Natural History Miscellany

Temporal but Not Spatial Environmental Variation Drives Adaptive Offspring Sex Allocation in a Plural Cooperative Breeder

Dustin R. Rubenstein[*]

Department of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853

ABSTRACT: Cooperatively breeding birds have been used frequently to study sex allocation because the adaptive value of the sexes partly depends upon the costs and benefits for parents of receiving help. I examined patterns of directional sex allocation in relation to maternal condition (Trivers-Willard hypothesis), territory quality (helper competition hypothesis), and the number of available helpers (helper repayment hypothesis) in the superb starling, *Lamprotornis superbus*, a plural cooperative breeder with helpers of both sexes. Superb starlings biased their offspring sex ratio in relation to prebreeding rainfall, which was correlated with maternal condition. Mothers produced relatively more female offspring in wetter years, when they were in better condition, and more male offspring in drier years, when they were in poorer condition. There was no relationship between offspring sex ratio and territory quality or the number of available helpers. Although helping was male biased, females had a greater variance in reproductive success than males. These results are consistent with the Trivers-Willard hypothesis and suggest that although females in most cooperatively breeding species make sex allocation decisions to increase their future direct reproductive success, female superb starlings appear to base this decision on their current body condition to increase their own inclusive fitness.

Keywords: Trivers-Willard, maternal condition, cooperative breeding, sex allocation, spatiotemporal environmental variation, environmental unpredictability.

Offspring sex ratio manipulation occurs in a variety of mammals and birds (Frank 1990). When the reproductive values (Fisher 1930) of male and female offspring differ,

* Present address: Department of Integrative Biology and Museum of Vertebrate Zoology, University of California, Berkeley, California 94720-3140; e-mail: drubenstein@berkeley.edu.

mothers are expected to bias their offspring toward the sex that contributes more to parental fitness relative to its production costs (hereafter, the more valuable sex; Trivers and Willard 1973). Females are predicted to do this as long as they have the resources to do so and the investment has a high probability of enabling the more valuable sex to achieve its fitness potential (Charnov 1982). Cooperatively breeding birds have received particular attention in studies of sex allocation because, in these species, the adaptive value of the sexes partly depends upon the costs and benefits for parents of receiving help (Pen and Weissing 2000; Komdeur 2004). Most cooperatively breeding species live in kin-based family groups where members of the philopatric sex—typically males in birds (Greenwood 1980)—often delay reproduction and help raise the offspring of conspecifics, generally their siblings (Brown 1987; Cockburn 1998).

In cooperatively breeding species where dispersal and helping are sex biased, patterns of offspring sex allocation can be explained by three hypotheses that are related to (i) maternal condition (Trivers-Willard hypothesis), (ii) the positive effects on parental fitness by helpers (helper repayment hypothesis), or (iii) the negative effects on parental fitness by helpers (helper competition hypothesis). According to the Trivers-Willard hypothesis, because the condition of young at the end of the period of parental investment will tend to be correlated with the condition of the mother during the period of parental investment, maternal condition can influence sex allocation (Trivers and Willard 1973; Rosenfeld and Roberts 2004). The more valuable sex is typically the one with the higher variance in reproductive success (Trivers and Willard 1973); this tends to be the male sex in most polygynous species (Bateman 1948). In birds, mothers in good condition often bias their offspring toward being males, the sex that needs more investment to reach its full reproductive potential, but the bias is toward females when the mothers are in poor condition (e.g., Lessels and Avery 1987; Nager et al. 2000; Whittingham and Dunn 2000; Rosenfeld and Roberts 2004; Love et al. 2005).

In contrast, the helper repayment and helper competition hypotheses are specific to cooperative breeders and make contrasting predictions about the direction of sex allocation according to whether helpers have a positive or negative effect on parental fitness (Clark 1978). The helper repayment hypothesis (ii) suggests that when the presence of helpers has a positive effect on parental fitness, parents should overproduce the helping and more philopatric sex when that sex is rare in their group (local resource enhancement hypothesis; Gowaty and Lennartz 1985; Emlen et al. 1986; Lessels and Avery 1987; Pen and Weissing 2000; Griffin et al. 2005). In contrast, when the presence of helpers has a negative effect on parental fitness (e.g., often on lower-quality territories), the helper competition hypothesis (iii) suggests that parents should overproduce the nonhelping and dispersive sex when that sex is common in their group in order to reduce competition over resources (local resource competition hypothesis; Clark 1978; Clutton-Brock and Iason 1986; Dickinson 2004) or mates (mate competition hypothesis; Hamilton 1967).

Most studies of sex ratio variation in cooperatively breeding species assume that sex allocation decisions are related to territory quality (Komdeur 1996*a*, 1998, 2003; Komdeur et al. 1997; Hasselquist and Kempenaers 2002) because territory quality often constrains dispersal and independent breeding, leading to the formation of cooperative groups (Koenig and Pitelka 1981; Emlen 1982; Koenig et al. 1992). Cooperative species showing the greatest offspring sex ratio bias are expected to live in spatially variable environments, where resource levels differ among territories and where helpers will have the greatest effect on parental fitness (Julliard 2000). Both the helper repayment and helper competition hypotheses assume that territory quality is predictable across time periods and that mothers make sex allocation decisions by considering whether offspring will enhance or diminish future parental fitness by contributing to or competing for territorial resources. Although differences in habitat quality among territories (spatial environmental variation) are generally consistent among years (Komdeur 1996*b*, 1996*c*) and can influence reproductive decisions in many species of cooperative breeders (Stacey and Ligon 1991), temporal environmental variation, such as year-to-year variation in rainfall that is unpredictable and independent of seasonality, can also influence reproductive decisions, breeding roles, and maternal condition (Emlen 1982; Rubenstein 2007). One key prediction that is often overlooked in studies of avian sex allocation is that although birds living in spatially variable environments, which are predictable in time, should adjust offspring sex ratio to maximize their own future direct reproductive success on their territories (Komdeur 1996*b*, 1996*c*), birds living in temporally variable and unpredictable environments should not adjust

offspring sex ratio for future helping needs. Rather, they should invest to take advantage of current conditions when they are good or, conversely, make the best of a bad situation when conditions are poor, because future conditions are unpredictable.

