Estimating Repeatability of Egg Size

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ABSTRACT.—Measures of repeatability have long been used to assess patterns of variation in egg size within and among females. We compared different analytical approaches for estimating repeatability of egg size of Black Brant. Separate estimates of repeatability for eggs of each clutch size and laying sequence number varied from 0.49 to 0.64. We suggest that using the averaging egg size within clutches results in underestimation of variation within females and thereby overestimates repeatability. We recommend a nested design that partitions egg-size variation within clutches, among clutches within females, and among females. We demonstrate little variation in estimates of repeatability resulting from a nested model controlling for egg laying sequence and a nested model in which we assumed laying sequence was unknown.

Egg size represents the unit of investment in a reproductive attempt for birds. Accordingly, researchers have long been interested in variation in egg size and its adaptive significance. Repeatability is a measure of the proportion of variation in a trait that is due to differences among individuals. Therefore, estimates of repeatability of egg size split the total phenotypic variation in egg size into within-individual and among-individual components. Falconer (1989) describes that partitioning of the phenotypic variance in terms of the origin of the different variance components. Variation in egg size among individuals is caused by a combination of genetic and environmental differences. Variation in egg size within an individual among years is caused by temporary environmental differences between reproductive attempts. Falconer (1989) goes on to demonstrate that estimates of repeatability are useful in the context of quantitative genetics because they set upper limits to levels of heritable variation.

Falconer (1989) states that estimates of repeatability can be based on measures of a characteristic that are repeated in either time or space. Following Falconer's (1989) descriptions, egg size is repeated in both time and space—where time refers to different nesting attempts and space refers to eggs within a specific nesting attempt (i.e. clutch). In many cases researchers estimating repeatability of egg size use the average egg size within clutches (e.g. Lessells et al. 1989, Flint and Sedinger 1992, Flint and Grand 1996). However, that approach ignores some of the phenotypic variation in egg size within individuals (e.g. within clutches) and may have been used because researchers were uncertain how to deal with multiple measurements within clutches statistically. Other researchers have used a nested design to partition variance among females, within females among years, and within females within years (i.e. clutches) (LeBlanc 1987, Erikstad et al. 1998, Flint and Grand 1999).

In this paper, we compare those two approaches in terms of estimates of repeatability. We decompose each estimate into its variance components and discuss each estimate in the context of quantitative genetics. We also discuss those estimates relative to the assumptions of repeatability analyses. Finally, we recommend approaches for estimating repeatability of egg size given specific data sets.

Methods.—This study was conducted at the Tutakoke River Black Brant (*Branta bernicla nigricans*) colony along the outer coastal fringe of the Yukon-Kuskokwim Delta from 1987–1997. Breeding Black Brant have been banded at that site since 1986, providing a large sample of identifiable females on which to measure repeatability. Methods of nest searching, measuring of eggs, and estimation of egg volume have been described by Flint and Sedinger (1992). Estimation of repeatability requires multiple measurements of individuals among years, and that large data set allowed partitioning to many different levels (i.e. specific clutch size and egg sequence numbers). In each analyses, we deleted individuals for which we did not have multiple measurements; therefore, sample sizes vary for each analyses. Further, we restricted our analyses to clutches of 3, 4,

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TABLE 1. Estimated repeatability of egg size by clutch size and egg sequence number for Black Brant nesting on the Yukon-Kuskokwim Delta, Alaska.

Clutch size	Egg-sequence number				
	1	2	3	4	5
3	0.6394 (96:209)[a]	0.5544 (95:204)	0.6069 (91:197)		
4	0.5482 (354:814)	0.5371 (346:804)	0.5505 (353:815)	0.5287 (349:805)	
5	0.5532 (280:674)	0.5021 (280:675)	0.5698 (271:655)	0.5308 (277:671)	0.4929 (267:642)

[a] Sample sizes for each estimate are given in parentheses as: (number of females:number of eggs).

or 5 eggs because those are the most common clutch sizes. Clutches <3 and >5, are likely the result of partial nest predation and intraspecific nest parasitism, respectively. By excluding those clutches, we attempted to reduce the possibility that either partial predation or intraspecific nest parasitism influence our determination of clutch size, egg sequence number, or both.

