ORIGINAL PAPER

Progeny sex ratios in a short-lived lizard: seasonal invariance despite sex-specific effects of hatching date on fitness

Davnah Urbach · Robert M. Cox · Ryan Calsbeek

Abstract   When fitness returns are sex-specific, selection should favor the facultative adjustment of offspring sex ratios. Seasonal shifts in offspring sex ratios are predicted to be particularly beneficial in short-lived, sexually dimorphic species in which hatching date is linked to adult size, which is related to fitness in a sex-specific fashion. We used four time series of hatching dates and progeny sex ratios in the brown anole (*Anolis sagrei*), a short-lived lizard with male-biased sexual size dimorphism, to test for such a seasonal shift in progeny sex ratio. In 2 of the 4 years, we also released hatchlings to their natural environment to test for sex-specific effects of hatching date on juvenile survival and adult size. We found that the relationship between hatching date and size the following year was significantly steeper in males than in females, and previous work has shown that adult size is more strongly tied to fitness in males than in females. Based on those results and on further evidence linking hatching date and body size to sex-specific survival and reproductive success, we predicted that sex ratios should shift from male- to female-biased as the breeding season progressed. Contrary to our prediction, we detected no clear seasonal shift in progeny sex ratio. Furthermore, although juvenile survival was correlated with hatching date, this relationship did not consistently differ between the sexes. The observation that progeny sex ratios are seasonally invariant despite several apparent links to adult fitness suggests that the evolution of a seasonal sex-ratio bias is either inherently constrained or requires a stronger selective advantage with respect to juvenile survival.

Keywords   Hatching date · Natural selection · Sex allocation · Sex ratio · Seasonal shift · Brown anole

D. Urbach · R. M. Cox · R. Calsbeek (✉)
Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA
e-mail: ryan.calsbeek@dartmouth.edu

R. M. Cox
Department of Biology, University of Virginia, Charlottesville, VA 22904, USA

⦿ Springer

Introduction

Sex-allocation theory predicts that when the fitness returns of producing daughters and sons are comparable, natural selection should favor identical investment in the sexes (Fisher 1930) and sex ratios should consequently be unbiased amongst individuals and across populations. However, when fitness returns are sex-specific, females should bias their investment into one or the other sex (Charnov 1982; Clutton-Brock and Iason 1986; Frank 1990; Hardy 1997; West 2009). Biased sex allocation is likely to be adaptive in a number of cases, including when sex allocation is condition-dependent and the correlation between maternal investment and reproductive return is sex-specific (Trivers-Willard hypothesis, Trivers and Willard 1973; Clutton-Brock 1994; Leimar 1996; Hewison and Gaillard 1999; Veeroja et al. 2010), when the operational sex ratio is imbalanced (Werren and Charnov 1978; Werren and Taylor 1984; Warner and Shine 2007; Robert et al. 2010), when attractiveness is heritable and the correlation between attractiveness and reproductive success is sex-specific (Burley 1981; Ellengren et al. 1996; Cockburn et al. 2002; Korsten et al. 2006; Karino and Sato 2009), or when local resource competition between mothers and their offspring favors overproduction of the sex with greater dispersal (Clark 1978; Taylor 1981; Silk 1983; Hewison and Gaillard 1996).

One further case of adaptive sex allocation occurs when offspring sex ratio changes in a systematic manner over the course of the breeding season because one sex experiences a higher fitness gain from hatching earlier than does the other sex (e.g., Dijkstra et al. 1990; Weatherhead et al. 1998; Pen et al. 1999; Korpimaki et al. 2000; Laaksonen et al. 2004; Husby et al. 2006). Seasonal shifts in progeny sex ratio have been described in a variety of species, including long-lived birds (see e.g., Daan et al. 1996; Andersson et al. 2003) and mammals (Clutton-Brock and Iason 1986). However, sex-specific selection on hatching date is likely to be particularly strong in (1) short-lived species in which hatching or emergence time is strongly correlated with the timing of maturation and size at maturity, and (2) sexually dimorphic species in which age at maturity, size at maturity, or the relationship between adult size and fitness differs between the sexes (e.g., Uller and Olsson 2006; Blouin-Demers and Weatherhead 2007; Uller and Olsson 2010).

An example of a species that fits these predictions is the brown anole lizard, *Anolis sagrei*. Brown anoles reach sexual maturity within a year of hatching and most adults die before reaching a second breeding season (Calsbeek et al. 2008; Cox and Calsbeek 2010c). Brown anoles lay single-egg clutches at 1–2 week intervals over a 5–6 month breeding season (Cox and Calsbeek 2010b), such that variation in hatching date is considerable and size at the beginning of the subsequent breeding season also varies substantially. This species also exhibits extreme sexual size dimorphism in which adult males average 30 % longer and 150 % heavier than adult females (Cox et al. 2009; Cox and Calsbeek 2010c). This sexual size dimorphism is associated with dramatic sex differences in growth rate and size at maturity (Lee et al. 1989; Cox et al. 2009).

In brown anoles, a suite of observations suggests that early hatching date should be more strongly tied to fitness in males than in females. First, early hatching date appears to be strongly correlated with survival in juvenile males, but this relationship is weak and non-significant in juvenile females (Cox and Calsbeek 2010a). Second, survival of adult males is positively correlated with body size at the beginning of the breeding season, whereas viability selection favors intermediate size with no strong directional component in adult females (Cox and Calsbeek 2010b). Third, sexual selection favors large body size in male–male competition for high-quality breeding territories (Tokarz 1985) and possibly also via cryptic female preference for large mates (Cox and Calsbeek 2010a). Fourth,

although the frequency of egg production appears to increase modestly with maternal body size (Cox and Calsbeek 2011), fecundity selection for large female size is thought to be relatively weak in anoles due to their single-egg clutch. Thus, multiple aspects of selection acting via juvenile survival, adult survival, and reproductive success all seemingly favor early hatching date and large adult size more strongly in males than in females.

Collectively, these observations suggest that female anoles would benefit from preferentially producing sons early in the year to maximize their survival to, and size at, the beginning of the first breeding season. Moreover, recent evidence suggests that female brown anoles differentially produce sons and daughters as a function of sire body size and condition, which implies that progeny sex ratios can be adaptively adjusted in this species (Calsbeek et al. 2008; Cox et al. 2009; Cox et al. 2011). Although the basis of sex determination is not precisely known for *A. sagrei*, all members of this genus that have been studied exhibit some form of chromosomal, rather than temperature-dependent, sex determination. Most also exhibit some form of male heterogamety (XY, XXY or XXXY), even though many also lack heteromorphic sex chromosomes (Gorman and Atkins 1966; Janzen and Phillips 2006). Here, we test the prediction that progeny sex ratios exhibit a seasonal shift from male-biased to female-biased using 4 years of data on hatching date and offspring sex collected in captive brown anoles. In 2 of these 4 years, we also tracked the survival and growth of the progeny in their natural environment to determine whether hatching date has sex-specific effects on juvenile survival and adult size, a predictor of adult fitness.