The three hypotheses for sex allocation in cooperative breeders make different predictions about which is the more valuable sex (table 1). The Trivers-Willard hypothesis predicts that the sex with a higher variance in reproductive success is more valuable when a female is in good condition and the opposite when she is in poor condition (Trivers and Willard 1973; Cameron and Linklater 2002), whereas the helper repayment and helper competition hypotheses assume that the more valuable sex is determined by the effect that helpers have on parental fitness (reviewed in Komdeur 2004; table 1). Males tend to have a higher variance in reproductive success in most noncooperatively breeding species (Bateman 1948), but females tend to have a higher variance in reproductive success in cooperatively breeding species (Hauber and Lacey 2005). This creates a potential paradox for cooperatively breeding species with male-biased helping, such as most avian cooperative breeders (Greenwood 1980). For species with male-biased helping and female-biased dispersal, the Trivers-Willard hypothesis predicts that mothers should adaptively overproduce female offspring when they are in good condition and male offspring when they are in poor condition. In contrast, the helper repayment hypothesis predicts that mothers should adaptively overproduce male helpers when helpers are rare because helpers can increase parental fitness, essentially discounting their production cost (Emlen et al. 1986). Finally, the helper competition hypothesis predicts that mothers should adaptively overproduce female offspring, or the primary dispersing sex, when they live on poor-quality territories (but male offspring when they live on high-quality territories) because the presence of additional group members could reduce parental fitness (Griffin et al. 2005).

Here, I examine offspring sex allocation and test contrasting predictions of the Trivers-Willard, helper repayment, and helper competition hypotheses in the superb starling *Lamprotornis superbus*, a plural cooperative breeder with helpers of both sexes (Feare and Craig 1999; Fry et al. 2000). Superb starlings are endemic to East Africa and live in a temporally variable and unpredictable environment where dry season rainfall varies greatly among years and influences not only breeding roles but also the body condition of breeding birds (Rubenstein 2007). Additionally, they live in a spatially variable environment where differences in territory quality influence mating decisions such that females on low-quality territories seek more extrapair fertilizations than those on high-quality territories (Rubenstein, forthcoming *b*). I examined the

Table 1: The Trivers-Willard, helper repayment, and helper competition hypotheses make alternative predictions about sex allocation in relation to temporal environmental variation (prebreeding rainfall and maternal condition), spatial environmental variation (territory quality), and group size (the number of available helpers)

		Direction of sex bias					
	Assumption of helper effect on parental fitness	Maternal condition: prebreeding rainfall		Territory quality: habitat quality (% vegetation cover)		Available helpers: group size and no. surviving offspring	
Hypothesis		Bad year	Good year	Low quality	High quality	Few helpers	Many helpers
General predictions:							
Trivers-Willard[a]	NA	Sex with lower variance in RS	Sex with higher variance in RS	No bias	No bias	No bias	No bias
Helper repayment[b]	Positive effect	No prediction	No prediction	Helping sex	No bias	Helping sex	No bias
Helper competition[c]	Negative effect	No prediction	No prediction	Dispersing sex	Helping sex	Helping sex	Dispersing sex
Specific to superb starlings:							
Trivers-Willard[a]	NA	Males	Females	No bias	No bias	No bias	No bias
Helper repayment[b]	Positive effect	No prediction	No prediction	Males	No bias	Males	No bias
Helper competition[c]	Negative effect	No prediction	No prediction	Females	Males	Males	Females

Note: Shown are general predictions from the three hypotheses for sex allocation in cooperative breeders assuming that (i) temporal environmental variation (e.g., prebreeding rainfall) has short-term effects on maternal condition that vary from year to year, and (ii) there is spatial environmental variation, which influences territory quality and has long-term effects on territory quality. Thus, maternal condition may vary from year to year, whereas territory quality is predictable across time. Also shown are predictions specific to superb starlings. Because (i) superb starling helpers have a positive effect on parental fitness and (ii) females have a higher variance in reproductive success than males, the Trivers-Willard, helper repayment, and helper competition hypotheses make specific predictions about sex allocation in relation to maternal condition, territory quality, and the number of available helpers. Superb starlings do not meet the primary assumption of the helper competition hypothesis, namely, that helpers have a negative effect on parental fitness. RS = reproductive success.

[a] After Trivers and Willard 1973.
[b] After Gowaty and Lennartz 1985; Emlen et al. 1986; Lessels and Avery 1987.
[c] After Clark 1978; Clutton-Brock and Iason 1986; Emlen 1997; Hasselquist and Kempenaers 2002.

effect that helpers had on parental fitness, determined whether helping was sex biased, and evaluated sex differences in variance in reproductive success. I then examined patterns of directional sex allocation in relation to (i) temporal environmental variation, which was related to maternal condition, (ii) territory quality, and (iii) the number of available helpers (see table 1).

Methods

Study System and Species

Breeding activities of seven superb starling social groups were monitored over five long-rain and five short-rain breeding periods from April 2001 to December 2005 at the Mpala Research Centre, Laikipia, Kenya (0°17′N, 37°52′E). One additional group was added in January 2002, and another was added in January 2003. Groups of 10–35 (mean = 21) individuals defended year-round territories, and birds bred during both the long (March–May) and short rains (November). Up to six breeding pairs per group (mean = 3.5) bred during the long rains, and up to four breeding pairs per group (mean = 1.7) bred during the short rains (Rubenstein 2006). There was no behavioral or genetic evidence of intraspecific brood parasitism (i.e., egg dumping) by female superb starlings (Rubenstein, forthcoming *a*). Pairs frequently renested multiple times during the long rains—but not during the short rains—and it was common for pairs to lay three or

four clutches of eggs in a season if clutches laid early failed or were depredated. Some pairs successfully fledged up to three broods in a single season, but many pairs failed to fledge any young in a season.

The main dry season (hereafter, the prebreeding period) occurs between the short- and long-rain breeding seasons and generally lasts from December through February. Daily rainfall data were collected continuously during the study using a Hydrological Services TB3 tipping bucket rain gauge located at the Mpala Research Centre. Prebreeding rainfall was calculated as the sum of the daily rainfall during December, January, and February of each year. This period represented the (i) 3 months with the lowest average cumulative monthly rainfall, (ii) three (of four) months where the variance in mean monthly rainfall was greater than the mean value, and (iii) three (of five) months when superb starlings did not initiate new clutches of eggs (the other two months were August and September, or the brief dry period before the short rains). Thus, cumulative rainfall from December through February gives a relative measure of rainfall intensity for the prebreeding dry season that is comparable across years (Rubenstein 2006). There was no relationship between prebreeding rainfall and breeding rainfall (correlation: $F = 0.57$, df $= 1, 3$, $P = .51$, $r = 0.40$), and while prebreeding rainfall varied greatly over the course of this study (coefficient of variation [CV] = 93.6), breeding rainfall varied little (CV = 38.6).

