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Original Article

Food availability and offspring sex in a monogamous seabird: insights from an experimental approach

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Sex allocation theory predicts that parents should favor offspring of the sex that provides the greatest fitness return. Despite growing evidence suggesting that vertebrates are able to overcome the constraint of chromosomal sex determination, the general pattern remains equivocal, indicating a need for experimental investigations. We used an experimental feeding design to study sex allocation during 3 years in black-legged kittiwakes (*Rissa tridactyla*). Intense male–male competition for securing a breeding site is common in this species in which males are heavier and larger than females. Hence, we hypothesized that parents producing fledglings in better than average condition, as supplementarily fed pairs do, would increase their fitness return by producing sons. Conversely, producing daughters would be a better tactic for Unfed parents. Hence, we predicted that Fed parents produce more sons than Unfed parents. This prediction is particularly expected if sexual dimorphism arises as early as during chick rearing, suggesting strong selective pressures for optimal male development. Our results showed that 1) males were heavier and larger than females prior to fledging and that 2) Fed parents produced relatively more male hatchlings than Unfed parents. We interpret this result in terms of a Trivers–Willard-type process. Furthermore, our data revealed that Unfed parents significantly overproduced female hatchlings, whereas offspring sex ratio was balanced among Fed parents. Because the 3 reproductive seasons we considered were particularly poor food years, Unfed parents may have overproduced daughters to avoid the apparent higher reproductive costs of raising sons. *Key words:* black-legged kittiwake, chick growth, experimental feeding, genetically monogamous, reproductive cost hypothesis, sex allocation, sex ratio, sexual dimorphism, Trivers–Willard hypothesis. *[Behav Ecol]*

INTRODUCTION

Sex allocation is one of the most compelling, yet controversial, theories in evolutionary biology. It refers to the way parents should invest in male and female functions, with an expected bias toward offspring of the sex providing the higher fitness return under current conditions (Frank 1990). As an example, in species where body condition impacts the reproductive success of males more than females and assuming that some females are better able to produce offspring in good condition than others (condition enduring into adulthood), such females are expected to bias their offspring sex ratio toward males (Trivers and Willard 1973). Whereas sex allocation predictions have been confirmed by empirical studies in some taxa such as parasitic Hymenoptera (reviewed in Ode and Hunter 2002), the factors shaping those patterns remain confused for others, such as birds and mammals (Cockburn et al. 2002).

In birds, the availability of molecular sex identification techniques (Griffiths et al. 1998; Fridolfsson and Ellegren 1999) has led to a growing number of studies reporting adaptive sex ratio biases (reviewed in Alonso-Alvarez 2006; Blanchard et al. 2007), suggesting an ability of some birds to overcome the constraint imposed by chromosomal sex determination (West et al. 2002). However, inconsistent results are also commonly reported, including contradictory results within the same species (e.g., Rosivall et al. 2004; Maddox and Weatherhead 2009), lack of sex ratio bias when theoretically expected (e.g., Leech et al. 2001; Cockburn and Double 2008), and counterintuitive biases (e.g., Doutrelant et al. 2004; Dietrich-Bischoff et al. 2006). Hence, as highlighted by West et al. (2002), further investigations are required to improve our understanding of sex ratio patterns. In particular, because most published studies are based on correlational studies and focus on species with polygynous mating systems or with helpers at the nest, in line with classical theoretical

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expectations (Cockburn et al. 2002), there is a clear need for new studies using experimental approaches (Komdeur and Pen 2002) and targeting species with contrasting mating systems.

In this paper, we describe an experiment aimed at investigating sex ratio patterns in a genetically monogamous seabird (Helfenstein, Tirard, et al. 2004), the black-legged kittiwake (*Rissa tridactyla*). Several life-history and behavioral characteristics of this species comprise potential selective pressures that may have led to the evolution of adaptive sex allocation tactics. Males typically arrive at the breeding colonies earlier than females and compete intensely for the best nesting sites (Cullen 1957; Coulson 1968; Wooller and Coulson 1977). The outcome of such competition may impact the onset of reproduction and the length of the rearing period, a key determinant for chick postfledging survival and future reproductive performance (Cam et al. 2003). Accordingly, within a breeding season, males starting their reproduction earlier appear to be heavier and better competitors (Coulson 1968; Wooller and Coulson 1977; Coulson and Thomas 1985). Moreover, nest characteristics affect reproductive success (Coulson 1968; Regehr et al. 1998; Massaro et al. 2001), and male–male competition has been reported to be all the more intense at the most attractive nesting sites (Wooller and Coulson 1977). The male's physical ability to defend the nest may also be important during chick rearing (see Moe et al. 2002; Leclaire et al. 2010), when prospecting behavior (Cadiou and Monnat 1996; Cadiou 1999) and/or predation are common (Massaro et al. 2001), as suggested by the higher nest attendance of males observed during this period (Leclaire et al. 2010). Altogether, given that 1) rearing conditions determine body mass (BM) and structural size at fledging (thereafter referred to as "condition" and considered as proxies of a male ability to obtain/defend a breeding site) (Gill et al. 2002) and given that 2) these characteristics are further expected to have long-term fitness consequences in this species (Cam et al. 2003), these observations suggest that rearing conditions may be more important determinants of male than female subsequent reproductive success (for similar expected fitness return pathway, see also Albrecht and Johnson 2002). Indeed, adult male kittiwakes are heavier and larger than females (BM: 10.2%, tarsus length [TL]: 3.5%; wing length: 2.8%, Helfenstein, Danchin, et al. 2004), with large heavy males likely favored by sexual selection (as expected in other seabird species with terrestrial displays and between males competition; Serrano-Meneses and Szekely 2006).

