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## INDIVIDUAL AND RESOURCE-DEPENDENT VARIATION IN ABILITY TO LAY SUPRANORMAL CLUTCHES IN RESPONSE TO EGG REMOVAL

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**ABSTRACT.**—We investigated individual and resource-dependent variation in ability of female Zebra Finches (*Taeniopygia guttata*) to lay supranormal clutches in response to egg removal, and effect of continuous laying on (1) egg composition and (2) plasma yolk precursor levels. Egg removal significantly increased the number of eggs that individual females laid, but that response was diet-dependent: on a high-quality (egg-supplemented) diet, females laid  $12.4 \pm 1.0$  more eggs compared with their pretreatment clutch size; whereas on the low-quality (seed-only) diet, females laid only  $4.9 \pm 1.2$  more eggs. Removal clutch size (i.e. total number of eggs laid in response to egg removal) was positively correlated with pretreatment mean egg mass and clutch size on the low-quality diet, but not on the high-quality diet. That suggests that there is interindividual variation in egg-laying ability (“large-egg” females had a greater capacity to respond to egg removal than “small-egg” females), but that higher resource levels can overcome individual differences. Egg mass did not vary with laying sequence in supranormal clutches (up to 22 eggs); however, there was a significant decrease (6%) in yolk protein content of additional eggs that was apparent by the tenth egg laid (i.e. only 4–5 more than the normal clutch size). Plasma levels of the two yolk precursors, vitellogenin and very-low-density lipoprotein (VLDL), were independent of diet and did not differ in individual birds at the 1 egg stage versus the 14 egg stage. However, there was a systematic change in relationship between yolk lipid content and plasma VLDL levels, from nonsignificant for third-laid eggs to significant and positive for sixteenth-laid eggs. We propose a possible mechanism linking female condition and egg-laying ability: good quality females, capable of laying extended clutches, are able to maintain production of generic VLDL for their own metabolic needs, as well as producing yolk-targeted VLDL, whereas poor quality females are not. Received 13 February 2002, accepted 17 November 2002.

**RÉSUMÉ.**—Nous avons étudié les variations individuelles et la dépendance aux ressources chez le Diamant mandarin (*Taeniopygia guttata*) lors de la ponte de couvées supranormales survenues suite à un retrait d’œufs. Nous avons également examiné l’effet d’une ponte continue sur (1) la composition des œufs et (2) le niveau plasmatique des précurseurs du vitellus. Le retrait d’œufs a augmenté significativement le nombre d’œufs produits par une femelle, mais la réponse était sous la dépendance de la diète. Avec une diète de bonne qualité (supplément d’œufs), les femelles ont pondu  $12,4 \pm 1,0$  œufs de plus en comparaison aux tailles de couvées avant traitement. Par contre, pour une diète de faible qualité (seulement des graines), les femelles ont pondu seulement  $4,9 \pm 1,2$  œufs de plus. La taille de couvée après retrait (i.e. le nombre total d’œufs pondus en réponse au retrait d’œufs) était corrélée positivement avec la masse moyenne des œufs et la taille de couvées avant traitement dans le cas d’une diète de faible qualité, mais ce n’était pas le cas pour la diète de bonne qualité. Cela suggère qu’il existe une variation interindividuelle dans l’aptitude à pondre des œufs (les femelles pondant des gros œufs avaient une meilleure aptitude de réponse au retrait d’œufs que les femelles pondant des petits œufs). Par ailleurs, les résultats montrent que des ressources plus importantes peuvent palier ces différences individuelles. La masse des œufs n’a pas varié avec la séquence de ponte pour les couvées supranormales (jusqu’à 22 œufs). Néanmoins, il y avait une diminution significative (6%) du contenu en protéines du vitellus pour les œufs supplémentaires, et ce à partir du 10ème œuf pondu (i.e. seulement 4–5 en plus que la taille de couvée normale). Les niveaux plasmatiques des deux précurseurs du vitellus, la vitellogénine et la lipoprotéine très basse densité (LTBD), étaient indépendants de la diète et ne différaient pas chez les individus entre la ponte du 1er œuf et la ponte du 14ème œuf. Néanmoins, il y avait un changement

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systématique dans la relation entre le contenu en lipides du vitellus et les niveaux plasmatiques en LTBD allant d'une relation non significative pour le 3ème œuf pondu à une relation significative et positive pour le 16ème œuf pondu. Nous proposons donc un mécanisme qui pourrait permettre de relier la condition des femelles à leur aptitude à pondre des œufs: les femelles de bonne qualité, aptes à pondre des couvées plus grandes, seraient capables de maintenir une production générique de LTBD pour leurs propres besoins métaboliques, ainsi que de produire de la LTBD spécifiquement destinée au vitellus, alors que les femelles de faible qualité n'en seraient pas capables.

