

Maternal effects and offspring performance: in search of the best method

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Traditionally, maternal effects have been treated as a source of troublesome environmental variance that confounds our ability to accurately estimate the genetic basis of the traits of interest. However, the adaptive significance of maternal effects is currently at the centre of the attention of ecologists. Thus, in turn, the genetic basis of traits has become a troublesome source of the genetic resemblance that confounds our ability to accurately estimate the maternal effects of interest. This fact is, however, less widely realized among ecologists. We demonstrate this on the example of studies investigating egg-size effects on offspring performance in birds. Traditionally this relationship is being studied by cross-fostering of eggs or young and it is claimed that this design is able to separate the effects of egg size per se. However, a positive covariation between the direct effects of genes and the maternal effects exists for many studied traits, which may result in overestimation of the egg-size effects on offspring performance in cross-fostering studies. Within-clutch comparisons or direct experimental manipulation of the egg size are the approaches that do not suffer from such covariation and therefore give less biased estimates of the egg-size effects than cross-fostering studies.

Maternal effects in animal ecology

Offspring phenotype is determined by genes and the environment. Besides the direct effect of genes and the environment, maternal effects often play a significant role. Previously, maternal effects have been treated as the source of confusion in determining precisely quantitative genetic parameters (Falconer 1989). However, it is now widely appreciated that they can play an important role in driving the dynamics of evolution and population growth. Specifically, by introducing time lags into both these processes, they may lead to unpredictable evolutionary trajectories (Kirkpatrick and Lande 1989) and destabilization of population dynamics, e.g. in the form of population cycles or decaying oscillations (Ginzburg 1998, Beckerman et al. 2002). Moreover, at the individual level, maternal effects may influence offspring fitness and thus serve both offspring and parents as adaptations. This adaptive significance of maternal effects has recently become a popular focus of ecological

and evolutionary studies (Mousseau and Fox 1998). Traditionally, maternal effects have been studied in domesticated species by complex analyses (Lynch and Walsh 1998), which is not easily applicable to free-ranging animals. Moreover, these analyses just partition the variance in the focal trait and determine which part of this variance can be ascribed to maternal effects in general. Lande and Price (1989) devised a regression method based on Kirkpatrick and Lande (1989) that is able to isolate maternal effects specific for certain maternal traits. However, this method requires that all the maternal characters exerting maternal effects be included in the analyses (rather unrealistic condition) and is not readily applicable to sex-limited characters. Below we evaluate other methods for studying maternal effects employed in wild populations, including sex-limited characters, on the example of the effects of egg size on offspring performance in birds.

Studying egg-size effects on offspring performance is important for two areas of evolutionary ecology. One, life-history theory predicts a trade-off between number and quality of offspring produced from limited resources. Two, potentially adaptive allocation of limited resources among siblings within a clutch is widely studied in a broad range of species. The critical assumption in both these cases is that the amount of resources allocated to an egg has an effect on offspring performance. Consequently, egg-size effects on offspring performance are among the most frequently studied topics in the area of maternal effects and birds are the taxon in which these effects have been studied most often. We are aware of at least 60 studies dealing with this question in birds, 41 of which were reviewed by Williams (1994). However, the approaches usually employed do not control for potential confounding factors. Consequently, despite high research attention, results of many studies estimating egg-size effects on offspring performance may be biased.

Quantitative genetic framework

To demonstrate how the effect of egg size per se on offspring performance can be derived, it is useful to frame the problem in the quantitative genetics terms. From the quantitative genetics perspective, the phenotypic value of each trait can be partitioned into the components attributable to genes (genotypic value) and the environment (environmental deviation) (Falconer 1989). The genotypic value can be further partitioned into the breeding value (determined by additive effect of genes), the dominance deviation (interactions of alleles within the same locus) and the interaction deviation (interactions of alleles between loci, i.e. epistasis). In this basic framework, maternal effects are subsumed under the environmental effects, because they are defined as the non-genetic influence of the maternal phenotype on the offspring's phenotype (Kirkpatrick and Lande 1989).

