

Thomas W. Pike

Sex ratio manipulation in response to maternal condition in pigeons: evidence for pre-ovulatory follicle selection

Received: 5 August 2004 / Revised: 24 January 2005 / Accepted: 8 February 2005 / Published online: 20 April 2005
© Springer-Verlag 2005

Abstract A number of recent reports have documented offspring sex ratio biases in birds. However, to date the potential mechanisms that have been put forward to explain the proximate basis for these deviations are entirely speculative. Using a captive population of domestic pigeons (*Columba livia domestica*), I tested the hypothesis that mothers in relatively poor physical condition should overproduce daughters by manipulating maternal body condition around the time of egg laying by continuous egg removal and differing feeding regimes. During treatment, females were fed a controlled quantity of food. This, combined with the high energetic costs of repeated egg production caused a significant reduction in maternal body weight. In contrast, during control when food was available ad libitum, maternal body weight did not decline, despite repeated egg production. No significant deviation from parity was evident in the sex ratio of either the first or second eggs during control, whereas during treatment a significant female bias was evident in not only the first egg, but also in the second egg. The absence of single-egg clutches, the rarity of infertile eggs and the lack of laying delays between eggs strongly suggests that the mechanism of sex ratio adjustment in pigeons occurs prior to ovulation. The highly skewed sex-distribution within the two-egg clutches and the unexpectedly large amount of variation in the yolk weight of eggs produced during treatment (but not control)

are consistent with the expectations of pre-ovulatory selective resorption of ‘wrong’ sex ovarian follicles.

Keywords Selective resorption · Primary sex ratio · Pre-ovulation manipulation

Introduction

A number of recent studies have suggested that breeding female birds can adjust the primary sex ratio (the sex ratio at laying) of their offspring (e.g. Komdeur et al. 2002) in relation to the social and environmental conditions experienced during breeding (e.g. Komdeur et al. 1997; Sheldon et al. 1999; Badyaev et al. 2002). However, although manipulation of offspring sex appears widespread throughout avian taxa (Pike and Petrie 2003), birds possess no known physiological or genetic mechanisms for skewing the sex ratio at laying (Krackow 1999) and so the processes underlying the observed deviations in sex ratio remain a mystery.

Importantly, in birds, females are the heterogametic sex (producing Z- and W-bearing ova) and this has led to the suggestion that mechanisms of sex ratio adjustment could potentially be under maternal control (Oddie 1998). It has been postulated that deviations from equality in the primary sex ratio could occur through non-random segregation of the sex chromosomes during meiosis (segregation distortion; e.g. Petrie et al. 2001; but see Krackow 1999 for a critique), differential maturation of follicles (yolk sacs bearing ova, which exist in a hierarchy within the ovary) to influence the order in which they are ovulated (asynchronous follicular development; Krackow 1995; see also Ankney 1982), or as a consequence of sex-specific abortion and hence non-random ovulation of Z and W ova within the ovulation sequence (selective resorption; Krackow 1995; see Pike and Petrie 2003 for a review). Because not all ova mature into eggs (Wood-Gush and Gilbert 1970), any predilection of Z or W ova toward developmental failure could be the basis for facultative bias in the sex ratio at ovulation (Krackow 1995). Emlen (1997) further suggested that birds that lay

Communicated by M. Leonard

T. W. Pike (✉)
Evolution and Behaviour Research Group, School of Biology,
University of Newcastle upon Tyne,
Newcastle upon Tyne, NE2 4HH, UK
e-mail: t.pike@bio.gla.ac.uk
Tel.: +44-(0)141-330-2430
Fax: +44-(0)141-330-5971

Present address:

T. W. Pike
Institute of Biomedical and Life Sciences, Division of
Environmental and Evolutionary Biology, University of
Glasgow,
Glasgow, G12 8QQ, UK

clutches of more than one egg could bias the sex of the first laid egg and then let the rest of the clutch be determined randomly by the Mendelian 'luck-of-the-draw'. For species that lay small clutches this could be a very effective strategy (Pike and Petrie 2003), with the effect diminishing as clutch size increases. For instance, in the noisy miner (*Manorina melanocephala*), which lays between one and four eggs, the overall sex ratio was approximately even but 17 out of 18 of the first laid eggs were male (Arnold et al. 2001). However, it is assumed that repeated selective resorption would require skipping a day when an egg could have been laid (because of the distinct maturational hierarchy among developing follicles), resulting in either delayed clutch completion or a smaller clutch. Although post-ovulation (secondary) sex manipulation mechanisms may also act, for example through sex-specific embryo mortality (Pike and Petrie 2003) or infanticide (Kilner 1998; Williams 1999), pre-ovulation control has been favoured by authors because the resources provisioned to an egg would not be wasted (resorption of yolk, on the other hand, is likely to incur only minor costs since much of the invested energy will be reabsorbed) and would therefore be the more efficient mechanism of control. In turn, more efficient mechanisms should broaden the scope for adaptive sex ratio adjustment because the fitness differential between sons and daughters need not be as great to balance the costs of adjustment (Pen and Weissing 2000).

