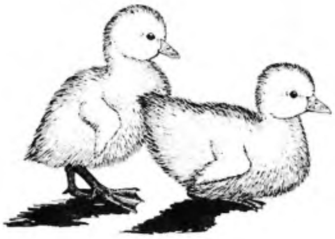
The effects of genotypic and yearly variations on the egg volumes of the Mute Swan *Cygnus olor*



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Measurements of Mute Swan eggs are highly replicable between different observers. Analysis of the egg volumes of 119 clutches of Mute Swans measured in four years gave a mean volume of 332 cm^3 (SD = 27) and partitioned the variance into three random components (within clutch; between bird; bird/year-interaction) and two 'fixed' effects (four year-to-year differences and three female genotypes at a biochemical marker locus). The same females consistently laid similar sized eggs in different years, but it was concluded that there were small but real differences between eggs laid by the different genotypes. It is unnecessary to measure every egg in a clutch to estimate adequately the average egg volume of that clutch. Other studies show that larger eggs would, in Mute Swans more than most species, be expected to confer better cygnet survival, but there is presently no direct evidence that this is so.

It has been shown for several bird species that the volumes of eggs affect the survival prospects of chicks hatching from those eggs, with larger eggs conferring better survival prospects. This is true for several species of wildfowl; for example, Lessells (1981) demonstrated that goslings of Canada Geese *Branta canadensis* hatched from larger eggs survive better during their first months of life. As it is known that egg volumes are heritable (60-80% of the variation in Great Tit egg volumes is heritable; van Noordwick 1981) it is clear that eggs volumes are of considerable evolutionary importance.

In the Mute Swan *Cygnus olor* Bacon (1980) showed that laying dates and clutch sizes varied between females depending on their genotype at a biochemical marker locus: SS homozygous females laid larger clutches earlier. In the absence of countering selection favouring the alternative allele, F, this would imply that the proportion of S alleles should rise and the whole population should eventually become homozygous SS, instead of the observed 5:4:1 SS:SF:FF ratio. A suggestion that mates of heterozygous SF males laid larger clutches at all times of year (Bacon 1980) was not confirmed by subsequent work further elucidating the effects of individual females, female genotypes and year differences on clutch sizes (Birkhead, Bacon & Walter 1983).

More recently it has been shown (Walter, Bacon & Sears 1991) that cygnet survival from hatching to fledging is affected by both male and female genotypes, but it is unclear how this is achieved. However, Bacon (1980) noted that the average egg volumes of the different females (FF>SF>SS) was in the reverse order to the average clutch sizes (SS>SF>FF) (note the herozygote is always intermediate). Thus there might be a counter-balancing effect favouring the F allele if cygnets from larger eggs survived better. Even if this effect were small in terms of absolute survival, Bacon & Beekman (1991) suggest it could have a considerable effect on the preferred laying dates and clutch sizes of individual females.

We here analyse previously unpublished data from the Oxford study area to assess whether the average egg volumes differ for the esterase genotypes and are in the order FF>SF>SS. Such analysis is complicated by three factors: first, the eggs were measured by three different observers in four years; second, there were differences between the years; third, the data are not independent, as the same female's eggs (on the same territories with the same mates) were measured in different years. This paper describes an analysis which copes with these difficulties.

Methods

The study area consisted of the upper Thames and its tributaries around Oxford, and is described in Bacon (1980b). Egg volumes were calculated from a formula modified from Stonehouse (1963), which Bacon (1980) has shown to be applicable to Mute Swans. Measurements of egg length and breadth were highly replicable for Mute Swans, but variations in shape, as indexed by the position of the maximum diameter along the egg's length (see Fig. 1), are not reliable (Bacon 1980a). As Mute Swan eggs are not perfectly circular in the plane of their maximum diameter, the minimum diameter was estimated from single measures of maximal diameters following Bacon (1980) according to the formula:

$$^{D}\mathrm{min} = {}^{D}\mathrm{max} * 0.99571$$

and the volume calculated from a modification of Stonehouse's formula:

$$\mathrm{Volume} = 0.512 * \mathrm{Length} * ((^{D}\mathrm{max} + {}^{D}\mathrm{min}) / 2)^2$$

where 0.512 is a shape correction factor.