Pacific populations of kittiwakes have been experiencing chronic breeding failure at many colonies since the late 1970s (Gill and Hatch 2002). In our population, a supplemental feeding experiment has been carried out since 1996 on a sample of birds to determine whether food availability limits productivity (Gill and Hatch 2002). This hypothesis was strongly supported by the difference in breeding parameters between supplemented (provided with ad libitum food through the entire breeding season) and nonsupplemented pairs (Gill and Hatch 2002; Gill et al. 2002), as expected given that the chick-rearing stage is highly food limited (Gill and Hatch 2002). In 1996–1997, annual productivity averaged only 0.48 chicks per nest while it reached 1.15 when birds were given extra food (Gill and Hatch 2002). More specifically, chicks from Fed parents had greater mass gain and wing chord, attained heavier peak mass, and survived better until fledging than chicks from Unfed parents (Gill et al. 2002). Hence, Fed parents are better able to provide offspring with good rearing conditions, and ultimately to produce fledglings in good condition, than Unfed parents in this food-restricted population.

In this theoretical context, we tested 2 predictions. Because natural and sexual selection are expected to favor large and heavy adult males, we first predicted that 1) sexual dimorphism arises during chick rearing, with males being larger and heavier than females close to fledging, as commonly reported in dimorphic seabirds (common tern: Becker and Wink 2003; blue-footed booby: Torres and Drummond 1999; wandering albatross: Weimerskirch et al. 2000). Although several previous studies have analyzed chick growth in kittiwakes (e.g., Coulson and Porter 1985; Suryan et al. 2002; Jodice et al. 2008), none have compared the growth of male and female chicks. Furthermore, in the context of the supplemental feeding experiment that allowed us to increase interpair variability in the ability to provide care (a key determinant of sex allocation in a Trivers–Willard context, e.g., Hewison et al. 2002; Blanchard et al. 2005) and assuming that condition at fledging impacts adult condition (Trivers and Willard 1973), as expected in this species (Cam et al. 2003), we predicted that 2) Fed parents should produce a greater proportion of male chicks than Unfed parents. Indeed, we expected Fed parents to increase their fitness return by producing high quality competitive sons (Gill et al. 2002), whereas daughters would confer a higher fitness gain to Unfed parents, whose sons would probably become poorer competitors (Trivers and Willard 1973; Albrecht and Johnson 2002). This second prediction is all the more expected if prediction (1) is met, that is, if selective pressures for an increase in male size and BM and, more generally, for optimal rearing conditions for males, are stronger.

To test these predictions, we 1) compared the growth parameters of male and female chicks (from nests that were not part of the feeding experiment) from hatching to close to fledging and 2) compared the sex ratios of clutches at hatching between nests where parental ability to invest in their chicks (Gill et al. 2002) was experimentally increased by food supplementing during 3 breeding seasons (from several weeks prior to fertilization until fledging) with those of nonsupplemented nests, using molecular techniques (Fridolfsson and Ellegren 1999) and controlling for laying order.

MATERIALS AND METHODS

Study site and years

The study was carried out from 2006 to 2009 on a population of black-legged kittiwakes (*R. tridactyla*) nesting on an abandoned U.S. Air Force radar tower on Middleton Island (lat 59°26'N, long 146°20'W), Gulf of Alaska. The tower is a 12-walled polygon where artificial nest sites have been created on the upper walls, permitting observations from inside the tower through sliding one-way windows (Gill and Hatch 2002), allowing us to capture and easily monitor the breeders and their chicks. Each year, nests were checked twice daily during the entire breeding season to determine nest content (i.e., egg/chick numbers). Laying date was recorded, and each egg was individually marked (A for the first-laid egg and B for the second egg, 2 being the typical clutch size) with nontoxic waterproof ink within 12 h of laying. Chicks were also marked on the head at hatching with nontoxic marker to identify their hatching rank.