Almost a century ago, Riddle (1917) reported being able to manipulate the offspring sex ratios of domestic pigeons (*Columba livia domestica*) in favour of females by continually removing eggs shortly after laying, although to my knowledge this work has not been repeated in light of recent advances such as the development of molecular sexing techniques (e.g. Griffiths et al. 1998; Fridolfsson and Ellegren 1999). More recently, sex ratio manipulation has also been reported in another Columbiform species, the mourning dove (*Zenaida macroura*; Hanson and Kosack 1963; Edmunds and Ankney 1987; but see MacGregor 1958) as well as in response to changes in maternal condition in a wide variety of other avian species (Bradbury and Blakey 1998; Kilner 1998; Nager et al. 1999; Whittingham and Dunn 2000; Kalmbach et al. 2001; Clout et al. 2002).

In this study, I experimentally manipulated maternal body condition around the time of egg production in a captive population of domestic pigeons in an attempt to induce biases in the sex ratio (following Riddle 1917) which could then be analysed to look for clues as to the underlying mechanism of manipulation. I predicted that, consistent with Riddle (1917), experimentally reducing female body condition via continuous egg removal would induce them to overproduce daughters. I then looked at the sex-composition of clutches (i.e. the number of MM, MF, FM and FF clutches produced), the time between the laying of the first and second eggs, and egg and yolk weight within and between clutches in order to investigate potential mechanisms of sex ratio manipulation. Pigeons were chosen because they provide an ideal species in which to investigate these mechanisms: in particular they lay an almost invari-

able clutch size of two eggs (Levi 1945) approximately 44 h apart (Abs 1983) making it possible to compare differences in egg weight and the timing of oviposition without confounding factors such as variable clutch size or laying sequence gaps. Furthermore, pigeons are known to produce more than the two follicles needed to supply the two eggs which they lay at any one laying attempt (Levi 1945), although the purpose of multiple follicles in a species that almost invariably lays two eggs has not been explained.

Methods

General methods

Pigeons were housed and bred in captivity between August 2002 and June 2003. The lighting pattern was 14 h of light, 10 h of darkness (photophase 0800–2000 hours) and the temperature was $22 \pm 4^\circ\text{C}$. Natural daylight was available during the day, and artificial lighting used in the early mornings and evenings. All birds used in the experiment were sexually experienced and obtained from various sources across the UK, and were thus as outbred as possible. Individuals were identified by a unique combination of coloured plastic leg rings. Sixteen pairs of birds were housed in individual wooden cages measuring 50 cm in width, 50 cm in depth and 100 cm in height in which a nest bowl with straw was constantly present. All cages were kept in the same room and so the birds were within visual and acoustic, but not physical, contact at all times. Mixed seeds (wheat 50%, maize 25%, millet 25%), water and oyster-shell grit were available throughout the experiment and refreshed daily.

Experimental design

Each female ($n=16$) was randomly assigned a mate, and these pairs were further subdivided equally into two groups, A and B. The experiment consisted of two phases (each lasting 4 months), during which each group alternated between treatment and control. Thus eight pairs received the treatment diet (see below) and eight pairs the control diet for 4 months, before alternating. This cross-over design meant that each female acted as her own control and allowed me to increase the effective sample size whilst controlling for temporal changes in sex allocation. Pairs were initially allocated randomly to either treatment or control. In between phases, all birds were group housed in a large room for 2 months and allowed access to food ad libitum. This acted as a 'recovery' period because access to suitable nesting sites was restricted and so no recorded eggs were laid.

During treatment, each caged pair was fed a strictly controlled diet of 56 g of feed daily (28 g is approximately the average adult daily requirement; Hawkins et al. 2001) and female body condition manipulated by continuous egg removal. When a clutch of eggs is lost, pigeons compensate by laying an additional clutch; a process that can continue

indefinitely in a captive population under artificial day lengths (Levi 1945; Riddle 1917). Therefore, by removing fresh eggs immediately following clutch completion, it was possible to induce females to lay between 3 and 9 (median 5) additional clutches over a 4-month period. Egg production is an energetically costly process (Houston et al. 1983; Monaghan et al. 1998; Veasey et al. 2001), and so egg removal combined with a controlled diet significantly decreased female body weight (females were weighed, to the nearest 0.01 g, by allowing them to perch on digital scales at the beginning and end of treatment; start weight (mean across both treatments): 223.08 ± 6.27 g, end weight: 214.79 ± 5.10 g; paired t -test: $t_{15} = 2.78$, $p = 0.014$). It is likely that the decrease in body condition was due to the cost of egg production because male body weight did not decline significantly during treatment (start weight: 235.95 ± 5.96 g, end weight: 228.40 ± 3.73 g; $t_{15} = 1.58$, $p = 0.134$).