Adult Captures

Adult starlings were captured annually during the dry season with baited wire traps. Each individual was given a unique set of color leg bands and a numbered metal leg ring. Birds were weighed to the nearest 0.5 g, and tarsus and wing cord length were measured to the nearest 0.1 cm and 0.5 cm, respectively. Body mass was regressed against tarsus length to calculate standardized body mass, an index of body mass corrected for body size (i.e., residuals). Separate linear regressions were used for each year in order to compare individuals within seasons; each regression was positive and significant. Because it was not possible to capture each breeding female in each year, standardized body mass was averaged for all of the breeding females caught in a given year. Twelve unique breeding females were captured during prebreeding in 2002, eight in 2003, 12 in 2004, and six in 2005. No birds were captured during the prebreeding period in 2001 because prebreeding trapping was not conducted during the first year of the study.

Nestling Sampling

Active nests were checked every 1–3 days during the incubation and nestling stages. Nest predation events typically resulted in the entire clutch disappearing and often in the nest being destroyed. Nest failure was attributed to starvation if one or more nestlings disappeared over an extended period of time during the nestling stage and there was no evidence of nest predation (Rubenstein 2006). Only nests from the long-rain breeding periods were used for sex ratio analyses in this study in order to isolate the effect of prebreeding rainfall on offspring sex ratio. Blood was collected from 204 nestlings from 79 nests from nine social groups that spanned five long-rain breeding seasons from April 2001 through July 2005. During this period 895 eggs were laid in 276 nests, but only 204 survived long enough to be sampled for DNA analysis (i.e., at least to day 8 after hatching). There were 19 nests sampled in 2001, 12 nests in 2002, 15 nests in 2003, 18 nests in 2004, and 15 nests in 2005. To minimize the role that starvation may have played in influencing offspring sex ratio, a reduced data set that included only nests where three or four nestlings survived long enough to be sexed was also used (mean clutch size of all nests = 3.5; Rubenstein 2006). There was no difference in the sex ratio between the different types of nests in any of the years (ANOVA: nest type: $F = 1.70$, df = 1, 69, $P = .20$; year: $F = 1.50$, df = 4, 69, $P = .21$; interaction: $F = 1.67$, df = 4, 69, $P = .17$), suggesting that both the full and reduced data sets give an unbiased estimation of secondary sex ratio. In the reduced data set of 158 nestlings, there were 10 nests in 2001, eight

nests in 2002, 11 nests in 2003, 14 nests in 2004, and five nests in 2005. Every superb starling was sexed using polymerase chain reaction primers (Griffiths et al. 1998) after the accuracy of this method for superb starlings was confirmed using adult birds of known sex.

Behavioral Observations

Focal observations (1–2 h) at nests were conducted during the nest building and incubation stages to identify the social breeders. Observers used spotting scopes and were hidden under a tree or behind a blind >30 m from the nest. A social mother was identified as the female at a nest that incubated eggs, and a social father was defined as one that remained near the incubating female while the female was on the nest. After eggs hatched, focal observations (1–3 h) were used to identify helpers at the nest. Although most nests were observed multiple times over the course of the nestling stage, repeated observations were not always possible due to extremely high nest predation rates (nearly 75% of nests failed, with >90% of the failures caused by nest predation; Rubenstein 2006). All of the birds that came within about 30 m of the nest were identified, and each bird that brought food to the nest was recorded. Breeders were defined as the social parents of the nest, whereas helpers were defined as those individuals other than the parents that brought food to a nest.

The number of helpers at superb starlings nests did not influence the fledging success of a nest (Rubenstein 2006). However, since nest predation explains >90% of nest failures, this analysis did not account for the role that helpers play in preventing nestling starvation. To determine the impact that helper provisioning had on parental fitness by reducing nestling starvation, the number of helpers per hour and the number of helper provisioning trips per hour were compared at nests in which either all of the offspring that hatched fledged or one or more offspring that hatched subsequently died of starvation. To minimize variation in nestling number, only nests where three or four eggs hatched were considered. To compare helping effort between male and female helpers, the number of helpers of each sex that visited a nest per hour were compared, as well as the number of provisioning trips per hour per nestling that each sex made.

Estimating Variance in Reproductive Success

Reproductive success was estimated as the number of offspring fledged for each individual in each year. Paternity analysis with microsatellite markers was used to confirm parentage in all cases (Rubenstein, forthcoming *a*). Separate values were calculated for males and females. Data from the short and long rains were combined for each

year to give a true estimate of annual reproductive success. Censuses were performed before and after each breeding period to determine which individuals were still alive and part of each group. Data from 2001 were excluded because not all of the birds in each group were captured that year, the first year of the study. Once it was determined which individuals were alive in each group in each year and what their individual reproductive success was for that year, the standardized variance in reproductive success (I) was calculated separately for all males and females in each group as the variance in reproductive success $(RS)/(mean\ RS)^2$ (sensu Weatherhead and Boag 1997). Approximately 7% of all fertilizations were with extragroup extrapair males that presumably were dominant breeders in nearby unmarked groups (Rubenstein, forthcoming *a*). These rare paternity losses to extragroup males were assumed to cancel out with the breeding males' own chances for gaining extragroup extrapair fertilizations and thus were not factored into the analysis of reproductive success. However, the analysis did account for extrapair fertilizations with males from inside the group, which also accounted for approximately 7% of all offspring (Rubenstein, forthcoming *a*).

Testing Hypotheses for Offspring Sex Ratio Allocation

Because (i) prebreeding rainfall was known to be correlated with standardized body mass in breeders ($r = 0.32$; Rubenstein 2007), (ii) no females were captured in 2001, and (iii) not every female was captured in each year, prebreeding rainfall was used as a proxy for maternal condition in all models. Habitat quality, or percent vegetation cover, was used as a measure of territory quality because this has been shown to be correlated with the amount of *Cynadon* grass, the dominant grass species in the grassland glades where superb starlings concentrate their breeding and foraging; the grass is also an indicator of nutrient-rich soils (Augustine 2003) and elevated insect biomass (Rubenstein, forthcoming *b*). Additionally, habitat quality is related to female extrapair mating decisions in superb starlings such that females in lower-quality territories seek more extrapair fertilizations than those in higher-quality territories (Rubenstein, forthcoming *b*). Percent vegetation cover was estimated using vegetation transects (see methods in Rubenstein, forthcoming *b*). Two measures of the number of available helpers were used: (i) the number of surviving offspring a female had from the previous 2 years, because this measure is related to extrapair mating decisions in superb starlings in that females are more likely to seek extrapair fertilizations with males from inside the group when they have fewer surviving offspring (Rubenstein, forthcoming *a*); and (ii) group size, because superb starling helpers are not only offspring but also other mem-

bers of the group that may be less related or even unrelated to the breeding pair (Rubenstein 2006). Both measures give an estimate of the number of available helpers at the time of copulation and are more appropriate than simply measuring the number of helpers that came to a nest during the nestling period because the number of helpers at a nest varies with nestling age (Rubenstein 2006). The two measures of the available number of helpers were not correlated (correlation: $F = 0.10$, df $= 1, 30$, $P = .75$, $r = 0.06$).

