

# Adaptive seasonal trend in brood sex ratio: test in two sister species with contrasting breeding systems

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## Abstract

Evolutionary theory predicts adaptive adjustment in offspring sex ratio by females. Seasonal change in sex ratio is one possibility, tested here in two sister species, the Common sandpiper and the Spotted sandpiper *Actitis hypoleucos* and *A. macularia*. In the monogamous Common sandpiper, males are the most competitive sex. In each of 3 years, there was a change from mainly sons in early clutches to mainly daughters in late clutches. This seasonal adjustment of clutch sex ratio took place within the female before the eggs were laid, not by differential egg or chick survival. The sex of all eggs laid in the clutches used here was determined molecularly from chick blood taken at the time of hatching. The Spotted sandpiper in contrast is polyandrous, with partly reversed sex roles. There was no seasonal trend from sons to daughters in this species. When tested together, the two species differed significantly as predicted by the hypothesis of adaptive sex ratio adjustment by females.

## Introduction

Evolutionary theory predicts that females can benefit from adjusting brood sex ratio in relation to ecological or social factors (e.g. Hamilton, 1967; Trivers & Willard, 1973; Charnov, 1982; Frank, 1990). In some taxa there is strong evidence that females adjust the sex ratio, but in others the situation is less clear and much debated (reviewed by Cockburn *et al.*, 2002; West & Sheldon, 2002). There are some convincing examples of adaptive sex ratio control (e.g. Komdeur *et al.*, 1997, 2002; Sheldon *et al.*, 1999; Badyaev *et al.*, 2002), but many other aspects remain debated (reviewed, e.g. by Hardy, 1997; Sheldon, 1998; Cockburn *et al.*, 2002; Hasselquist & Kempenaers, 2002; Komdeur & Pen, 2002).

One such aspect concerns seasonal trends in offspring sex ratios (e.g. Komdeur & Pen, 2002). If early birth is more likely to reduce the age at first breeding in, say, males than in females, then early broods should be biased

towards production of sons (Wright *et al.*, 1995; Daan *et al.*, 1996; Pen *et al.*, 1999). There is evidence from several raptors for seasonal trends in birth sex ratios, which seem to differ depending on body size (reviewed by Daan *et al.*, 1996; Komdeur & Pen, 2002). Among small falcons the proportion of sons decreases over the season (Dijkstra *et al.*, 1990; Tella *et al.*, 1996; Smallwood & Smallwood, 1998; Korpimäki *et al.*, 2000), but in several larger raptors the proportion of daughters decreases (e.g. Olsen & Cockburn, 1991; Zijlstra *et al.*, 1992; Daan *et al.*, 1996). Effects on the age at first breeding have been demonstrated in some species (Dijkstra *et al.*, 1990; Smallwood & Smallwood, 1998; also see Arroyo, 2002). Further tests of the assumptions and predictions of the hypothesis are needed (Pen *et al.*, 1999; Komdeur & Pen, 2002), particularly in taxa other than raptors. The hypothesis has been corroborated in a recent test of a passerine bird (Cordero *et al.*, 2001).

Here we test sex ratio trends in two sister species of tringine waders, the socially monogamous Eurasian Common sandpiper *Actitis hypoleucos* and its polyandrous North American sister species, the Spotted sandpiper *A. macularia*. Sister species are more likely to share

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background traits than less closely related forms, which reduces the risk that unrecognized differences other than those under study will influence the result. Polyandrous shorebirds for several reasons are of particular interest in relation to brood sex ratio adaptation (Hasselquist & Kempenaers, 2002).

In Common sandpipers males are the most competitive sex, advertising the territory by song, and fighting over female or territory with other males (Cramp & Simmons, 1983). Therefore, sons hatching early may have an advantage from a longer growth season, reaching better condition and starting to breed at an earlier age than late sons (see above). Better condition also may be advantageous in reproductive competition for other reasons (e.g. Trivers & Willard, 1973; Dijkstra *et al.*, 1990).

If this assumption is correct, the seasonal trend in offspring sex ratio of the monogamous Common sandpiper should differ in a predictable way from that of its sister species, the Spotted sandpiper. This polyandrous species has partly reversed sex roles, females arriving earlier than males in spring, advertising their territories by song, and even fighting vigorously over males (Oring *et al.*, 1997). Spotted sandpipers are therefore predicted to show a reduced or even opposite seasonal trend in offspring sex ratio compared with the Common sandpiper, a prediction that is tested here.