During control, eggs were removed after lay (as during treatment) but food was available ad libitum. Egg removal induced females to lay 3–8 (median 4.5) additional clutches, but no decline in body weight was evident in control females (start weight: 219.68 ± 5.01 g, end weight: 222.73 ± 4.56 g; $t_{15} = 1.08$, $p = 0.296$).

Egg collection and treatment

The first and second eggs of each clutch were individually marked with a non-toxic marker (date, time of lay to the nearest 15 min and laying female), and both eggs were allowed to be incubated naturally for three days before being collected, weighed (to the nearest 0.01 g using digital scales) and frozen at -20°C for up to 3 months before analysis. Spot checks did not reveal any incubation prior to laying the second egg, so both eggs are likely to have received a similar length of incubation. The analyses were restricted to the final clutch laid within the 4-month period since the start of each phase since this final clutch would be expected to exhibit the most extreme sex ratio biases. It is unlikely that the number of clutches laid before this final clutch affected the results, since there was no significant difference between the total number of clutches laid during control and treatment (Wilcoxon signed-ranks test: $w_{16} = 33.0$, $p = 0.667$). For analysis, each egg was defrosted and the shell, albumin, yolk and all visible embryonic tissue separated and individually weighed (to the nearest 0.01 g using digital scales). Genomic DNA was then extracted

from the embryonic tissue using a proteinase K digestion followed by sodium chloride extraction and ethanol precipitation (Bruford et al. 1998), and the polymerase chain reaction (PCR) used to amplify part of the W-linked avian *CHD* gene (*CHD-W*) in females, and its non-W-linked homologue (*CHD-Z*) in both sexes using primers 2718R and 2550F (Fridolfsson and Ellegren 1999). PCR products were separated on 2% agarose gels and visualised with ethidium bromide. In all cases DNA extracted from known sex individuals was used as standards, and all samples were sexed blind and in a random order.

Statistical analysis

All females contributed two 2-egg clutches to the analysis. However, during treatment one of the eggs was infertile. Sexing of eggs that had not developed a visible embryo was inconsistent, and it is not clear if the results obtained are reliable (Arnold et al. 2003). Therefore, in the analyses I assumed this egg to be of the rarer sex (male); the 'worst-case' scenario. A fit between two clutch compositions was tested using Pearson's correlations (where a good fit would yield a significant relationship) since the data did not meet the requirements for alternative goodness-of-fit tests such as chi-square (expected values, for example, were often <5). Data are presented as means \pm SE with n denoting number of eggs, unless otherwise stated, and sex ratios are given as the proportion of males. All statistical tests are two-tailed and the significance level was set at 5%.

Results

Sex ratio

Female body weight declined significantly between control and treatment (control: 219.68 ± 5.01 g; treatment: 214.79 ± 5.10 g; paired t -test: $t_{15} = 6.77$, $p < 0.001$), although there was no evidence that this had any effect on clutch size, since no one- or three-egg clutches were produced. During treatment, the sex ratio of both first (Fisher's exact test: $p = 0.003$) and second ($p = 0.031$) eggs was significantly more female-biased than the sex ratio produced by the same females during control (Table 1). Furthermore, the sex-composition of clutches laid during treatment (Table 2) did not match the control clutch composition ($r_p = -0.43$, $p = 0.571$).

Table 1 Observed sex allocation in domestic pigeons (*Columba livia domestica*) (number of males and females, and sex ratio) to first- and second-laid eggs during treatment and control, and the expected compositions based on the predictions of the 'selective resorption' model

	First eggs			Second eggs		
	Males	Females	Sex ratio	Males	Females	Sex ratio
Treatment	1	15	0.06	4 ^a	12	0.25
Control	9	7	0.56	10	6	0.63
'Selective resorption' model	0	16	0.00	4	12	0.25

^aThis group contained an infertile egg, which is assumed to be of the rarer sex (male)

Table 2 Observed clutch composition (frequency of male-male (MM), male-female (MF), female-male (FM) and female-female (FF) clutches) of pigeon broods during treatment and control, and based on the predictions of the 'selective resorption' model

	MM	MF	FM	FF
Treatment ^a	1	0	3 ^b	12
Control	6	3	4	3
'Selective resorption' model ^a	0	0	4	12

^aClutch composition differs significantly from parity ($p < 0.05$)

^bThis group contained an infertile egg, which is assumed to be of the rarer sex (male)

Egg and yolk weight

The median time period between laying the first and second eggs was 44 h 15 m, and this did not differ between treatment and control (Wilcoxon signed-ranks test: $w_{16} = 68.0$, $p = 0.671$ Fig. 1).

