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Forum

Experience counts: lessons from studies of differential allocation

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One of the most fundamental decisions that female birds face after pairing is how much to invest in a particular reproductive attempt (Zhang et al., 1996). According to differential allocation (DA) theory, females are able to adjust their investment according to factors that affect the value of the breeding attempt, such as the attractiveness of their mate. A higher value is placed on matings with attractive males because of direct and/or indirect fitness benefits (Andersson, 1982; Hamilton and Zuk, 1982; Hoelzer, 1989; Norris, 1990), and therefore females should be willing to bear greater costs and invest more in offspring fathered by such males (Burley, 1988; Sheldon, 2000). However, in direct contrast is the compensation hypothesis, in which a female is predicted to compensate for reduced offspring viability when paired to a lower quality male, and the reverse allocation pattern occurs (Bluhm and Gowaty, 2004a,b; Michl et al., 2005; Saino et al., 2002a).

Avian research on DA began with Burley's now classic work on zebra finches (*Taeniopygia guttata*), in which females mated to more attractive males fed their offspring more frequently than females mated to unattractive males (Burley, 1988). Evidence in support of DA has since accumulated in a number of species (primarily in birds) and in a variety of different forms, with most recent work focusing on primary reproductive investment. For example, females have been found to alter their investment at egg laying by producing more, larger or higher quality eggs when mated to an attractive male (Cunningham and Russell, 2000; Gil et al., 1999; Petrie and Williams, 1993; Saino et al., 2002b).

Fundamental to DA are three basic assumptions. First, that females do allocate differentially. Second, that in order to do so they are able to respond in a flexible manner to environmental and social cues. Third, that a trade-off exists between current and future reproductive effort, such that elevated investment during one reproductive episode will be traded-off against a future episode (Sheldon, 2000). This trade-off is fundamental to understanding why a female should withhold investment in a current breeding attempt if a potentially more valuable breeding attempt should later present itself. Experimental manipulations are necessary to provide convincing evidence of DA (Sheldon, 2000), and crossover designs are widely used in such experiments (e.g., Balzer and Williams, 1998; Cunningham and Russell, 2000; Gil et al., 1999; Petrie and Williams, 1993). Here, individuals are assigned randomly to one of two treatments in the first experimental round, and then treatments are swapped in the second round, thus using each individual as its own control (Ruxton and Colegrave, 2003). Crossover designs are particularly useful in studies of DA because as well as being statistically powerful they provide a test of individual flexibility, which is necessary for DA to have evolved. In this article we use examples from our own zebra finch research and other studies to discuss how crossover designs can often produce complex carryover effects. These complicate the interpretation of the results (a warning made by Ruxton and Colegrave, 2003) but may themselves prove informative with respect to issues not previously considered in DA. For example, further investigation of carryover effects and the effects of prior female experience in general on current reproductive decisions may reveal interesting adaptations and provide some insight into the subtleties behind DA.

Example 1: manipulations of male attractiveness

In the first of our experiments (Rutstein et al., 2004), we used a crossover design to compare egg size when zebra finch females (with previous breeding experience) were mated with attractive and unattractive males. In the first breeding round, we found evidence of DA with respect to egg size (Figure 1a). Females mated to attractive males laid heavier eggs, and there was a positive relationship between female mass and egg mass, such that heavier females laid heavier eggs. This relationship did not hold for females mated to unattractive males, suggesting that heavy females were suppressing their investment when paired with an unattractive mate, in line with predictions from the DA hypothesis (Sheldon, 2000). Surprisingly, however, in the second breeding round (after a four week break from the first male) individual females maintained the same pattern of investment as in the first round (Figure 1b), even though the attractiveness of their mates had been reversed—a classic carryover effect. Therefore, although the second round could have reflected a switch in female allocation patterns to a compensatory strategy (Bluhm and Gowaty, 2004b), the more likely explanation is that female investment in egg size was inflexible in response to changes in mate attractiveness. In addition, there was no evidence of a trade-off in terms of egg size in the second breeding round, thus undermining a second fundamental assumption of DA.

Although this lack of individual flexibility appears completely contrary to the expectations of DA, the carryover effect between breeding rounds may be a consequence of the experimental design. Data from wild zebra finches suggest that when breeding conditions are favorable, females breed several times in a year, but subsequent breeding attempts are likely to be with the same male (Zann, 1996). In this situation females would not need to change their investment in the second breeding attempt. However, zebra finches may live up to 5 years in the wild, and females may mate with several different males (Zann, 1996), so DA would be predicted under these circumstances. Because of the greater likelihood of mortality between (when compared to within) seasons, females are more likely to have a new mate at the start of a new breeding season. A change in resource allocation therefore might have occurred in our experiment if females were left for a longer period between breeding rounds.