## Methods

### Study populations and data collection

Common sandpipers were studied during 1998–2000 along 4 km of the river Sävån, 30 km NE of Gothenburg, Sweden (55°47' N, 12°20' E), where about 10 pairs were distributed along the river each year. Some of the pairs nested on islands, others on the wooded banks 5–50 m from the shore. The study area and population will be described in more detail elsewhere. Most breeding adults were caught in mistnets or nest traps, blood-sampled and individually colour-ringed. Chicks were caught by hand and blood-sampled in or near the nest on the day of hatching.

In all, 40 adults and 65 offspring were sexed using molecular methods at the lab in Göteborg. Sixty of the offspring were chicks, five were embryos, four of which came from a clutch abandoned near the time of hatching. We collected 25–50 µL of blood from adults and 15–25 µL from chicks in capillary tubes after puncturing the brachial vein (adults) or the meta-tarsal vein (chicks). Samples were suspended in Queen's lysis buffer (Seutin *et al.*, 1991). Nuclear DNA was extracted from blood using proteinase K and phenol-chloroform-isoamylalcohol. For molecular sexing we used primers 3007 and 3112, following the protocol of Ellegren & Fridolfsson (1997) for the PCR profile, but we used an annealing temperature of 46 °C instead of 55 °C. The amplified product was loaded on 2% agarose gels in 1× TBE buffer

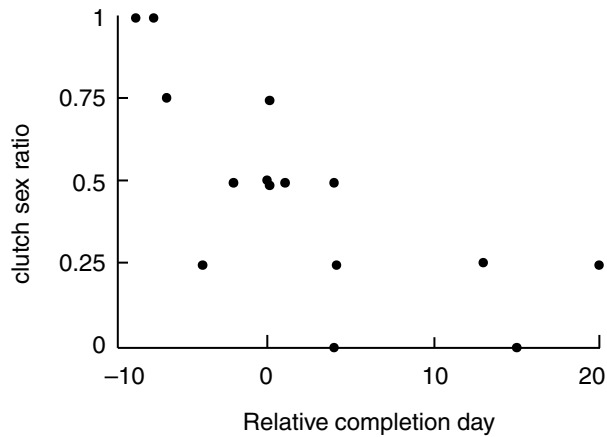
and electrophoresed for approximately 100 min at 4.9 V cm<sup>-1</sup>. After staining males were consistently represented by one band at about 340 bp and females by two bands at about 340 and 360 bp. In addition, sexing was double-checked in six adults by also using primers P2/P8 (Griffiths *et al.*, 1998) in a study of parentage (Blomqvist *et al.*, 2002), and in further five adults by observing their roles in copulation. There was no evidence suggesting incorrect sex determination.

Spotted sandpipers were studied during 1975–91 on Little Pelican Island (1.6 ha), Leech Lake, Minnesota, USA (47° 07' N, 97° 22' W) where six to 20 females bred annually (Oring *et al.*, 1991a, b). All breeding adults were mist-netted and colour-ringed. DNA was derived from chicks in 1990 and from incubator-maintained eggs in 1991. DNA was extracted as described in Oring *et al.* (1992). In all, 129 chicks or embryos were molecularly sexed at the Smithsonian Institution by amplifying the sex-linked CHD gene. For 91 offspring, we used primers P2 and P3, following the protocol in Griffiths *et al.* (1996); for 40 offspring we used primers P2 and P8 following Griffiths *et al.* (1998). Products amplified with P2 and P3 were digested with restriction enzyme *Hae III* prior to running on a 2% agarose gel. Females could be identified by a larger, undigested fragment (W-homologue) and two smaller, digested fragments from the autosomal copy (three bands in all). Males had only the smaller two fragments (two bands). The P2/P8 products were run on an ABI 377 DNA sequencer with ROX internal size standard (Applied Biosystems Inc., Foster City, CA, USA). In this case, males had a single, autosomal band whereas females had two (autosomal and W). For each method, up to 15 known-sex parents were included as positive controls. In all cases, sex was identified correctly, with either or both methods.