A double repeated-measures ANOVA revealed a significant interaction between group (treatment or control) and egg number (first or second) on egg and yolk weights (eggs: $F_{1,45} = 8.92$, $p = 0.005$; yolks: $F_{1,45} = 4.82$, $p = 0.033$). Post-hoc analyses showed that during control, the second eggs of a clutch were significantly heavier than the first eggs (first eggs: 17.53 ± 0.19 g; second eggs: 18.27 ± 0.22 g; paired t -test: $t_{15} = 12.43$, after Bonferroni-adjustment significant at $p < 0.001$ level) although the same pattern did not hold for the yolks (first eggs: 3.56 ± 0.05 g; second eggs: 3.57 ± 0.05 g; $t_{15} = 0.26$, $p = 0.802$). However, despite no difference in the weight of first- and second-laid eggs during treatment (first eggs: 16.44 ± 0.19 g; second eggs: 16.04 ± 0.22 g; $t_{15} = 1.08$, $p = 0.298$), yolks of second-laid eggs were significantly lighter than those of first-laid eggs (first eggs: 3.36 ± 0.03 g; second eggs: 3.22 ± 0.04 g; $t_{15} = 3.20$, $p = 0.012$). The frequency distribution of standardised second-yolk weights from these eggs laid during treatment shows distinct bimodality (Fig. 2), with a cluster around the expected weight and a second cluster significantly

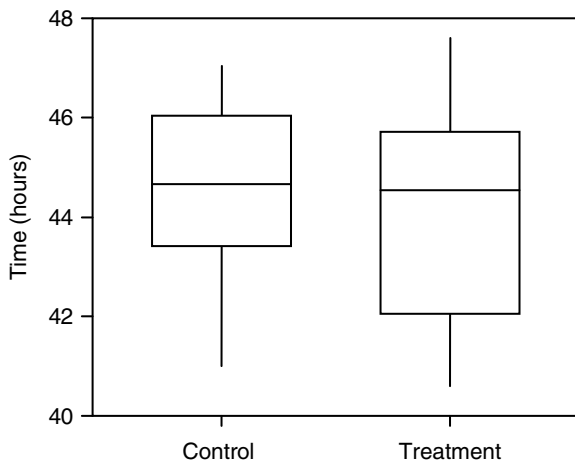


Fig. 1 Distribution of laying delays between first- and second-laid eggs of domestic pigeons (*Columba livia domestica*) during treatment and control ($n = 16$ in each group)

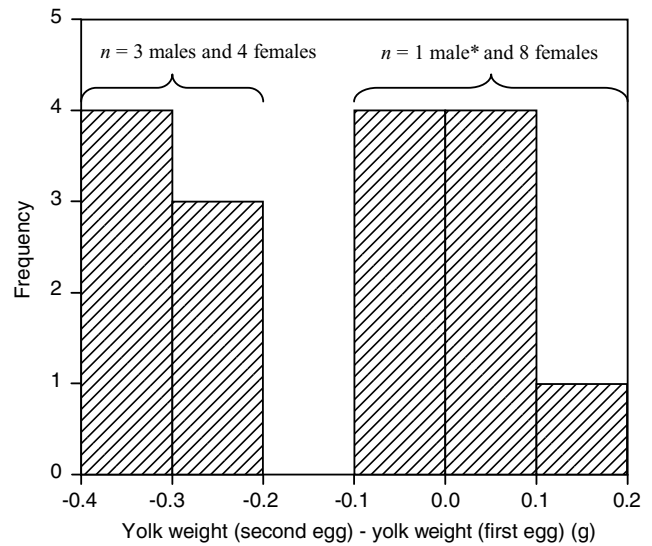


Fig. 2 Frequency distribution of the difference between the mass of the second- and first-laid egg-yolk of each clutch produced during treatment (which departs significantly from normality, Anderson-Darling test: $A^2 = 0.72$, $p = 0.049$). During control, there was a highly significant, positive correlation between first- and second-laid yolk weights ($r_p = 0.91$, $p < 0.001$), and so this figure presents the deviation from the expected weight for second-laid yolks during treatment. * the infertile egg is assumed to be of the rarer sex (male)

cantly lighter than expected (one-sample t -test (test mean = 0): $t_{16} = 3.20$, $p = 0.006$).