For the growth monitoring, we measured chicks from another part of the colony that were not involved in the experimental setting. We further restricted our analyses to 2008, the only good year regarding environmental conditions and thus providing enough B-chicks that survived until fledging. This allowed us to get a complete understanding of the pattern given that chick rank is known to impact growth parameters in this species (Gill et al. 2002). Nevertheless, similar patterns were found in the other years when focusing on A-chicks only.

For the experiment aimed at investigating the influence of parental supplemental feeding on chick sex, 4 years were initially considered: 2006–2009. However, we excluded the year 2008 from our analyses because only 8 and 10 chicks were sexed from Unfed and Fed pairs, respectively, in that year.

Measuring chick growth

Chicks were measured every 5 days from the day of hatching until they were 35 days old. Although fledging usually occurs at an older age (mean fledging age = 41.4 days in 2008; see also Mulard and Danchin 2008), we did not manipulate chicks after 35 days old to avoid precocious fledging. We weighed chicks to the nearest 0.1 g using an electronic scale and measured TL to the nearest 0.1 mm with a caliper. Chicks were banded and blood sampled at 25 days ($N = 116$; A-chicks: $N = 66$; B-chicks: $N = 50$) from the brachial vein with a syringe and a sterile needle for molecular sexing (see below). Blood samples were kept in Longmire buffer (Longmire et al. 1988).

We used hatching BM, hatching TL, maximum BM and maximum TL (i.e., maximum recorded measurements for a given chick: this usually occurs at 35 days, but possibly at 30 days) as growth parameters. One chick was excluded from the analyses related to TL as it was not measured. We further used well-established growth functions to extrapolate 2 other reliable parameters: maximum growth rate (maximum slope of the growth curve) and asymptotic value for both BM and TL (where the slope of the growth curve reaches zero) (Richards 1959; Ricklefs 1968). Chick growth patterns are best characterized by a sigmoid equation (Richards 1959). Many special cases have been developed to fit growth data, such as the Gompertz, logistic, or Richards equations (Ricklefs 1968). We fitted a growth curve from these 3 growth functions for each individual chick using a nonlinear least squares technique. Then the best fit was assessed based on the Akaike information criterion (AIC) model selection procedure, and growth parameters were extracted from the best model. This procedure has been automated in the *grofit* R library (Kahm et al. 2010). The logistic model provided the best fit overall (for BM: logistic: 54% of the individual chicks considered, Gompertz: 32%, and Richards: 14%; for TL: Richards: 43%, logistic: 41%, and Gompertz: 16%), in agreement with previous studies on kittiwake chick growth (Suryan et al. 2002). Therefore, we chose to force the procedure to fit only the logistic model to allow interindividual comparisons (Weimerskirch et al. 2000). Performing the analysis with the parameters estimated from the best model for each chick led to similar results (not shown).

For some chicks, the slope of the growth curve did not reach zero at 35 days, leading to an overestimation of asymptotic values. For this reason, we excluded 1 and 8 chicks for BM and TL analyses, respectively, because the difference between the estimated asymptotic value and the maximum value was more pronounced than for the other chicks. We based our decision rule on the breaks in the histogram of the differences between estimated asymptotic and maximum measured values plotted for all chicks. Results were similar when analyses were performed on the entire data set (not shown).

Experimental feeding design for sex ratio analysis

As mentioned above, a feeding experiment has been carried out since 1996 on a sample of kittiwakes (Gill and Hatch 2002) divided into 2 treatments: Fed (i.e., the experimental group, all nests on 3 walls) and Unfed (i.e., the control group, all nests on 2 walls). The 2 walls of Unfed birds alternated with the 3 walls of Fed birds so that environmental conditions (e.g., wind, rain, and sun exposure) were similar for both treatments. Moreover, the artificial nesting ledges are the same on the 5 plot walls and thus do not covary with feeding treatment.

Table 1

Sample sizes for each year according to feeding treatment and egg rank

Treatment	Egg rank	2006	2007	2009
Fed	A	31	14	23
	B	30	14	26
Unfed	A	39	9	19
	B	23	7	11
Total		123	44	79

Experimental birds were fed with capelin (*Mallotus villosus*) 3 times a day (at 9:00, 14:00, and 18:00) from inside the tower. During each feeding session, fish were given singly through a plastic tube passing through the wall at each nest site. Feeding continued until the parent(s) present at the nest were satiated.