Materials and methods

Lizard rearing

We gathered data on hatching date and progeny sex during the years 2004, 2007, 2009 and 2010. Each year involved a unique breeding cohort of male and female brown anole lizards, *Anolis sagrei*. All breeding cohorts were derived from populations near Georgetown, Great Exuma, The Bahamas (23°30′N, 75°45′W). All data on hatching date and offspring sex were collected from dams that mated naturally in the wild and were then captured and held in captivity for several months until they ceased egg production, presumably because they depleted their stores of viable sperm. Hatchlings were collected from 47, 69, 59 and 79 dams, respectively.

All lizards were housed separately in 10-gallon glass terraria (ca. 50 × 28 × 30.5 cm, 38 L). Individual 40-W incandescent bulbs nested in aluminum hoods were placed above each terrarium to provide heat (diurnal temperature range = 26–35 °C) and we used two Repti Glo 5.0 full-spectrum fluorescent bulbs for ultraviolet radiation (5 % UVB, Hagen, Montreal). The incandescent heat lamps were illuminated for 10 h/day and the fluorescent UVB lights for 12 h/day, turning on 1 h before and turning off 1 h after the heat lamps. We fed lizards an ad libitum diet of fruit flies (*Drosophila*, for hatchlings) and crickets (*Acheta*, for juveniles and adults). We dusted food with vitamin and mineral supplements once per week (Repta-Vitamin, Fluker Farms, Port Allen, LA).

Field-collected brown anoles use stored sperm to lay fertilized eggs at 1–2 week intervals for at least 3 months following mating (Calsbeek et al. 2007, 2008). Because females can produce up to 16 successive progeny following isolation, no additional access to males was needed in our study. Females oviposited in potted plants placed in their terraria and we left the eggs undisturbed until hatching. We have previously had higher

hatching success using this method compared to removal and relocation of eggs to standardized incubation conditions. All potted plants were connected to a central, automatic watering system and placed directly beneath the 40-W incandescent bulb. Thus, incubation conditions were standardized across cages. Progeny sex was diagnosed on the basis of enlarged post-anal scales (present only in males) and dorsal color patterns (present only in females; Calsbeek et al. 2010). This method is completely accurate for sexing *A. sagrei* hatchlings in this population.

Seasonal variation in sex ratio

We tested for seasonal variation in sex ratios by assuming that changes occurred either (i) linearly, (ii) nonlinearly but smoothly, or (iii) stepwise. Linear (i) and stepwise (iii) analyzes were performed using generalized linear mixed models (GLMM, Krackow and Tkadlec 2001; Wilson and Hardy 2002) and nonlinear analyzes (ii) were performed using generalized additive mixed models with smoothing splines (GAMM, Venables and Ripley 1999). Linear mixed models were fit using the R library lme4 and the function glmer. Additive mixed models were fit using the gamm4 library and the function gamm. Sex was treated as a binary response (1 for males and 0 for females) and we used individual offspring as our unit of observation (Wilson and Hardy 2002). Errors were modeled using a binomial distribution. We accounted for non-independence among observations within dams by including dam identity as a random intercept in all models (e.g., Pinheiro and Bates 2000). Under the assumptions of both linearity and non-linearity, we analyzed sex ratios on a daily basis by defining Julian hatching date as a continuous variable. Under the assumption of stepwise variation, we divided time into intervals of identical length and considered these intervals as categorical predictors. Intervals were created using the R package Hmisc and the cut2 function, which divides ranges of data into quantiles. We divided the data into either three or five intervals and used the information theoretic approach (Burnham and Anderson 2002) and the Akaike Information Criterion (AIC) to select the most parsimonious of these two models. AIC scores were systematically lower with three intervals ($0.9 \leq \Delta AIC \leq 59.7$). To estimate the predictive power of all these models, we tested each of them against a null model assuming no temporal variation. Comparisons were performed using likelihood ratio tests (Pinheiro and Bates 2000).

An important assumption of the models described above is that patterns of seasonal variation in sex ratios are comparable across dams and that the average slope for the population is therefore representative of all dams. To test for the existence of variation among dams in patterns of seasonal variation, we added random slopes to our linear mixed effects models by nesting dams within date (Schielzeth and Forstmeier 2008; Van de Pol and Wright 2009). We compared these latter, more general models to the more restricted models including those without random slopes using a likelihood ratio test (Pinheiro and Bates 2000). Data were analyzed both on a yearly basis and with models applied to all years at once. When data were combined and under the assumption of linearity (i) only, we included "year" as a fixed factor in our model and tested for interactions between year and hatching date. This model was compared to a model with no interaction terms and to a model with hatching date as the only predictive variable, using the information-theoretic approach (Burnham and Anderson 2002) and the Akaike Information Criterion (AIC). The selected model included hatching date, year and the interaction between the two ($1184.4 < AIC < 1218.2$).

Hatching date and juvenile survival

We tested for sex-specific fitness effects of hatching date by tracking the survival of juvenile males and females in their natural environment on Kidd Cay, a small (1,600 m^2) island in The Bahamas (23°30′N, 75°49′W) on which we have studied natural selection for seven consecutive years (Calsbeek 2009; Calsbeek et al. 2009; Cox and Calsbeek 2010c). In May of 2009 and 2010, we captured gravid adult females from this population and returned them to our captive breeding facility. Over the ensuing 4 months, we collected all progeny produced by these dams (see above) and then returned hatchlings to Kidd Cay in a series of three sequential releases per year, which we conducted at approximately monthly intervals (August, September, October). Sequentially releasing juveniles within several weeks of hatching eliminated any overall correlation between hatching date and size at release (this study: $r^2 = 0.002$; $n = 526$; $P = 0.31$), which had complicated our inter-pretations in a previous study using a single release date (previous study: $r^2 = 0.74$; $n = 116$; $P < 0.001$; see (Cox and Calsbeek 2010a). However, because the hatchlings within each release group varied by several weeks in age, weak correlations between hatching date and size at release were still present within each release group ($r^2 = 0.11$–0.38; all $P < 0.002$). We therefore included size at release as a covariate in our analyses (see below). Including age at release as a covariate generated similar results, and we focus on analyses including size as a covariate. To reduce the potentially confounding effects of maternal nest site, we randomly assigned each hatchling to one of 10 prede-termined release locations on Kidd Cay and ensured that each location received a similar number, sex ratio, and size distribution of hatchlings at each release date. We released a total of 237 hatchlings (123 males, 114 females) from 59 dams in 2009, and 290 hatchlings (151 males, 139 females) from 81 dams in 2010. Prior to release, we measured each hatchling's snout-vent length (SVL, nearest 0.5 mm) and marked it with a unique toe-clip for subsequent identification. Mean age at release (± SD) was 13.71 ± 10.09 days.