Analyses.—We used SAS (PROC NESTED) to estimate the true variance components for each data set (SAS Institute 1990). Therefore, consistent with Lessells and Boag (1987), our estimates of repeatability were based on variance components extracted from mean squares of the various models. We performed four main analyses. First, to control for egg-size variation among clutch sizes and due to egg-sequence number, and to avoid statistical problems associated with multiple measures within females, we partitioned the data set by clutch size and egg sequence number. We then estimated the repeatability for each egg-sequence number within clutch sizes using a series of one-way ANOVA models. Second, we replicated previous studies and calculated the average egg size for each clutch and estimated the repeatability of average egg size from multiple samples across years using a one-way ANOVA. Third, we used the complete data set and partitioned the variance among females, within females among years, and within clutches using a nested ANOVA design. Finally, we controlled for the combination of clutch size and egg-sequence number, then partitioned the remaining variance within and among females using a nested ANOVA.

To interpret the differences among those approaches to estimating repeatability estimates, we must examine the contribution of the different variance components. From Falconer (1989), we use the following: V_p = phenotypic variation at the population level, V_g = variation in egg size due to genetic differences among individuals, V_{eg} = general environmental variation among individuals arising from permanent or nonlocalized circumstances, and V_{Es} = special environmental variation within individuals arising from annual variation in circumstances. As we noted above, egg size within individuals is repeated on several scales (i.e. among years and within

clutches). Thus, we add the following: V_c = variation within clutches. Based on those definitions, when using the approach of averaging egg size within clutches or when restricting analyses to a specific egg-sequence number from a specific clutch size:

$$V_p = V_g + V_{eg} + V_{Es} \tag{1}$$

Alternatively, when using a nested design:

$$V_p = V_g + V_{eg} + V_{Es} + V_c \tag{2}$$

Given these definitions, repeatability as defined by Falconer (1989) is:

$$r = (V_g + V_{eg}) / V_p \tag{3}$$

Results and discussion.—Estimates of repeatability of egg size vary considerably depending on how they are calculated (Tables 1 and 2). Using Equations 1 and 2, it is clear that estimates of repeatability based on the nested design will always be less than or equal to those based on average egg size within clutches because of the additional term (V_c) in the denominator of Equation 3. In cases where there is no variation in egg size within clutches (i.e. $V_c = 0$), estimates of repeatability based on average egg size and the nested design will be equal. Thus, ignoring within-clutch variation in egg size, if it exists, inflates estimates of repeatability. In addition, using the average of multiple measurements will inflate the estimated repeatability because the mean of two or more measures has a higher heritability than does a single measurement (Falconer 1989). Therefore, the use of average egg size to estimate repeatability will result in estimates that are biased high. Finally, estimates of repeatability rely on the assumption that different measurements reflect what is genetically the same character (Falconer 1989). All eggs in a clutch originate from the same female, and in that sense there is no genetic variation in egg size within clutches. However, in Black Brant, egg size varies with the combination of clutch size and egg-laying sequence (Flint and Sedinger 1992) and a similar pattern of egg-size variation within clutches has been shown for other species of geese (Cooper 1978, Leblanc 1987, Owen and West 1988, Robertson and Cooke 1993, Williams et al. 1993, Cooke et al. 1995). Additionally, intraclutch patterns of egg size have been shown for many species of birds with altricial

TABLE 2. Partitioning phenotypic variance in egg size of Black Brant nesting on the Yukon-Kuskokwim Delta, Alaska within and among females using ANOVA.