IN HIS SEMINAL paper on the significance of clutch size Lack (1947) argued quite clearly that limitation of clutch size was not due to a (physiological) inability of females to produce more eggs, but rather was due to other factors determining cessation of laying. That idea of costs of egg production *per se* being relatively minor has dominated the literature until recently (Monaghan and Nager 1997), in part supported by the fact that many species of bird will lay additional eggs in response to egg removal (called "indeterminate layers"). The extensive literature on effects of egg removal on clutch size has been reviewed previously (Kennedy 1990, Haywood 1993a, Winkler and Walters 1993), but the focus of those reviews has been on patterns of interspecific variation (e.g. which species or taxa tend to be determinate vs. indeterminate) and how that relates to life-history variation. It seems intuitive, given significant costs of egg production *per se* (e.g. Heaney and Monaghan 1995, Monaghan et al. 1998), that (1) some individuals should be better able to sustain the costs of continuous, extended laying (i.e. the response should be state dependent), or that (2) the same individual should be better able to sustain those costs when environmental conditions are more favorable (i.e. the response should be resource dependent). Despite the intuitive nature of those predictions few studies have explicitly considered factors influencing interindividual (intraspecific) variation in response to egg removal (i.e. the differential ability of individuals to meet the demands of continuous or extended laying).

In general, manipulations of egg number have focused on egg size, and have not considered effects on egg composition (Nager et al. 2000). However, recent studies of gulls (Laridae) and terns (Sternidae) have shown that even though birds can lay extended clutches in response to egg removal the quality (composition) of eggs decreases with increasing egg number (Heaney et al. 1998, Nager et al. 2000).

That provides further support for the idea of substantial costs of egg production (and for physiological limitations on clutch size; cf. Lack 1947), although how widespread those patterns are is not known.

In this article, we first show that the ability of individual female Zebra Finches (*Taeniopygia guttata*) to respond to egg removal by laying additional eggs is resource dependent, and also related to individual quality. We then demonstrate a decrease in egg quality with increasing egg number, but for a different component than that reported in previous studies: yolk protein. Finally, we investigate the physiological basis of the response to egg removal by measuring changes in yolk precursor levels that occur with increasing egg number.

## METHODS

*Animal husbandary and breeding.*—Zebra Finches were maintained at a temperature of 19–23°C, humidity 35–55%, and on a constant lighting schedule of 14L:10D (lights on at 0700 hours PST). All birds had *ad libitum* access to seed (*Panicum* and white millet), water, grit and cuttlefish bone (calcium). Protocol for breeding experiments followed that described in Williams (1996a, b): breeding pairs were housed singly in 61 × 46 × 41 cm cages, each with a single external nest box (11.5 × 11.5 × 11.5 cm), and nest boxes were checked daily to monitor egg laying and to weigh eggs. Pairs were assigned randomly to one of two breeding diets: (1) a low-quality, seed-only diet ( $n = 14$ ), and (2) a high-quality, egg-food supplemented diet ( $n = 15$ ; 6 g egg food daily: 60 g whole egg, 13 g cornmeal, 13 g bread crumbs; 20.3% protein; 6.6% lipid; see Williams 1996b for further details). All females were more than six months old, and for most birds ( $n = 12$  for each diet) we had information on "pretreatment" reproductive history, that is, mean egg and clutch size from previous breeding attempts on the same diet as for the egg-removal clutches (hereafter, "pretreatment" clutch and egg size).