For our purposes, egg size is singled out as the maternal effect of interest, whereas all other maternal effects (e.g. parental feeding, concentration of testosterone in the egg) and pure environmental effects (e.g. weather, food supply) are grouped together as the offspring environment. Consequently, the phenotypic value of each offspring's trait (z_x) can be viewed as being determined by three sources: the direct effect of genes (G_{ox}), the offspring's environment E_x , and the egg size (S_m) which is itself compounded of an environmental (E_{mw}) and a genetic (G_{mw}) component (Fig. 1). Here, subscript x denotes an offspring and w mother; o is direct pathway of determining offspring phenotype, m denotes maternal (indirect) pathway through egg size. Throughout, we assume that direct and indirect genetic

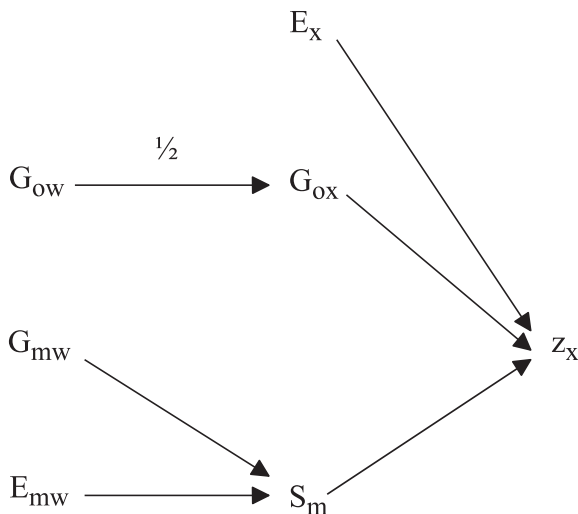


Fig. 1. Path diagram depicting the determination of the phenotype z_x of an individual x by direct genetic effects G_{ox} , environmental effects E_x (including also all parental effects except of egg size) and egg size S_m . The mother of x is denoted by w. O denotes direct pathway, m denotes maternal pathway. Modified from Lynch and Walsh (1998).

effects, G_{ox} and G_{mw} , contain additive effects but do not exhibit dominance or epistasis. Note that E_x has neither o nor m subscript, because it includes both direct and maternal effects, and S_m has neither w nor x subscript, because it is the trait of both the mother and the offspring.

If the three sources determining the traits of offspring were uncorrelated, a simple statistical technique such as the linear regression would give a reliable estimate of egg-size effects on any of offspring traits (except of daughter's egg size). However, when these sources are correlated, experimental or statistical techniques will be needed to separate egg-size effects per se.

A review of methods

In 1990 it was suggested that the size of the egg a female lays might be positively correlated with the quality of her territory or subsequent parental feeding rate to young (i.e. positive $CovE_xS_m$ exists) and that this correlation can be removed by experimental swapping of clutches/broods between nests – a technique known as cross-fostering (Amundsen and Stokland 1990, Reid and Boersma 1990). In both these studies, the authors found that the size of the eggs which foster mothers originally laid was a more influential determinant of offspring traits at fledging than the size of the egg from which the offspring actually hatched. This suggests that when cross-fostering is not performed, egg-size effects on offspring performance are highly overestimated. Therefore cross-fostering became a very popular technique to study egg-size effects on offspring performance (we know of 16 such studies performed to date).

However, although cross-fostering decouples much of correlation of egg size with offspring environment ($CovE_xS_m$, below, Table 1), it does not deal with the possible covariation between direct effect of genes and egg size ($CovG_{ow}S_m$). Yet, this covariation is likely to be large in many cases because of the choice of 'fitness' measure usually employed in studies of this kind. In practice, instead of studying directly the effects of egg size on offspring fitness, we usually study the effects on some correlate of fitness. Morphological traits of fledglings such as tarsus length or body weight are frequently used as these correlates. However, female size is frequently positively correlated with the size of eggs she lays (Christians 2002) and at the same time morphological traits are highly heritable (Merilä and Sheldon 2001). This means that the size of the trait in the offspring is highly influenced by direct effect of additive genes but this effect is ascribed (to the extent to which additive genes for mother body size are involved in the correlation between female size and egg size) to the effect of egg size in the cross-fostering design. In principle this may be a problem in every studied trait including offspring

Table 1. Summarization of relative merits and shortcomings of the individual approaches. $CovE_xS_m$ is a covariance between environmental effects and egg size, $CovG_{ow}S_m$ is a covariance between direct additive genetic effects and egg size.