Using a similar crossover design, Gil et al. (1999) found that female zebra finches altered their yolk steroid deposition depending on the attractiveness of their mate. Females deposited greater quantities of yolk androgens in their eggs when mated to a more attractive male in both breeding rounds of the experiment. An important difference between our experimental design and the experimental design of Gil et al. (1999) was that the experiment of Gil et al. left females with their mates only

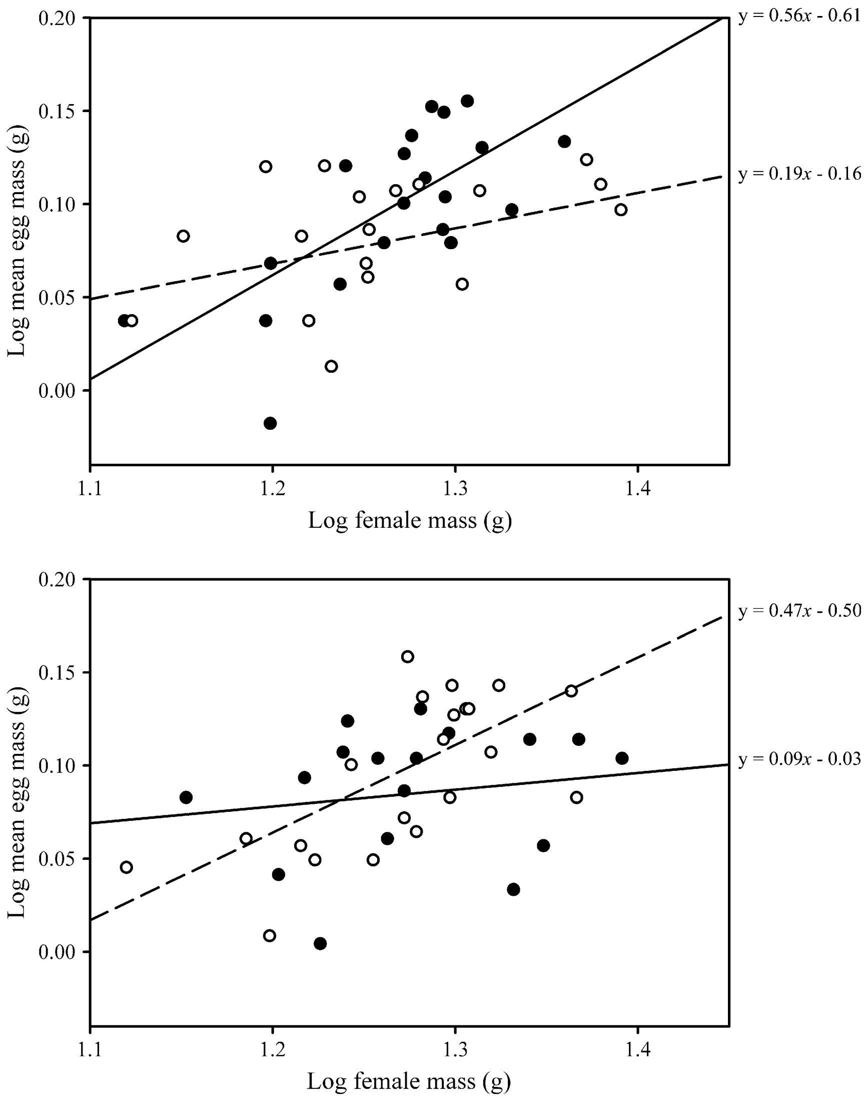

Figure 1

Allometric relationship between mean log egg mass and log female mass for females mated to red- and green-ringed males in (a) round one and (b) round two. The regression equations are given in the form: $y = a + bx$, where y = mean log egg mass, x = log female mass. Females mated to red-ringed males are shown by filled circles and solid regression lines. Females mated to green-ringed males are shown by open circles and dashed regression lines. In a paired, repeated-measures general liner model for both breeding rounds ($n = 39$ females) there was a significant interaction between ring color, female body mass, and breeding round with respect to egg mass ($F_{1,37} = 6.30$, $p = .02$). In round one there was a significant positive relationship between egg mass and body mass for females mated to red-ringed males ($F_{1,20} = 16.30$, $p = .0007$) but not for females mated to green-ringed males ($F_{1,15} = 1.99$, $p = .18$). In round two, there was a significant positive relationship between egg mass and body mass for females mated to green-ringed males ($F_{1,20} = 16.15$, $p = .0007$) but not for females mated to red-ringed males ($F_{1,14} = 0.57$, $p = .46$).

for the time it took to lay a single clutch, and no chicks were reared. In contrast, in our study, pairs were together for 2 months while they reared chicks. In the experiment of Gil et al. (1999), the removal of the first clutch may have had an effect similar to that of a failed breeding attempt in the wild, and this may have critically altered the female's perception of its mate. These two experiments highlight an important problem with experimental tests of DA. Different conclusions could be drawn as a result of subtle differences in experimental design and/or because the response variable being measured (egg size or yolk androgens) differs in plasticity.