Our feeding treatment began in early May in 2006 and 2007, in April in 2009 (i.e., at least 20 days before fertilization) and lasted until chicks fledged (or pairs failed to nest, or lost their eggs or chicks). On hatching, eggshells were collected from as many nests as possible, including both Fed and Unfed pairs. The presence of blood vessels in the egg membrane allowed us to sex chicks molecularly in order to estimate the sex ratio. We were only able to sex 1 unhatched egg because usually no blood vessels were apparent in their egg membrane, and/or unhatched eggs were more frequently lost or depredated. Hence, we thereafter consider sex ratio at hatching and not primary sex ratio (i.e., at fertilization). Sample sizes for each year in relation to egg rank and treatment are presented in Table 1.

Chick sexing

Chick sex for growth analysis was determined from blood samples ($N = 116$). Total DNA was extracted from these samples (≈25 µl of blood in conservation buffer) with the DNeasy Blood and Tissue Kit protocol (QIAGEN group) following the supplier's guidelines. We performed 2 elutions with 100 µl of AE Buffer. DNA concentrations ranged between 0.5 and 15 ng/µl. We used the polymerase chain reaction (PCR) to amplify a part of the CHD1 gene including an intron of different size on the W-chromosome and on the Z-chromosome. We used the universal primers MSZ1R (ATCCATCAAGTCTCTAAAGAG; Sehgal et al. 2005) and 2550F (GTTACTGATTCGTCTACGAGA; Fridolfsson and Ellegren 1999). Twenty microliters reaction mixture contained 2–30 ng of genomic DNA, 0.5 units of GoTaq DNA Polymerase (Promega), 4.0 µl of 5X Green GoTaq reaction Buffer (Promega), 200 µM of each dNTP (Promega), and 0.4 µM of each primer. The thermal profile consisted of denaturation at 95 °C for 3 min, followed by 36 cycles of 95 °C denaturation for 30 s, 50 °C annealing for 45 s, and 72 °C elongation for 45 s. Then a final elongation at 72 °C for 5 min was performed. PCR products were visualized on 1% agarose gels stained with ethidium bromide and visualized under UV light. We checked the reliability of the PCR protocol for sex determination on blood samples from adults of known sex (2 males and 2 females). As expected, heterogametic females (ZW) had upper and lower bands (≈650 and ≈450 bp), whereas homogametic males had a single upper band (≈650 bp). When in doubt (i.e., PCR bands not clearly visible), we used a second set of primers (P2 and P8; Griffiths et al. 1998), which are less easy to use (only 10 bp differences between bands), for cross checking. We were able to determine the sex of all chicks from the blood samples.

Chick sex for the feeding experiment was obtained from eggshells following the modified QIAGEN extraction protocol of Bush et al. (2005), with an overnight incubation for years 2007 and 2009. For year 2006, we used a salt extraction protocol following Aljanabi and Martinez (1997). We collected a total of 333 eggshells over the 4 years (including 2008); however, we were unable to sex 80 of them because of low quality material (see Table 1).

Statistical analyses

In both the chick growth and the sex ratio analyses, we had broods comprising 2 chicks with either 1 or 2 sexed chicks. In a first step, for every dependent variable, we began by testing the significance of nest identity as a random effect (i.e., whether within-nest variance was high), focusing only on complete broods (i.e., 2 sexed chicks), using either a simulation-based likelihood ratio test (LRT) (20 000 simulations) with the *RLRsim* package (Scheipl et al. 2008) for growth parameters or a simulation-based bootstrap approach (4000 bootstrap replicates) with the *glmmML* package (Broström 2009) for sex ratio analysis. When the random effect was significant, we restricted our analyses to those nests with a complete brood, including nest identity as a random effect ($N = 54$ for hatching BM and BM maximum growth rate; $N = 52$ for maximum TL and asymptotic TL), because there is obviously no within-nest variance for one-sexed chick broods. However results were similar when we used the whole data set without a random effect (not shown). When the random term was not significant, we used the complete data set, without including a random effect ($N = 116$ for maximum BM; $N = 115$ for asymptotic BM; $N = 115$ for hatching TL and TL maximum growth rate; sex ratio analysis: $N = 253$).

For the growth parameters analysis, we accounted for the effect of laying order as this is known to affect chick growth (Velando et al. 2002). We found a significant random effect of nest identity for hatching BM, maximum BM growth rate, maximum TL, and asymptotic TL. We thus investigated the relationships between chick sex, rank, and their interaction with growth parameters using mixed effects models in the *lme4* package (Bates and Maechler 2010) with a maximum likelihood estimator, with nest identity as a random term. We first fitted the complete model (i.e., with an interaction between chick rank and sex) and then removed each term successively, starting with the interaction. The significance of a term in the model was determined by assessing the change in deviance after removal of that term (LRT), using a chi-square test with the appropriate degrees of freedom (df), as deviance differences are chi-square distributed. For the other parameters (i.e., maximum BM, asymptotic BM, hatching TL, TL maximum growth rate), we ran analysis of variance using the same procedure (with *F*-tests) to assess the effects of sex, rank, and their interaction.