Approach	Controls for	Remains uncontrolled	Main use
Observational	Nothing	$CovE_xS_m$ $CovG_{ow}S_m$	Do not use
Cross-fostering	Partially $CovE_xS_m$	$CovE_xS_m$ (pre-laying parental effects and differential feeding of young) $CovG_{ow}S_m$	Use with caution (egg size-number trade-off)
Within-clutch	Partially $CovE_xS_m$ Fully $CovG_{ow}S_m$	$CovE_xS_m$ (pre-laying parental effects and differential feeding of young)	Within-clutch adaptive allocation of resources
Manipulation	Partially $CovE_xS_m$ Fully $CovG_{ow}S_m$	$CovE_xS_m$ (differential feeding of young)	For all purposes

survival. Cross-fostering thus does not reveal effects of egg size per se, which was realized only rarely (Magrath 1992, Styrsky et al. 1999) and was not mentioned in the most recent cross-fostering studies (Hipfner et al. 2001, Bize et al. 2002, Pelayo and Clark 2003). The covariation $CovG_{ow}S_m$ can arise either through $CovG_{ow}G_{mw}$ if, for example, the same gene facilitates the conversion of food into yolk in a female and food into flesh in a nestling (Magrath 1992) or through $CovG_{ow}E_{mw}$ if, for example, larger females or males (more precisely individuals with larger breeding values for body size) attain better territories which enable females to produce larger eggs. These covariations have not been quantified in birds so far, however, in mammals it has been found that the genetic covariation between direct and maternal pathways of determining the offspring phenotype might be high (Riska et al. 1985, McAdam et al. 2002). Thus it does not seem reasonable to assume that a similar covariation does not exist in other taxa.

The direct effect of additive genes may be controlled statistically by including the midparent value of the trait as a covariate in the analysis of egg-size effects on the same trait in offspring. Although this has been done with the maternal value of the trait in some observational studies (Larsson and Forslund 1992, Potti and Merino 1994), it has never been done in any cross-fostering study investigating egg-size effects on offspring performance. In some cases addition of such a covariate may be relatively easy – for example when investigating egg-size effects on fledging tarsus length, which is fully grown at the time of fledging in many species. However, this might be very burdensome when investigating traits that can be measured only in offspring (e.g. growth rate) and impossible when investigating survival of offspring up to recruitment, because all parents were successfully recruited.

However, two other approaches that do not suffer from $CovG_{ow}S_m$ can be used to investigate egg-size effects on offspring performance. First, effects of egg size on offspring performance may be compared within clutches. So far, this approach has been used less often

than the cross-fostering approach (we know of nine studies using the within-clutch approach, e.g. Dawson and Clark 1996, Amat et al. 2001), and its advantage over cross-fostering was not mentioned in any of these studies. Among-clutch variation in egg size may be removed by centring egg sizes within clutches, i.e. by subtracting mean egg size of the clutch from the actual size of every egg in the clutch. Resulting values represent within-clutch variation and as such are then used in the statistical analyses. Given that chromosomes segregate at random in meiosis, $CovG_{ox}S_m$ is zero among full-sibs. Non-zero $CovG_{ox}S_m$ could arise if the female was able to recognize which allele of an allelic pair had come to the ovum in meiotic division and targeted resources accordingly or to control the outcome of meiosis in relation to the size of ovum to be ovulated. Such high female control, however, seems highly unlikely for alleles on autosomes or homologous parts of sex-chromosomes. On the other hand, targeting of resources might perhaps work in relation to genes that are located at non-homologous parts of sex-chromosomes, such as sex-determining genes, as suggested by studies demonstrating differences in egg size between the sexes (Cordero et al. 2000).

In the within-clutch approach, territory and parents are the same for all sibs and that is why also $CovE_xS_m$ is controlled to a similar degree as in cross-fostering design (Table 1). Strictly speaking, however, $CovE_xS_m$ need not be zero both in within-clutch and cross-fostering design. Firstly, egg size may be correlated with other pre-laying maternal effects, for example concentration of hormones, antibodies or carotenoids in the egg. In this case the amount of these compounds would increase allometrically with egg size (slope of the regression of the amount of a compound on egg size would differ from one) contrary to the situation when it would increase isometrically with egg size (slope would equal one). In the latter case, the amount of these compounds may be treated as being a part of the egg size. Secondly, parents may feed more intensely small (or large) young which hatched from small (or large) eggs. This effect may be