Statistics

Most data were found to be normally distributed; nonparametric tests were used in cases where they were not. To determine whether helpers had an effect on parental fitness, *t*-tests were used to compare the number of helpers per hour and the number of helper provisioning trips per hour at nests in which either all of the offspring that hatched eventually fledged or at nests where one or more offspring that hatched eventually died. Spearman rank correlations were used to compare these measures of helping with the proportion of eggs fledged from the nests. To examine the standardized variance in reproductive success in males and females, *t*-tests were used to compare the ratio of female to male variance values to a mean value of 1, which is the expected ratio when male and female standardized variance in reproductive success is equal (sensu Hauber and Lacey 2005). To compare helping effort between male and female helpers, paired *t*-tests were used to assess the number of helpers per hour of each sex as well as the proportion of helping trips done by each sex per nestling per hour.

To examine among-year variation in offspring sex ratios, data were analyzed using generalized linear models with a binomial error distribution and logit link function in the SAS GENMOD procedure (SAS, ver. 8.02, SAS Institute; Wilson and Hardy 2002). To test the effects of (i) maternal condition (prebreeding rainfall), (ii) territory quality, and (iii) the number of available helpers on offspring sex ratios, "males/clutch size" was set as the response variable (clutch sex ratio is weighted by the number of eggs in the clutch); "mother" was set as a repeated subject to account for differences between laying females; and prebreeding rainfall (maternal condition), habitat quality, (territory quality), group size (available helpers), and number of surviving offspring (available helpers) were included as predictor variables. There was no relationship between group size and habitat quality (regression: $F = 0.82$, df $= 1, 24$, $P = .38$, $R^2 = 0.03$). Although there was a relationship between group size and prebreeding rainfall (regression: $F = 12.97$, df $= 1, 31$, $P = .0011$, $R^2 = 0.30$), tests of multicollinearity showed that none of the

independent variables were strongly correlated (all variance inflation factors < 1.58). After running the full model containing all four predictor variables, nonsignificant predictor variables ($P > .05$) were sequentially removed until only significant predictors remained (Wilson and Hardy 2002). Because all interaction terms were nonsignificant, they were not reported. A model containing only the significant predictor variables was then run using a reduced data set that included only nests in which three and four nestlings survived until sampling. All test statistics were reported for likelihood ratio tests. Although the reduced data gave a better approximation of primary sex ratio than the full data set, 16 of 48 (33%) of the nests from the reduced data set had lost one nestling to starvation before blood samples could be taken, and hence offspring sex ratio could still only be estimated as the secondary sex ratio. Moreover, there was no bias in the sex of the starved nestlings in any of the years (ANOVA: nest type: $F = 0.25$, df $= 1, 38$, $P = .62$; year: $F = 1.99$, df $= 4, 38$, $P = .12$; interaction: $F = 0.79$, df $= 4, 38$, $P = .54$). A χ^2 test was used to compare the offspring sex ratio in the driest and wettest years.

Results

Helper Effects on Parental Fitness

There was a positive effect of helper provisioning on parental fitness; there were more helpers at nests that did not lose nestlings to starvation than at those that did (*t*-test: $t = 2.34$, df $= 19$, $P = .03$; fig. 1*A*), and there was a nonsignificant tendency for helpers to make more provisioning trips at nests that did not lose nestlings to starvation than at those that did (*t*-test: $t = 1.97$, df $= 19$, $P = .064$; fig. 1*B*). Additionally, there was a positive relationship between the proportion of eggs fledged per nest and both the number of helpers (Spearman rank correlation: $S_r = 0.57$, $n = 21$, $P = .0074$) and the number of helper provisioning trips (Spearman rank correlation: $S_r = 0.49$, $n = 21$, $P = .023$). However, there was no relationship between the number of parent provisioning trips and the proportion of eggs fledged per nest (Spearman rank correlation: $S_r = -0.038$, $n = 21$, $P = .87$), suggesting that parents could not compensate for the number of helper provisioning trips and increase their own number of provisioning trips at either nests where offspring starved (regression: $F = 1.28$, df $= 1, 10$, $P = .29$, $R^2 = 0.15$) or did not starve (regression: $F = 1.19$, df $= 1, 10$, $P = .30$, $R^2 = 0.11$).

Sex Valuation

I examined the standardized variance in annual reproductive success for each sex in each group in each year. The mean $\pm$ SE for the ratios of female to male standardized variance in reproductive success at each site for each year was 1.27 ± 0.12 ($n = 33$); females had a higher standardized variance in reproductive success than males (*t*-test: $t = 2.21$, df $= 32$, $P = .035$).

Sex-Biased Helping Patterns

There were more male than female helpers at nests (paired *t*-test: $t = 2.58$, df $= 61$, $P = .012$; fig. 2*A*), and male helpers did a greater proportion of nest provisioning than did females (paired *t*-test: $t = 3.41$, df $= 54$, $P = .0012$; fig. 2*B*). Approximately one-third of the nest provisioning (i.e., helping) was done by females, and two-thirds was by males (fig. 2*B*).

Environmental Variation and Maternal Body Condition

Prebreeding rainfall was negatively correlated with prebreeding maternal standardized body mass such that the mother weighed relatively more in drier years than in wetter years (regression: $F = 7.02$, df $= 1, 36$, $P = .012$, $R^2 = 0.16$). Although gaining body mass during harsh conditions seems counterintuitive, it is a well-documented response to unpredictable food supply in European starlings *Sturnus vulgaris* and may be linked to reduced activity and metabolic expenditure as well an increase in fat stores to endure the period of food shortage (Witter et al. 1995; Cuthill et al. 2000; Buchanan et al. 2003). There was no relationship between habitat quality and maternal standardized body mass (regression: $F = 0.15$, df $= 1, 25$, $P = .70$, $R^2 = 0.0061$).

