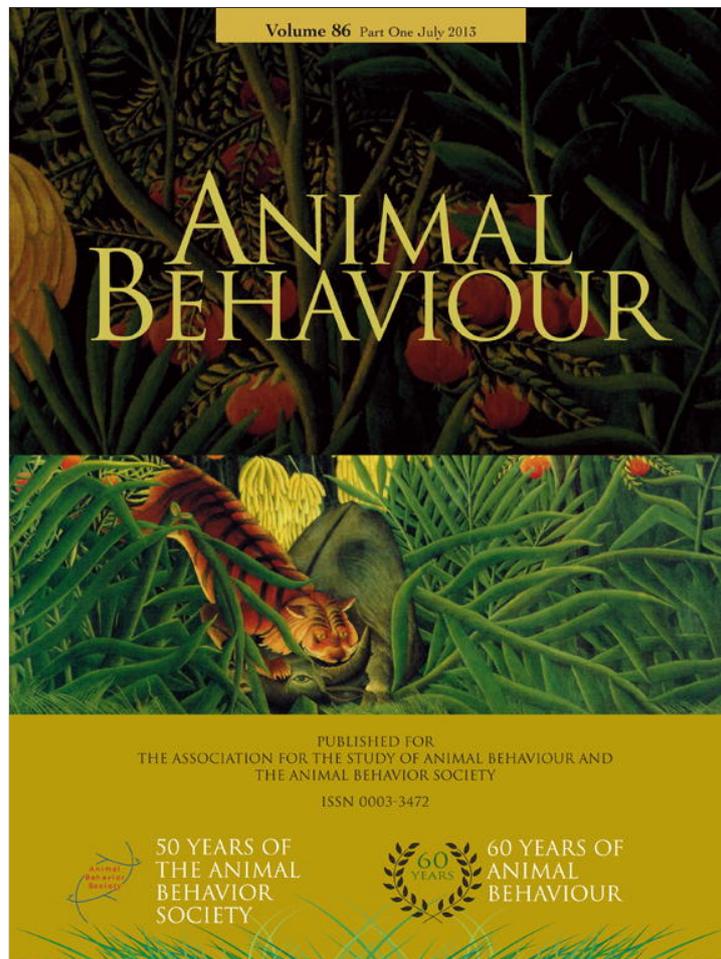


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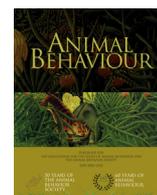
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Variation in female mate choice and mating success is affected by sex ratio experienced during early life



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Females vary in their mate choice and consequent fitness outcomes. Individual differences may be explained by conditions experienced early in life. We tested whether the sex ratio at which young pheasants, *Phasianus colchicus*, were reared affected their adult sexual behaviour. Females reared in equal sex ratios discriminated strongly between males of differing attractiveness in choice tests and had the lowest variance in mating success. Conversely, females reared in female-biased sex ratios showed little discrimination between males based on their attractiveness, and exhibited highly skewed mating success with the majority gaining no copulations, but a quarter each gaining more copulations than any other female in the study. Early life environmental determination of variation in female choice could explain the lack of uniformity in mate choice and hence maintain variation in male traits in the face of directional sexual selection.

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Individual females typically differ from one another in their mating preferences (Jennions & Petrie 1997). Differences in their preferences may be driven by external factors that exert their effects early in life, with consequences only seen as individuals become sexually mature (Lindström 1999). Components of an individual's early social environment affect mate preference functions. These can include the presence of adults of one sex (Adkins-Regan & Krakauer 2000), tickling and play (Paredes-Ramos et al. 2011, 2012) and being reared in groups of single-sex young (Mansukhani et al. 1996).

One component of the early social environment likely to affect adult sexual behaviour is the sex ratio at rearing. This may act directly by affecting the process of sexual imprinting (ten Cate & Vos 1999). In extreme cases, individuals reared in single-sex groups switch their sexual preferences to exhibit homosexuality (Field & Waite 2004). Alternatively, or in addition, the sex ratio early in life may act indirectly on mate preference, modulating the individual's hormone profiles by altering the levels of intrasexual and intersexual competition that an individual experiences (Dhondt 1970; Oddie 2000) leading to differential hormonal levels

being experienced during development (Hirschenhauser & Oliveira 2006). Steroid hormone levels early in development affect development of partner choice in mammals (Henley et al. 2011) and birds (Burley & Foster 2004).