After releasing hatchlings to the wild, we left them undisturbed until the following May, at which point we thoroughly searched Kidd Cay for 1 week and attempted to recapture all survivors. Most males and females had attained the minimum size of sexual maturity by then, so our recapture interval encompassed the majority of the period over which viability selection acted on juveniles. Within each year, we used logistic regressions with survival (0 or 1) as the dependent variable to test for sex-specific fitness effects of hatching date, which we inferred from significant interactions between sex and hatching date. Across years, we conducted similar analyses including year as an effect, along with all two- and three-way interactions involving year. To estimate the strength of selection on hatching date, we standardized hatching date to the population mean in units of standard deviation and calculated relative fitness by dividing individual survival (0 or 1) by the population mean (Arnold and Wade 1984). To estimate selection within each sex, we standardized trait values and relative fitness using separate population means for each sex. To estimate selection across all progeny, we pooled the sexes and used mean trait values and survival rates for the entire population.

We estimated directional selection gradients as the partial regression coefficients ($\beta \pm$ SEM) from ordinary least squares regressions of relative fitness on standardized hatching date and size at release in each sex. Models used to estimate linear gradients did not include quadratic terms. We estimated quadratic selection gradients ($\gamma_{1,1} \pm$ SEM) by doubling the quadratic regression coefficients (and their associated SEM) from models that included both linear and quadratic (i.e., squared) terms for hatching date and size at release (Phillips and Arnold 1989; Stinchcombe et al. 2008). Robust estimates of correlational

selection require large sample sizes (Brodie et al. 1995), and inclusion of cross-product terms (hatching date × size at release) occasionally altered the fit of linear and quadratic terms in our models in a non-informative and potentially spurious fashion. Hence, we did not attempt to estimate correlational selection, nor did we include cross-product terms when estimating quadratic selection within each sex and year. However, we found no evidence for correlational selection on combinations of hatching date and size at release when pooling data across sexes and years ($\gamma_{1,2} = -0.036 \pm 0.118$; $n = 527$; logistic: $P = 0.70$). We visualized fitness surfaces for hatching date by fitting cubic splines with binomial fitness distributions (Schluter 1988). To find the best-fit cubic spline, we searched a range of possible smoothing parameters ($-10 \leq \lambda \leq 10$) and selected the value of λ that minimized the generalized cross validation (GCV) score.

Hatching date and adult size

In addition to directly measuring juvenile viability, we indirectly estimated the potential for hatching date to influence adult fitness by examining body size (SVL) at recapture near the beginning of the breeding season (May) as a function of hatching date in the previous year. As discussed above, several lines of evidence link body size at the beginning of the breeding season to subsequent adult fitness for both males and females in this population. We analyzed SVL in May as the response variable in linear mixed effect models that included hatching date and sex as fixed effects and year as a random effect. We specifically tested for a sex × hatching date interaction to determine whether the relationship between hatching date and adult size differed between sexes.

Results

Seasonal variation in sex ratio

Our data provided essentially no support for our prediction that female brown anoles shift offspring sex ratios over the course of the reproductive season. All likelihood ratio tests comparing models with and without main effects of hatching date on sex ratios favored the null model (i.e., no seasonal bias in sex ratio—2004: $\chi_1^2 = 0.57$, $P = 0.45$; 2007: $\chi_1^2 = 2.47$, $P = 0.12$; 2009: $\chi_1^2 = 0.34$, $P = 0.60$; 2010: $\chi_1^2 = 2.95$, $P = 0.09$). Accordingly, when sex ratios were analyzed on a daily basis, linear effects of hatching date on sex ratio were extremely weak and shifted signs between positive and negative, the former indicating an increasing bias towards males and the latter an increasing bias towards females (2004: $\beta = 0.0055 \pm 0.0073$, $P = 0.45$; 2007: $\beta = -0.0084 \pm 0.0052$, $P = 0.11$; 2009: $\beta = 0.0035 \pm 0.006$, $P = 0.56$; 2010: $\beta = -0.0094 \pm 0.0055$, $P = 0.09$). That is, the probability of producing a male increased over time in 2004 and 2009 by 0.14 and 0.09 % each day, respectively, and decreased over time in 2007 and 2010 by 0.02 and 0.24 %, respectively. Probabilities were calculated using the "divide by 4" rule (Gelman 2007; Hamel et al. 2012). Non-linear effects of hatching date on sex ratio were also non-significant (2004: $F_{1,195} = 0.56$, $P = 0.46$; 2007: $F_{1,275} = 2.61$, $P = 0.11$; 2009: $F_{1,247} = 0.28$, $P = 0.60$; 2010: $F_{1,310} = 2.79$, $P = 0.10$), regardless of the complexity of the fitted spline (i.e., the number of degrees of freedom associated with the curve, $d.f._{\text{spline}}$; here $d.f._{\text{spline}} = 1$). When time was divided into categorical intervals, no statistically significant deviation from equilibrium was detected at any point in time in 2004, 2007, or

2009, but sex ratios tended to be female-biased late in the 2010 hatching season (Table 1). This pattern was congruent with the marginally significant P values ($P = 0.09$) estimated with the linear model (see above). Finally, likelihood ratio tests systematically favored models with no random slope, i.e., they favored models detecting no among-female differences in patterns of seasonal variation, both when data were analyzed on a daily basis (2004: $\chi_2^2 < 0.01$, $P = 1.0$; 2007: $\chi_2^2 = 1.47$, $P = 0.48$; 2009: $\chi_2^2 = 0.03$, $P = 0.99$; 2010: $\chi_2^2 = 0.02$, $P = 0.99$) and when they were analyzed by discrete time periods (2004: $\chi_5^2 = 0.39$, $P = 0.99$; 2007: $\chi_5^2 = 3.65$, $P = 0.60$; 2009: $\chi_5^2 = 0.66$, $P = 0.99$; 2010: $\chi_5^2 = 1.00$, $P = 0.96$). Mean temporal variation in offspring sex ratio is shown in Fig. 1. The overall absence of a seasonal effect was confirmed by the absence of any effect of hatching date on sex ratio in analyses performed using all four data sets combined. When applied to the pooled data set, the comparison between a linear model with and without a main effect of hatching date on sex ratios favored the null model ($\chi_7^2 = 6.12$, $P = 0.53$). The null hypothesis was also favored when comparing models with and without a random slope (linear model: χ_3^2 0.01, $P \sim 1$, stepwise model: $\chi_5^2 = 0.37$, $P \sim 1$). When sex ratios were analyzed on a daily basis under the assumption of linearity, none of the terms of the interaction model were significant and effect sizes were small: $\beta_{date} = 0.0055 \pm 0.0073$ (0.14 %), $P = 0.45$, (reference level: first hatching day in year 2004); β_{year}—2007: 3.06 ± 2.16, $P = 0.15$; 2009: 0.93 ± 2.86, $P = 0.72$; 2010: 2.94 ± 2.2, $P = 0.18$; $\beta_{date \times year}$—2007: -0.012 ± 0.009 (0.3 %), $P = 0.18$; 2009: -0.0023 ± 0.011 (0.06 %),