In all analyses, we checked for normality and homoscedasticity of data and residuals. When at least one of the assumptions was not met, we used Box–Cox transformation (Box and Cox 1964) in the *MASS* package (Venables and Ripley 2002). This method was designed to find the value of λ in the function of the observed data with: $f(y) = (y^{-\lambda} - 1)/\lambda$ if $\lambda \neq 0$, that maximizes the likelihood of the function. The resulting λs were: hatching BM: $\lambda = -1.1$, asymptotic BM: $\lambda = 7.2$, and maximum TL growth rate: $\lambda = 0.2$. When the random effect was significant, we also tested for normality of intercepts. We then used the transformed variable with the procedure mentioned above.

For the feeding experiment, we investigated the effect of parental feeding treatment on chick sex at hatching, accounting for year and chick rank. Because nest identity was not significant as a random effect ($P = 0.53$), we investigated the relationship between parental feeding treatment and chick sex (specifying a logit function with 0 = female and 1 = male) with a generalized linear model (GLM), following the same procedure as for growth parameters. As data were not overdispersed, we did not have to control for this (i.e., estimated scale parameter = 1). Hence, we compared the deviance between models with and without a term (LRT) using a chi-square test with the appropriate df (Wilson and Hardy 2002), as differences of deviances are chi-square distributed. As a possible confounding effect could arise from the same walls being Fed and Unfed across years, we tested the wall effect on sex ratio using a GLM model with sex as the binary dependent variable and year and wall as fixed effects for each treatment. There was no wall effect in either treatment (Fed: difference in deviance = 4.89, difference in df = 1, $P = 0.09$; Unfed: difference in deviance = 0.05, difference in df = 1, $P = 0.82$).

Sex ratio deviations were tested against the null expectation of 0.5 using a chi-square goodness-of-fit test. Results are shown with mean ± standard error, and significance is evaluated against a 5% threshold. All tests were run with R 2.12.1 (R Development Core Team 2010).

RESULTS

Chick sexual dimorphism

Hatching BM and TL

There was no effect of sex or of the interaction between sex and rank on BM or on TL at hatching (all $P > 0.11$). However, A-chicks were significantly heavier and larger than B-chicks (BM: A-chicks 39.19 ± 0.88 g, $N = 27$; B-chicks 35.44 ± 0.70 g, $N = 27$, $\chi^2_1 = 24.746$, $P < 0.0001$; TL: A-chicks 19.23 ± 0.15 mm, $N = 66$; B-chicks 18.74 ± 0.17 mm, $N = 49$ $F_{112,113} = 5.0072$, $P = 0.027$).

Chick growth: BM

Maximum growth rate was higher in males, and this was especially true for B-chicks (interaction sex × rank: $\chi^2_1 = 4.99$, $P = 0.025$). Hence, male B-chicks had a higher maximum growth rate than all other types of chicks (21.48 ± 0.81 g/day, $N = 14$ as compared with male A-chicks: 20.26 ± 0.71 g/day, $N = 15$; female A-chicks: 19.15 ± 0.86 g/day, $N = 12$; female B-chicks: 17.28 ± 1.17 g/day, $N = 13$).

Males also reached a significantly higher BM asymptotic value than females (447.74 ± 3.31 g, $N = 54$ as compared with 416.94 ± 3.86 g, $N = 61$, respectively; $F_{112,113} = 32.093$, $P < 0.0001$; Figure 1). Furthermore, maximum measured BM was 7.7% higher in males (450.51 ± 3.35 g) than females (418.39 ± 3.82 g; $F_{112,113} = 39.14$, $P < 0.0001$; Figure 1). There was no effect of rank or of its interaction with sex (all $P > 0.16$) on the asymptotic or maximum BM.

Chick growth: TL

Maximum growth rate tended to differ in relation to chick sex, with male chicks (1.60 ± 0.04 mm/day, $N = 54$) showing a higher tarsus maximum growth rate than female chicks (1.52 ± 0.04 mm/day, $N = 61$; $F_{112,113} = 3.56$, $P = 0.061$). There was no effect of the interaction or of chick rank (all $P > 0.19$).

