Female Zebra Finches (*Taeniopygia guttata*) Are Chronically but Not Cumulatively "Anemic" during Repeated Egg Laying in Response to Experimental Nest Predation

Ketterson and Nolan 1999), which produce longer-term effects—for example, a slower recovery—that persist beyond the period of egg production itself (Wagner et al. 2008*b*).

It remains unknown whether reproductive anemia is proportional to reproductive effort such that females that lay more or larger eggs or that lay eggs more frequently (e.g., in replacement clutches) become more anemic and, therefore, potentially pay higher costs of egg production. Kalmbach et al. (2004) showed that experimentally increasing egg production in female great skuas (*Stercorarius skua*) increased anemia in terms of a greater reduction in hematocrit and red blood cell number compared with control females, and they suggested that anemia was proportional to egg-laying effort rather than there being a "fixed" decline. In contrast, Wagner et al. (2008*a*) suggested that female zebra finches breeding on a low-quality diet traded off reproduction and hematological status, maintaining hematocrit, hemoglobin, and red blood cell number at some minimum functional level at the cost of reduced reproductive investment (see also Garcia et al. 1986). If anemia is positively correlated with reproductive effort, with an "additive" effect of increased or repeated egg production, this could have widespread significance in many free-living birds, for example, where birds initiate a second clutch while still rearing offspring from a first brood (Verhulst and Hut 1996; Grüebler and Naef-Daenzer 2008; Wagner et al. 2008*b*) or where females produce many successive clutches in response to high levels of nest predation (Grzybowski and Pease 2005; Zanette et al. 2006).

In this article, we investigated the effect of repeated cycles of egg production on hematological traits in the female zebra finch (*Taeniopygia guttata*). Specifically, we caused birds to lay either two or three successive clutches by egg removal (experimental nest predation) with or without the opportunity for physiological recovery during incubation. We predicted that if the negative effect of egg production on hematocrit and hemoglobin concentration was additive or cumulative, then (1) females laying three successive clutches with no incubation (recovery) would show the greatest reproductive anemia and (2) females laying two successive clutches with an intervening period of incubation would show lower reproductive anemia at the end of their replacement clutch than females laying two clutches with no incubation. All birds were provided with a high-quality diet to reduce the effect of resource availability, since we were primarily interested in anemia related to repeated cycles of exposure to reproductive hormones (estrogens) during egg production. After females laid two to three clutches, we let birds rear chicks to investigate the effect of re-laying on hematocrit and hemoglobin concentrations after a phase of chick rearing just before or at fledging of the first-brood chicks.

Material and Methods

General Husbandry and Breeding

Zebra finches (*Taeniopygia guttata*) were housed under controlled environmental conditions (temperature 19°–23°C, hu-

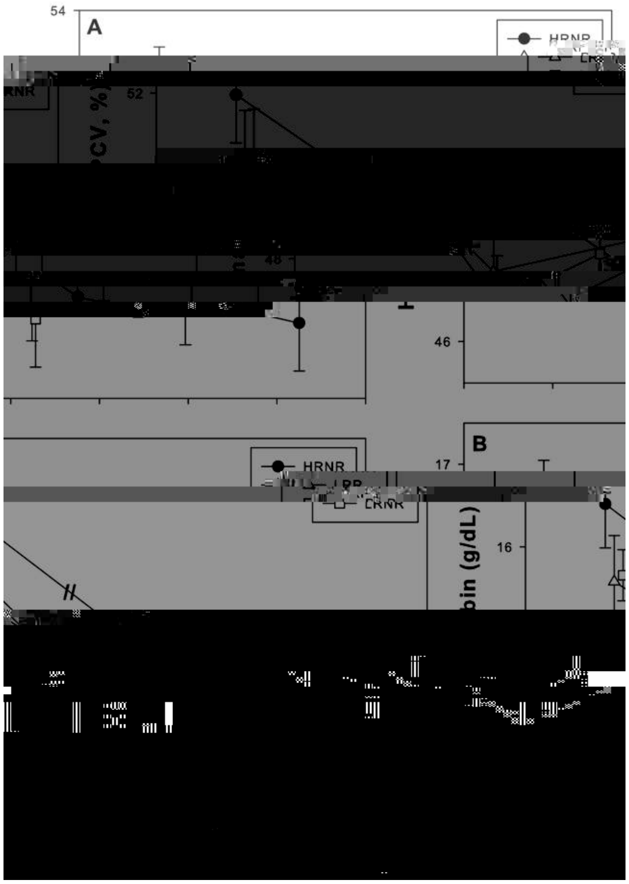

Figure 2. Variation in hematocrit (*A*) and hemoglobin (*B*) concentration over time in females laying two or three successive clutches: high renesting/no recovery (HRNR) females laid three successive clutches with no recovery; low renesting/recovery (LRR) females laid two successive clutches with 10 d for physiological recovery during incubation; low renesting/no recovery (LRNR) females laid two successive clutches with no recovery. Values are least squares means ± SE.

cases) and at clutch completion ($P > 0.50$ in all cases), so we do not control for body mass in subsequent analyses.