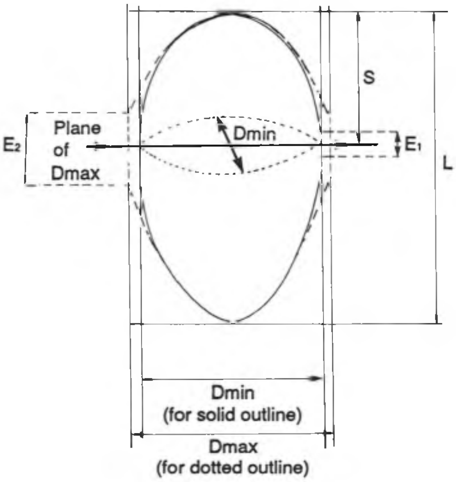

Figure 1. Measurements describing Mute Swan eggs: Measures of D max and L are highly repeatable. D min may be appreciably less than D max, so the maximum should be carefully chosen. For some eggs the surface is very flat (solid outline) near the plane of maximum diameter. Because of this the error E_2, on the position of the D max plane (S/L) is highly variable and is not adequately repeatable.

The eggs were measured using Vernier callipers to an accuracy of 0.01 cm and all volumes are given in cubic centimetres.

Results

Test of technique

A test of technique was carried out to estimate the differences between the measurements made by the three observers who took part during the four years of fieldwork. Four eggs, from the same clutch which was flooded shortly after completion, were each measured four times by each of the three observers. The mean volumes are given in Table 1 and the Analysis of Variance in Table 2.

Table 1. Mute Swan egg volumes and means as measured by the three observers

Observer	Egg 1	Egg 2	Egg 3	Egg 4	Mean
PJB	341.2	352.5	323.0	305.2	330.5
DKS	340.1	354.8	322.6	296.3	328.5
MEB	337.8	351.0	323.3	296.0	327.0
Means	339.7	352.8	323.0	299.2	328.7

Table 2. The measuring of Mute Swan eggs: technique test; Analysis of Variance

Source of variation	D.F.	M.S.
Between observers	2	48.5
Between eggs	3	768.6
Egg* observer	6	29.5
Repeatability	36	8.4

There is no suggestion of any difference between observers in their measurements. (Observer MS/'observer*egg interaction MS' = 48.5/29.5 = 1.64 = $F_{2,6}$, NS). This conclusion is reinforced by the relative values of the estimates of the variance components when the three observers are regarded as random sample from a population of observers. The estimates of the variance components, obtained by equating the observed mean squares to their expected values are given in Table 3, together with their percentage contributions to the total variance.

Table 3. The measuring of Mute Swan eggs: technique test. Estimated components of variation.

Factor	Var. Comp	% Variance
Observer	1.2	0.2
Egg	533.0	97.3
Observer - Egg	5.3	1.0
Repeatability	8.4	1.5

Thus we can see from Table 3 that the total observer and repeatability errors represent less than 3% of the within clutch variance: moreover it is shown below that the within clutch variance is small compared to the between clutch variance; relative to the overall variability observer differences are negligible.

Analysis for the four years separately

The examination of differences between the genotypes began with a separate analysis of each year's data; as replacement clutches were not included, each female was represented only once each year. The analysis takes into account the familial similarity of the eggs within the same clutch which vary about an average value depending on the female which laid them; this average volume varies from female to female, as expressed by:-

$$V = U + E1 + E2$$

where U is a mean value dependent on genotype

E1 is the female effect common to all eggs within a clutch but which varies between different females

E2 is a measure of the variation of eggs within a clutch.