Table 1 Generalized linear mixed-effect model coefficients (ß), standard errors (SE), P values (P) and probabilities of producing a male for each period (%) for models of variation in sex ratios over successive time intervals in four independent data sets (2004, 2007, 2009, 2010) and the pooled data set (Pooled) of hatching date and offspring sex ratio in brown anoles

Year	Interval (N)	ß	SE	P	%
2004	1 (60)	−0.18	0.25	0.45	4.5*
	2 (58)	−0.22	0.36	0.54	5.5*
	3 (57)	0.31	0.35	0.38	7.8
2007	1 (61)	0.20	0.20	0.31	5.0
	2 (49)	−0.22	0.30	0.46	5.5*
	3 (51)	−0.48	0.31	0.13	12.0*
2009	1 (88)	−0.01	0.20	1.00	0.25*
	2 (71)	0.34	0.31	0.28	8.5
	3 (74)	0.15	0.30	0.61	3.8
2010	1 (99)	0.34	0.20	0.09	8.5
	2 (112)	−0.32	0.27	0.23	8.0*
	3 (85)	−0.57	0.29	0.05	14.3*
Pooled	1 (288)	0.13	0.11	0.24	3.3
	2 (310)	−0.15	0.15	0.31	3.8*
	3 (241)	−0.18	0.16	0.27	4.5*

Time series were divided into three intervals (samples sizes are given in *parenthesis*). Intervals were defined as factors and the first one was used as reference level for comparison with effect sizes in successive intervals. Coefficients differing significantly from the reference level ($P \leq 0.05$) are in bold. Patterns of ß shifting from *positive* to *negative* indicate that sex ratios are male-biased first and female-biased later. Hence values identified with a * correspond to a decrease in the probability of producing a male
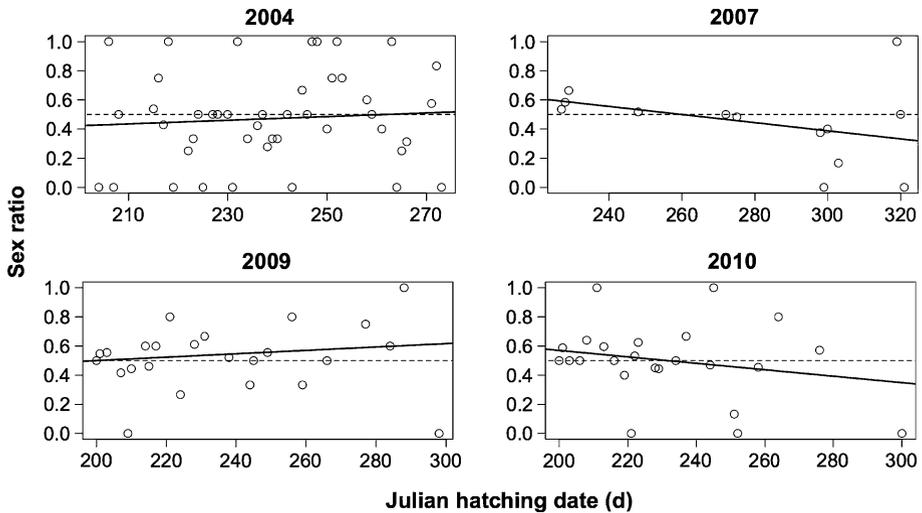
Fig. 1 Sex ratio (male/total) as a function of time in four independent data sets. *Values* represent daily means over all dams, *dashed lines* correspond to a 1:1 sex ratio and *plain lines* serve to guide the eye only. A *negative slope* indicates that sex ratios are becoming biased towards females along time

$P = 0.83$; 2010: -0.012 ± 0.009 (0.4 %), $P = 0.21$. This remained the case when time was divided into intervals (Table 1). Under the assumption of non-linearity, no significant effect of hatching date on sex ratio was detected either ($F_{1,1027} = 1.95$, $P = 0.16$).

The comparison among our four independent data sets revealed that there was no relationship between the effect sizes we estimated with our various analyses (see above) and the size of the data sets (2004: 175 eggs laid by 47 dams; 2007: 161 eggs laid by 69 dams; 2009: 233 eggs laid by 59 dams; 2010: 269 eggs laid by 79 dams), indicating that the absence of temporal variation in sex ratio is unlikely to be attributable to a lack of power. This was further confirmed by the results obtained based on the analyses we performed on the pooled data, which also showed small effect sizes and no sign of a significant seasonal shift in hatchling sex ratios.

Hatching date and juvenile survival

Early hatching dates were associated with higher survival in 2009 ($\chi_1^2 = 14.30$; $P < 0.001$; Fig. 2a, b) and 2010 ($\chi_1^2 = 4.94$; $P = 0.026$; Fig. 2c, d). Juvenile survival was higher for females than for males in 2009 ($\chi_1^2 = 11.89$; $P < 0.001$), but the sexes did not differ in survival in 2010 ($\chi_1^2 = 0.26$; $P = 0.61$). Males and females did not differ in the relationship between hatching date and survival in either 2009 (sex × hatching date: $\chi_1^2 = 0.92$; $P = 0.34$) or 2010 (sex × hatching date: $\chi_1^2 = 0.08$; $P = 0.78$). Combining both years into a single analysis revealed a strong association between hatching date and survival ($\chi_1^2 = 14.88$; $P < 0.001$) that did not differ between the sexes (sex × hatching date: $\chi_1^2 = 0.76$; $P = 0.38$) or between years (year × hatching date: $\chi_1^2 = 1.62$; $P = 0.20$). Survival was marginally higher in females ($\chi_1^2 = 3.30$; $P = 0.069$), but this trend was driven by data from 2009 and was not consistent across years (sex × year: $\chi_1^2 = 7.48$; $P = 0.006$). Survival did not differ across years ($\chi_1^2 = 1.16$; $P = 0.28$), nor was it
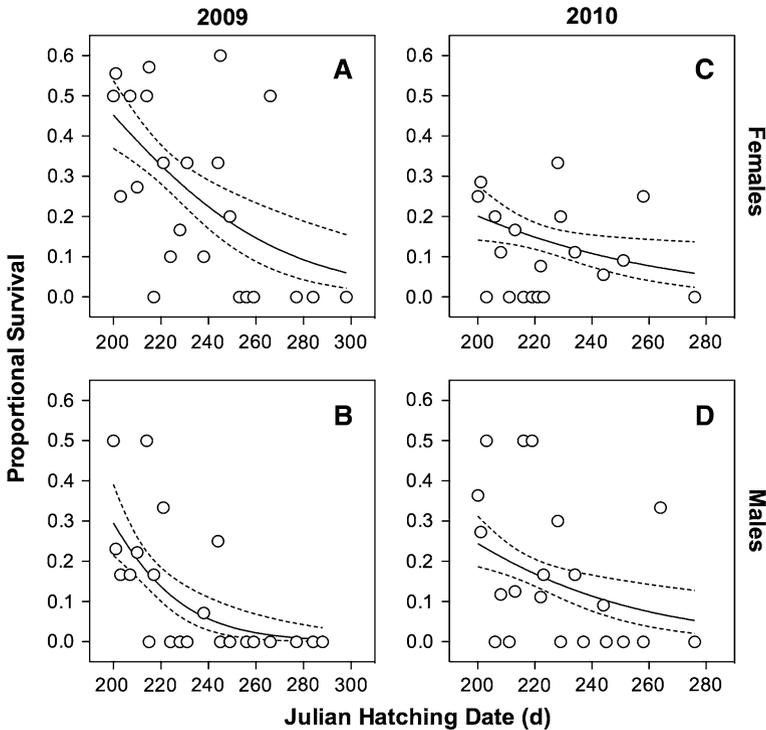
Fig. 2 Best-fit cubic splines (± 1 SEM) for survival as a function of Julian hatching date in juvenile females (*top*) and males (*bottom*) from two cohorts (2009, 2010). Each *symbol* reports the proportional survival of all males or females with a given hatching date, although individual animals with binomial survival outcomes were used to fit splines. Selection favored early hatching date in both sexes and in both years. See text for details and Fig. 3 for an alternative presentation of these data that emphasizes sex differences in relative fitness as a function of early or late hatching date