Neither albumin (first egg: 11.53 ± 0.15 g, second egg: 11.29 ± 0.31 g; $t_{15} = 0.74$, $p = 0.474$) nor shell (first egg: 1.56 ± 0.02 g, second egg: 1.53 ± 0.03 g; $t_{15} = 0.93$, $p = 0.368$) weights differ significantly between first- and second-eggs laid during treatment, and there was no evidence that male and female eggs differed in weight (two-sample t -test: $t_{15} = 1.40$, $p = 0.172$).

Discussion

The results presented here strongly suggest that a reduction in maternal body condition, brought about by an experimental increase in reproductive effort, causes pigeons to overproduce daughters (see also Riddle 1917). There may be selective pressure on poor condition females to avoid producing low quality sons which, as adults, may be less able to compete for access to prime nesting sites (Levi 1945). Alternatively, if sons are more expensive to rear than daughters (perhaps because they have a higher metabolic rate or show more rapid initial growth: Levi 1945; Kotov 1978) then poor condition females may overproduce daughters because they will be unable to meet the costs of successfully rearing sons (Fiala 1981; Charnov 1982; Teather 1987; Teather and Weatherhead 1988). While this latter explanation may be a mechanism to ensure that at least one offspring survives to independence (Johnston and Janiga 1995), it may simply represent a reproductive constraint

rather than an adaptive example of sex ratio manipulation (Cockburn et al. 2002).

In most studies that report sex ratio biases at hatching, it is not clear exactly when the observed bias may have occurred (although see Komdeur et al. 2002). Studies which have shown a relationship between laying order and sex ratio or provide evidence that deviations from parity continue beyond the first-laid egg certainly suggest the operation of pre-ovulation mechanisms, although in these studies not enough information was available to completely rule out later mechanisms, or authors did not explicitly test for deviation from parity beyond that of the first egg. In this study, however, it is unlikely that mechanisms operating after ovulation could have caused the observed deviations. All females invariably laid two-egg clutches and the rate of infertility was very low (only 1 infertile egg out of 64). Even assuming the ‘worst-case’ scenario in which the infertile egg was of the rarer sex (male), the sex ratio produced during treatment was still significantly biased towards females and, crucially, there was a significant bias in the second egg (although care must be taken with this conclusion given the small sample sizes involved as it is possible that significant biases might have arisen by chance and hence may not reflect an adaptive strategy (Ewen et al. 2004)). As this egg was laid approximately 44 h after the first in all cases and no laying sequence gaps were evident, pre-ovulation, or primary, mechanisms are likely to contribute to the sex ratio biases observed (Komdeur et al. 2002).

A number of potential pre-ovulation mechanisms of sex ratio manipulation have been proposed (see Pike and Petrie 2003 for a review). It is possible that manipulation occurred through segregation distortion at the first meiotic division (Oddie 1998), or by influencing the order in which follicles bearing ova of different sexes are ovulated (Ankney 1982; Krackow 1995). However, mechanisms of sex ratio adjustment involving sex-differential abortion, but without the constraints of laying delays cannot be completely ruled out. For example, Emlen (1997) suggested that birds could bias the sex of the first laid egg and then let the rest of the clutch be determined randomly. For species that lay small clutches, such as pigeons (but also other species in which dramatic sex ratio biases have been observed, such as the Seychelles warbler (*Acrocephalus sechellensis*), Komdeur et al. 1997 and eclectus parrots *Ecelectus roratus*, Heinsohn et al. 1997), this could be a highly effective strategy. However, I could find no evidence for it in this study. Even though the sex ratio of the first eggs laid during treatment was significantly biased towards females, the sex ratio of second-laid eggs was similarly biased (rather than having an equal number of males and females as would be predicted). Consequently, females must actively manipulate the sex of the second egg as well, although potential mechanisms which currently exist to explain how this could happen are purely speculative (Pike and Petrie 2003). What is needed is an alternative mechanism which explains how pigeons can manipulate the sex of both eggs without incurring the costs of clutch-size reduction or laying gaps, and without the need to induce non-random segregation of sex chromosomes at meiosis (for which little positive evidence

exists: Krackow 1999; Pike and Petrie 2003). To fill this void, I developed a post-hoc sex mechanistic model that could explain both the deviations in yolk weight and the sex ratio skews observed during treatment (but which were absent during control).