	Proportion of phenotypic variance		
Method of analysis[a]	Among females[b]	Within females among years	Within clutches
One-way ANOVA based on average egg size per clutch	0.7099	0.2901	
Nested ANOVA—assuming egg sequence numbers unknown	0.5118	0.1180	0.3702
Nested ANOVA—controlling for clutch size and egg sequence number	0.5584		

[a] Sample size equals 12,506 eggs from 1,246 females.
[b] Repeatability.

young (Lack 1968, Slagsvold et al. 1984). Those patterns, repeated across many species, raise the possibility that intraclutch egg-size variation may be adaptive (Lack 1968, Slagsvold et al. 1984, Williams et al. 1993, Flint et al. 1994, Williams 1994, Erikstad et al. 1998). If so, the level and pattern of variation in egg size within clutches may be genetically determined. Thus, estimating the repeatability by averaging egg size within clutches and using a one-way ANOVA would result in averaging across characters that may not be the same genetic character. Under those conditions, Falconer (1989:141) states that "repeatability becomes a somewhat vague concept, without precise meaning in relation to the components of variance." The genetic basis and genetic influence on intraclutch egg-size variation deserves further study. Therefore, we caution researchers to temper inferences drawn from estimates of repeatability that are based on mean egg size within clutches because those estimates are likely biased.

When we partitioned our data and estimated repeatability of egg size by sequence number, there was little difference in the estimated repeatability among clutch sizes or egg sequence numbers. Most of the variation among those partitioned estimates of repeatability was likely caused by sampling error. Clearly those estimates are free from bias associated with averaging multiple measurements. Further, those estimates would not be biased if all eggs within a clutch are not genetically the same character. Therefore, we conclude that the true value of egg size repeatability for Black Brant lies between 0.49 and 0.64. However, for comparative purposes, it is difficult to have a separate estimate of repeatability for each egg sequence number in each clutch size. Also, that approach can only be used if egg-laying sequence is known. For many species of birds, determination of laying sequence in field studies is not practical. The estimated repeatabilities from the two nested ANOVA (Table 2) fall within the range predicted from the individual estimates by clutch size and egg-sequence number (Table 1). We believe either approach correctly models the multiple measurements within clutches and results in a good approximation of repeatability. We suggest future studies should examine patterns of egg-size variation with egg sequence when possible. If consistent patterns of egg-size variation exist, then estimates of egg size repeatability could control for egg sequence and partition the remaining variance within and among females. If the pattern of variation with egg-sequence number varies among clutch sizes (e.g. as it does for Black Brant), then estimates of repeatability should control for the combination of clutch size and egg sequence. In the absence of data on egg size by laying sequence, we suggest the nested design partitioning variance among females, within females among years, and within clutches is most appropriate for estimating repeatability. It is useful to note that the proportion of variance attributed to differences among females (i.e. repeatability) is similar in both models. Thus, the nested design without egg-sequence numbers could be used in all cases and gives the clearest picture regarding the distribution of the variance within females.

Estimation of repeatability from large data sets can be computationally tedious (Lessells and Boag 1987), particularly if the design is unbalanced. Previous researchers have used program SAS (PROC NESTED; SAS Institute 1990) to partition the phenotypic variation in egg size (Erikstad et al. 1998, Flint and Grand 1999). That procedure is useful because it calculates the actual variance components in addition to the mean square errors (Lessells and Boag 1987). However, it is important to realize that nested ANOVA is a hierarchical analysis and results will vary depending on the order in which variables are listed in the model (Sokal and Rohlf 1981). Erikstad et al. (1998) examined variation in egg size with a nested model in the following order: year, females, clutch size, egg sequence, error. It is clear that variation within clutches (i.e. egg-sequence number) must be considered as nested within clutch sizes; however, clutch sizes should not be nested within females. Therefore, it is essential to consider the hierarchical structure of the variance when using a nested ANOVA. We recommend the following hierarchical orders (1) when egg sequence is known: clutch size, egg sequence number, female; (2) when egg sequence is unknown: female, nest attempt.