Example 2: manipulation of diet

A second situation in which females are predicted to adjust their reproductive investment is in relation to resource quality. Dietary studies differ somewhat from those on mate attractiveness because there are well-established nutritional constraints acting on egg size and quality (Bolton et al., 1992; Houston et al., 1995; Smith et al., 1993; Williams, 1996). However, the pattern of smaller, lighter clutches when on a poorer quality diet may also result from maternal DA. When resources are limited, reproduction is more costly in terms of a female's body condition and future reproductive potential. In addition, fewer offspring can be reared (Perrins, 1970), and these offspring may be of lower reproductive value (Hochachka, 1992; Hussell, 1972).

Carryover effects have also been reported in dietary experiments. Williams (1996) found that female zebra finches on a protein-supplemented diet continued to lay heavier eggs for 3 weeks after the diet was stopped (Williams, 1996). His explanation was that females might store some limiting amino acid after the diets were switched. We have found similar carryover effects in zebra finch diet crossover experiments in relation to yolk androgen investment as well as egg size (Rutstein et al., 2005). Such carryover effects may not only be physiological but might reflect the female's perception of environmental stability and thus the future rearing diet of the chicks. When diets are swapped after a relatively long period of time on one experimental diet, females may perceive this as only a temporary change in resource quality at the time of egg laying and so may not adjust their primary investment.

In summary, the carryover effects in our experiments appear to undermine two basic assumptions behind DA. First, although DA is evident in the experiments' first rounds, females do not appear to be flexible to subsequent changes in their social or nutritional conditions. Second, the strong carryover effects are in contrast with the expectations of a trade-off. Carryover effects reveal a lack of plasticity in allocation in the context of DA, and therefore they are clearly phenomena that require exploration in order to properly understand the adaptive flexibility of females and to understand how female experience affects current reproductive decisions. We therefore believe that it is important to find out how widespread carryover effects are, under what circumstances they persist, and the timescale over which they operate. Some aspects of reproductive investment will be more flexible than others. Behaviors such as chick provisioning are probably more flexible than, for example, altering egg size, which is likely to be more physiologically constrained. In addition, the size and the duration of the carryover effect are likely to depend on the species, the treatments, and the experimental design. For example, in dietary experiments on zebra finches, the carryover effect seems more pronounced when females are not permitted to rear chicks compared with experiments where females do rear chicks (Rutstein, unpublished data). This may be because females in the former situation maintain better body condition as chick rearing is a highly demanding activity (Monaghan and Nager, 1997). Furthermore, chick rearing may directly affect the female's perception of the breeding attempt and of her mate. In many DA studies eggs are removed as soon as the clutch is laid (e.g., Balzer and Williams, 1998; Cunningham and Russell, 2000; Gil et al., 1999; Zann and Runciman, 2003). This is likely to critically alter the female's perception of the quality of her first mate and therefore her investment when paired with her second mate.

The importance of female experience in other areas of behavioral ecology, such as mate choice has been well documented (e.g., Collins, 1995; Downhower and Lank, 1994; Ophir and Galef, 2004; Qvarnstrom et al., 2004). Previous experience of a particular male has been found to have a significant effect on current mate preferences. However, the effects of female experience with a previous mate continues to be overlooked in studies of DA, and its possible influences have not been explicitly tested to date. This is in spite of the fact that current resource allocation is predicted to depend crucially on the future expected quality of mates (Sheldon, 2000), which in turn is likely to be determined by past experience. For this reason we believe that careful consideration of experimental design in DA studies is necessary, and we recommend that future research should be directed towards explicitly testing the influence of female experience on current resource allocation decisions.

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REFERENCES

Andersson S, 1982. Sexual selection, natural selection, and quality advertisement. Biol J Linn Soc 17:375–393.

Balzer AL, Williams TD, 1998. Do female zebra finches vary primary reproductive effort in relation to mate attractiveness? Behaviour 135:297–309.

Bluhm CK, Gowaty PA, 2004a. Social constraints on female preferences in mallards, *Anas platyrhynchos*, decrease offspring viability and mother productivity. Anim Behav 68:977–983.