influenced by the three-way interaction between sex, year, and hatching date ($\chi_1^2 = 0.31$; $P = 0.58$)

Because the proportion of females that survived (0.31) was more than double that of males (0.14) in 2009, the relative fitness of a surviving female (1/0.31 = 3.22) was less than half that of a surviving male (1/0.14 = 7.14). Consequently, although selection favored early hatching date in both sexes, it was nearly twice as strong in males as in females for this year (Table 2). In 2010, survival was low for both females (0.14) and males (0.17) and selection favoring early hatching date was comparable in both sexes (Table 2). Hence, patterns of sex-specific relative fitness in 2009 were similar to those observed in a previous study (Cox and Calsbeek 2010a; redrawn in Fig. 3a of this study) and suggested that dams should preferentially produce sons early and daughters late in the breeding season (Fig. 3b). However, this sex-specific fitness effect was not significant in 2009, and it was entirely absent in 2010 (Fig. 3c). In general, selection on size at release was weaker than selection on hatching date and was never statistically significant (Table 2). We did not detect quadratic selection on hatching date or size at release in either males or females (Table 2).

Table 2 Directional (β) and quadratic (γ) selection on hatching date and size (SVL) at release in 2009 and 2010

Year	Sex	N	Trait	Directional selection $\beta \pm$ SE	Quadratic selection $\gamma \pm$ SE
2009	Female	114	Hatching date	-0.335 ± 0.146*	0.007 ± 0.250
			Size at release	0.174 ± 0.139	-0.135 ± 0.135
	Male	123	Hatching date	-0.646 ± 0.242*	0.373 ± 0.375
			Size at release	0.151 ± 0.332	-0.162 ± 0.654
	Both	237	Hatching date	-0.408 ± 0.121*	0.104 ± 0.207
			Size at release	0.055 ± 0.121	-0.056 ± 0.149
2010	Female	139	Hatching date	-0.303 ± 0.221	0.014 ± 0.359
			Size at release	0.095 ± 0.217	-0.060 ± 0.239
	Male	151	Hatching date	-0.352 ± 0.190	0.168 ± 0.316
			Size at release	0.272 ± 0.180	0.019 ± 0.242
	Both	290	Hatching date	-0.333 ± 0.139*	0.087 ± 0.222
			Size at release	0.198 ± 0.136	-0.016 ± 0.159
Both	Female	253	Hatching date	-0.322 ± 0.137*	0.028 ± 0.220
			Size at release	0.133 ± 0.133	-0.094 ± 0.134
	Male	274	Hatching date	-0.449 ± 0.147*	0.199 ± 0.236
			Size at release	0.176 ± 0.159	0.061 ± 0.223
	Both	527	Hatching date	-0.365 ± 0.097*	0.080 ± 0.155
			Size at release	0.196 ± 0.099*	-0.046 ± 0.112

Relative fitness and standardized trait values were calculated separately within each sex for "male" and "female" selection estimates and collectively for all progeny in analyses including "both" sexes. Asterisks indicate significant ($P < 0.05$) selection, as determined by separate logistic regressions with survival (0 or 1) as the response variable. See text for details
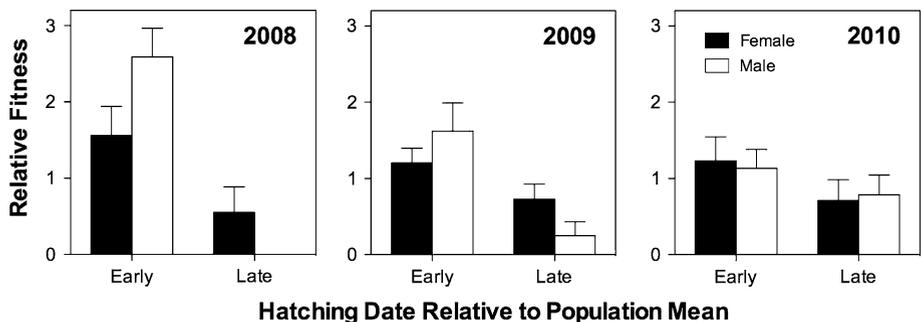
Fig. 3 Mean ($\pm$ 1 SEM) relative fitness for early- and late-hatching males and females in three cohorts. Data from 2008 are redrawn from Cox and Calsbeek (2010a) and are presented here for comparison, although we note that the site of that study and other details of the experimental design differ from those reported in the present study. Data from 2009 and 2010 are from the present study. Relative fitness was calculated by dividing individual survival (0 or 1) by the sex-specific population mean survival rates for males and females in each year. For graphical clarity, individuals are dichotomized into "early-" and "late-" hatching dates relative to the population mean hatching date in each year

Hatching date and adult size

Body size at the beginning of the reproductive season was strongly influenced by hatching date the previous year ($F_{1,66} = 8.79$; $P = 0.004$). Males had grown significantly larger than females by this point ($F_{1,66} = 66.16$; $P < 0.001$), and the relationship between hatching date and body size the following year was significantly steeper in males than in females (sex $\times$ hatching date: $F_{1,66} = 5.69$; $P = 0.019$). Based on these regressions, the difference in adult body size between an early- and a late-hatching female (Julian hatching dates 200 vs. 260) is relatively small (2.02 mm), whereas the predicted difference between an early- and a late-hatching male over this same interval is substantial (12.84 mm; Fig. 4).