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LITERATURE CITED

COOKE, F., R. F. ROCKWELL, AND D. B. LANK. 1995. The Snow Geese of La Pèrouse Bay: Natural Selection in the Wild. Oxford University Press, New York.

COOPER, J. A. 1978. The history and breeding biology of the Canada Geese of Marshy Point, Manitoba. Wildlife Monographs, no. 61.

ERIKSTAD, K. E., T. TVERAA, AND J. O. BUSTNES. 1998. Significance of intraclutch egg-size variation in Common Eider: The role of egg size and quality of ducklings. Journal of Avian Biology 29:3–9.

FALCONER, D. S. 1989. Introduction to Quantitative Genetics, 3rd ed. John Wiley and Sons, New York.

FLINT, P. L., AND J. B. GRAND. 1996. Variation in egg size of the Northern Pintail. Condor 98:162–165.

FLINT, P. L., AND J. B. GRAND. 1999. Patterns of variation in size and composition of Greater Scaup eggs: Are they related? Wilson Bulletin 111:465–471.

FLINT, P. L., M. S. LINDBERG, M. C. MACCLUSKIE, AND J. S. SEDINGER. 1994. The adaptive significance of hatching synchrony of waterfowl eggs. Wildfowl 45:248–254.

FLINT, P. L., AND J. S. SEDINGER. 1992. Reproductive implications of egg size variation in the Black Brant. Auk 109:896–903.

LACK, D. 1968. Ecological Adaptations for Breeding in Birds. Methuen, London.

LEBLANC, Y. 1987. Intraclutch variation in egg size of Canada Geese. Canadian Journal of Zoology 56: 3044–3047.

LESSELLS, C. M., AND P. T. BOAG. 1987. Unrepeatable repeatabilities: A common mistake. Auk 104:116–121.

LESSELLS, C. M., F. COOKE, AND R. F. ROCKWELL. 1989. Is there a trade-off between egg weight and clutch size in Lesser Snow Geese (*Anser c. caerulescens*)? Journal of Evolutionary Biology 2:457–472.

OWEN, M., AND J. WEST. 1988. Variation in egg composition in semi-captive Barnacle Geese. Ornis Scandanavica 19:58–62.

ROBERTSON, G. J., AND F. COOKE. 1993. Intraclutch egg-size variation and hatching success in the Common Eider. Canadian Journal of Zoology 71:544–549.

SAS INSTITUTE. 1990. SAS/STAT User's Guide, version 6. SAS Institute Inc., Cary, North Carolina.

SLAGSVOLD, T., J. SANDVIK, G. ROFSTAD, Ö. LORENTSEN, AND M. HUSBY. 1984. On the adaptive value of intraclutch egg-size variation in birds. Auk 101:685–697.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry, 2nd ed. W. H. Freeman and Company, New York.

WILLIAMS, T. D. 1994. Intraspecific variation in egg size and egg composition in birds: Effects on offspring fitness. Biological Reviews of the Cambridge Philosophical Society 68:35–59.

WILLIAMS, T. D., D. B. LANK, AND F. COOKE. 1993. Is intraclutch egg-size variation in the Lesser Snow Goose adaptive? Oikos 67:250–256.

Effect of Investigator Disturbance on Nest Attendance and Egg Predation in Eurasian Oystercatchers

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ABSTRACT.—Eurasian Oystercatchers (*Haematopus ostralegus*) breeding on the salt marsh of Schiermonnikoog (Dutch Wadden Sea) lose many eggs to predators, mainly Herring (*Larus argentatus*) and Mew gulls (*L. canus*). We estimated that the probability for an egg to survive from laying until hatching was 69%. Daily egg mortality was higher during the laying period than during the incubation period. When researchers were present in the study area, oystercatchers spent more time at greater distances from the nest. We investigated whether human disturbance resulted in more eggs being lost to predators. Two experimental areas were in turn visited at high and at low frequency. From a preliminary analysis, we estimated higher daily egg mortality rates when nests were checked three times per day instead of once every oth-

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