stronger in within-clutch approach, because offspring are raised in the same nest and larger sibs may monopolize resources supplied by parents. Moreover, the problem that is specific for the within-clutch approach is that within-clutch (and also within-female) variation in egg size is usually much smaller than inter-female variation. On average, differences in egg size within clutches explain only 30% of the total egg-size variation (Christians 2002). Thus effects of great differences in egg size, which exist at the population level, cannot be directly estimated by within-clutch approach. On the other hand, there are many studies investigating adaptive allocation of resources among individual eggs within a clutch in relation to laying order (Slagsvold et al. 1984) and sex (Cordero et al. 2000). These studies rely on the assumption that within-clutch variability in egg size has some consequences for offspring performance. This assumption may be properly tested by the within-clutch approach outlined above. Possible monopolization of resources by larger siblings and small differences in egg size within clutches are not problems in this context, because they are inherent features of the relationships among young within a clutch.

The second approach to remove $CovG_{ow}S_m$ is the direct manipulation of egg volume. This method is the best way to elucidate potential effects of egg size on offspring performance (Sinervo et al. 1992). In birds, it was used, to our best knowledge, only twice on domesticated species under laboratory conditions (Hill 1993, Finkler et al. 1998). In these studies, certain part of the albumen or yolk of unincubated eggs was removed by a syringe and a needle. Such an egg size manipulation removes also the potential correlation between egg size and other pre-laying maternal effects, which is an additional advantage compared to the other approaches. Given the strengths of this approach, it could become a powerful tool in elucidating effects of egg size on offspring performance also in populations of wild-ranging birds. However, although invasive egg sampling and manipulation have been successfully applied to some wild bird species (Lipar 2001, Saino et al. 2003), rather high egg mortality encountered in the study on hens mentioned above (Finkler et al. 1998) seems to question broad applicability of this method. Moreover, we have no information on how big changes in egg volume in comparison with the natural egg-size variability are within the reach of this method, while at the same time keeping egg mortality within acceptable limits. Both these issues remain to be addressed in studies on wild species. The manipulative approach also suffers from the possibility that parents may feed their young selectively with respect to their size, which also means to the size of the egg they hatched from. This could be controlled for by statistically controlling for the amount of food brought to individual offspring by their

parents or by hand-rearing of the young (Anderson et al. 1997).

Conclusions

In this comment we evaluated relative merits and shortcomings of the different approaches to the study of egg-size effects on offspring performance in birds (Table 1). It has been accepted that the cross-fostering design is better than the simple observational approach and thus it became a standard methodological tool. We argue that there are even better approaches that should give less biased estimates of egg-size effects: the within-clutch approach and the direct experimental manipulation of egg-size. These approaches are relatively readily applicable to free-ranging populations of animals and plants. Therefore, further studies using these approaches would be valuable for better understanding of the evolution and impact of maternal effects and also for the evaluation of how much the traditional approaches for studying adaptive maternal effects suffer from uncontrolled confounding factors. We focused our attention on egg-size effects in birds because this is one of the best-studied systems in the area of maternal effects and much effort has been devoted to it. However, general logic of our argument applies equally well to other traits and other taxa.

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References

- Amat, J. A., Fraga, R. M. and Arroyo, G. M. 2001. Intraclutch egg-size variation and offspring survival in the kentish plover *Charadrius alexandrinus*. – *Ibis* 143: 17–23.
- Amundsen, T. and Stokland, J. N. 1990. Egg size and parental quality influence nestling growth in the shag. – *Auk* 107: 410–413.
- Anderson, D. J., Reeve, J. and Bird, D. M. 1997. Sexually dimorphic eggs, nestling growth and sibling competition in american kestrels *Falco sparverius*. – *Funct. Ecol.* 11: 331–335.
- Beckerman, A., Benton, T. G., Ranta, E. et al. 2002. Population dynamic consequences of delayed life-history effects. – *Trends Ecol. Evol.* 17: 263–269.
- Bize, P., Roulin, A. and Richner, H. 2002. Covariation between egg size and rearing condition determines offspring quality: an experiment with the alpine swift. – *Oecologia* 132: 231–234.
- Christians, J. K. 2002. Avian egg size: variation within species and inflexibility within individuals. – *Biol. Rev.* 77: 1–26.
- Cordero, P. J., Griffith, S. C., Aparicio, J. M. et al. 2000. Sexual dimorphism in house sparrow eggs. – *Behav. Ecol. Sociobiol.* 48: 353–357.