*Egg removal and blood sampling.*—Nest boxes were checked daily between 0930 and 1030 hours for newly

laid eggs and all eggs were removed on the day of laying (i.e. no eggs were left in the nest). All eggs were weighed ( $\pm 0.001$  g) and the third, tenth, and sixteenth eggs were stored frozen for egg composition analysis. The 16th egg was replaced with a Zebra Finch egg of approximately the same size and birds were then allowed to complete egg laying without further egg removal. Some females skipped one or more days (i.e. they laid no egg) during their complete laying sequence. Because we were interested in stimulating continuous, extended egg formation (e.g. yolk or albumen deposition, not oviposition *per se*), and to maximize sample sizes, we only excluded birds with skips of four or more days. Given a four-day egg development period (three days for rapid yolk development plus one day for albumen and shell formation; Haywood 1993b), birds with skips of three days or less could have "stopped" egg formation for at most one day of yolk development. Egg composition was analyzed as previously described (Balzer and Williams 1998). Basically, eggs were boiled for 2 min, separated into shell, albumen, and yolk; those components were then dried to constant weight at 60°C and yolks were then lipid extracted using Soxhlet apparatus.

Females were blood sampled (200  $\mu$ L, from the brachial vein) on the day they laid their first, eighth, and fourteenth egg, between 0930 and 1030 hours. Blood was centrifuged at 5,000 rpm and plasma frozen at -20°C until assayed for yolk precursors. Given the timing of egg development (see above), on the day of blood sampling females were depositing most of the yolk for the egg which would be laid two days later. We therefore compared plasma yolk precursor levels measured on the first, eighth, and fourteenth egg day with egg composition for the third, tenth, and sixteenth eggs, respectively (i.e. in each case we matched the blood sample with the composition of the egg forming when that particular blood sample was obtained). Plasma vitellogenin (VTG) was assayed indirectly using the zinc method developed for the domestic hen (Mitchell and Carlisle 1991) that we have validated for passerines (e.g. Williams 1999, Williams and Martyniuk 2000). However, because we repeatedly blood-sampled birds over the 16-day interval, we could only obtain enough blood to assay for total zinc (i.e. we omitted the depletion step to determine VTG-bound zinc; Mitchell and Carlisle 1991). Previously, we have shown that total zinc and VTG-bound zinc are positively correlated ( $r = 0.96$ ; Williams and Martyniuk 2000) so the former measure provides a reliable index of plasma VTG (see also Mitchell and Carlisle 1991). Plasma triglycerides were assayed using a triglyceride kit (Wako Chemicals, Richmond, Virginia) and were used as an index of plasma very-low-density lipoprotein (VLDL) (Williams 1999, Williams and Martyniuk 2000, following the method of Mitchell and Carlisle 1991). Interassay variation was 6.6% ( $n = 11$ ) and 4.8% ( $n = 7$ ) for VTG and triglyceride, respectively.

All statistical analyses were carried out using SAS (SAS Institute 1989). Where possible we used a repeated measures design (paired *t*-test or ANOVA including pretreatment egg size or clutch size values as covariates as relevant). For some analyses, residual egg mass was calculated by regressing egg mass against mean egg mass for each individual, for eggs of different laying sequences. Plasma yolk precursor levels were normalized by  $\log_{10}$  transformation, and body mass was included as a covariate in analyses of plasma VLDL (or residual VLDL was calculated by regressing VLDL on body mass). Values are presented as means  $\pm$  SE unless otherwise stated.

## RESULTS

**Clutch and egg size.**—Egg removal significantly increased the number of eggs that individual females laid (repeated measures analysis,  $F = 129.7$ ,  $df = 1$  and 22,  $P < 0.001$ ), but that response was diet-dependent (time  $\times$  diet interaction,  $F = 24.3$ ,  $df = 1$  and 22,  $P < 0.001$ ; Fig. 1). On the high-quality (egg-supplemented) diet females laid on average  $12.4 \pm 1.0$  more eggs compared with their pretreatment clutch size (paired *t*-test,  $t = 12.6$ ,  $df = 12$ ,  $P < 0.001$ ), whereas on the