Sex Ratio Allocation

Among-year variation in offspring sex ratio was related to prebreeding rainfall (maternal condition) but not to territory quality or to either measure of the number of available helpers (table 2). When all nonsignificant predictor variables were sequentially removed from the model, prebreeding rainfall was still the only significant predictor of offspring sex ratio (likelihood ratio test: $\chi^2 = 4.59$, df $= 1$, $P = .032$). A similar relationship between sex ratio and prebreeding rainfall was found when using the reduced data set; prebreeding rainfall was the only significant predictor of offspring sex ratio (likelihood ratio test: $\chi^2 = 4.43$, df $= 1$, $P = .035$; fig. 3). The total proportion of male offspring likewise was significantly lower in 2004,

A

B
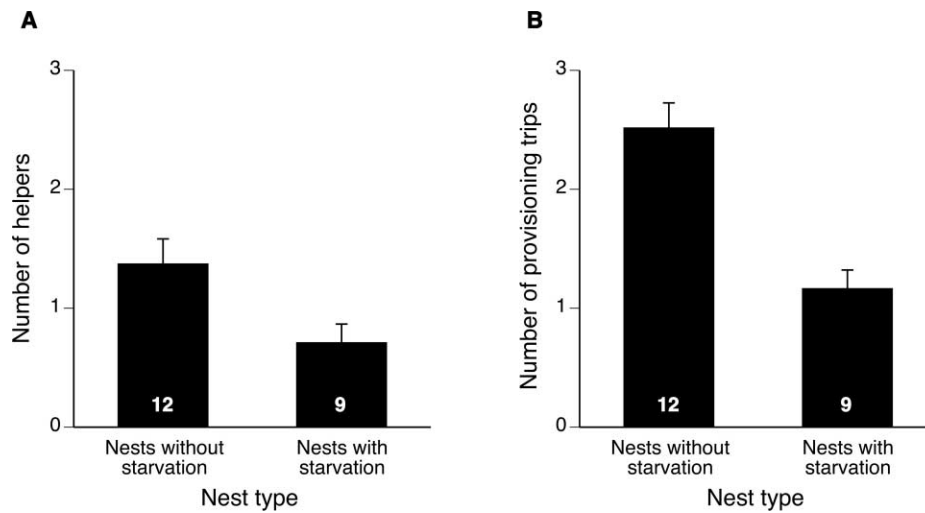
Figure 1: Helpers had a positive effect on parental fitness. There were significantly more helpers (individuals per hour; *A*), and helpers tended to do more provisioning (trips per hour; *B*) at nests that fledged all of the nestlings that hatched than at those where at least one of the nestlings died of starvation. Each bar represents the mean ± SE for nests in which three or four eggs hatched. Sample sizes are indicated at the bottom of each bar.

the wettest year of the study, than in 2002, the driest year of the study ($\chi^2 = 13.84$, df $= 1$, $P = .0002$).

Discussion

Offspring sex ratio was negatively related to prebreeding rainfall, which was correlated with maternal condition. Although in most cooperative breeders the pattern of sex allocation is related to the effect that helpers have on parental fitness, which is often related to territory quality and the number of available helpers (reviewed in Komdeur 2004; Griffin et al. 2005), there was no relationship between offspring sex ratio and territory quality or the number of available helpers in superb starlings. Overall, these results do not support either of the two hypotheses that most often explain sex ratio variation in cooperative breeders, the helper repayment and competition hypotheses. Instead, they are most consistent with the Trivers-Willard hypothesis because mothers produced relatively more female offspring in wetter years, when they had greater access to resources and presumably were in better condition; they produced more male offspring in drier years, when they had less access to resources and presumably were in poorer condition.

Female superb starlings that became breeders weighed relatively more during the prebreeding period in drier years and relatively less in wetter years. Many species of birds, including European starlings, are known to increase body mass during periods of unpredictable food supply, which may be linked to reduced activity and metabolic

expenditure as well as to an increase in fat stores to endure the period of food shortage (Witter et al. 1995; Cuthill et al. 2000; Buchanan et al. 2003). Unpredictable food availability is associated not only with weight gain in many avian species but also with elevations in baseline corticosterone, which has been linked to maternal condition and sex allocation as a possible mechanism for sex manipulation in birds (Pike and Petrie 2006). In European starlings, deposition of yolk corticosterone that influences embryonic mortality may be a bet-hedging strategy for unpredictable environments where environmental conditions at egg laying may be different from those during the nestling stage (Love et al. 2005). Although previous work in superb starlings has shown that prebreeding corticosterone was not related to prebreeding rainfall in female breeders (Rubenstein 2007), further examination of corticosterone in mothers immediately before egg laying and in egg yolk may help in understanding the relationships among environmental variation, female body mass and condition, and sex allocation.

Most studies of sex allocation in avian cooperative breeders assume that the more valuable sex is determined by the parental fitness effects of helping. Because helping is generally male biased in birds (Greenwood 1980), males are assumed to be the more valuable sex when helpers have a positive effect on parental fitness (reviewed in Komdeur 2004; Griffin et al. 2005). Most studies of sex allocation in cooperative breeders, however, do not consider the direct future reproductive output of the offspring, which is related to the average offspring variance in re-

productive success (Pen and Weissing 2000; Komdeur 2004). Variance in reproductive success is an indirect measure, or predictor, of what is possible in the future, based on current investment. Although males have a higher variance in reproductive success in most noncooperatively breeding species (Bateman 1948), females tend to have a higher variance in reproductive success in cooperatively breeding species (Hauber and Lacey 2005). This was true in superb starlings; females had a higher variance in reproductive success than males. This result probably explains why mothers who were in better condition during wetter years produced significantly more female offspring and why mothers who were in poorer condition during drier years produced relatively more male offspring. Similar patterns of sex allocation might be expected in any cooperatively breeding species with male-biased helping and in which females are the sex with the higher variance in reproductive success. Most avian cooperative breeders fit these requirements; the only avian species reported by Hauber and Lacey (2005) to have a higher variance in reproductive success in males than in females was the Seychelles warbler, which is also the only species in their study with exclusively female helpers (Komdeur 2003). It will be important to examine how patterns of sex allocation are related to maternal condition in Seychelles warblers and other avian cooperative breeders where offspring sex allocation has been linked to territory quality and/or the number of available helpers (Griffin et al. 2005).