Pheasants, *Phasianus colchicus*, operate a polygynous, nonresource-based mating system in which males defend a harem of females. Females appear to make free choices among males, probably based on male morphology (Göransson et al. 1990; Mateos & Carranza 1995), courtship behaviours (Mateos & Carranza 1999) or complementary MHC (Baratti et al. 2012). Despite the opportunity for free choice, and males being able to hold a harem of five or more females, the distribution of females between males reveals that few individual males have especially large harems; instead, many have just one or two females (Ridley & Hill 1987). Clutches comprise an average of 13 eggs, but occasionally include over 25 eggs (Hill & Robertson 1988). Pheasant broods typically have an even sex ratio on hatching (Rodgers 1984), but this can vary (Latham 1947; Dale 1952). Chicks are precocial and remain with their mother for 7–8 weeks (Hill & Robertson 1988). During this period around a third of the brood will die, often from predation, farming machinery or exposure (Riley et al. 1998). Such stochastic events can lead to biases in the sex ratio of the remaining brood. We tested how three different early sex ratios (male biased, female biased and equal) experienced by pheasants in the first 7 weeks of

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life affected their patterns of mate choice and subsequent mating success as adults, 10 months later.

METHODS

Rearing Birds at Different Sex Ratios

Nine hundred 1-day-old pheasant chicks were obtained in June 2010 from a commercial hatchery and were sexed using wattle presence as a cue (Woehler & Gates 1970). Our accuracy of sexing birds, confirmed by comparing their putative sex at 1 day old with their sex determined on release at 7 weeks old when adult plumage was well established and visual sexing uncontroversial, was high: we misclassified 38/888 (4.3%) birds that survived to 7 weeks. All individuals were marked using numbered plastic patagial wing tags (Roxan Ltd, Selkirk, U.K., www.roxan.co.uk). Individuals were allocated randomly to one of three rearing treatments in which they remained for the first 7 weeks: a male-biased sex ratio, with 20 males and 10 females per pen; an equal sex ratio, with 15 males and 15 females per pen; and a female-biased sex ratio, with 10 males and 20 females per pen. Each treatment had 10 replicates in visual, but not auditory isolation from the other replicates. For the first 2 weeks, chicks were confined to an enclosed, heated shed (130 × 130 cm). After 2 weeks they were allowed access to an open grass run (130 × 680 cm), as well as the shed. All treatments were supplied with identical diets (commercial, age-appropriate feeder pellets provided ad libitum in standard plastic feeders, and water supplied ad libitum through standard plastic drinkers) and tended by staff at the game farm who were naïve to the hypotheses being tested. Any birds that died during rearing were replaced by a sex-matched individual drawn from a pool of replacement birds that were reared at approximately equal sex ratio. A total of 75 birds died during rearing. These deaths were spread fairly evenly across treatments (27 male bias, 19 female bias and 29 equal), with nine male bias replicates, eight female bias replicates and 10 equal-sex replicates containing replacement birds. These replacement birds were simply there to maintain the experimental rearing ratios and were individually marked. They were excluded from our subsequent analyses because they had not spent all their early rearing in the experimental treatments. The work was approved by the University of Exeter Psychology Ethics Committee (Approval 2007/015).

Releasing and Recapturing Birds

At 7 weeks, the birds from all of the treatments and replicates were mixed together and placed in a large (ca. 70 × 60 m) open-topped pen in woodland in mid-Devon, U.K. The pen contained feeders and drinkers that provided ad libitum access to food and water, along with natural and artificial shelter. Birds could disperse from or re-enter the pen at will by flying over the fence or using gates in the fence.

Birds were recaptured by the gamekeeper at the end of the shooting season 2011 using large walk-in traps (3 × 3 m and 1.2 m high), baited with grain and containing food, water and natural cover. The traps were visited each day by the gamekeeper. These birds were taken to a commercial game farm for breeding in the forthcoming year.

Thirty of our experimental adult females were captured and used by the gamekeeper for breeding the following season. These comprised 12 hens from the female-biased treatment, and nine from each of the equal and male-biased treatments. At the end of March 2011, females were placed in laying pens measuring 4 × 12 m, which were roofed with netting, had a grass floor and contained perch sites and shelters. Each pen contained six hens

comprising two hens from the female-biased treatment, one hen that had not been in any of our treatments, and either one or two hens from the equal and male-biased treatments; three pens had two hens from the equal treatment and one from the male-biased treatment and three pens had two hens from the male-biased treatment and one from the equal treatment. A cock pheasant was introduced to the pen on 1 April. All pens were supplied throughout the observation period with commercial poultry food ad libitum, as well as water and ground shells to provide a calcium and nutrient source during egg production. All birds were returned to the wild by the gamekeeper at the end of May 2011.