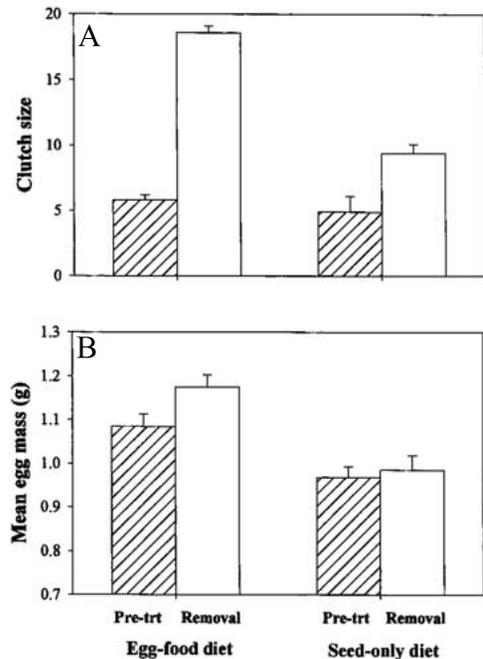


FIG. 1. Effect of egg removal and diet on clutch size and mean egg mass in female Zebra Finches in relation to pretreatment (nonmanipulated) clutch and egg size.

low-quality (seed-only) diet females laid on average only  $4.9 \pm 1.2$  more eggs ( $t = 4.25$ ,  $df = 12$ ,  $P < 0.01$ ). Mean clutch size was  $9.4 \pm 1.2$  eggs for birds on the low-quality diet and  $18.6 \pm 0.7$  eggs for birds on the high-quality diet.

Mean egg mass was also greater in egg removal clutches compared with pretreatment clutches ( $F = 5.46$ ,  $d = 1$  and  $22$ ,  $P < 0.05$ ; Fig. 1). That effect was independent of diet (time  $\times$  diet interaction,  $P > 0.10$ ), although there was a diet effect within treatment: females on the egg food diet laid larger eggs for both the pretreatment and removal clutches ( $F = 14.9$ ,  $df = 1$  and  $22$ ,  $P < 0.001$ ; Fig. 1). Because egg size increases with laying sequence in Zebra Finches (e.g. Williams 1996a, 2000) those egg-mass effects might have been due to extended clutches containing more, larger, later-laid eggs, resulting in a higher mean egg mass. However, although residual egg mass increased with laying sequence for eggs 1–6 (Fig. 2), that increase did not continue for later-laid eggs and residual egg mass was independent of laying sequence in birds laying at least 18 eggs ( $n = 11$ ,  $P > 0.15$ ; Fig. 2). For those individual birds laying more than 18 eggs there was no difference in the average size of eggs 1–6 ( $1.201 \pm 0.032$  g) compared with the average size of eggs 14–22 laid late in the extended sequence ( $1.209 \pm 0.033$  g; paired  $t$ -test,  $t = 0.67$ ,  $df = 11$ ,  $P > 0.50$ ). An alternative explanation is that individual “large-egg” females had a greater capacity to respond to egg removal, in that they laid larger extended clutches than “small-egg” females. In support of that, removal clutch size

was positively correlated with pretreatment mean egg mass ( $r = 0.67$ ,  $df = 12$ ,  $P < 0.025$ ) and pre-treatment clutch size ( $r = 0.64$ ,  $df = 12$ ,  $P < 0.05$ ) in birds on the low-quality diet, although not in birds on the high-quality diet ( $P > 0.15$  in both cases; Fig. 3).

*Yolk precursor levels.*—Diet had no effect on the mean plasma level of either yolk precursor at any stage of laying: At the 1-, 8-, or 14-egg stage ( $P > 0.05$  in all cases), so data were pooled for subsequent analyses. Individual plasma VTG levels were positively correlated in birds sampled at the 1-egg and 8-egg stages ( $r = 0.52$ ,  $df = 19$ ,  $P < 0.05$ ) but not between the 1- and 14-egg stages ( $r = -0.05$ ,  $df = 18$ ,  $P > 0.85$ ). In contrast, plasma VLDL levels were positively correlated between the 1 egg and 8 egg stages ( $r = 0.63$ ,  $df = 22$ ,  $P < 0.01$ ) and between the 1 and 14 egg stages ( $r = 0.71$ ,  $df = 18$ ,  $P < 0.001$ ).