Effect of Re-Laying, with and without Recovery, on Hematocrit and Hemoglobin

There was no effect of treatment ($F_{2,25} = 0.04$, $P > 0.95$) or a treatment × time interaction ($F_{4,50} = 1.07$, $P > 0.30$) on hematocrit (Fig. 2). However, there was a highly significant time effect ($F_{2,50} = 12.92$, $P < 0.001$). Hematocrit was significantly lower at clutch completion for both bleed 2 (Tukey-Kramer adjusted, $P < 0.001$) and bleed 3 ($P < 0.001$) compared with pretreatment values, but mean hematocrit for bleeds 2 and 3 were not significantly different ($P > 0.40$). Overall, hematocrit decreased by 7.5% from 50.9% ± 0.7% in pretreatment females to 47.4% ± 0.7% at bleed 2 (Fig. 1*A*). There was no effect of treatment ($F_{2,24.3} = 0.15$, $P > 0.85$) or a treatment × time interaction ($F_{4,48.8} = 0.95$, $P > 0.40$) on hemoglobin concentration. However, there was a highly significant time effect ($F_{2,48.8} = 9.40$, $P < 0.001$). Hemoglobin was significantly lower at both clutch completion bleeds 2 ($P < 0.001$) and 3 ($P < 0.001$) compared with pretreatment values (Fig. 1*B*), but mean hemoglobin for bleeds 2 and 3 were not significantly different ($P > 0.90$). Overall, hemoglobin concentration decreased by 10.6% from prebreeding (15.92 ± 0.31 g/dL) to bleed 2 (14.39 ± 0.30 g/dL; Fig. 1*B*). Females maintained low hematocrit and hemoglobin for between 20–40 d depending on the number of clutches they laid (Fig. 2).

Total number of eggs laid had no significant effect on the change in hematocrit ($F_{1,27} = 0.01$, $P > 0.90$; treatment and interaction term, $P > 0.40$) or hemoglobin concentration ($F_{1,26} = 0.00$, $P > 0.90$; treatment and interaction term, $P > 0.90$) between pretreatment and clutch completion for the final clutch (Fig. 3). Similarly, change in hematocrit and hemoglobin was independent of the total mass of eggs laid ($P > 0.60$ in both cases).

Effect of Re-Laying, with and without Recovery, on Reproductive Traits

There was no difference in mean egg mass, clutch size, or clutch mass for the first breeding attempts among treatment groups

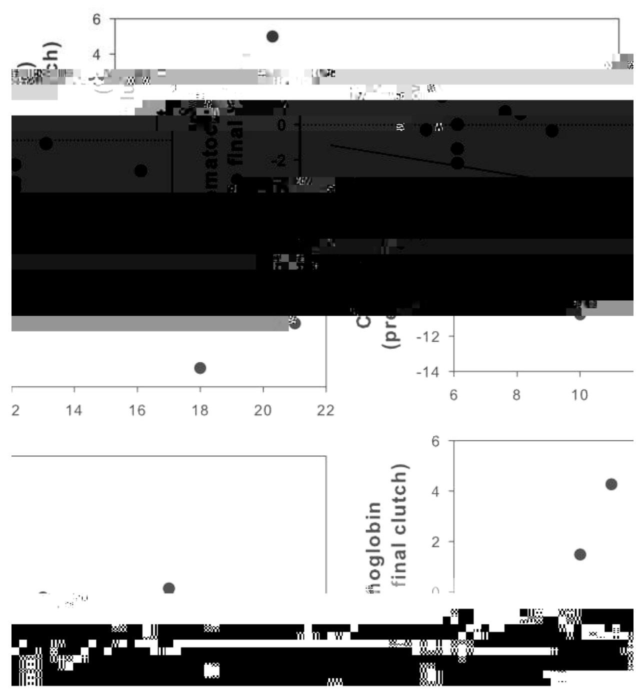

Figure 3. Relationship between total number of eggs laid and change in hematocrit (*top*) and change in hemoglobin (*bottom*) between pretreatment and clutch completion for the final clutch laid.

($P > 0.10$ in all cases). Time to laying for the first clutch was almost twice as long in HRNR females (8.1 ± 1.2 d) compared with LRR females (4.9 ± 1.3 d) and LRNR females (4.5 ± 1.2 d), but this difference was not significant ($F_{2,26} = 2.61$, $P = 0.094$). However, laying interval was independent of prebreeding hematocrit, treatment, or the hematocrit × treatment

Table 1: Reproductive output in relation to treatment and clutch number

Treatment and Clutch	*n*	Egg Mass (g)	Clutch Size	Clutch Mass (g)
HRNR	10			
1		1.132 ± .029	6.1 ± .3	6.72 ± .49
2		1.169 ± .029	5.7 ± .3	6.70 ± .49
3		1.168 ± .029	5.7 ± .3	6.78 ± .49
LRR	8			
1		1.129 ± .032	5.3 ± .3	6.11 ± .55
2		1.149 ± .032	5.8 ± .3	6.79 ± .55
LRNR	10			
1		1.091 ± .029	6.0 ± .3	7.29 ± .49
2		1.112 ± .028	5.1 ± .3	5.55 ± .49

Note. HRNR, high renesting/no recovery females; LRR, low renesting/recovery females; LRNR, low renesting/no recovery females. Values are estimates (least squares means ± SE) from proc MIXED model.

males laying more clutches or laying successive clutches without recovery during incubation would show greater reproductive anemia (lower hematocrit and hemoglobin concentration). In contrast, if females maintain hematocrit and hemoglobin concentration at some minimum functional level independent of reproductive effort (Wagner et al. 2008*a*), then we predicted