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<tr>
<td>SVL at release</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>F</td>
<td>54</td>
<td>0.74±0.46</td>
<td>−0.94±1.76</td>
</tr>
<tr>
<td>M</td>
<td>62</td>
<td>2.08±0.48*</td>
<td>−0.62±1.06</td>
</tr>
<tr>
<td>Both</td>
<td>116</td>
<td>1.41±0.33*</td>
<td>−0.45±0.84</td>
</tr>
<tr>
<td>Mass at release</td>
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<tr>
<td>F</td>
<td>54</td>
<td>1.11±0.38*</td>
<td>−1.80±1.04*</td>
</tr>
<tr>
<td>M</td>
<td>62</td>
<td>2.05±0.32*</td>
<td>−0.62±0.66</td>
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<tr>
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<td>0.38±0.31</td>
</tr>
<tr>
<td>M</td>
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<td>0.22±0.18</td>
<td>0.03±0.19</td>
</tr>
<tr>
<td>Both</td>
<td>116</td>
<td>0.36±0.14*</td>
<td>0.22±0.14</td>
</tr>
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</table>

sex × maternal condition; weighted: $\chi^2 = 12.23; P = 0.001$; unweighted: $\chi^2 = 5.31; P = 0.021$. Paternal condition was not related to the survival of either daughters (weighted: $\chi^2 = 0.30; P = 0.58$; unweighted: $\chi^2 = 1.13; P = 0.29$; Fig. 4B) or sons (weighted: $\chi^2 = 0.01; P = 0.97$; unweighted: $\chi^2 = 0.03; P = 0.87$; Fig. 4D), nor did relationships between paternal condition and progeny survival in the wild differ between sons and daughters (weighted: $\chi^2 = 0.19; P = 0.65$; unweighted: $\chi^2 = 0.83; P = 0.36$).

Juvenile males grew significantly more than females with respect to both SVL ($F_{1,18} = 4.35; P = 0.05$) and body mass ($F_{1,18} = 11.22; P = 0.003$). Growth in SVL was weakly influenced by the interaction between progeny sex and paternal condition ($F_{1,18} = 2.48; P = 0.13$), and growth in body mass was weakly influenced by the interaction between progeny sex and maternal condition ($F_{1,18} = 2.71; P = 0.12$). In each case, the growth of sons tended to increase with maternal or paternal condition, whereas the growth of daughters was unrelated to maternal or paternal condition.

**Discussion**

At the population level, brown anoles produce offspring in an approximately balanced sex ratio (Calsbeek and Bonneau 2008; Cox et al. 2009; this study). At the individual level, however, we found that sex allocation was biased such that the production of sons increased sharply with the body condition of the sire. By contrast, the production of daughters was independent of the sire’s body condition. Consequently, dams produced a female-biased sex ratio when mating with sires in poor condition and a male-biased sex ratio when mating with sires in good condition. Previous studies have shown that female lizards, including brown anoles, bias sex allocation as a function of paternal body size (Calsbeek and Sinervo 2004; Calsbeek and Bonneau 2008; Cox and Calsbeek 2010), but ours is the first to demonstrate such a relationship for condition, a trait that is statistically uncorrelated with body size.

There are at least three mechanisms by which paternal condition can affect the fitness of progeny and thereby influence sex-allocation strategies. First, fathers in good condition may be better able to provision their offspring (e.g., Korpimaki et al. 2000). This explanation is not applicable to brown anoles, which lack parental care. Second, paternal body condition may signal direct benefits, such as high-quality territory, which could provide a favorable environment for progeny growth and survival. Brown anoles are territorial, and body size (although not condition per se) is known to confer an advantage in male–male competition (Tokarz 1985). Territory size or quality could also impact the fitness of sons more than that of daughters because it is a primary determinant of male reproductive success in anoles and other lizards (Trivers 1976; Hews 1990; Tokarz 1998; Lebas 2001). The sires in our experiment were raised in captivity, so we could not assess the correlation between their condition and the size or quality of their territories, nor could we examine the fitness of progeny with respect to paternal territories. Thus, direct benefits correlated with paternal condition remain a plausible but untested explanation for the observed sex-ratio bias.