If S^2_1 and S^2_2 are the variances of the error terms E1 and E2 then the variance of the mean of a clutch of *n* eggs is equal to $S^2_1 + S^2_2/n$. As clutches are of different sizes, allowances must be made for the differing accuracies of the estimates of the means of different clutches. The details of the analysis appropriate to this type of data are described by Healy (1972). The resulting means and their errors are shown in Fig. 2.

Table 4. Significance level of differences between genotypes: Single Years; based on 119 clutches laid by 65 different female Mute Swans

	Clutches			Comparison between		
	SS	SF	FF	SSvSF	SSvFF	SFvFF
1977	11	8	4	n.s.	x	n.s.
1978	25	13	5	n.s.	*	n.s.
1979	17	5	1	n.s.	n.s.	n.s.
1980	19	8	3	n.s.	*	n.s.

n.s. = not significant at 10% level
x = significant at 10%
* = significant at 5%

Table 5. Genotypic differences from the overall analysis of all four years data on Mute Swans egg volumes.

Genotype contrast	Differences in volumes	Standard error	t-value
SS v SF	- 5.7	5.8	-0.98
SS v FF	-17.9	8.5	-2.07
SF v FF	-12.3	9.1	-1.33

In three of the four years the anticipated ordering of the mean egg volumes (FF>SF>SS) was obtained. The exception was 1979, when only a single FF clutch was measured. The significance of the genotypic effect remains in doubt, as formal significance (5%) was not always obtained (Table 4).

Combined analysis of all four years data simultaneously

Differences between esterase genotypes .

To make the test for a genotypic effect more powerful the complete data set was subject to a single overall analysis.

Briefly the method of analysis, known as the Fitting Constant method, estimates the various components of variance by equating the expected Mean Square terms from the Analysis of Variance model with the calculated values derived from the data. Given these variance components the differences between years and genotypes can be derived, together with their standard errors and significances. Figure 2 suggests that the differences between genotypes are consistent from year to year. Numerical analysis, using the method outlined, confirmed that the genotype year interactions is not significantly different from zero. Thus the genotypic effects and the yearly effects are directly and separately estimable.

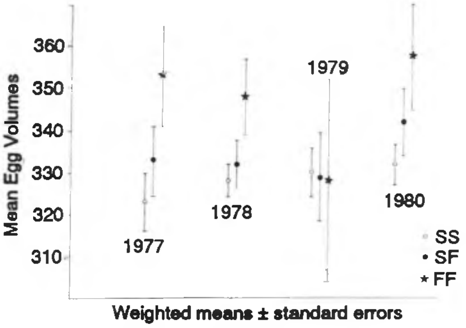

Figure 2. Mean egg volumes per clutch, for three different Mute Swans genotypes in four years, with associated standard errors.

In reporting the results of the analysis we first present the estimates of the genotypic effects and consider the significances of the differences between the genotypes. Figure 3(a) shows the estimated genotypic effects and their 95% confidence intervals; the estimates follow the anticipated order SS<SF<FF. Table 5 gives the mean values and standard errors of the various pairwise differences between the genotypes. The genotypic contrast of prior and primary interest, SS with FF, has a t-value of -2.07 with 111 degrees of freedom; this values is signifi-

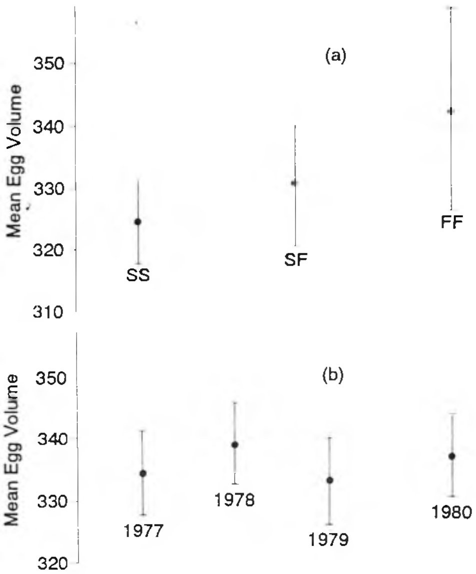

Figure 3. Estimates of mean Mute Swan egg volumes, with 95% confidence limits for both genotypes and years: (a) Genotypic effect, corrected for Year differences (b) Yearly effects, corrected, for genotype frequencies.

cant at the 2% level; the ordering SS<SF<FF is equally significant.