Observing Adult Mating Behaviour

We conducted a series of 12 observation periods on each pen, each lasting 30 min, between 20 April 2011 and 13 May 2011, at the peak of the mating and egg-laying period. One set of observations was collected from each pen on the same day, between 0830 and 1600 hours with the times and order in which pens were observed being randomized between days. During these observation periods, we recorded a total of 49 copulations with our treatment females. These were highly conspicuous, typified by the cock pheasant performing a display and then jumping on the hen's back, and beating his wings vigorously as he copulated, followed by his descent from the female to crow and in some cases attacking the female (pecking at her) as she stood shaking her wings. Females could escape this male harassment by moving to other areas of the pen. We did not observe any injuries arising from these interactions.

Measuring Mate Choice by Adult Hens

We exposed six hens from each treatment group to binary mate choice tests on 15 April 2011. Each hen was placed in a central choice chamber (400 × 150 cm). At either end was a male, separated from the female by plastic mesh, so that she could see both males simultaneously. The choice chamber was divided, by external markings on the frame, into three equal-sized areas, with one corresponding to each male and the central area reflecting a zone of indecision. The males had not encountered the test females in the previous 4 months, although as they had been caught from the same shooting estate we cannot be certain that they had not encountered the females previously. Each hen was given 5 min to acclimatize and then observed for a further 5 min; the time that she spent in each of the three areas was recorded. We used three different pairs of males, with two females from each treatment being tested with each pair.

Within each pair of males, we assigned one as the preferred male and one as the nonpreferred male. Our assignment was based on two methods. Prior to testing, we collected morphometrics from each male and compared them between males. As female choice of pheasants is based on multiple morphometric and behavioural elements (Göransson et al. 1990; Mateos & Carranza 1995, 1999; Baratti et al. 2012) we considered three measures that were simple to collect from live birds and have previously been correlated with mating success: mass, spur length and wattle height. We corrected for body size for the spur length and wattle height by dividing each of these measures by the male's tarsus length. We did not measure tail length as four of six males had broken tails. We designated the preferred male as the one that was larger, had longer spurs and a larger wattle. In one pairing, one male was larger than the other in all three measures, while in the other two pairs, one male was larger than the other in two of the three measures. Following testing, we compared the total amounts of time across all females that were spent next to each male during the test and designated as

preferred the male in each pair next to which females across all treatments spent the longest time. In all three pairs, males designated as preferred based on their morphometrics were also those designated as preferred based on total time that females spent next to them. Therefore, we are confident that our assignment of preferred and nonpreferred males is meaningful.

We had previously tested (11 April 2011) whether this test paradigm accurately represented choice behaviour by hens. Adult female pheasants associate preferentially with other females during the breeding season, probably to reduce harassment by males (Hill & Robertson 1988). We ran a similar set of experiments with the same 18 hens in which we recorded their behaviour when offered the choice of associating with an unknown male or female. As expected, hens spent more time next to the unknown female than the male (sex: $F_{1,18} = 5.04$, $P = 0.04$). Hens from different treatments did not vary in the total time they spent next to males or females (treatment: $F_{2,18} = 0.94$, $P = 0.41$) and there was no indication that hens from different treatments chose between males or females differently (sex * treatment: $F_{2,18} = 0.67$, $P = 0.53$).

Statistical Analysis

For analyses of female preferences in choice tests we used repeated measure ANOVAs to test whether females spent more time with one alternative (male versus female or preferred male versus nonpreferred male) than the other. These two alternatives were exclusive, but not exhaustive, with the female being able to spend time in the neutral central third of the test chamber. Tests did not violate Mauchly's tests of sphericity. For analyses of copulation events, we used nonparametric statistics owing to the small number of events observed and strong skew in the data. We used a chi-square test to ask whether the three treatments differed in the distribution of copulations among females. Owing to the small number of copulations observed (30) and their broad distribution, we pooled our data into categories of 0, 1–3 and >3 copulations. We used a Kruskal–Wallis test to ask whether the median number of copulations per female differed between treatments.