### Statistical analysis

The sample from the Common sandpiper is small, and that from the Spotted sandpiper includes repeated sampling of some females, with different numbers of eggs sexed (see Appendix). To efficiently extract the full information without committing pseudoreplication we developed a Monte Carlo randomization test (e.g. Davison & Hinkley, 1997; Manly, 1997), which is suitable in the present case (S. Holm, personal communication). (An alternative approach is generalized linear mixed models, e.g. Wilson & Hardy, 2002). The test is applied first to the Common sandpiper, then to the Spotted sandpiper, and finally the difference between the two species is tested.

There are 15 different females in the data set for the Common sandpiper, each contributing a single clutch, from either of the 3 years. In addition to its size, the day of completion is given for each clutch (Appendix A). In Fig. 1, the day is given relative to the median day of clutch completion for all 3 years combined (164), which is set to 0 in the figure, splitting the data set into two parts



**Fig. 1** Common sandpiper clutch sex ratio (proportion sons) in relation to clutch completion day, relative to the median clutch completion day (164), which is set to 0 in the figure (the point at 0/0.5 represents two observations; see Appendix A).

for statistical analysis. A measure of seasonal change in brood sex ratio is the ratio  $EL$  between the early proportion of sons,  $CE$  (clutches completed before the median day), and the late proportion of sons,  $CL$  (clutches completed after the median day);  $EL = CE/CL$ . This ratio is used as a test statistic to measure the discrepancy between the empirically observed data and the null hypothesis of no seasonal change, which predicts that  $EL$  will take a value of about 1. The observed early proportion of sons  $CE$  is calculated as the sum of all sons from early clutches (and correspondingly for the late proportion,  $CL$ ). For the Common sandpiper the empirically observed early-to-late ratio is  $EL = 2.616$ .

Under the null hypothesis of no seasonal sex ratio trend all clutches represent the same statistical population.  $H_0$  can therefore be tested against the empirically observed ratio by randomly drawing half the number of clutches to form an early sample, then drawing the remaining clutches to form a late sample. From these samples a new, simulated value of the ratio  $EL$  is calculated. Repeating this process  $R$  times and counting the number of occasions,  $k$ , on which the simulated value equals or exceeds the empirically observed one, the Monte Carlo  $P$ -value is obtained as  $P = (k + 1)/(R + 1)$  (e.g. Davison & Hinkley, 1997). If  $P$  is sufficiently small, the null hypothesis is rejected in favour of the alternative hypothesis, which here predicts that the proportion of sons decreases over the season. All tests here are two-tailed and use  $R = 100\,000$  simulations. Two-tailed probabilities are calculated by counting also the runs in which the simulated value exceeds the empirically observed one, but in the direction opposite to that predicted by the alternative hypothesis.

To obtain the best estimate of mid-season location, the median date of clutch completion for the Spotted sandpiper (162) includes some nests not sampled for DNA analysis. Most females are represented by several clutches (Appendix B), which are all likely to be influenced by her individual characteristics. Females, not clutches, are therefore treated as independent sample units, and in each of the  $R$  Monte Carlo simulations one of the clutches from each female is randomly drawn and included. In calculating the empirically observed sex ratio trend  $EL = 0.8307$ , all clutches of a female are included, but depending on their numbers they are discounted by a weight factor, standardizing the influence of each female so that it corresponds to one clutch. Thereafter, the Monte Carlo  $P$ -value is calculated under the null hypothesis that all clutches represent the same statistical population.

Testing for a difference in seasonal sex ratio trend between Common and Spotted sandpiper, a suitable test statistic is the ratio  $CS$  between the trends of the two species.  $ELC$  is the ratio between early and late proportions of sons for the Common sandpiper, and  $ELS$  is so for the Spotted sandpiper. Using the observed values above, the empirically observed ratio between the two species is  $CS = ELC/ELS = 2.616/0.8307 = 3.15$ . The null hypothesis is that all clutches from both species represent the same statistical population as regards sex ratio trend. The data sets from Common and Spotted sandpiper under the null hypothesis are therefore merged into a common pool, from which clutches are sampled randomly in the simulations. The appropriate numbers of clutches are allocated to each of the two species and periods, and the Monte Carlo  $P$ -value is calculated in analogy with the previous tests.