A third possibility is that body condition may signal genetic quality by reflecting the overall performance of a male’s genotype in a given environment, such that females receive genetic benefits for their progeny by mating with males in good condition. Other vertebrates are known to bias progeny sex ratio with respect to various phenotypic indicators of paternal quality, such as nuptial coloration, ornamentation, and body size (Ellegren et al. 1996; Rowe and Houle 1996; Svensson and Nilsson 1996;
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Figure 3. Natural selection favored (A) large snout-vent length, (B) large body mass, and (C) high body condition in juvenile females (top) and males (bottom). Survival was measured over an eight-month period in the wild. Datapoints are average survival probabilities for each phenotypic value. Symbol size corresponds to the number of individuals contributing to each estimate (range: 1–11). Fitness surfaces are best-fit cubic splines ±1SE from 500 bootstrap iterations. For illustrative clarity, splines were fit using bivariate analyses and therefore do not account for effects of hatch date. See Table 2 for linear and quadratic selection gradients that account for effects of hatch date.

Kolliker et al. 1999; Calsbeek and Sinervo 2004; Olsson et al. 2005; Roed et al. 2007; but see Leech et al. 2001). Although many of these studies have demonstrated correlations between sex-ratio bias and the fitness of the sire, most have not actually tested the predicted adaptive consequences by measuring the resultant fitness of progeny (Calsbeek and Sinervo 2004; Olsson et al. 2005; Cox and Calsbeek 2010).

We tested the adaptive significance of sex-ratio bias by tracking the survival of sons and daughters immediately after hatching in captivity, and as juveniles when released to their natural habitat. Despite strong selection for large size and high condition, the fitness of both sons and daughters was unrelated to paternal condition. This is perhaps not surprising, given that sires in good condition did not produce sons or daughters of significantly larger size or better condition at hatching. We did detect a nonsignificant trend for the growth of sons, but not daughters, to increase as a function of paternal condition. Given the importance of body size in territory acquisition and mating success of males (Tokarz 1985, 1998), this suggests a possible adaptive explanation for the observed sex-ratio bias. However, we consider this interpretation tentative due to the small number of offspring and correspondingly weak statistical support. Thus, despite a strong relationship between progeny sex ratio and paternal condition, we have no direct evidence for an adaptive function of this sex-ratio bias.

We propose two potential, nonexclusive explanations for this result. First, although condition may provide a reliable index of paternal quality among wild males, its relevance to heritable fitness variation in our simplified laboratory common-garden environment is debatable. Future studies would benefit from similar analyses of paternal condition and progeny fitness against a common environmental background, preferably in wild populations. Second, any genetic benefits conferred by sires in good condition may be manifest primarily with respect to adult mating success, whereas our selection analyses were based on juvenile viability. Indeed, the sex-specific benefits of maternal investment originally envisioned by Trivers and Willard (1973) are based upon the assumption that males have a higher reproductive value than daughters due to their greater variance in adult reproductive success. However, it is noteworthy that several studies of lizards have documented adaptive consequences of sex-ratio bias with respect to juvenile growth, juvenile survival, and the timing of maturation (Calsbeek and Sinervo 2004; Uller et al. 2004; Olsson et al. 2005; Cox and Calsbeek 2010), rather than adult reproductive success per se.

Contrary to the predictions of Trivers and Willard (1973), we found no evidence that females biased the sex ratio of their progeny on the basis of their own condition. One possibility is that our single measure of maternal condition at the onset of breeding failed to capture relevant fluctuations in condition as successive progeny were fertilized. However, even when considering only the first offspring produced by each dam, maternal condition was unrelated to progeny sex. Another concern is that
simple morphological estimates of body condition may be poor predictors of sex-ratio bias (Sheldon and West 2004). Nonetheless, we detected a strong sex-ratio bias using an identical measure of paternal condition. A third possibility is that females base their sex-allocation decisions on a comparative assessment of their own condition relative to other females in the population. Our breeding design isolated dams from other females, so further studies would be required to address this possibility.

Although support for the Trivers–Willard hypothesis has been reported for birds and mammals (Olsen and Cockburn 1991; Bradbury and Blakey 1998; Nager et al. 1999; Cameron 2004; Cockburn et al. 2004; Sheldon and West 2004), individual studies often give highly divergent results (Kosmider and Pen 2002; Sheldon and West 2004). Other studies of lizards report no correlation between maternal condition and progeny sex ratios (Lovern and Passek 2002; Uller et al. 2006), or contrasting patterns in different study seasons (Olsson and Shine 2001; Calsbeek and Sinervo 2004; Olsson et al. 2005; Uller et al. 2006; Warner et al. 2007; Calsbeek and Bonneau 2008; Cox and Calsbeek 2010), this group currently provides only weak and inconsistent support for the original prediction of the Trivers–Willard hypothesis.