RESULTS

As expected, across treatments, females spent more time next to the preferred male than the nonpreferred male (repeated measures ANOVA: preference: $F_{1,15} = 7.94$, $P = 0.013$), and the total time that a female spent next to any male did not differ across treatments (treatment: $F_{2,15} = 1.50$, $P = 0.25$). However, the time that a female spent next to either a preferred or nonpreferred male varied according to her early rearing environment (preference * treatment: $F_{2,15} = 5.51$, $P = 0.016$; Fig. 1). Females reared at equal sex ratios and in a male-biased sex ratio spent longer next to generally preferred males whereas females from female-biased treatments tended to spend more time with nonpreferred males.

The distribution of copulations among females varied according to rearing treatment ($\chi^2_2 = 14.16$, $P = 0.007$; Fig. 2), with copulation rates being fairly evenly distributed across females reared in male-biased or equal sex ratios, but having a highly skewed distribution among females reared in female-biased ratios. Most females reared in the female-biased environment (7/12) received no copulations, but three of the 12 females received five or more copulations. In contrast, only one or two of the nine females reared under male-biased or equal sex ratios gained no copulations, and no female from these treatments gained more than three copulations. Although the distribution of copulations between females differed across treatments, the number of copulations that females from each treatment received as adults did not differ (Kruskal–Wallis test: $H_2 = 2.18$, $P = 0.34$).

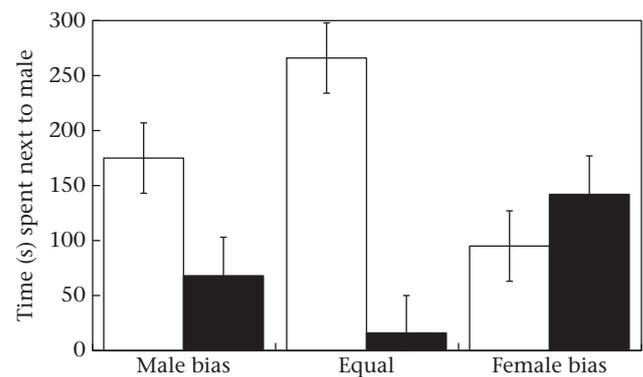


Figure 1. Mean time spent by female pheasants reared at three different sex ratios next to preferred (white bars) and nonpreferred (black bars) males during a 5 min choice test. Error bars indicate 1 SE.

DISCUSSION

The sex ratio at which female pheasants were reared for the first 7 weeks of life had long-term consequences for their adult sexual behaviour and hence their adult mating success and subsequent fitness. We detected two, interrelated, processes by which this could happen. First, females reared in equal sex ratio replicates discriminated more strongly between preferred and nonpreferred males, spending about 13 times as long next to the preferred male as the nonpreferred male. Females reared in male-biased replicates spent about 2.2 times as long next to the preferred male as the nonpreferred male. In contrast, females reared in female-biased sex ratios spent about 1.5 times longer next to the nonpreferred male than the preferred male. This may result in their choosing suboptimal males as mates in the wild. Second, females specifically reared in female-biased sex ratios experienced extremely skewed mating success when compared to other females, with the majority gaining no copulations, but a quarter gaining more copulations than all other females in the study. These differences in copulation rates may be a consequence of the distorted female preferences seen in our choice tests. Females from female-biased ratios spent more time with nonpreferred males than preferred males, and longer with nonpreferred males overall than other females. Alternatively, they may have generally failed to solicit copulations, or in a minority of cases solicited less discerningly or at an unusually high rate, owing to their sexual behaviour being confounded by

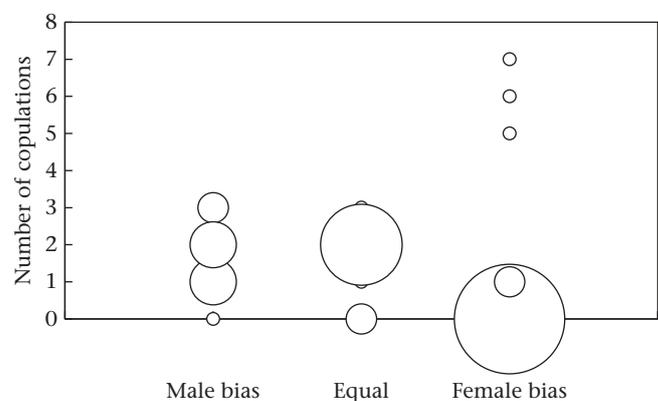


Figure 2. Differences in the numbers of copulations that female pheasants reared at three different sex ratios experienced as adults. The size of the data point indicates the number of females represented by that point.

their early development. Our observations cannot separate these mechanisms.