We tested for effects of extended laying on plasma yolk precursor levels in two ways. First, we compared plasma levels in birds blood sampled at the 1-egg and 14-egg stage ( $n = 16$ ; we excluded birds that laid their last egg within two days of the last blood sample, because those birds were likely to have terminated yolk development and plasma VTG declines rapidly at that time; Challenger et al. 2001). There was no significant time effect for either plasma VTG (Wilks'  $\lambda$ ,  $F = 1.44$ ,  $df = 1$  and  $15$ ,  $P > 0.20$ ) or plasma VLDL ( $F = 1.53$ ,  $df = 1$  and  $14$ ,  $P > 0.20$ ; mass was included as a covariate for VLDL and there was a significant mass effect,  $F = 12.3$ ,  $df = 1$  and  $14$ ,  $P < 0.01$ ; Table 1). Second, we compared plasma levels at the 1-egg stage with those in birds sampled 2–5 days before laying their last egg ( $n = 8$ , pooling 8- and 14-egg stage samples); those birds should still have been forming yolk but would be close to clutch completion. Again there was no significant time effect for either plasma VTG ( $F = 1.64$ ,  $df = 1$  and  $7$ ,  $P > 0.20$ ) or plasma VLDL ( $F = 1.42$ ,  $df = 1$  and  $6$ ,  $P > 0.20$ ; Table 1).

*Egg composition.*—For females that laid at least 16 eggs ( $n = 14$ ), there was no difference in mean egg mass comparing egg number 3 and egg number 16 in the laying sequence (paired  $t$ -test, Table 2; because that was a repeated measures analysis we included data from both diets). The only egg component for which there was a significant change in proportional egg composition was yolk protein ( $t = 3.23$ ,  $df = 14$ ,  $P < 0.01$ ) which decreased by 6.7% between egg 3 and 16 (Table 2).

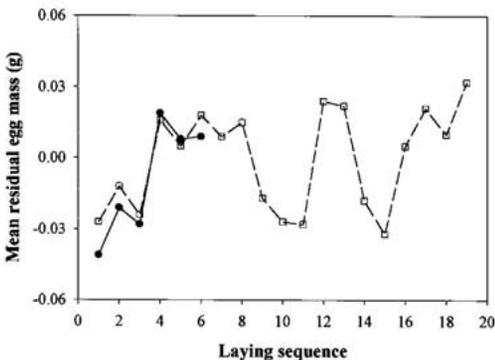


FIG. 2. Variation in mean residual egg mass (controlling for individual differences in mean egg mass per clutch) with laying sequence for eggs 1–6 (all birds laying  $>5$  eggs; closed circles), and eggs 1–19 (all birds laying  $>18$  eggs; open squares).