Differences between years

Turning now to a consideration of the annual variations in egg volumes the relative yearly effects and their 95% confidence limits are illustrated in Figure 3(b). The overall F test for differences between years gives a 10% significance level. Thus there is some suggestion that year-to-year differences in egg volumes are significant, and that these differences are not simply due to different observers measuring in different years, but are partly due to environmental effects.

Sources of variation

An important feature of the final analysis is the estimation of three components of variation. The model estimates three variances: between eggs within clutches, Se; the bird x year interaction, Sd; and between birds, Sb. Their relative magnitudes indicate the major sources of variation. The estimates are:-

within clutch	$S^2e = 213$
female x year interaction	$S^2d = 53$
between females	$S^2b = 399$

We first consider the effect on estimation of taking measurements on less than the full number of eggs in each clutch.

The variance of a mean volume-per-clutch based on the measurement of *n* eggs from the same clutch equals $S^2b + S^2d + S^2e/n = 452 + 213/n$ (= 480 for a typical clutch).

This variance, and hence the precision of measurements, is only marginally influenced by the number of eggs measured per clutch, *n*. For example the gain in precision by measuring all ten eggs in a clutch is only 41% more than that from measuring a single egg chosen at random, and only 18% more than selecting two eggs at random. In fact similar precisions should be obtained by measuring randomly selected pairs of eggs from 12 clutches as from measuring all ten eggs from 12 '10 egg' clutches.

As the greater part of the overall variation in egg volume is between the clutch means of different females rather than between eggs of the same clutch, the precision of estimates of genotypic and annual effects are mainly determined first by the number of clutches that are measured and secondly by whether or not the same female provides estimates in more than one year. Genotypic effects are best examined by comparing the clutches of different, rather than the same, birds in successive years. In any one year the difference in mean clutch volume between a pair of females of different genotype has variance approximately equal to $2(S^2_b + S^2_d + S^2_c)$, = 960. If the same pair of birds are included in a second year the variance of the average difference is reduced, only slightly, to $2S^2_b + S^2_d + S^2_c/7$, (= 880). If a quite different pair of birds figure in the second year the variance of the average difference between genotypes is now reduced to $S^2_b + S^2_d + S^2_c/7$, (= 480). There is scarcely any gain in precision if the same birds are measured in different years, but 100% gain in precision if different birds are measured.

Conversely, when estimating differences between years, the most accurate measures are obtained when the same females are measured in different years. The variance of a comparison of one female between two years is approximately $2(S^2_d + S^2_c/7) \simeq 160$, whereas the comparison for a different female in each year would be $2\ (S^2_b + 2^2_d + 2^2_c/7) \simeq 960$, a 6-fold increase.

Discussion

The complete data set showed that Mute Swans around Oxford had a mean egg volume of 332 cm³ with a standard deviation of 27. The same female laid eggs of very similar sizes in different years, but there were some small year to year differences and larger differences between the average egg volumes of females with different esterase genotypes. It is interesting to note that Birkhead, using a sub-set of this data comprising four randomly chosen eggs from ten clutches that were all measured in each of the four years, obtained a probability level of $P<0.025$ for differences between years (Birkhead, Bacon & Walter 1983, Birkhead 1984). Some caution must be used in interpreting this result, as the ten females were not selected at random but on the basis of how many times each had been measured, which was determined both by fieldwork restrictions in the different years and which birds survived and re-nested. The present analysis attributed only a 10% significance to the yearly differences in mean egg volumes. Taken together the two analyses suggest that the yearly differences are real but relatively small: over the four years the range of the difference is 6 cm^3 or only 1.5% of the average egg volume.