Females make adaptive sex allocation decisions to maximize their future reproductive success (Frank 1990). There is one critical difference between how the Trivers-Willard and the helper repayment and competition hy-

potheses explain the adaptive benefits of making sex allocation decisions; whereas the Trivers-Willard hypothesis suggests that mothers bias their offspring sex ratio to increase their future indirect reproductive success (i.e., inclusive fitness), the helper repayment and competition hypotheses suggest that mothers bias their offspring sex ratio to increase their own future direct reproductive success by adjusting the number of helpers in the group. The results of this study suggest that even in cooperatively breeding species with sex-biased helping, females can make sex allocation decisions based on their current body condition in order to increase their own inclusive fitness rather than to increase their own future direct reproductive success. Further support for this explanation will require testing the three critical assumptions of the Trivers-Willard hypothesis in this and other cooperatively breeding species: (i) the condition of the young at the end of parental investment will be correlated with the condition of the mother during parental investment, (ii) differences in the condition of the young at the end of the period of parental investment will tend to endure into adulthood, and (iii) one sex will be differentially helped in reproductive success by slight advantages in condition (Trivers and Willard 1973).

The helper repayment and helper competition hypotheses may not explain patterns of sex allocation in superb starlings because the temporal environment (i.e., environmental unpredictability) may have more of a causative role in driving reproductive decisions than the spatial environment (i.e., variation in territory quality; Rubenstein 2007). There was a significant relationship between maternal body condition and prebreeding rainfall (temporal
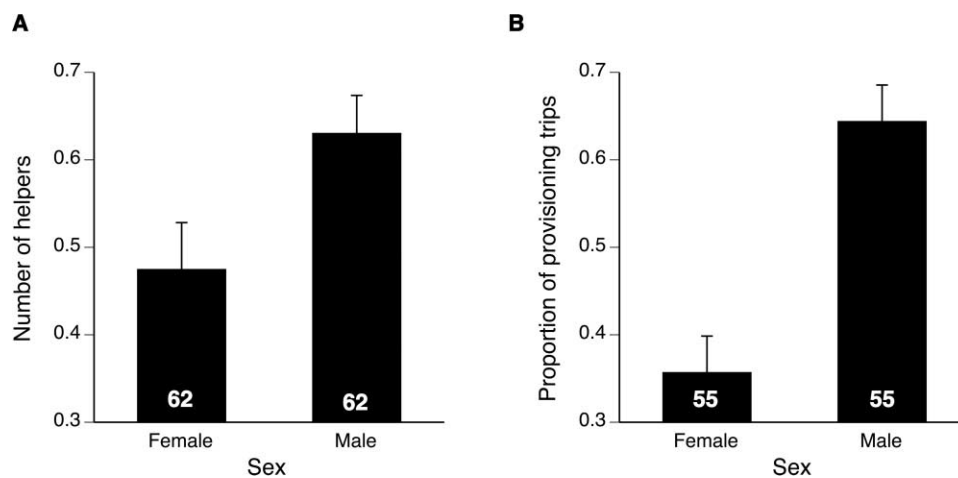
Figure 2: Helping was male biased in superb starlings. *A,* A greater number of male helpers (individuals per hour) provisioned nestlings than did female helpers. Moreover, (*B*) male helpers did a greater proportion of the nestling provisioning (trips per nestling per hour) than did female helpers. Each bar represents the mean ± SE. Sample sizes are indicated at the bottom of each bar.

Table 2: Results from analysis on sex allocation in superb starlings

| Effect	df	χ^2	P
Prebreeding rainfall (maternal condition)	1	4.74	.03
Habitat quality (territory quality)	1	.08	.78
Group size (the number of available helpers)	1	.12	.73
Surviving offspring (the number of available helpers)	1	2.41	.12

Note: Test statistics for likelihood ratio tests are reported.

environmental variation) but no relationship between maternal body condition and territory quality (spatial environmental variation). Moreover, the relationship between sex allocation and prebreeding rainfall, as well as a lack of a relationship between sex allocation and territory quality, is different from that in many temperate birds, which show a strong relationship between sex allocation and territory quality or food availability during the breeding season (Wiebe and Bortolotti 1992; Appleby et al. 1997; Suorsa et al. 2003; Millon and Bretagnolle 2005; Stauss et al. 2005). Where the environment is unpredictable, mothers should benefit more from investing in the sex that they can most affect at the time of fertilization (females in superb starlings) rather than planning helpers for future reproductive events.

The prebreeding period is an important life-history stage that influences reproductive decisions in many birds (Jacobs and Wingfield 2000), particularly those living in unpredictable arid and semiarid environments (du Plessis et al. 1995), where variation in rainfall is highest during the drier months (Ward et al. 2004; Rubenstein 2006). Few studies of sex allocation have been conducted in tropical species living in arid and unpredictable environments, and those that were done generally failed to find evidence of sex ratio variation or at least support for any of the sex allocation hypotheses. For instance, food availability during the breeding season did not influence sex allocation in wild zebra finches *Taeniopygia guttata* (Zann and Runciman 2003) even though it did in captive finches (Bradbury and Blakey 1998; Kilner 1998; Rutkowska and Cichon 2002). Similarly, whereas increasing food availability during the breeding season influenced breeding roles in tropical cooperatively breeding sociable weavers *Philetairus socius* (Covas et al. 2004), patterns of sex allocation were opposite those predicted by the helper repayment hypothesis (Doutrelant et al. 2004). Sociable weavers are one of the few other avian plural cooperative breeders to show evidence of sex ratio variation (Doutrelant et al. 2004); most of the rest are singular breeders (reviewed in Komdeur 2004; Griffin et al. 2005). It will be important for future studies of these and

other avian species—particularly plural cooperative breeders—living in arid and semiarid environments to examine sex allocation in relation to environmental conditions and maternal body condition in the prebreeding dry period leading up to the breeding season.