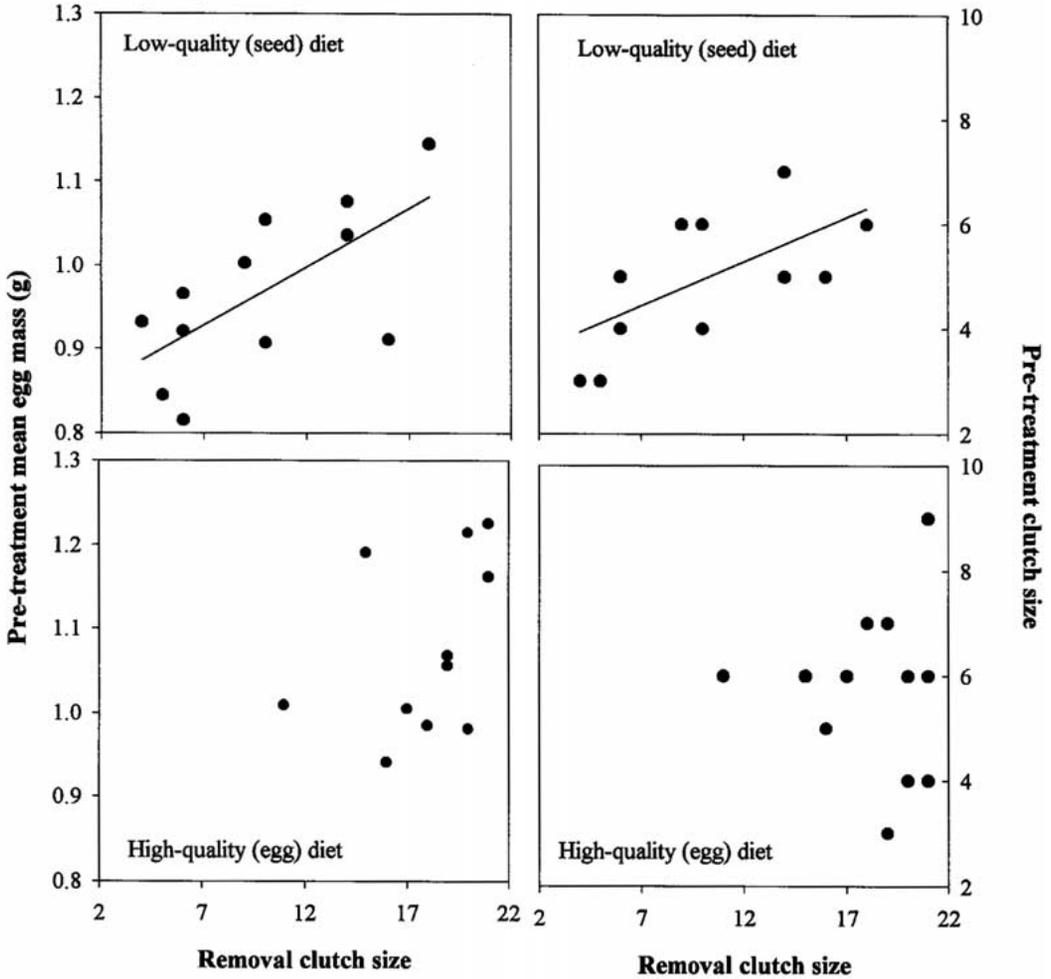


FIG. 3. Relationship between clutch size laid in response to egg removal and pre-treatment egg mass and clutch size on low- and high-quality diets.

Analysis of the larger sample of birds that laid at least 10 eggs ( $n = 21$ ) confirmed that result: only proportional yolk protein changed with laying sequence ( $3.89 \pm 0.05\%$  vs.  $3.64 \pm 0.05\%$ ,  $t = 3.84$ ,  $df = 21$ ,  $P < 0.001$ ), decreasing by 6.4% between egg 3 and 10 (all other components  $P > 0.15$ ).

Yolk protein mass was independent of plasma VTG levels at all egg stages (1, 8, and 14 egg;  $P > 0.25$  in all cases; diets were pooled because "diet" and "diet  $\times$  precursor" interaction term were not significant). In contrast, there was a strong positive relationship between yolk lipid mass and residual plasma VLDL for egg stage 14 ( $r^2 = 0.37$ ,  $F = 7.10$ ,  $df = 1$  and  $13$ ,  $P < 0.025$ ), although not at egg stage 1 or 8 ( $P > 0.20$  in both

cases; Fig. 4). That was not due to "selection" of birds, or to different individuals being included in the analyses at different time points, with time because there was no relationship between yolk lipid mass and VLDL at the 1-egg stage when analyzed only for those birds that subsequently laid 14+ eggs ( $F = 0.03$ ,  $df = 1$  and  $13$ ,  $P > 0.50$ ; Fig. 4).

DISCUSSION

Ability of female Zebra Finches to lay additional eggs in response to egg removal was highly dependent on the resources (i.e. diet) that females had to meet the demands of egg

TABLE 1. Plasma yolk precursor concentrations in relation to extended egg laying in female zebra finches. See text for details.

Stage of laying	<i>n</i>	Log plasma VTG( $\mu$ g/mL)	Log plasma VLDL (mg/mL)
1st egg	16	0.59 $\pm$ 0.02	1.20 $\pm$ 0.07
14th egg	16	0.55 $\pm$ 0.04	1.12 $\pm$ 0.06
Start of laying (one-egg)	8	0.57 $\pm$ 0.02	1.16 $\pm$ 0.05
Clutch completion	8	0.52 $\pm$ 0.05	1.17 $\pm$ 0.07

production: females on the low-quality (seed) diet laid eight eggs fewer than those on the high-quality diet. However, continuous laying was also related to individual "quality": on the low-quality diet, females that had previously laid large eggs produced larger supranormal clutches in response to egg removal than females that previously laid small eggs. That relationship was not apparent on the high-quality diet, which suggests that a higher level resource somehow overcomes individual differences, increasing the ability of lower quality, "small egg" birds to lay additional eggs (see Fig. 3). In a previous study of Zebra Finches following the same removal protocol, Haywood (1993b) reported a mean clutch size of 13.4 eggs for Zebra Finches on a seed diet with no egg food supplement. Our lower clutch size for birds on a seed-diet (9.4 eggs) is, in part, due to our stopping egg removal at the 16th egg (cf. Haywood 1993b), although in our study only 2 of 14 females laid >15 eggs on the seed diet. However, mean clutch size on the egg food diet (18.6 eggs) was greater than that reported by Haywood (1993b), even though we did not keep removing eggs, confirming the effect of resource or diet on egg-laying ability. Haywood (1993b) also noted marked interindividual variation in the clutch sizes laid in response to egg removal (SD = 4.4 eggs), with one female laying 39 eggs.