The model is outlined in Fig. 3 and, assuming that during treatment the desired sex was females, is as follows. Assuming that the breeding female can detect the sex of an ovum following the completion of meiosis, she could potentially abort male ova until such a time as a follicle bearing a female ovum is ready to be ovulated. However, rather than leave the sex of the subsequent egg to chance (cf. Emlen 1997) if the next follicle in the hierarchy is female then it would be ovulated as normal but if it is male then it will be aborted and the next follicle, regardless of sex, ovulated in its place. According to King’s (1973) model of follicular growth (Fig. 4), by the time one follicle reaches maturity, the subsequent follicle has almost completed formation and would only be a fraction smaller than if it had been allowed to fully complete development. Hence because pigeons rarely lay one-egg clutches, the time between laying the first- and second-eggs is almost invariably constant and the rate of infertility is low, if the second follicle is aborted then the next follicle in the hierarchy must be ovulated in its place. Smaller follicles (further

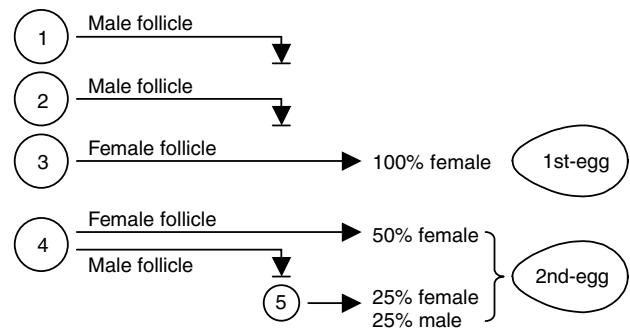


Fig. 3 Under the ‘selective abortion’ model, male follicles (1, 2) are aborted until a female follicle (3) can be ovulated. If the subsequent follicle (4) is female then it will be ovulated, but a male follicle will be aborted and the next follicle in the hierarchy (5), regardless of sex, ovulated in its place. Thus all first-laid eggs will be female, the M:F ratio in second eggs will be 1:3 and half of all second-laid eggs will contain smaller than average yolks in which the M:F ratio will be 1:1

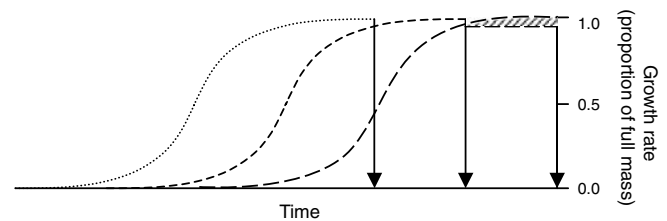


Fig. 4 The growth rate of individual F_1 (solid line), F_2 (dashed line) and F_3 (dotted line) follicles (which form the future egg yolks) as a function of time (after King 1973). If, during production of the second egg, the follicle is aborted, the subsequent follicle will be ovulated in its place. However, since it has not attained its full mass it will be slightly smaller than a ‘normal’ second-yolk (indicated by the shaded region). Vertical arrows indicate ovulation events

down the hierarchy) would probably be too underdeveloped to undergo ovulation (Sturkie 1986). The predictions of this 'selective resorption' model are highly consistent with the data collected during treatment, but not control:

1. All first-laid eggs will be female and the M:F ratio in second-laid eggs will be 1:3 (treatment: $r_p > 0.99$, $p = 0.002$; control: $r_p = -0.80$, $p = 0.200$; Table 1), and the MM:MF:FM:FF clutch composition will thus be 0:0:1:3 (treatment: $r_p = 0.99$, $p = 0.011$; control: $r_p = -0.50$, $p = 0.500$; Table 2).
2. Half of all second-laid eggs (during treatment) will contain smaller than average yolks (Fisher's exact test: $p = 0.260$; Fig. 2) in which the M:F ratio will be 1:1 ($p = 0.392$; Fig. 2).

This model neatly explains the observed skew towards female-female clutches as well as the disparity in yolk (but not egg) weight during treatment, both of which were absent during control. It is also plausible inasmuch as the life-history of pigeons is favourable to such a mechanism (for instance, they exhibit considerable temporal plasticity with respect to clutch initiation: Levi 1945) and the costs involved are likely to be minimal, thus broadening the scope for adaptive sex ratio adjustment because the fitness differential between sons and daughters need not be as great to balance the costs of the adjustment (Pen and Weissing 2000). However, it must be stressed that since this model was developed post-hoc, it is not surprising that it fits the data so well and as a result to be taken seriously evidence is needed from other species that similar mechanisms could be operating. In peafowl it has been found that during the final third of a breeding season, poor condition females tend to overproduce daughters (unpublished data). When the yolk weights and sex ratios of eggs laid during this time are examined, we find that not only are the yolks (but again, not the eggs themselves) of male eggs significantly lighter than those of female eggs, but the M:F ratio is 7:17; remarkably similar to the 6:18 ratio that would be predicted by this model. However, it is also possible that differences in yolk weight came about through an entirely different process. For instance, a higher embryonic growth rate in some eggs may have depleted yolk reserves more rapidly, or poor maternal condition may have either precluded the production of a full yolk for both eggs or altered incubation behaviour so that one egg received more incubation than the other. This model thus provides a testable hypothesis for future work on mechanisms of sex ratio manipulation in birds.