Males had a significantly higher asymptotic TL than females (respectively, 37.99 ± 0.36 mm, $N = 28$ and 36.56 ± 0.35 mm, $N = 24$; $\chi^2_1 = 5.82$, $P = 0.016$; Figure 2), a difference that averaged 3.9%. There was also a significant effect of rank on asymptotic TL (A-chicks: 37.74 ± 0.39 mm, $N = 26$; B-chicks: 36.93 ± 0.36 mm, $N = 26$; $\chi^2_1 = 7.39$, $P = 0.006$; Figure 2). A-chicks had tarsi

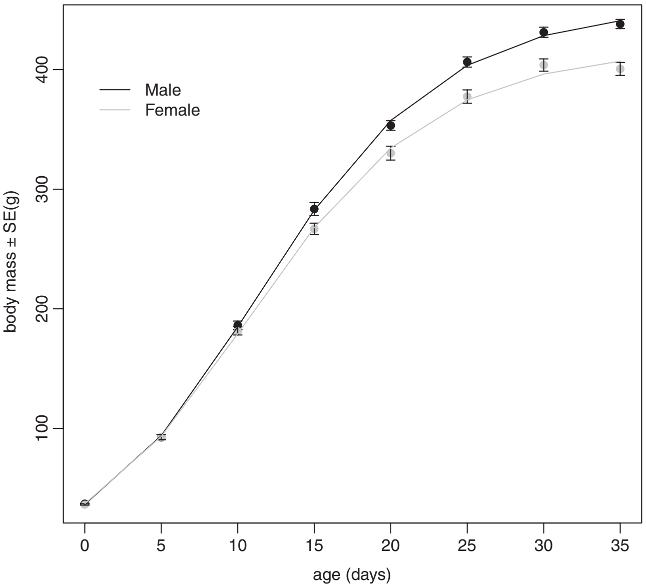

Figure 1
Male and female mass growth curves (logistic model) (males: black curve; females: gray curve) and field data (males: black points; females: gray points) in relation to sex. Males reached a higher mass than females close to fledging. As the random effect of nest identity was not significant, we considered the complete data set (for statistical details, see text).

that were on average 2.2% longer than those of B-chicks. The sex × rank interaction was not significant ($P > 0.4$).

Males had a longer maximum TL than females, but the degree of difference depended on chick rank (sex × rank interaction: $\chi_1^2 = 3.84$, $P = 0.05$): maximum TL did not differ significantly between sexes among A-chicks (males: 37.70 ± 0.43 mm, as compared with females: 37.36 ± 0.50 mm), whereas B-females had significantly lower maximum TL compared with B-males (respectively, 35.62 ± 0.46 vs. 37.83 ± 0.37 mm).

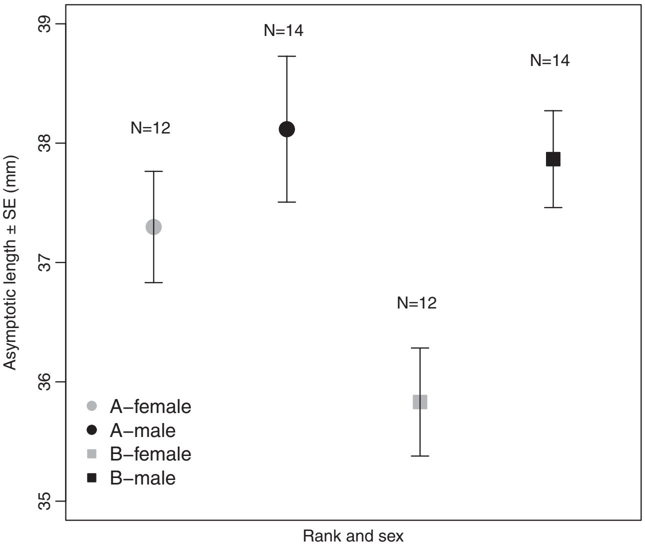

Figure 2
Asymptotic TL in relation to chick rank and sex. Males had longer tarsi than females, and A-chicks had longer tarsi than B-chicks. The interaction between sex and chick rank was not significant. We considered only nests with 2 chicks because nest identity was a significant random factor in the model.

Sex ratio and the feeding experiment

As predicted, chick sex at hatching was significantly related to feeding treatment: the probability of producing a male hatchling was higher for Fed parents than for Unfed parents (difference in deviance = 5.41, difference in df = 1, $P = 0.02$; Figure 3). Egg rank ($P = 0.38$), year ($P = 0.51$), and all interactions (all $P > 0.23$) were not significantly related to chick sex. Overall, 70 of 138 chicks from the Fed parents were males versus 34 of 98 from the Unfed parents. The overall male/female ratio was 1.94 times higher among Fed parents.

Within treatments, chicks born from Fed parents had a balanced sex ratio (all years together: number of males/total number of chicks = 0.507, $\chi_1^2 = 0.03$, $P = 0.86$; all 3 $P > 0.31$ when each year is considered independently), whereas chick sex ratio from Unfed parents differed from parity: they produced significantly more females than males (all years together: number of males/total number of chicks = 0.347, $\chi_1^2 = 9.18$, $P = 0.002$; when each year is considered independently: 2006: $P = 0.069$; 2007: $P = 0.045$; 2009: $P = 0.068$).