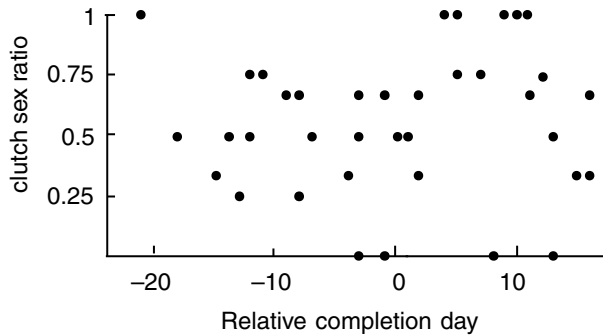
We wrote and ran the simulation programs in Resampling Stats (Bruce *et al.*, 1997).

## Results

### Common sandpiper

There is a strong seasonal trend in offspring sex ratio at the time of hatching in the Common sandpiper (Table 1, Fig. 1), expressed in the empirically observed ratio  $EL = 2.616$  between the early and late proportions of sons. The Monte Carlo test of the null hypothesis of no seasonal trend (see Methods) gives  $P < 0.009$ .  $H_0$  is therefore refuted, corroborating the alternative that offspring sex ratio (proportion of sons) at the time of hatching decreases from the early to the late half of the laying season.

Moreover, the change has the same direction in each of the 3 years studied (data in Table 1; also see Methods), the ratio  $EL$  being  $>1$  for each year: 1.09, 5.25 and 5.0, respectively. Therefore, the significant result of the 3 years tested together is not an outcome of an unusual



**Fig. 2** Spotted sandpiper clutch sex ratio (proportion sons) in relation to clutch completion day, relative to the median clutch completion day in the population (162), which is set to 0 in the figure. (the point at 1/0.5 represents three observations, and those at -14/0.5, 4/1 and 13/0.5 represent two observations; see Appendix B).

result in one of the years; the trend has the same direction in all 3 years.

We tested whether brood sex ratio was correlated with female size (wing length or head + bill length), but there was little indication of such a relationship (wing length: Spearman  $r_s = -0.03$ , two-tailed n.s.,  $n = 14$ ; head + bill:  $r_s = 0.12$ , two-tailed n.s.,  $n = 15$ ). Our data do not permit meaningful estimation of condition, as individual body mass varies greatly over the breeding period and needs to be measured at a standardized time, which was not possible.

### Spotted sandpiper

In the Spotted sandpiper (Fig. 2) the empirically observed ratio  $EL = 0.83$  between the early and late proportions of sons in the Monte Carlo test gives  $P = 0.40$ . The weak trend towards more sons is therefore far from statistically significant.

### Comparison between Common and Spotted sandpiper

Testing the difference in sex ratio trends between the two species as described in the Methods section results in  $P = 0.0088$ . The null hypothesis of the same sex ratio trend in the two species is therefore refuted: the sex ratio change from early to late half of the season differed between Common and Spotted sandpiper. In the Common sandpiper there was a significant change from mainly sons to mainly daughters produced in the early and late part, respectively (Fig. 1). In the Spotted sandpiper there was no significant change in sex ratio (see above and Fig. 2).

### Discussion

Common and Spotted sandpipers differed in seasonal sex ratio trends among offspring, in the direction predicted if

females adaptively adjusted the sex of the eggs produced (see Introduction). The trend in the Spotted sandpiper was, however, not clearly reversed compared with the Common sandpiper: the weak trend was far from statistically significant. It is uncertain how much the Spotted sandpiper should be expected to differ in seasonal sex ratio trend from its sister species. Although Spotted sandpipers show reversed sex differences in several ways (see Introduction), they do not do so in all respects. For example, whereas Common sandpipers have male-biased philopatry (Holland & Yalden, 1991), in Spotted sandpiper there is little or no sex bias in philopatry (Reed & Oring, 1993). Moreover, in the Spotted sandpiper not only female but also male competition may be intense (Oring *et al.*, 1997). For these reasons it is not certain that daughters will benefit more than sons from being born early in this species. It is clear, however, that female competition is much stronger than in the Common sandpiper, and that the Spotted sandpiper therefore should show a reduced or opposite seasonal trend in offspring sex ratio compared with its sister species.