In summary, female superb starlings biased their offspring sex ratio in relation to prebreeding rainfall such that mothers produced relatively more female offspring in wetter years, when they were in better condition, and more male offspring in drier years, when they were in poorer condition. Although patterns of sex allocation in most cooperatively breeding species with sex-biased helping are linked to territory quality or the number of available helpers because females make decisions to increase their future direct reproductive success, female superb starlings appear to make sex allocation decisions based on their current body condition in order to increase their own inclusive fitness. Maternal body condition during the prebreeding period is likely to be an important factor affecting sex allocation in other cooperatively breeding birds, particularly in species living in arid and semiarid environments, because this life-history stage is characterized by highly unpredictable rainfall and food availability. Where the environment is unpredictable, mothers should benefit more
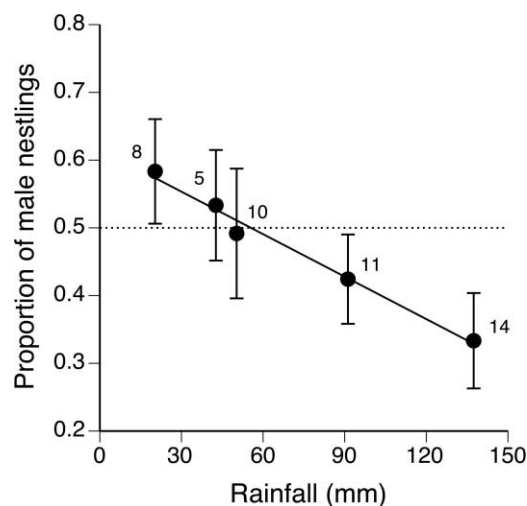
Figure 3: Offspring sex ratio was negatively related to prebreeding rainfall. The dotted line represents an even 50 : 50 sex ratio. Breeding females produced a greater proportion of male offspring following drier prebreeding periods and a greater proportion of female offspring following wetter prebreeding periods. The figure presents data from the reduced data set containing only nests where three or four nestlings survived until capture because the logistic regression weights the proportion of male offspring per nest by the total number of offspring in that nest, and this cannot be shown graphically using the full data set. Each point represents the mean ± SE proportion of male nestlings per nest. The number of nests sampled in each year is indicated above each point.

from investing in the sex that they can most affect at the time of fertilization rather than planning helpers for future reproductive events. It will be important for other studies of sex ratio variation in cooperative breeders with male-biased helping to consider whether females have a higher variance in reproductive success than males and how this might impact sex allocation patterns in relation to territory quality or the number of available helpers. For cooperatively breeding species living in spatially variable environments with large among-territory differences in quality, local competitive or helping effects would be expected to influence offspring sex allocation, whereas for species living in temporally variable environments with high among-year variation in food and rainfall, a female's current condition should influence sex allocation.

Acknowledgments

Literature Cited

Appleby, B. M., S. J. Petty, J. K. Blakey, P. Rainey, and D. W. MacDonald. 1997. Does variation of sex ratio enhance reproductive success of offspring in tawny owls (*Strix aluco*)? Proceedings of the Royal Society B: Biological Sciences 264:1111–1116.

Augustine, D. J. 2003. Long-term, livestock-mediated redistribution of nitrogen and phosphorus in an East African savanna. Journal of Applied Ecology 40:137–149.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity 2:349–368.

Bradbury, R. R., and J. K. Blakey. 1998. Diet, maternal condition, and offspring sex ratio in the zebra finch, *Poephila guttata*. Proceedings of the Royal Society B: Biological Sciences 265:895–899.

Brown, J. L. 1987. Helping and communal breeding in birds: ecology and evolution. Princeton University Press, Princeton, NJ.

Buchanan, K. L., K. A. Spencer, A. R. Goldsmith, and C. K. Catchpole. 2003. Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). Proceedings of the Royal Society B: Biological Sciences 270:1149–1156.

Cameron, E. Z., and W. L. Linklater. 2002. Sex bias in studies of sex bias: the value of daughters to mothers in poor condition. Animal Behaviour 63:F5–F8.

Charnov, E. L. 1982. The theory of sex allocation. Princeton University Press, Princeton, NJ.

Clark, A. B. 1978. Sex ratio and local resource competition in a prosimian primate. Science 201:163–165.

Clutton-Brock, T. H., and G. R. Iason. 1986. Sex ratio variation in mammals. Quarterly Review of Biology 61:339–374.

Cockburn, A. 1998. Evolution of helping behaviour in cooperatively breeding birds. Annual Review of Ecology and Systematics 29:141–177.

Covas, R., C. Doutrelant, and M. A. du Plessis. 2004. Experimental evidence of a link between breeding condition and the decision to breed or help in a colonial cooperative bird. Proceedings of the Royal Society B: Biological Sciences 271:827–832.

Cuthill, I. C., S. A. Maddocks, C. V. Weall, and E. K. M. Jones. 2000. Body mass regulation in response to changes in feeding predictability and overnight energy expenditure. Behavioral Ecology 11:189–195.

Dickinson, J. L. 2004. Facultative sex ratio adjustment by western bluebird mothers with stay-at-home helpers at the nest. Animal Behaviour 68:373–380.

Doutrelant, C., R. Covas, A. Caizergues, and M. du Plessis. 2004. Unexpected sex ratio adjustment in a colonial cooperative bird: pairs with helpers produce more of the helping sex whereas pairs without helpers do not. Behavioral Ecology and Sociobiology 56:149–154.

du Plessis, M. A., W. R. Siegfried, and A. J. Armstrong. 1995. Ecological and life-history correlates of cooperative breeding in South African birds. Oecologia (Berlin) 102:180–188.

Emlen, S. T. 1982. The evolution of helping. 1. An ecological constraints model. American Naturalist 119:29–39.

———. 1997. When mothers prefer daughters over sons. Trends in Ecology & Evolution 12:291–292.

Emlen, S. T., J. M. Emlen, and S. A. Levin. 1986. Sex-ratio selection in species with helpers-at-the-nest. American Naturalist 127:1–8.

Feare, C., and A. Craig. 1999. Starlings and mynas. Princeton University Press, Princeton, NJ.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon, Oxford.

Frank, S. A. 1990. Sex allocation theory for birds and mammals. Annual Review of Ecology and Systematics 21:13–55.

Fry, C. H., S. Keith, and E. K. Urban. 2000. The birds of Africa. Academic Press, San Diego, CA.

Gowaty, P. A., and M. R. Lennartz. 1985. Sex ratios of nestling and fledgling red-cockaded woodpeckers (*Picoides borealis*) favors males. American Naturalist 126:347–353.

Greenwood, P. J. 1980. Mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour 28:1140–1162.

Griffin, A. S., B. C. Sheldon, and S. A. West. 2005. Cooperative

breeders adjust offspring sex ratios to produce helpful helpers. American Naturalist 166:628–632.

Griffiths, R., M. C. Double, K. Orr, and R. J. G. Dawson. 1998. A DNA test to sex most birds. Molecular Ecology 7:1071–1075.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science 156:477–488.

Hasselquist, D., and B. Kempenaers. 2002. Parental care and adaptive brood sex ratio manipulation in birds. Philosophical Transactions of the Royal Society B: Biological Sciences 357:363–372.