As for the genotypic effects we were looking for the possibility of a difference favouring FF females; the appropriate one-tailed test is significant at the 2.5% level. Additional data on twelve clutches from females of known esterase genotype from the 'Midlands' study area of Coleman & Minton (see Minton 1968, Coleman *et al* 1991), measured in 1978, did not show similar genotypic differences. There are however considerable habitat differences between the two areas: moreover, within the Oxford study area Bacon (1980a) and Walter (1981) found an association between habitat and genotype (which was not quite significant statistically). In short there is strong evidence that in the mainly riverine habitats of the Oxford area there are consistent differences in egg volumes between the different esterase genotypes, with a range of 18 cm^3 (6% of an average egg) separating the different genotypes.

It is interesting to compare the range differences of these fixed effects with the standard deviations of the within-clutch variance ($\sqrt{213} = 14$) and the between-female variance ($\sqrt{399} = 20$). The above variance of 399, representing 'between females within genotypes' is increased to a value of 488 when the genotypic classification is ignored (and then represents the variance between all females irrespective of their genotypes). Accordingly the variance in egg volumes associated with esterase genotypes would seem to represent some 12% of egg volume variance $(488-399)/(213 + 53 + 488)$, some 16% of mean egg volume per clutch variation $(488 - 399)/(213/7 + 53 + 488)$ or, if van Noordwick's estimate of 70% of between mean clutch variance being heritable is applicable to the Mute Swan, some 23% of the heritable variation of egg volume. The evidence thus suggests that the esterase locus is linked to a section of chromosome that has a considerable effect on egg volumes; four sections having similar effects might account for all the likely heritable variation.

The range of mean egg volumes between years, (6 cm^3) is small compared with the within-clutch variance ($\sqrt{213} = 14$ cm^3) and the esterase differences (18 cm^3). It is therefore rather difficult to accept that the small year-to-year differences observed for Mute Swan egg volumes represent an appreciable constraint on their breeding success.

The analysis also suggests that not all eggs in a clutch need be measured to achieve adequate discrimination between clutches; two or three eggs might be sufficient. With territorial Mute Swans, the largest amounts of time are spent getting from one nest to another, and the measurement time is relatively small, though it can be disturbing to timid birds with large clutches, and might usefully be reduced. However, in circumstances where Mute Swans nest in colonies (e.g. Denmark), the presence of an observer can disturb the whole colony, and in these circumstances reduced measuring time at every nest would benefit all birds. As the first and last eggs of a clutch are generally smaller (Reynolds 1972) but not always (Anderson-Harild pers. comm.), it might be worth excluding the first and last eggs (most deeply and least stained) and randomly choosing two eggs to measure from the remainder, if time is at a premium or if simplicity of analysis is desired. Eggs from first time breeders all tend to be small (Anderson-Harild pers. comm.) and greater accuracy for these, requiring more measurements, might be worthwhile.

In many bird species it is known that as the absolute volume of eggs increases as the relative proportion of yolk decreases, so that there are counteracting effects to a simple advantage from a larger egg. However Birkhead (1984) has shown that in the Mute Swan the proportion of yolk is actually relatively larger in larger eggs, implying a more straightforward advantage to large eggs (Birkhead 1984). A direct test of the hypothesis that cygnets from larger eggs should survive better was recently carried out in the Outer Hebrides, but failed to demonstrate any clear increase in survival prospects between hatching and fledging for cygnets hatching from small, medium and large eggs (C. Spray pers. comm.).

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A pair of nesting **Mute Swans.** *Photo: The Author*

Mute Swan nest-building. *Photo: The Author*