If this mechanism turns out to be widespread, then it may explain why the sex ratios observed in nature are rarely absolute (i.e. 100% females or 100% males), and suggests that selective resorption need not result in laying sequence gaps (see Introduction). However, this mechanism would be hard to detect without sacrificing the eggs, although there may be hormonal cues signifying multiple ovulations in close succession such as dual surges in luteinising hormone (the hormone most likely to provide the direct stimulus for germinal vesicle breakdown and subsequent ovulation; Johnson and Tienhoven 1980). Clearly, there is a need for

more studies on the processes of gamete formation and ovulation in species where sex ratio adjustment is known to occur, as well as to related factors that could contribute to the mechanism of sex ratio manipulation.

Acknowledgements I am grateful to the Biotechnology and Biological Sciences Research Council for financial support and to Marion Petrie and two anonymous referees for valuable comments which greatly improved the manuscript. The experiment outlined here complies with the current laws of the United Kingdom

References

- Abs M (1983) The physiology and behaviour of the pigeon. Academic Press, London
- Ankney CD (1982) Sex ratio varies with egg sequence in lesser snow geese. *Auk* 99:662–666
- Arnold KE, Griffith SC, Goldizen AW (2001) Sex-biased hatching sequences in the cooperatively breeding Noisy Miner. *J Avian Biol* 32:219–223
- Arnold KE, Orr KJ, Griffiths R (2003) Primary sex ratios in birds: problems with molecular sex identification of undeveloped eggs. *Mol Ecol* 12:3451–3458
- Badyaev AV, Hill GE, Beck ML, Dervan AA, Duckworth RA, McGraw KJ, Nolan PM, Whittingham LA (2002) Sex-biased hatching order and adaptive population divergence in a passerine bird. *Science* 295:316–318
- Bradbury RB, Blakey JK (1998) Diet, maternal condition, and offspring sex ratio in the zebra finch, *Poephila guttata*. *Proc R Soc Lond B* 265:895–899
- Bruford MW, Hanotte O, Brookfield JFY, Burke T (1998) Multilocus and single-locus DNA fingerprinting. In: Hoelzel AR (ed) *Molecular genetic analysis of populations: a practical approach*, 2nd edn. IRL Press, Oxford, pp 287–336
- Charnov EL (1982) Parent-offspring conflict over reproductive effort. *Am Nat* 119:736–737
- Clout MN, Elliott GP, Robertson BC (2002) Effects of supplementary feeding on the offspring sex ratio of kakapo: a dilemma for the conservation of a polygynous parrot. *Biol Conserv* 107:13–18
- Cockburn A, Legge S, Double MC (2002) Sex ratios in birds and mammals: can the hypotheses be disentangled? In: Hardy I (ed) *The sex ratio handbook*. Cambridge University Press, Cambridge
- Edmunds SR, Ankney CD (1987) Sex ratios of hatchling mourning doves. *Can J Zool* 65:871–874
- Emlen ST (1997) When mothers prefer daughters over sons. *Trends Ecol Evol* 12:291–292
- Ewen JG, Cassey P, Moller AP (2004) Facultative primary sex ratio variation: a lack of evidence in birds? *Proc R Soc Lond B* 271:1277–1282
- Fiala KL (1981) Sex ratio constancy in the red-winged blackbird. *Evolution* 35:898–910
- Fridolfsson AK, Ellegren H (1999) A simple and universal method for molecular sexing of non-ratite birds. *J Avian Biol* 30:116–121
- Griffiths R, Double C, Orr K, Dawson R (1998) A DNA test to sex most birds. *Mol Ecol* 7:1071–1075
- Hanson HC, Kosack CW (1963) The mourning dove in Illinois III. Dept. Conserv Tech Bull No. 4
- Hawkins P, Morton DB, Cameron D, Cuthill I, Francis R, Freire R, Gosler A, Healy S, Hudson A, Inglis I, Kirkwood J, Lawton M, Monaghan P, Sherwin C, Townsend P (2001) Laboratory birds: refinements in husbandry and procedures. *Lab Anim-UK* 35:S1–S163
- Heinsohn R, Legge S, Barry S (1997) Extreme bias in sex allocation in Eclectus parrots. *Proc R Soc Lond B* 264:1325–1329
- Houston DC, Jones PJ, Sibley RM (1983) The effect of female body condition on egg-laying in lesser back backed gulls *Larus fuscus*. *J Zool* 200:509–520