Hauber, M. E., and E. A. Lacey. 2005. Bateman's principle in cooperatively breeding vertebrates: the effects of non-breeding alloparents on variability in female and male reproductive success. Integrative and Comparative Biology 45:903–914.

Jacobs, J. D., and J. C. Wingfield. 2000. Endocrine control of life-cycle stages: a constraint on response to the environment? Condor 102:35–51.

Julliard, R. 2000. Sex-specific dispersal in spatially varying environments leads to habitat-dependent evolutionary stable offspring sex ratios. Behavioral Ecology 4:421–428.

Kilner, R. 1998. Primary and secondary sex ratio manipulation by zebra finches. Animal Behaviour 56:155–164.

Koenig, W. D., and F. A. Pitelka. 1981. Ecological factors and kin selection in the evolution of cooperative breeding in birds. Pages 261–280 *in* R. D. Alexander and D. W. Tinkle, eds. Natural selection and social behavior. Chiron, New York.

Koenig, W. D., F. A. Pitelka, W. J. Carmen, R. L. Mumme, and M. T. Stanback. 1992. The evolution of delayed dispersal in cooperative breeders. Quarterly Review of Biology 67:111–150.

Komdeur, J. 1996*a*. Facultative sex ratio bias in the offspring of Seychelles warblers. Proceedings of the Royal Society B: Biological Sciences 263:661–666.

———. 1996*b*. Influence of age on reproductive performance in the Seychelles warbler. Behavioral Ecology 7:417–425.

———. 1996*c*. Seasonal timing of reproduction in a tropical bird, the Seychelles warbler: a field experiment using translocation. Journal of Biological Rhythms 11:333–346.

———. 1998. Long-term fitness benefits of egg sex modification by the Seychelles warbler. Ecology Letters 1:56–62.

———. 2003. Daughters on request: about helpers and egg sexes in the Seychelles warbler. Proceedings of the Royal Society B: Biological Sciences 270:3–11.

———. 2004. Sex-ratio manipulation. Pages 102–116 *in* W. D. Koenig and J. Dickinson, eds. Ecology and evolution of cooperative breeding in birds. Cambridge University Press, Cambridge.

Komdeur, J., S. Daan, J. Tinbergen, and C. Mateman. 1997. Extreme adaptive modification in sex ratio of the Seychelles warbler's egg. Nature 385:522–525.

Lessels, C. M., and M. I. Avery. 1987. Sex ratio selection in species with helpers at the nest: some extensions of the repayment model. American Naturalist 129:610–620.

Love, O. P., E. H. Chin, K. E. Wynne-Edwards, and T. D. Williams. 2005. Stress hormones: a link between maternal condition and sex-biased reproductive investment. American Naturalist 166:751–766.

Millon, A., and V. Bretagnolle. 2005. Nonlinear and population-specific offspring sex ratios in relation to high variation in prey abundance. Oikos 108:535–543.

Nager, R. G., P. Monaghan, and A. I. Houston. 2000. Parental condition, brood sex ratio and differential young survival: an experimental study in gulls (*Larus fuscus*). Behavioral Ecology and Sociobiology 48:452–457.

Pen, I., and F. J. Weissing. 2000. Sex-ratio optimization with helpers at the nest. Proceedings of the Royal Society B: Biological Sciences 267:539–543.

Pike, T. W., and M. Petrie 2006. Experimental evidence that corticosterone affects offspring sex ratios in quail. Proceedings of the Royal Society B: Biological Sciences 273:1093–1098.

Rosenfeld, C. S., and R. M. Roberts. 2004. Maternal diet and other factors affecting offspring sex ratio: a review. Biology of Reproduction 71:1063–1070.

Rubenstein, D. R. 2006. The evolution of the social and mating systems of the plural cooperatively breeding superb starling, *Lamprotornis superbus*. PhD diss. Cornell University, Ithaca, NY.

———. 2007. Stress hormones and sociality: integrating social and environmental stressors. Proceedings of the Royal Society B: Biological Sciences 274:967–975.

———. Forthcoming *a*. Female extrapair mate choice in a cooperative breeder: trading sex for help and increasing offspring heterozygosity. Proceedings of the Royal Society B: Biological Sciences.

———. Forthcoming *b*. Territory quality drives intraspecific patterns of extrapair paternity. Behavioral Ecology.

Rutkowska, J., and M. Cichon. 2002. Maternal investment during egg laying and offspring sex: an experimental study of zebra finches. Animal Behaviour 64:817–822.

Stacey, P. B., and J. D. Ligon. 1991. The benefits-of-philopatry hypothesis for the evolution of cooperative breeding: variation in territory quality and group size effects. American Naturalist 137:831–846.

Stauss, M., G. Segelbacher, J. Tomiuk, and L. Bachmann. 2005. Sex ratio of *Parus major* and *P. caeruleus* broods depends on parental condition and habitat quality. Oikos 109:367–373.

Suorsa, P., H. Helle, E. Huhta, A. Jantti, A. Nikula, and H. Hakkarainen. 2003. Forest fragmentation is associated with primary brood sex ratio in the treecreeper (*Certhia familiaris*). Proceedings of the Royal Society B: Biological Sciences 270:2215–2222.

Trivers, R. L., and D. E. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90–92.

Ward, D., D. Saltz, and B. T. Ngairorue. 2004. Spatio-temporal rainfall variation and stock management in arid Namibia. Journal of Range Management 57:130–140.

Weatherhead, P. J., and P. T. Boag. 1997. Genetic estimates of annual and lifetime reproductive success in male red-winged blackbirds. Ecology 78:884–896.

Whittingham, L. A., and P. O. Dunn. 2000. Offspring sex ratios in tree swallows: females in better condition produce more sons. Molecular Ecology 9:1123–1129.

Wiebe, K. L., and G. R. Bortolotti. 1992. Facultative sex ratio manipulation in American kestrels. Behavioral Ecology and Sociobiology 30:379–386.

Wilson, K., and I. C. W. Hardy. 2002. Statistical analysis of sex ratios: an introduction. Pages 48–92 *in* I. C. W. Hardy, ed. Sex ratios: concepts and research methods. Cambridge University Press, Cambridge.

Witter, M. S., J. P. Swaddle, and I. C. Cuthill. 1995. Periodic food availability and strategic regulation of body mass in the European starling, *Sturnus vulgaris*. Functional Ecology 9:568–574.

Zann, R., and D. Runciman. 2003. Primary sex ratios in zebra finches: no evidence for adaptive manipulation in wild and semi-domesticated populations. Behavioral Ecology and Sociobiology 54:294–302.