Why may sex ratios experienced early in life have an impact on adult mate preferences? First, different sex ratios may affect the mechanisms of sexual imprinting or the window during which such imprinting can take place (ten Cate & Vos 1999). Alternatively, skewed sex ratios may influence early life hormonal levels which in turn drive sensory biases towards particular cues (Adkins-Regan 1998). We do not believe that the differences in patterns of mate choice we observed in pheasants were the result of misplaced sexual imprinting during the early rearing period. Although in female-biased replicates there were twice as many females as males, all females had ample opportunity to view a range of conspecific males. In addition, the males that they viewed during this period were in juvenile plumage, lacking many morphological and behavioural features that sexually mature cock pheasants exhibit, which serve as targets of female choice (Göransson et al. 1990; Mateos & Carranza 1995, 1999). Once these features developed in males, the birds were already well mixed after release into the woodland. It is possible that the windows of imprinting for females reared in biased environments were shorter than those reared in equal sex ratios: females from biased environments may have little or no window of overlap with males in mature plumage postrelease whereas the longer windows of females from equal ratios encompass the period during which males acquire their morphological traits.

Variation in patterns of mate choice and the resulting skew in copulation rates determined early in life by nongenetic, stochastic, factors have two broader consequences. First, although there can be clear benefits to the parent in biasing sex ratios (Uller et al. 2007; West 2009), the long-term consequences for offspring may be more complicated. An unusual pattern of mate choice driven by biased sex ratios may, as we report, lead to females courting generally less preferred males and risking very low levels of copulations. Such costs of a skewed sex ratio may offset benefits accrued to the mother through enhanced investment in one sex of offspring. Second, we suspect consequences for sexual selection on male traits in this polygynous, lek-like mating system. Skew in male mating success is likely to be reduced when females reared in female-biased broods choose normally nonpreferred males as partners. This effect of stochastic events early in life affecting the sex ratio at rearing with downstream consequences for female preference and mating success provides a novel and perhaps more generally applicable resolution to the lek paradox.

We have shown how variation in female preferences and skew in female mating success may be established by social factors early in life that are the product of stochastic events. Chance events or strategic allocation can lead to biased sex ratios under natural conditions for pheasants (Riley et al. 1998). Our manipulations of sex ratio (33%/50%/66% female bias) were not extreme. In natural-sized broods of 12 pheasant chicks, with no sex ratio manipulation by the parents, there is a 22% chance that the primary sex ratio will be at least two-thirds biased towards one sex or the other. Because females from the different treatments did not, overall, differ in the numbers of copulations that they received, selection on this response to stochastic factors may be weak. It may also be difficult for a female pheasant to produce equal sex ratio broods reliably, because of difficulties in both her own control of the sex ratio at laying (Rubolini et al. 2007) and her control of sex bias in hatching success and posthatching survival. Therefore, variation in patterns of female preference may be maintained in the population by chance.