The maturation time hypothesis (Daan *et al.*, 1996) is not the only possible adaptive explanation for seasonal trends in offspring sex ratio. For example, in species where mainly males compete over mates, the most successful males will obtain mates first. This should apply whether mating competition takes the form of mate choice or contests over mates or territories (Andersson, 1994). To the extent that traits leading to competitive success are heritable (e.g. Pomiankowski & Møller, 1995; Rowe & Houle, 1996; Merilä & Sheldon, 1999), females mating with successful males should mainly produce sons. Because competitively successful males will also tend to be among the earliest males in the season to obtain a mate and breed, females breeding early in the season should then usually produce male-biased broods (e.g. Sheldon *et al.*, 1999). Females breeding late should produce female-biased broods, as they will usually be mated to males with lower competitive success. In species with partly reversed sex roles, these sex differences may be reduced or even reversed, favouring less male-biased or even female-biased broods early in the season.

Another possible explanation for the observed sex ratio trends is that the females breeding earliest in the season are in the best condition and that such females, for reasons suggested by Trivers & Willard (1973; also see Leimar, 1996), should overproduce the sex that benefits most from being born early.

There are now observations of seasonal sex ratio trends in a number of birds, some of which provide evidence for skewed population sex ratios at the time of hatching (reviewed by Sheldon, 1998; Komdeur & Pen, 2002; Komdeur *et al.*, 2002). The sex ratio in the Common sandpiper is additional evidence, as the sex was determined at the time of hatching for all but five eggs (see Methods) in the clutches used here. The sex ratio skew therefore was present at hatching and did not arise from

egg mortality, so the skew was probably achieved inside the female. How female birds control the sex of offspring before the egg is laid is not known, but there is now evidence for nonrandom sex allocation also in other than the first laid egg (Komdeur & Pen, 2002), demonstrating preovulation control of offspring sex (Komdeur *et al.*, 2002).

Although it is not known how they were achieved by females, the patterns of seasonal offspring sex ratios in two sister species with contrasting mating systems, Common and Spotted sandpiper, differed in the direction predicted by hypotheses of female adaptive adjustment of offspring sex ratios. Future work should test additional assumptions and relationships to clarify the fitness consequences of the patterns found here, for example the reproductive success of offspring in relation to their hatching date.

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### References

Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton.

Arroyo, B.E. 2002. Fledgling sex ratio variation and future reproduction probability in Montagu's harrier, *Circus pygargus*. *Behav. Ecol. Sociobiol.* **52**: 109–116.

Badyaev, A.V., Hill, G.E., Beck, M.L., Dervan, A.A., Duckworth, R.A., McGraw, K.J., Nolan, P.M. & Whittingham, L.A. 2002. Sex-biased hatching order and adaptive population divergence in a passerine bird. *Science* **295**: 316–318.

Blomqvist, D., Andersson, M., Küpper, C., Cuthill, I.C., Kis, J., Lanctot, R.B., Sandercock, B.K., Székely, T., Wallander, J. & Kempenaers, B. 2002. Genetic similarity between mates and extra-pair parentage in three species of waders. *Nature* **419**: 613–615.

Bruce, P., Simon, J. & Oswald, T. 1997. *Resampling Stats User's Guide*. Resampling Stats Inc, Arlington, VA, USA.

Charnov, E.L. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton.

Cockburn, A., Legge, S. & Double, M.C. 2002. Sex ratios in birds and mammals: can the hypotheses be disentangled?. In: *Sex Ratios: Concepts and Research Methods* (I.C.W. Hardy, ed.), pp. 266–286. Cambridge University Press, Cambridge.

Cordero, P.J., Viñuela, J., Aparicio, J.M. & Veiga, J.P. 2001. Seasonal variation in sex ratio and sexual egg dimorphism favouring daughters in first clutches of the spotless starling. *J. Evol. Biol.* **14**: 829–834.

Cramp, S. & Simmons, K.E.L. 1983. *Handbook of the Birds of Europe, the Middle East and North Africa*, Vol. 3. Oxford University Press, Oxford.

Daan, S., Dijkstra, C. & Weissing, F.J. 1996. An evolutionary explanation for seasonal trends in avian sex ratios. *Behav. Ecol.* **7**: 426–430.