Discussion

Previous studies suggested that male brown anoles benefit more than females from (1) early hatching dates, which are associated with higher juvenile survival, and (2) large body size at the onset of the breeding season, which is favored by both natural and sexual selection and is associated with early hatching dates in the previous year (Calsbeek and Cox 2010; Cox and Calsbeek 2010a). Accordingly, the present study tested the hypothesis that offspring sex ratio should shift from male-biased early in the season to female-biased later in the season. We found essentially no evidence to support this hypothesis in our four independent data sets documenting progeny sex ratios across 3–4 months of variation in hatching dates. This general lack of seasonal shift in sex ratio of brown anoles in our study corroborates previous studies in other reptiles, such as the green anole *Anolis carolinensis* (Lovern and Passek 2002), the mallee dragon *Ctenophorus fordi* (Uller and Olsson 2006), and the meadow viper *Vipera ursinii* (Baron et al. 2010). However, seasonal shifts in sex-ratios have been documented in some reptiles, including the jacky dragon *Amphibolurus murcatus* (Warner and Shine 2008), the snow skink *Niveoscincus ocellatus* (Wapstra et al. 2004; Pen et al. 2010), and other short-lived lizards (Warner et al. 2009).

Several factors may help to explain why sex ratios appear to be seasonally invariant in brown anoles. First, the sex-specific fitness benefits of early hatching date may not be as strong or as consistent as inferred from previous studies (Fig. 3a). To address this possibility, we supplemented and expanded upon our previous study (Cox and Calsbeek 2010a) by

Fig. 4 Body size (snout-vent *length*, SVL) near the beginning of the breeding season (May) as a function of hatching date in the previous year. Data from two cohorts (2009, 2010) are shown. The relationship between body size and hatching date is significantly steeper in males (*open symbols*, *dashed line*) than in females (*filled symbols*, *solid line*)
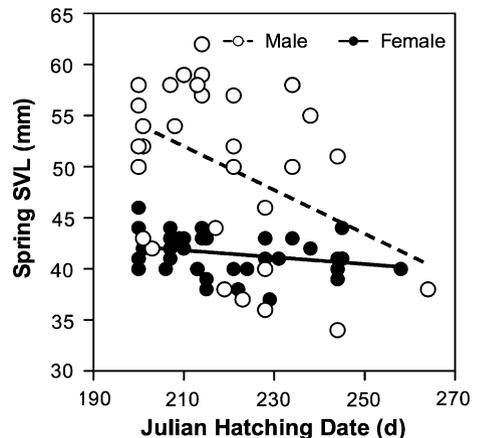
tracking juvenile viability for two additional cohorts using larger sample sizes and an experimental design that decoupled hatching date from size at release. Although patterns of juvenile survival in 2009 were qualitatively consistent with our previous finding that males benefit more from early hatching dates than do females, this result was not significant (Fig. 3b). Moreover, we did not find any evidence for such a pattern in 2010 (Fig. 3c). This suggests that sex differences in the relationship between juvenile viability and hatching date are generally weak and/or variable across years, and that a seasonal shift in sex ratio might therefore not be consistently adaptive. However, we also investigated the potential effects of hatching date on adult fitness by examining size at the beginning of the breeding season as a function of hatching date in the previous year. At the beginning of the breeding season, nearly every female had attained the minimum size of reproduction of 38 mm (Cox and Calsbeek 2011), and variation in female size was only weakly related to hatching date (Fig. 4). By contrast, variation in male size was substantial and negatively related to hatching date (Fig. 4). Because both survival and mating success of adult males increase with body size in *A. sagrei* and related species (Trivers 1976; Tokarz 1998; Cox and Calsbeek 2010c), this result suggests that the overproduction of males early in the season could be adaptive even in the absence of sex-specific effects of hatching date on juvenile survival.

A second factor that may explain why anole sex ratios appear to be seasonally invariant is that progeny sex has also been shown to vary as a function of sire phenotype in this species, with the production of sons increasing significantly with the size and condition of the sire (Calsbeek and Bonneaud 2008; Cox and Calsbeek 2010a). Hence, these sex-allocation decisions based on individual sire phenotypes could mask any additional allocation biases occurring seasonally. Although previous work has shown that adult body size is heritable from father to son (Calsbeek and Bonneaud 2008), there is no evidence for a significant association between the size or condition of an adult male and that of his sons or daughters at hatching (Cox et al. 2011). We currently have no data to suggest a correlation between the sex ratio produced by a dam and the condition of her sons. Moroever, maternal condition does not appear to affect sex allocation decisions (Cox et al. 2011), and is therefore unlikely to have prevented us from detecting seasonal sex ratio adjustments.

A third consideration is that facultative sex-ratio adjustment should only be favored if the fitness benefits of such behavior outweigh its costs (West and Sheldon 2002). The costs of biasing sex ratios are difficult to estimate (Burley 1982; Godfray and Werren 1996) and our limited understanding of the mechanisms of progeny sex allocation in the brown anole exacerbates these difficulties. However, the existence of physiological costs to the adjustment mechanism itself is well-supported (e.g., West et al. 2002) and residual reproductive values are expected to decrease with sex ratio manipulation based on principles of energy allocation (Burley 1982). The cost of sex-ratio control may also explain the absence of sex-ratio adjustment in other reptile species (Baron et al. 2010). Moreover, in species with genetic sex determination, the costs of sex-allocation adjustments are likely to be intrinsically high (Charnov 1982; Hardy 1997; Pen and Weissing 2002), and are likely to limit the situations where facultative adjustment is adaptive (West and Sheldon 2002). Accordingly, even though seasonal variation in sex ratio exists in species with genetic sex determination (see e.g., Baron et al. 2010), it is typically observed in species with temperature-dependent sex determination (e.g., Wapstra et al. 2004; Warner et al. 2009; Pen et al. 2010). Though the exact mechanism remains unclear, sex determination in brown anoles appears to have a genetic basis (Gorman and Atkins 1966), and sex ratio adjustment might therefore be inherently constrained. Accordingly, the benefits gained from seasonal adjustments might be too small or temporally inconsistent for such allocation biases to be profitable.

Finally, transferring females from their natural environment to the laboratory during oviposition may have deprived them of information on operational sex ratio and local resource competition, both of which can trigger adaptive sex allocation, (e.g., Hardy 1997; Olsson and Shine 2001) and eliminated the appropriate seasonal variation in environmental cues such as day length, temperature, rainfall, and food abundance. If these cues influence female sex allocation decisions, it may be unrealistic to expect a seasonal bias in offspring sex ratio in captivity. However, excluding operational sex ratio and local resource competition in a laboratory common-garden served to rule out these two factors as potential explanation for any putative bias. Although it would be informative to examine seasonal patterns in sex ratios of free-living anoles, the frequency with which females lay their single-egg clutches, along with the secrecy of their nesting sites, makes it extremely difficult to precisely track the sequential production of offspring in the wild. We also note that our removal of dams from the natural field environment necessitated the use of stored sperm for most fertilization decisions, as females were not allowed to re-mate in captivity. Contrary to results from another lizard in which females produce relatively more sons when fertilizing their eggs with stored sperm (Olsson et al. 2007), the lack of a seasonal pattern in sex-ratio bias suggests that there is no inherent tendency for older sperm to produce male progeny in brown anoles. Moreover, given the absence of a relationship between sample size and effect size in our four independent data sets and in the pooled data set, our results are unlikely to be attributable to a lack of statistical power.