In part, this weak support for the Trivers–Willard hypothesis may reflect important differences between the reproductive biology of lizards and the original assumptions derived from large mammals. Foremost among these differences are the general absence of postpartum maternal investment and the prevalence of multiegg clutches or litters in many lizards, the latter of which could permit mothers in good condition to increase their fitness by investing in offspring quantity rather than offspring quality (Fox and McCoy 2000; Warner et al. 2007). Each of these features would generally act to weaken the link between maternal condition and progeny condition. Indeed, we found no evidence for such a link in our study. Moreover, we found no evidence that dams in good condition produced sons with high fitness. By contrast, we found a positive correlation between maternal condition and the survival of daughters. If these effects on juvenile viability were representative of the effects of maternal condition on the total lifetime fitness of sons and daughters, then general sex-allocation theory would predict that female anoles in good condition should produce a female-biased sex ratio, rather than the male-biased sex ratio predicted by the original Trivers–Willard hypothesis. This underscores the importance of establishing the actual relationships between maternal condition and progeny fitness prior to making predictions about adaptive progeny sex ratios. Unfortunately, data linking progeny fitness to maternal condition are lacking for most studies of sex allocation (West 2009).

Although our data show that brown anoles produce biased sex ratios as a function of paternal phenotype, we currently lack a detailed mechanistic understanding of how this bias occurs. Because we only measured “secondary” sex ratios at hatching, we cannot determine whether the observed bias was caused by differential fertilization (i.e., “primary” sex-ratio bias), differential embryonic viability, or a combination of these effects. Moreover, any differential fertilization could occur as a form of cryptic female choice, or because sires in good condition contribute more sperm with male sex-determining loci. In other lizards, male-biased sex ratios occur because sperm with male sex-determining loci exhibit increased viability during storage in the female reproductive tract (Olsson et al. 2007). Because offspring in our study were produced primarily with stored sperm, this is an intriguing possibility, especially if the viability of sperm is also related to
parental condition. Although the mechanism of sex determination is unknown for brown anoles, other Anolis species exhibit XY and XXY male heterogamy or genetic sex determination without heteromorphic sex chromosomes (Gorman and Atkins 1966; Janzen and Phillips 2006). If this pattern of genetic sex determination is conserved in brown anoles, then our results add to the growing body of evidence contradicting Williams’ (1979) suggestion that chromosomal sex determination should constrain adaptive sex-ratio bias (Ellegren et al. 1996; Sheldon et al. 1999; Calsbeek and Sinervo 2004; Olsson et al. 2005). However, even in Anolis species with genetic sex determination, maternal effects (e.g., provisioning of the yolk with testosterone) are thought to influence offspring sex, possibly by altering the fertilization success of male- and female-inducing sperm (Lovern and Wade 2003). Collectively, these unanswered questions suggest a number of promising avenues for future research into the mechanistic basis of sex-ratio bias and its relation to both maternal and paternal effects.

In summary, we have shown that brown anoles bias the production of sons as a function of parental condition. This bias could reflect direct (e.g., high-quality territory) and/or indirect benefits (i.e., sex-specific good genes) associated with parental condition, each of which would presumably increase the fitness of sons more so than that of daughters. We did not investigate direct benefits in this study, but our tests for indirect genetic benefits did not strongly support an adaptive interpretation for the observed sex-ratio bias. Further studies of wild populations are required to disentangle these possibilities and to provide a rigorous test for the presumed adaptive significance of sex-ratio bias. Although classic sex-allocation theory has focused on paternal effects on progeny sex ratio, our results emphasize the importance of also considering the paternal phenotype, even in species where parental care is absent.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


Supporting Information

The following supporting information is available for this article:

Appendix S1. Equations for multivariate selection analyses on progeny phenotypes.

Figure S1. Frequency distributions for the number of offspring produced by individual (A) dams, and (B) sires. Box-and-whisker plots show the median (line), quartiles (box), and 10–90% quantiles (whiskers).

Table S1. PCR amplification conditions modified from Bardeleben et al. (2004).

Supporting Information may be found in the online version of this article.

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