- Dawson, R. D. and Clark, R. G. 1996. Effects of variation in egg size and hatching date on survival of lesser scaup *Aythya affinis* ducklings. – *Ibis* 138: 693–699.
- Falconer, D. S. 1989. Introduction to quantitative genetics. – Longman Scientific & Technical.
- Finkler, M. S., Van Orman, J. B. and Sotherland, P. R. 1998. Experimental manipulation of egg quality in chickens: influence of albumen and yolk on the size and body composition of near-term embryos in a precocial bird. – *J. Comp. Physiol. B* 168: 17–24.
- Ginzburg, L. R. 1998. Inertial growth: population dynamics based on maternal effects. – In: Mousseau, T. A. and Fox, C. V. (eds), *Maternal effects as adaptations*. Oxford Univ. Press, pp. 42–53.
- Hill, W. L. 1993. Importance of prenatal nutrition to the development of a precocial chick. – *Develop. Psychobiol.* 26: 237–249.
- Hipfner, J. M., Gaston, A. J. and Storey, A. E. 2001. Food supply and the consequences of egg size in the thick-billed murre. – *Condor* 103: 240–247.
- Kirkpatrick, M. and Lande, R. 1989. The evolution of maternal characters. – *Evolution* 43: 485–503.
- Lande, R. and Price, T. 1989. Genetic correlations and maternal effect coefficients obtained from offspring–parent regression. – *Genetics* 122: 915–922.
- Larsson, K. and Forslund, P. 1992. Genetic and social inheritance of body and egg size in the barnacle goose (*Branta leucopsis*). – *Evolution* 46: 235–244.
- Lipar, J. L. 2001. Yolk steroids and the development of the hatching muscle in nestling european starlings. – *J. Avian Biol.* 32: 231–238.
- Lynch, M. and Walsh, B. 1998. *Genetics and analysis of quantitative traits*. – Sinauer Associates, Inc.
- Magrath, R. D. 1992. The effect of egg mass on the growth and survival of blackbirds: a field experiment. – *J. Zool.* 227: 639–653.
- McAdam, A. G., Boutin, S., Réale, D. et al. 2002. Maternal effects and the potential for evolution in a natural population of animals. – *Evolution* 56: 846–851.
- Merilä, J. and Sheldon, B. C. 2001. *Avian quantitative genetics*. – In: Nolan, V. (ed.), *Current ornithology*, Kluwer Academic/Plenum Publishers.
- Mousseau, T. A. and Fox, C. W. 1998. *Maternal effects as adaptations*. – Oxford Univ. Press.
- Pelayo, J. T. and Clark, R. G. 2003. Consequences of egg size for offspring survival: a cross-fostering experiment in ruddy ducks (*Oxyura jamaicensis*). – *Auk* 120: 384–393.
- Potti, J. and Merino, S. 1994. Heritability estimates and maternal effects on tarsus length in pied flycatchers, *Ficedula hypoleuca*. – *Oecologia* 100: 331–338.
- Reid, W. J. and Boersma, P. D. 1990. Parental quality and selection on egg size in the magellanic penguin. – *Evolution* 44: 1780–1786.
- Riska, B., Rutledge, J. J. and Atchley, W. R. 1985. Covariance between direct and maternal genetic effects in mice, with a model of persistent environmental influences. – *Genet. Res.* 45: 287–297.
- Saino, N., Ferrari, R., Romano, M. et al. 2003. Experimental manipulation of egg carotenoids affects immunity of barn swallow nestlings. – *Proc. R. Soc. Lond. B* 270: 2485–2489.
- Sinervo, B., Doughty, P., Huey, R. B. et al. 1992. Allometric engineering: a causal analysis of natural selection on offspring size. – *Science* 258: 1927–1930.
- Slagsvold, T., Sandvik, J., Rofstad, G. et al. 1984. On the adaptive value of intraclutch egg-size variation in birds. – *Auk* 101: 685–697.
- Styrsky, J. D., Eckerle, M. P. and Thompson, C. F. 1999. Fitness-related consequences of egg mass in nestling house wrens. – *Proc. R. Soc. Lond. B* 266: 1253–1258.
- Williams, T. D. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. – *Biol. Rev.* 69: 35–59.