DISCUSSION

Sex allocation theory predicts that parents should produce more offspring of the sex with the highest fitness return given their own phenotypic characteristics and their environment (Frank 1990). In birds, a growing number of studies have now found adaptive sex ratio biases (reviewed in Alonso-Alvarez 2006), but general patterns remain equivocal. Here, we used an experimental approach to investigate sex ratio variation in the black-legged kittiwake, a genetically monogamous gull (Helfenstein, Tirard, et al. 2004). In this species, the documented adult sexual dimorphism (Helfenstein, Danchin, et al. 2004) as well as several life-history and behavioral traits led us to expect a stronger effect of BM and structural size on fitness in males than in females. Accordingly, we first expected sexual size dimorphism (with males bigger than females) to arise during rearing. Our results confirmed this first prediction. In particular, male chicks reached a higher maximum and asymptotic BM and TL than females. Following on from

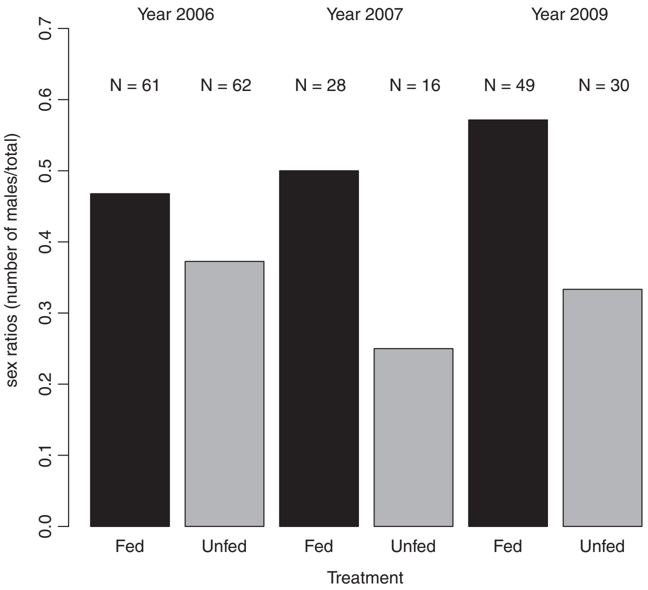

Figure 3
Sex ratios (number of males/total number of chicks) within treatment for each year. Sample sizes are shown above each bar.

these first results, because pairs that are able to produce better than average condition fledglings should increase their fitness return by producing sons (Trivers and Willard 1973), we predicted that experimentally Fed parents, which produce better condition chicks than Unfed parents (Gill and Hatch 2002; Gill et al. 2002), should produce relatively more sons at hatching than Unfed parents. Our data revealed that the probability of producing a male hatchling was significantly higher for Fed parents, controlling for egg rank, thereby supporting our second prediction.

Chick growth and sexual dimorphism: prediction (1)

Our data showed that males were significantly heavier and larger than females close to fledging (for both maximum and asymptotic measures—except for maximum TL for A-chicks). BM increased faster in males than females, especially among B-chicks. This likely buffered the significant effect of rank on BM at hatching as chicks from second laid eggs were on average lighter than first-laid chicks, leading to the observed sex difference in BM close to fledging. Males were on average 7.7% heavier than females close to fledging, which is less than the expected 8.8% sex difference in mass among adults in our study population the same year (our unpublished data), suggesting that sexual dimorphism continues to increase after this stage.

TL, which constitutes a good estimator of overall body size (Rising and Somers 1989), tended to grow faster in males than females. Unlike for BM, the rank effect on TL found at hatching remained significant close to fledging. Thus, we found that A-chicks reached a significantly higher asymptotic TL than B-chicks and that males reached a significantly higher asymptotic TL than females. This rank-related dimorphism close to fledging could potentially affect postfledging survival, which has been shown to be lower in B- than A-chicks (Cam et al. 2003). As for BM, the degree of sexual dimorphism in TL in chicks close to fledging (3.9%, this study) was lower than the one found in adults in the same population and year (5%, our unpublished data).

It therefore seems, as expected, that most of the sexual dimorphism observed among adults develops during the chick-rearing phase in kittiwakes. Our results are in line with previous studies on dimorphic species where sexual dimorphism (in BM and TL) has been shown to develop during the nest stage in a wide array of species, independently of which sex is bigger (for a review, see Richner 1991).

Our results suggest that selective pressures favor larger size in kittiwake males, probably as a result of male–male competition. Hence, assuming that large males do comparatively well in terms of reproductive success, parents that are able to increase their investment in males by providing them with more (Anderson et al. 1993) or higher quality food (Magrath et al. 2004) should be favored. Alternatively, and nonexclusively, an advantage may be conferred on male chicks that increase their begging rate (Kitaysky et al. 2001). However, chick dimorphism may not fully reflect the differences in food received (Anderson et al. 1993; McDonald et al. 2005). Chick dimorphism may even occur without detectable sex-biased provisioning (e.g., Torres and Drummond 1999). In kittiwakes, however, as chick growth rate has been previously shown to be impacted by meal delivery rate (Jodice et al. 2008), it seems reasonable to expect males to receive more food than females.