Davison, A.C. & Hinkley, D.V. 1997. *Bootstrap Methods and Their Application*. Cambridge University Press, Cambridge.

Dijkstra, C., Daan, S. & Buker, J.B. 1990. Adaptive seasonal variation in the sex ratio of kestrel broods. *Funct. Ecol.* **4**: 143–148.

Ellegren, H. & Fridolfsson, A.-K. 1997. Male-driven evolution of DNA sequences in birds. *Nat. Genet.* **17**: 182–184.

Frank, S.A. 1990. Sex allocation theory for birds and mammals. *Ann. Rev. Ecol. Syst.* **21**: 13–55.

Griffiths, R., Daan, S. & Dijkstra, C. 1996. Sex identification in birds using two CHD genes. *Proc. R. Soc. Lond. B.* **263**: 1251–1256.

Griffiths, R., Double, M., Orr, K. & Dawson, R. 1998. A DNA test to sex most birds. *Mol. Ecol.* **7**: 1071–1075.

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

Hardy, I.C.W. 1997. Possible factors influencing vertebrate sex ratios: and introductory overview. *Appl. Anim. Behav. Sci.* **51**: 217–241.

Hasselquist, D. & Kempenaers, B. 2002. Parental care and adaptive brood sex ratio manipulation in birds. *Phil. Trans. R. Soc. Lond. B* **357**: 363–372.

Holland, P.K. & Yalden, D.W. 1991. Population dynamics of Common sandpipers *Actitis hypoleucos* breeding along an upland river system. *Bird Study* **38**: 151–159.

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

Komdeur, J., Magrath, M.J.L. & Krackow, S. 2002. Pre-ovulation control of hatchling sex ratio in the Seychelles warbler. *Proc. R. Soc. Lond. B* **269**: 1067–1072.

Komdeur, J. & Pen, I. 2002. Adaptive sex allocation in birds: the complexities of linking theory and practice. *Phil. Trans. R. Soc. Lond. B* **357**: 373–380.

Korpimäki, E., May, C.A., Parking, D.T., Wetton, J.H. & Wiehn, J. 2000. Environmental- and parental condition-related variation in sex ratios of kestrel broods. *J. Avian Biol.* **31**: 128–134.

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

Manly, F.J. 1997. *Randomization, Bootstrap and Monte Carlo Methods in Biology*, 2nd edn, Chapman & Hall, London.

Merilä, J. & Sheldon, B.C. 1999. Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. *Heredity* **83**: 103–109.

Olsen, P.D. & Cockburn, A. 1991. Female-biased sex allocation in peregrine falcons and other raptors. *Behav. Ecol. Sociobiol.* **28**: 417–423.

Oring, L.W., Colwell, M.A. & Reed, J.M. 1991b. Lifetime reproductive success in the spotted sandpiper (*Actitis macularia*): sex differences and variance components. *Behav. Ecol. Sociobiol.* **28**: 425–432.

Oring, L.W., Fleischer, R.C., Reed, J.M. & Marsden, K.E. 1992. Cuckoldry through stored sperm in the sequentially polyandrous Spotted Sandpiper. *Nature* **359**: 631–633.

Oring, L.W., Gray, E.M. & Reed, J.M. 1997. Spotted Sandpiper (*Actitis macularia*). In: *Birds of North America*, no. 289. (A. Poole & F. Gill, eds). Academy of Natural Sciences, Philadelphia, PA.

Oring, L.W., Reed, J.M., Colwell, M.A., Lank, D.B. & Maxson, S.J. 1991a. Factors regulating annual mating success and reproductive success in spotted sandpipers (*Actitis macularia*). *Behav. Ecol. Sociobiol.* **28**: 433–442.

Pen, I., Weissing, F.J. & Daan, S. 1999. Seasonal sex ratio trends in the European kestrel: an evolutionarily stable strategy analysis. *Am. Nat.* **153**: 384–397.

Pomiankowski, A. & Møller, A.P. 1995. A resolution of the lek paradox. *Proc. R. Soc. Lond. B* **260**: 21–29.

Reed, J.M. & Oring, L.W. 1993. Philopatry, site fidelity, dispersal, and survival of Spotted Sandpipers. *Auk* **110**: 541–551.

Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B* **263**: 1415–1421.