Bluhm CK, Gowaty PA, 2004b. Reproductive compensation for offspring viability deficits by female mallards, *Anas platyrhynchos*. Anim Behav 68:985–992.

Bolton M, Houston DC, Monaghan P, 1992. Nutritional constraints on egg formation in the lesser black-backed gull: an experimental study. J Anim Ecol 61:521–532.

Burley N, 1988. The differential-allocation hypothesis—an experimental test. Am Nat 132:611–628.

Collins SA, 1995. The effect of recent experience on female choice in zebra finches. Anim Behav 49:479–486.

Cunningham EJA, Russell AF, 2000. Egg investment is influenced by male attractiveness in the mallard. Nature 404:74–77.

Downhower JF, Lank DB, 1994. Effects of previous experience on mate choice by female mottled sculpins. Anim Behav 47:369–372.

Gil D, Graves J, Hazon N, Wells A, 1999. Male attractiveness and differential testosterone investment in zebra finch eggs. Science 286:126–128.

Hamilton WD, Zuk M, 1982. Heritable true fitness and bright birds: a role for parasites? Science 218:384–387.

Hochachka WM, 1992. How much should reproduction cost? Behav Ecol 3:42–52.

Hoelzer GA, 1989. The good parent process of sexual selection. Anim Behav 38:1067–1978.

Houston DC, Donnan D, Jones PJ, 1995. The source of nutrients required for egg production in zebra finches *Poephila guttata*. J Zool (Lond) 235:469–483.

Hussell DJT, 1972. Factors affecting clutch size in Arctic passerines. Ecol Monogr 42:317–364.

Michl G, Torok J, Peczely P, Garamszegi LZ, Schwabl H, 2005. Female collared flycatchers adjust yolk testosterone to male age, but not to attractiveness. Behav Ecol 16:383–388.

Monaghan P, Nager RG, 1997. Why don't birds lay more eggs? Trends Ecol Evol 12:270–274.

Norris KJ, 1990. Female choice and the quality of parental care in the great tit *Parus major*. Behav Ecol Sociobiol 27:275–281.

Ophir AG, Galef BG, 2004. Sexual experience can affect use of public information in mate choice. Anim Behav 68:1221–1227.

Perrins CM, 1970. The timing of birds breeding seasons. Ibis 112: 242–255.

Petrie M, Williams A, 1993. Peahens lay more eggs for peacocks with larger trains. Proc R Soc Lond B 251:127–131.

Qvarnstrom A, Blomgren V, Wiley C, Svedin N, 2004. Female collared flycatchers learn to prefer males with an artificial novel ornament. Behav Ecol 15:543–548.

Rutstein AN, Gilbert L, Slater PJB, Graves JA, 2004. Mate attractiveness and primary resource investment in the zebra finch. Anim Behav 68:1087–1094.

Rutstein AN, Gilbert L, Slater PJB, Graves JA, 2005. Sex-specific patterns of yolk androgen allocation depend on maternal diet in zebra finches. Behav Ecol 16:62–69.

Ruxton, GD, Colegrave, N, 2003. Experimental design for the life sciences. Oxford: Oxford University Press.

Saino N, Bertacche V, Ferrari RP, Martinelli R, Moller AP, Stradi R, 2002a. Carotenoid concentration in barn swallow eggs is influenced by laying order, maternal infection and paternal ornamentation. Proc R Soc Lond B 269:1729–1733.

Saino N, Ferrari R, Martinelli R, Romano M, Rubolini D, Møller AP, 2002b. Early maternal effects mediated by immunity depend on sexual ornamentation of the father in the barn swallow (*Hirundo rustica*). Proc R Soc Lond B 269:1005–1009.

Sheldon BC, 2000. Differential allocation: tests, mechanisms and implications. Trends Ecol Evol 15:397–402.

Smith HG, Ottosson U, Ohlsson T, 1993. Interclutch variation in egg mass among starlings *Sturnus vulgaris* reflects female condition. Ornis Scand 24:311–316.

Williams TD, 1996. Variation in reproductive effort in female zebra finches (*Taeniopygia guttata*) in relation to nutrient-specific dietary supplements during egg laying. Physiol Zool 69:1255–1275.

Zann R, Runciman D, 2003. Primary sex ratios in zebra finches: no evidence for adaptive manipulation in wild and semi-domesticated populations. Behav Ecol Sociobiol 54:294–302.

Zann RA, 1996. The zebra finch. New York: Oxford University Press.

Zhang DY, Jiang XH, Zhao SL, 1996. Evolutionarily stable reproductive strategies in sexual organisms. 2. Dioecy and optimal resource allocation. Am Nat 147:1115–1123.