Feeding treatment and chick sex

Fed parents produce more male hatchlings than Unfed parents: prediction (2)

Whatever the underlying mechanism leading to chick sexual dimorphism, parents producing higher than average quality chicks, as Fed pairs do (Gill et al. 2002), are expected to increase their fitness return by producing more sons. Accordingly, we found that the probability of producing a male hatchling was significantly higher in experimentally fed than in Unfed pairs regardless of year and rank, thereby confirming our second prediction. As chick survival from hatching to the age of 35 days was not related to sex, this pattern holds for chicks close to fledging (our unpublished data).

Experimental feeding has already been used to study sex ratio variation in previous studies and notably in an experiment on a closely related species, the lesser black-backed gull (*Larus fuscus*) (Nager et al. 1999). In this species, the survival prospect of male offspring hatching from less well-provisioned eggs, such as those laid in compensatory clutches, is lower than for females. Accordingly, the authors reported that unfed females, but not experimentally fed females, adaptively overproduced daughters with increasing overall egg production, induced by egg removal.

The next step toward confirmation of the adaptive value of the pattern revealed by our second prediction would clearly be a long-term study to assess the reproductive output of individuals born from both Fed and Unfed parents in relation to sex. If our understanding of the underlying selective pressures is correct, we predict that males born to Fed parents should show on average a substantially higher reproductive output than males born to Unfed parents, whereas such a difference should be much less marked in the case of females.

Within treatment: Unfed pairs produce more female than male hatchlings

Our second and main prediction dealt with the relative difference between feeding treatments in the probability of producing a male, in line with models predicting a role of relative parental rearing capacities in shaping sex allocation patterns (Trivers and Willard 1973; Frank 1990; see also Hewison et al. 2002; Blanchard et al. 2005). However, in addition to these Trivers–Willard-type selective pressures, the magnitude of the sex ratio deviation within Unfed pairs we report, with a substantial bias toward females, is intriguing in itself.

Because hatching success was quite low for Unfed parents (2006: 66%; 2007: 50%; 2009: 46%), we cannot rule out the possibility that the bias toward females in hatchlings sex ratio in that group was the result of higher embryo mortality for males. When males are the larger sex, their greater sensitivity, exacerbated by poor environmental conditions, is classically attributed to their higher nutritional requirements (Clutton-Brock et al. 1985). Here, however, we did not find any sexual dimorphism at hatching. Although size-independent traits may also lead to an increased sensitivity in males (Fairbairn et al. 2007), this explanation seems unlikely as our data reveal comparable mortality rates for both sexes during rearing (females: 75%, males: 70%).

Alternatively, given that from an energetic costs point of view, "the earlier the adjustment, the better" (Trivers and Willard 1973), the bias toward females within Unfed pairs may instead have been generated at fertilization. Unbalanced sex ratios in poor years (or for poor quality parents) have been explained as the result of the differential costs of producing one sex or the other, with individual parents biasing their primary sex ratio toward the cheaper sex to diminish reproductive costs that may impact their own survival and/or subsequent reproduction (i.e., "the reproductive cost hypothesis" sensu Myers 1978; Wiebe and Bortolotti 1992). Sex ratio biases toward the less costly sex have been reported to correlate with poor environmental conditions (Weatherhead 2009). The Trivers–Willard and the reproductive cost hypotheses are not mutually exclusive. The former, as originally stated, focuses on expected offspring reproductive success in relation to parental capacity to invest (and may explain the relative difference between treatments, as suggested above in accordance with our second, main, prediction), whereas the latter focuses on the parental residual reproductive value (and may explain the strong bias toward females among Unfed parents). The 3 years included in our sex ratio analyses were clearly poor years in terms of environmental conditions. As proxies of environmental conditions, we calculated fledging success and B-chick survival, and both were much lower during these 3 years than in 2008 (not included in the present analyses because too few chicks were sexed, see Materials and Methods): fledging success: 2006: 43%; 2007: 66%; 2008: 81%; 2009: 29%; B-chick survival: 2006: 13%; 2007: 38%; 2008: 87%; 2009: 44%. If Unfed parents avoid producing males during poor years to limit reproductive costs, the skew toward females in sex ratio at hatching should be reduced during good years. Accordingly, although the sample size in 2008 is too low to allow any firm conclusions, the sex ratio at hatching was balanced in that year (4 males, 4 females). Further studies are needed to confirm that prediction and to investigate the cost to parents of producing offspring of either sex in relation to their ability to provide care as well as annual variation in food availability.

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