Seutin, G., White, B.N. & Boag, P.T. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Can. J. Zool.* **69**: 82–90.

Sheldon, B.C. 1998. Recent studies of avian sex ratios. *Heredity* **80**: 397–402.

Sheldon, B.C., Andersson, S., Griffith, S.C., Örnberg, J. & Sendecka, J. 1999. Ultraviolet colour variation influences blue tit sex ratios. *Nature* **402**: 874–877.

Smallwood, P. & Smallwood, J.A. 1998. Seasonal shifts in sex ratios of fledging American kestrels (*Falco sparverius paulus*): the early bid hypothesis. *Evol. Ecol.* **12**: 839–853.

Tella, J.L., Donazar, J.A., Negro, J.J. & Hiraldo, F. 1996. Seasonal and interannual variations in the sex ratio of lesser kestrel *Falco naumanni* broods. *Ibis* **138**: 342–345.

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

West, S.A. & Sheldon, B.C. 2002. Constraints in the evolution of sex ratio adjustment. *Science* **295**: 1685–1688.

Wilson, K. & Hardy, I.C.W. 2002. Statistical analysis of sex ratios: an introduction. In: *Sex Ratios: Concepts and Research Methods* (ed. I. C. W. Hardy), pp. 48–92. Cambridge University Press, Cambridge.

Wright, D.D., Ryser, J.T. & Kiltie, R.A. 1995. First-cohort advantage hypothesis: a new twist on facultative sex ratio adjustment. *Am. Nat.* **145**: 133–145.

Zijlstra, M., Daan, S. & Bruinenberg-Rinsma, J. 1992. Seasonal variation in the sex of marsh harrier *Circus aeruginosus* broods. *Funct. Ecol.* **6**: 553–559.

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**Appendix**

- (a) Common sandpiper broods in relation to clutch completion date.
- (b) Spotted sandpiper broods in relation to clutch completion date\*

Year and female	Day of clutch completion	No. of chicks	No. of sons
(a)			
1998 / 018	156	3	3
1999 / 047	157	4	4
2000 / 610	158	4	3
1998 / 038	160	4	1
2000 / 607	162	4	2
1998 / 341	164	4	2
1998 / 032	164	4	2

**Appendix Continued**

Year and female	Day of clutch completion	No. of chicks	No. of sons
1999 / 388	164	4	3
1998 / 033	165	4	2
1998 / 034	168	4	2
1999 / 452	168	4	1
2000 / 614	168	4	0
2000 / 613	177	4	1
1999 / 457	179	4	0
1999 / -	184	4	1
(b)			
Female and year	Day of clutch completion	No. of chicks	No. of sons
MA:GG91	144	4	2
MA:GG91	163	2	1
MA:GG91	173	4	4
MA:GG90	151	4	3
MA:GG90	173	3	2
MA:GG90	164	3	1
dGO:MA91	155	4	2
dGO:MA91	162	4	2
dGO:MA91	175	4	2
dGO:MA90	159	3	2
dBA:OO91	159	4	2
dBA:OO90	150	4	3
RA:OB91	141	4	4
RA:OB91	148	2	1
RA:OB91	161	1	0
RA:OB91	172	1	1
RA:OB90	167	4	3
BA:BO91	147	3	1
BA:BO91	175	2	1
MY:AY91	148	4	2
MY:AY91	170	3	0
MY:AY91	178	3	1
MY:AY90	166	1	1
RO:AR91	149	4	1
RO:AR91	161	3	2
RO:AR91	171	4	4
AY:YR91	153	3	2
AY:YR91	169	4	3
AY:YR91	177	3	1
AO:RW91	154	4	1
AO:RW91	167	2	2
AO:RW91	178	3	2
AO:RW90	175	2	0
OO:dGA91	158	3	1
OO:dGA91	163	2	1
OO:dGA91	174	4	3
dGA:RY91	164	3	2
RA:dBY90	150	4	2
WR:AdB90	159	2	0
WR:AdB90	163	4	2
AdB:YdB91	166	1	1
WA:RM91	154	3	2

\* There are repeat measures for most Spotted sandpiper females, within and between years. For example, female MA:GG is represented with three clutches in each of 1990 and 1991. In statistical testing, only one clutch is drawn randomly from each female for each run of the Monte Carlo simulation (see Statistical analysis).