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References

- Adkins-Regan, E. 1998. Hormonal mechanisms of mate choice. *American Zoologist*, **38**, 166–178.
- Adkins-Regan, E. & Krakauer, A. 2000. Removal of adult males from the rearing environment increases preference for same-sex partners in the zebra finch. *Animal Behaviour*, **60**, 47–53.
- Baratti, M., Dessi-Fulgheri, F., Ambrosini, R., Bonisoli-Alquati, A., Caprioli, M., Goti, E., Matteo, A., Monnanni, R., Ragionieri, L., Ristori, E., et al. 2012. MHC genotype predicts mate choice in the ring-necked pheasant *Phasianus colchicus*. *Journal of Evolutionary Biology*, **25**, 1531–1542.
- Burley, N. T. & Foster, V. S. 2004. Digit ratio varies with sex, egg order and strength of mate preference in zebra finches. *Proceedings of the Royal Society B*, **271**, 239–244.
- Dale, F. H. 1952. Sex ratios in pheasant research and management. *Journal of Wildlife Management*, **16**, 156–163.
- Dhondt, A. A. 1970. The sex ratio of nestling great tits. *Bird Study*, **17**, 282–286.
- Field, K. L. & Waite, T. A. 2004. Absence of female conspecifics induces homosexual behaviour in male guppies. *Animal Behaviour*, **68**, 1381–1389.
- Göransson, G., von Schantz, T., Fröberg, I., Helgee, A. & Wittzell, H. 1990. Male characteristics, viability and harem size in the pheasant, *Phasianus colchicus*. *Animal Behaviour*, **40**, 89–104.
- Henley, C. L., Nunez, A. A. & Clemens, L. G. 2011. Hormones of choice: the neuroendocrinology of partner preference in animals. *Frontiers in Neuroendocrinology*, **32**, 146–154.
- Hill, D. A. & Robertson, P. A. 1988. *The Pheasant: Ecology, Management and Conservation*. London: Blackwell Scientific.
- Hirschenhauser, K. & Oliveira, R. F. 2006. Social modulation of androgen levels in vertebrates: a meta-analysis of the challenge hypothesis. *Animal Behaviour*, **71**, 265–277.
- Jennions, M. D. & Petrie, M. 1997. Variation in mate choice and mating preferences: a review of the causes and consequences. *Biological Reviews*, **72**, 283–327.
- Latham, R. M. 1947. Differential ability of male and female game birds to withstand starvation and climatic extremes. *Journal of Wildlife Management*, **11**, 139–149.
- Lindström, J. 1999. Early development and fitness in birds and mammals. *Trends in Ecology & Evolution*, **14**, 343–348.
- Mansukhani, V., Adkins-Regan, E. & Yang, S. 1996. Sexual partner preference in female zebra finches: the role of early hormones and social environment. *Hormones and Behavior*, **30**, 506–513.
- Mateos, C. & Carranza, J. 1995. Female choice for morphological features of male ring-necked pheasants. *Animal Behaviour*, **49**, 737–748.
- Mateos, C. & Carranza, J. 1999. Effects of male dominance and courtship display on female choice in the ring-necked pheasant. *Behavioral Ecology and Sociobiology*, **45**, 235–244.
- Oddie, K. R. 2000. Size matters: competition between male and female great tit offspring. *Journal of Animal Ecology*, **69**, 903–912.
- Paredes-Ramos, P., Miquel, M., Manzo, J. & Coria-Avila, G. A. 2011. Juvenile play conditions sexual partner preference in adult female rats. *Physiology and Behavior*, **104**, 1016–1023.
- Paredes-Ramos, P., Miquel, M., Manzo, J., Pfaus, J. G., López-Meraz, M. L. & Coria-Avila, G. A. 2012. Tickling in juvenile but not adult female rats conditions sexual partner preference. *Physiology and Behavior*, **107**, 17–25.
- Ridley, M. W. & Hill, D. A. 1987. Social organisation in the pheasant: harem formation, mate selection and the role of mate guarding. *Journal of Zoology*, **211**, 619–630.
- Riley, T. Z., Clark, W. R., Ewing, D. E. & Vohs, P. A. 1998. Survival of ring-necked pheasant chicks during brood rearing. *Journal of Wildlife Management*, **62**, 36–44.
- Rodgers, R. D. 1984. Ring-necked pheasant. In: *Guidelines for Increasing Wildlife on Farms and Ranches* (Ed. by F. R. Henderson), pp. 89–94. Manhattan: Kansas State University Cooperative Extension Service.
- Rubolini, D., Martinelli, R., von Engelhardt, N., Romano, M., Groothuis, T. G., Fasola, M. & Saino, N. 2007. Consequences of prenatal androgen exposure for the reproductive performance of female pheasants (*Phasianus colchicus*). *Proceedings of the Royal Society B*, **274**, 137–142.
- ten Cate, C. & Vos, D. R. 1999. Sexual imprinting and evolutionary processes in birds: a reassessment. *Advances in the Study of Behavior*, **28**, 1–31.
- Uller, T., Pen, I., Wapstra, E., Beukeboom, L. W. & Komdeur, J. 2007. The evolution of sex ratios and sex-determining systems. *Trends in Ecology & Evolution*, **22**, 292–297.
- West, S. A. 2009. *Sex Allocation*. Princeton, New Jersey: Princeton University Press.
- Woehler, E. E. & Gates, J. M. 1970. An improved method of sexing ring-necked pheasant chicks. *Journal of Wildlife Management*, **34**, 228–231.