- Johnson AL, van Tienhoven A (1980) Plasma concentrations of six steroids and LH during the ovulatory cycle of the hen, *Gallus domesticus*. *Biol Reprod* 23:386–393
- Johnston RF, Janiga M (1995) Feral pigeons. Oxford University Press, New York
- Kalmbach E, Nager RG, Griffiths R, Furness RW (2001) Increased reproductive effort results in male-biased offspring sex ratio: an experimental study in a species with reversed sexual size dimorphism. *Proc R Soc Lond B* 268:2175–2179
- Kilner R (1998) Primary and secondary sex ratio manipulation by zebra finches. *Anim Behav* 56:155–164
- King JR (1973) Energetics of reproduction in birds. In: Farner DS (ed) *Breeding biology of birds*. National Academy of Sciences, Washington, D.C., pp 78–106
- 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
- Komduer J, Magrath MJL, Krackow S (2002) Pre-ovulation control of hatchling sex ratio in the Seychelles warbler. *Proc R Soc Lond B* 269:1067–1072
- Kotov AA (1978) Data on the ecology and behaviour of the rock dove in the Southern Urals and Western Siberia. *Byull Moskovsk O-va Isp Prirodi Otd Biol*, 83:71–80
- Krackow S (1995) Potential mechanism for sex ratio adjustment in mammals and birds. *Biol Rev* 70:225–241
- Krackow S (1999) Avian sex ratio distortions: The myth of maternal control. In: Adams N, Slotow R (eds) *22nd International Ornithological Congress*, vol. 1. Bird Life South Africa, Durban, pp 425–433
- Levi WM (1945) *The pigeon*. Bryan, Columbia, S.C.
- Monaghan P, Nager RG, Houston DC (1998) The price of eggs: increased investment in egg production reduces the offspring rearing capacity of parents. *Proc R Soc Lond B* 265:1731–1735
- MacGregor WC (1958) Non-unisexual broods in the mourning dove. *J Wildl Manage* 22:103
- Nager RG, Monaghan P, Griffiths R, Houston DC, Dawson R (1999) Experimental demonstration that offspring sex ratio varies with maternal condition. *Proc Natl Acad Sci USA* 96:570–573
- Oddie K (1998) Sex discrimination before birth. *Trends Ecol Evol* 13:130–131
- Pen I, Weissing FJ (2000) Optimal sex allocation: steps towards a mechanistic theory. In: Hardy I (ed) *The sex ratio handbook*. Cambridge University Press, Cambridge, pp 59–69
- Petrie M, Schwabl H, Brande-Lavridsen N, Burke T (2001) Sex differences in avian yolk hormone levels. *Nature* 412:489
- Pike TW, Petrie M (2003) Potential mechanisms of avian sex manipulation. *Biol Rev* 78:553–574
- Riddle O (1917) The control of sex ratio. *J Wash Acad Sci* 7:319–356
- Sheldon BC, Andersson S, Griffith SC, Ornborg J, Sendecka J (1999) Ultraviolet colour variation influences blue tit sex ratios. *Nature* 402:874–877
- Sturkie PD (1986) *Avian Physiology*. Springer, Berlin Heidelberg New York
- Teather KL (1987) Intersexual differences in food consumption by hand-reared great-tailed grackle (*Quiscalus mexicanus*) nestlings. *Auk* 104:635–639
- Teather KL, Weatherhead PJ (1988) Sex-specific energy-requirements of great-tailed grackle (*Quiscalus mexicanus*) nestlings. *J Anim Ecol* 57:659–668
- Veasey JS, Houston DC, Metcalfe NB (2001) A hidden cost of reproduction: the trade-off between clutch size and escape take-off speed in female zebra finches. *J Anim Ecol* 70:20–24
- Whittingham LA, Dunn PO (2000) Offspring sex ratios in tree swallows: females in better condition produce more sons. *Mol Ecol* 9:1123–1129
- Williams TD (1999) Parental and first generation effects of exogenous 17 β -estradiol on reproductive performance of female zebra finches (*Taeniopygia guttata*). *Horm Behav* 35:135–143
- Wood-Gush DGM, Gilbert AB (1970) The rate of egg loss through internal laying. *Br Poult Sci* 11:161–163