In conclusion, we found no evidence for a seasonal shift in offspring sex ratio in brown anoles, despite recent evidence that this species can adaptively adjust progeny sex allocation in response to other factors (Calsbeek and Bonneaud 2008; Cox and Calsbeek 2010a; Cox et al. 2011). Although results from the present study confirm that early hatching dates are associated with higher juvenile survival, they also reveal that this benefit is only weakly and inconsistently sex-specific. However, sex differences in the relationship between hatching date and adult size indirectly suggest that males are more likely than females to benefit from early hatching dates in terms of their adult survival and reproductive success. Hence, available data generally support the idea that a seasonal shift from male- to female-biased sex ratios would be adaptive, yet we see no evidence that such a seasonal pattern has evolved. By contrast, seasonal biases in progeny sex ratios have been documented in several lizards with temperature-dependent sex determination and may even provide an adaptive explanation for the evolution of this mode of sex determination (Wapstra et al. 2004; Warner and Shine 2008; Wapstra et al. 2009; Warner et al. 2009; Pen et al. 2010). Our results generally agree with those from several other reptiles exhibiting genotypic sex determination, in which sex ratios are often seasonally invariant even in situations where a seasonal bias would appear to be adaptive (Lovern and Passek 2002; Uller and Olsson 2006; Baron et al. 2010).

References

Andersson M, Wallander J, Oring L, Akst E, Reed JM, Fleischer RC (2003) Adaptive seasonal trend in brood sex ratio: test in two sister species with contrasting breeding systems. J Evol Biol 16:510–515
Arnold SJ, Wade MJ (1984) On the measurement of natural and sexual selection: applications. Evolution 38:720–734

Baron JP, Tully T, Le Galliard JF (2010) Sex-specific fitness returns are too weak to select for non-random patterns of sex allocation in a viviparous snake. Oecologia 164:369–378

Blouin-Demers G, Weatherhead PJ (2007) Allocation of offspring size and sex by female black ratsnakes. Oikos 116:1759–1767

Brodie IED, Moore AJ, Janzen FJ (1995) Visualizing and quantifying natural selection. Trends Ecol Evol 10:313–317

Burley N (1981) Sex ratio manipulation and selection for attractiveness. Science 211:721–722

Burley N (1982) Sex-ratio manipulation. Am Nat 120:81–107

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York

Calsbeek R (2009) Sex-specific adult dispersal and its selective consequences in the brown anole *Anolis sagrei*. J Anim Ecol 78:617–624

Calsbeek R, Bonneaud C (2008) Postcopulatory fertilization bias as a form of cryptic sexual selection. Evolution 62:1137–1148

Calsbeek R, Cox RM (2010) Experimentally assessing the relative importance of predation and competition as agents of selection. Nature 465:613–616

Calsbeek R, Smith TB, Bardeleben C (2007) Intraspecific variation in *Anolis sagrei* mirrors the adaptive radiation of Greater Antillean anoles. Biol J Linn Soc 90:189–199

Calsbeek R, Bonneaud C, Smith TB (2008) Differential fitness effects of immunocompetence and neighbourhood density in alternative female lizard morphs. J Anim Ecol 77:103–109

Calsbeek R, Buerman W, Smith T (2009) Parallel shifts in ecology and natural selection. BMC Evol Biol 9:3 (open access)

Calsbeek R, Bonvini L, Cox RM (2010) Geographic variation, frequency dependent selection, and the maintenance of a female limited polymorphism. Evolution 64:116–125

Charnov EL (1982) The theory of sex allocation. Princeton University Press, Princeton

Clark AB (1978) Sex ratio and local resource competition in a prosimian primate. Science 201:163–165

Clutton-Brock T (1994) The cost of sex. In: Short RV, Balaban E (eds) The differences between the sexes. Cambridge, Cambridge University Press, pp 347–362

Clutton-Brock TH, Iason GR (1986) Sex ratio variation in mammals. Quat Rev Biol 61:339–374

Cockburn A, Legge S, Double MC (2002) Sex ratios in birds and mammals: can the hypotheses be disentangled? In Hardy ICW (ed) Sex ratios: concepts and research methods. Cambridge, Cambridge University Press, pp 266–286

Cox RM, Calsbeek R (2010a) Cryptic sex-ratio bias provides indirect genetic benefits despite sexual conflict. Science 328:92–94

Cox RM, Calsbeek R (2010b) Severe costs of reproduction persist in Anolis lizards despite the evolution of a single-egg clutch. Evolution 64:1321–1330

Cox RM, Calsbeek R (2010c) Sex-specific selection and intraspecific variation in sexual size dimorphism. Evolution 64:798–809

Cox RM, Calsbeek R (2011) An experimental test for alternative reproductive strategies underlying a female-limited polymorphism. J Evol Biol 24:343–353

Cox RM, Stenquist DS, Calsbeek R (2009) Testosterone, growth, and the evolution of sexual size dimorphism. J Evol Biol 22:1586–1589

Cox RM, Duryea MC, Najarro M, Calsbeek R (2011) Paternal condition drives progeny sex-ratio bias in a lizard that lacks parental care. Evolution 65:220–230

Daan S, Dijkstra C, Weissing FJ (1996) An evolutionary explanation for seasonal trends in avian sex ratio. Behav Ecol 7:426–430

Dijkstra C, Daan S, Buker JB (1990) Adaptive seasonal variation in the sex ratio of kestrel broods. Funct Ecol 4:143–147

Ellengren H, Gustafsson L, Sheldon BC (1996) Sex ratio adjustment in relation to paternal attractiveness in a wild bird population. Proc Natl Acad Sci USA 93:11723–11728

Fisher RA (1930) The genetical theory of natural selection. Oxford University Press, Oxford

Frank SA (1990) Sex allocation theory for birds and mammals. An Rev Ecol Syst 21:13–55

Gelman A (2007) Interpreting the logistic regression coefficients. In: Gelman A, Hill J (eds) Data analysis using regression and multilevel/hierarchical models. Cambridge University Press, Cambridge, pp 81–85

Godfray HCJ, Werren JH (1996) Recent developments in sex ratio studies. Trends Ecol Evol 11:59–63

Gorman GC, Atkins L (1966) Chromosomal heteromorphism in some male lizards of the genus Anolis. Am Nat 100:579–583

Hamel S, Yoccoz NG, Gaillard JM (2012) Statistical evaluation of parameters estimating autocorrelation and individual heterogeneity in longitudinal studies. Statistical evaluation of parameters estimating autocorrelation and individual heterogeneity in longitudinal studies (in press)

Hardy ICW (1997) Possible factors influencing vertebrate sex ratios: an introductory overview. App Anim Behav Sci 51:217–242

Hewison AJM, Gaillard JM (1996) Birth-sex ratios and local resource competition in roe deer, *Capreolus capreolus*. Behav Ecol 7:461–464