In our study, on the low-quality diet, clutch size in response to egg removal was positively correlated with an individual's pretreatment mean egg and clutch size. Those traits therefore appear to indicate individual "quality" with regard to a bird's egg-laying ability (see also Sockman and Schwabl 2001, Williams et al. 2001). Many studies investigating fitness consequences of egg size have assumed that females laying larger eggs are higher quality females (e.g. when assigning birds to "high" and "low" quality groups for egg switching experiments; Blomqvist et al. 1997; Styrsky et al. 1999). However, our study is one of the few to provide direct, experimental evidence for a correlation between egg size and female quality. Consistent with our results several previous studies have shown that later laying, presumably lower quality, females lay fewer eggs in response to egg removal than do earlier laying individuals (Kennedy and Power 1990, Haywood 1993c).

Haywood (1993b) highlighted the importance of the tactile stimulus from eggs in the nest in sensory control of clutch size (see also Haywood 1993a). In Zebra Finches, there is a critical period between laying of the third and fourth egg where tactile contact with eggs is required to trigger disruption of ovarian follicular growth and cessation of laying, with the timing of that event determined on the second day of laying (Haywood 1993b). In Zebra Finches, with no egg removal, the timing of that critical period is relatively invariant (Haywood 1993b), in contrast to other species (e.g. Blue Tit [*Parus caeruleus*]; Haywood 1993c). Nevertheless, clutch size is variable in Zebra Finches (2–7 eggs; Williams 1996a) so variation in timing of the stimulus provided by eggs does not account for individual variation in clutch size (Haywood 1993b). In the current study, no birds obtained any tactile stimulus from eggs in the nest (except for <2 h between laying and egg removal), yet many females still terminated laying, and

TABLE 2. Effect of egg removal on egg composition of eggs 3 and 16 in the laying sequence. Values for egg components are expressed as percent of fresh egg mass.

Component	Egg # 3	Egg # 16	Paired- <i>t</i>	<i>P</i>
Fresh egg mass (g)	1.128 $\pm$ 0.029	1.131 $\pm$ 0.046	0.09	NS
Dry albumen (%)	7.48 $\pm$ 0.01	7.15 $\pm$ 0.04	0.76	NS
Dry shell (%)	5.38 $\pm$ 0.01	5.53 $\pm$ 0.03	0.59	NS
Yolk lipid (%)	6.20 $\pm$ 0.02	6.21 $\pm$ 0.02	0.16	NS
Yolk protein (%)	3.87 $\pm$ 0.05	3.62 $\pm$ 0.01	3.23	< 0.01

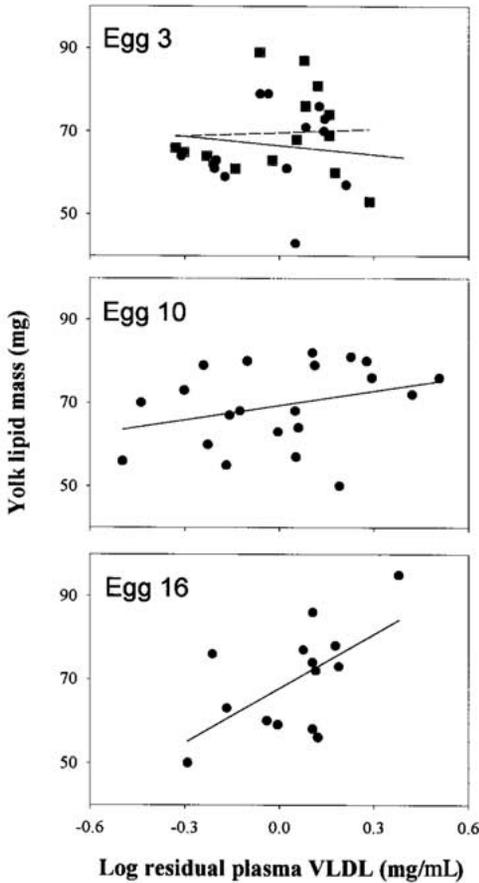


FIG. 4. Variation in the relationship between yolk lipid mass and plasma levels of the yolk precursor, very-low-density lipoprotein for the 3rd, 10th and 16th laid eggs in an extended laying sequence. Dashed line in the top panel indicates the relationship for those individual birds which went on to lay 16+ eggs (bottom panel).