Hewison AJM, Gaillard JM (1999) Successful sons or advantaged daughters? The Trivers-Willard model and sex-biased maternal investment in ungulates. Trends Ecol Evol 14:229–234

Husby A, Saether BE, Jensen H, Ringsby TH (2006) Causes and consequences of adaptive seasonal sex ratio variation in house sparrows. J Anim Ecol 75:1128–1139

Janzen FJ, Phillips PC (2006) Exploring the evolution of environmental sex determination, especially in reptiles. J Evolut Biol 19:175–1784

Karino K, Sato A (2009) Male-biased sex ratios in offspring of attractive males in the guppy. Ethology 115:682–690

Korpimaki E, May CA, Parkin DT, Wetton JH, Wiehn J (2000) Environmental- and parental condition-related variation in sex ratio of kestrel broods. J Avian Biol 31:128–134

Korsten P, Lessels CM, Mateman AC, Van Der Velde M, Komdeur J (2006) Primary sex ratio adjustment to experimentally reduced male UV attractiveness in blue tits. Behav Ecol 17:539–546

Krackow S, Tkadlec E (2001) Analysis of brood sex ratios: implications of offspring clustering. Behav Ecol Sociobiol 50:293–301

Laaksonen T, Lyytinen S, Korpimaki E (2004) Sex-specific recruitment and brood sex ratios of Eurasian kestrels in a seasonally and annually fluctuating northern environment. Evol Ecol 18:215–230

Lee JC, Clayton DG, Eisenstein S, Perez I (1989) The reproductive cycle of *Anolis sagrei* in southern Florida. Copeia 1989:930–937

Leimar O (1996) Life-history analysis of the Trivers and Willard sex ratio problem. Behav Ecol 7:316–325

Lovern MB, Passek KM (2002) Sequential alternation of offspring sex from successive eggs by female green anoles, *Anolis carolinensis*. Can J Zool 80:77–82

Olsson M, Shine R (2001) Facultative sex allocation in snow skink lizards (*Niveoscincus microlepidotus*). J Evol Biol 14:120–128

Olsson M, Schwartz T, Uller T, Healey M (2007) Sons are made from old stores: sperm storage effects on sex ratio in a lizard Biology Letters 3:491–493

Pen I, Weissing FJ (2002) Optimal sex allocation: steps towards a mechanistic theory. In: Hardy ICW (ed) Sex ratios: concepts and research methods. Cambridge University Press, Cambridge, pp 26–45

Pen I, Weissing FJ, Daan S (1999) Seasonal sex ratio trend in the European kestrel: an evolutionary stable strategy analysis. Am Nat 153:384–397

Pen I, Uller T, Feldmeyer B, Harts A, While GM, Wapstra E (2010) Climate-driven population divergence in sex-determining systems. Nature 468:436–438

Phillips PC, Arnold SJ (1989) Visualizing multivariate selection. Evolution 43:1209–1222

Pinheiro JC, Bates DM (2000) Mixed-effects models in S and S-PLUS. Springer, New York

Robert KA, Schwanz LE, Mills HR (2010) Offspring sex varies with maternal investment ability: empirical demonstration based on cross-fostering. Biol Lett 6:242–245

Schielzeth H, Forstmeier W (2008) Conclusions beyond support: overconfident estimates in mixed models. Behav Ecol 20:416–420

Schluter D (1988) Estimating the form of selection on a quantitative trait. Evolution 42:849–861

Silk JB (1983) Local resource competition and facultative adjustment of sex ratios in relation to competitive abilities. Am Nat 121:56–66

Stinchcombe JR, Agrawal AF, Hohenlohe PA, Arnold SJ, Blows MW (2008) Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? Evolution 62:2435–2440

Taylor PD (1981) Intra-sex and inter-sex sibling interactions as sex determinants. Nature 291:145–168

Tokarz R (1985) Body size as a factor determining dominance in staged agonistic encounters between male brown anoles (*Anolis sagrei*). Anim Behav 33:746–753

Tokarz RR (1998) Mating pattern in the lizard *Anolis sagrei*: implications for mate choice and sperm competition. Herpetologica 54:388–394

Trivers RL (1976) Sexual selection and resource-accruing abilities in *Anolis garmani*. Evolution 30:254–269

Trivers RL, Willard DE (1973) Natural selection of parental ability to vary sex ratio of offspring. Science 179:90–92

Uller T, Olsson M (2006) No seasonal sex-ratio shift despite sex-specific fitness returns of hatching date in a lizard with genotypic sex determination. Evolution 60:2131–2136

Uller T, Olsson M (2010) Offspring size and timing of hatching determine survival and reproductive output in a lizard. Oecologia 162:663–671

Van de Pol M, Wright J (2009) A simple method for distinguishing within- versus between-subject effects using mixed models. Anim Behav 77:753–758

Veeroja R, Kirk A, Tilgar V, Sade S, Kreitsberg M, Tonisson J (2010) Conception date affects litter type and foetal sex ratio in female moose in Estonia. J Anim Ecol 79:169–175

Venables WN, Ripley BD (1999) Modern applied statistics with S-plus. Springer, New York

Wapstra E, Olsson M, Shine R, Edwards A, Swain R, Joss JMP (2004) Maternal basking behaviour determines offspring sex in a viviparous reptile. Proc R Soc Biol Sci B 271:S230–S232

Wapstra E, Uller T, Sinn DL, Olsson M, Mazurek K, Joss JMP, Shine R (2009) Climate effects on offspring sex ratio in a viviparous lizard. J Anim Ecol 78:84–90

Warner DA, Shine R (2007) Reproducing lizards modify sex allocation in response to operational sex ratios. Biol Lett 3:47–50

Warner DA, Shine R (2008) The adaptive significance of temperature-dependent sex determination in a reptile. Nature 451:565–566

Warner DA, Uller T, Shine R (2009) Fitness effects of the timing of hatching may drive the evolution of temperature-dependent sex determination in short-lived lizards. Evol Ecol 23:281–294

Weatherhead PJ, Brown GP, Prosser MR, Kissner KJ (1998) Variation in offspring sex ratios in the northern water snake (*Nerodia sipedon*). Can J Zool 76:2200–2206

Werren JH, Charnov EL (1978) Facultative sex ratios and population dynamics. Nature 272:349–350

Werren JH, Taylor PD (1984) The effects of population recruitment to sex ratio selection. Am Nat 124:143–148

West S (2009) Sex allocation. Princeton University Press, Princeton

West S, Sheldon BC (2002) Constraints in the evolution of sex ratio adjustment. Science 295:1685–1688

West SA, Reece SE, Sheldon BC (2002) Sex ratios. Heredity 88:117–124

Wilson K, Hardy ICW (2002) Statistical analysis of sex ratios: an introduction. In: Hardy ICW (ed) Sex ratios. Concepts and research methods. Cambridge University Press, Cambridge, pp 48–92