thus ovulation, before the end of egg removal, especially on the low-quality diet. Variation in clutch size is therefore not determined by the tactile stimulus from eggs *per se*, but rather by the extent to which that affects or disrupts follicular growth (see Haywood 1993b). Although the mechanism(s) mediating that remains unknown it is clear from our study that it is highly dependent on individual state (i.e. quality) and resource availability.

Zebra Finches laid up to 20 eggs with egg removal in the present study, 4× the normal clutch size, but there was no change in egg mass for later-laid eggs (i.e. individual egg size was

maintained in supranormal clutches). However, composition of the additional eggs did change independent of egg size, with yolk protein content decreasing by an average 6%. Importantly, that change in yolk protein was evident by the tenth egg, on average only 4–5 eggs more than the modal clutch size in our population. That contrasts with the situation in gulls (Nager et al. 2000) where egg quality declined progressively as more eggs were laid, and became more variable towards the end of long laying sequences (in our study the coefficient of variation was 17–18% for yolk lipid and yolk protein for both the third egg and sixteenth egg). However, our study and that of Nager et al. (2000) provide support for physiological limitation in clutch-size determination in that egg quality decreases rapidly as additional eggs are laid greater than the normal clutch size (although further studies are required to show how important that effect is over the smaller range of clutch sizes typical of most populations). Three studies have so far reported changes in egg composition in response to egg removal but in each case the specific egg component has been different: decreased yolk protein (this study), decreased yolk lipid with increased water content (Nager et al. 2000) and decreased shell calcium (Heaney et al. 1998). Several studies have shown that egg production in Zebra Finches is protein limited (Houston et al. 1995, Williams 1996b), and the decrease in yolk protein content with extended laying is consistent with that. Gulls are also protein-limited and egg production is unaffected by lipid supplementation (Bolton et al. 1993), although nutrients limiting egg production in females might not be those limiting for chick growth and survival (Nager et al. 2000). In all three studies to date, changes in egg composition with extended laying have been relatively small (6–8%), consistent with low intraindividual variability of egg parameters in females; but Nager et al. (2000) have shown that that can translate into substantial effects on offspring survival (i.e. it does represent a significant decrease in egg quality).

Finally, we investigated whether changes in egg composition were mediated by decreases in circulating yolk precursor levels, perhaps indicating that females were unable to sustain costs of yolk precursor production. Plasma VTG and VLDL levels did not change within individuals in birds laying up to 18 eggs. That result is

perhaps not surprising given the marked inter-individual variation in plasma yolk precursor levels, and the generally weak relationships between plasma VTG or VLDL and egg size or egg composition that we have reported previously (e.g. Christians and Williams 2001, Williams et al. 2001). However, the relationship between yolk lipid content and plasma VLDL did change with extended laying, from nonsignificant for egg 3 to significant and positive for egg 16. Our measure of plasma VLDL (i.e. total triglyceride) includes both generic VLDL, which the female uses for her own maintenance requirements, as well as yolk-targeted VLDL (Walzem 1996) which is taken up by the developing ovarian follicles. During laying, synthesis of yolk-targeted VLDL leads to a 5–7 fold increase in plasma triglyceride, although female Zebra Finches continue to synthesize generic VLDL at least early in laying (K. G. Salvante pers. comm.). A possible explanation for the result in Figure 4 is therefore that extended laying causes a gradual depletion of generic VLDL, indicative of depressed female “condition”, leading to a better matching of triglyceride-rich yolk-targeted VLDL with yolk lipid. In other words, that could provide one mechanism linking female condition and egg-laying ability (Alisauskas 1986, Meathrel et al. 1987). Further work in our lab is directly addressing that issue by characterizing changes in generic versus yolk-targeted VLDL during egg laying in female Zebra Finches.

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