

Egg carotenoids in passerine birds introduced to New Zealand: relations to ecological factors, integument coloration and phylogeny

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Summary

1. Carotenoids are a diverse group of organic compounds that function as important antioxidants and immunostimulants and are of particular importance to developing embryos and young birds. In addition, they constitute the majority of yellow to red hues in the integumentary pigments of birds.

2. We investigated the maternally derived carotenoid concentrations and balances in the yolks of eight European passerine species successfully introduced and abundant in New Zealand. Specifically, we addressed whether variation in maternally derived yolk carotenoids were related to phylogeny, integument coloration, native *vs* introduced distribution, breeding habitat and laying sequence.

3. Across species, the concentration and balance of carotenoids deposited in yolks varied significantly. Egg carotenoid concentration was positively associated with the occurrence of male carotenoid pigmented body regions. Carotenoid concentrations differed between agricultural habitat types within New Zealand but not between samples from New Zealand and Europe. Controlling for the differences among species, and among clutches within species, increased egg carotenoid concentrations were significantly associated with decreasing fresh egg mass and eggs laid earlier in the laying sequence.

4. We conclude that variability in the concentration and balance of carotenoids deposited in the egg yolk imply different relative roles for ecological and phylogenetic factors that warrant further investigation, both within and across species.

Key-words: Egg yolk, integument pigments, introduced birds

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Introduction

Carotenoids are a diverse group of organic compounds that in birds are widely responsible for both integument pigmentation and antioxidant and immune defences. Carotenoids can only be synthesized by algae, bacteria, fungi and plants (Fox 1979; Brush 1981; Goodwin 1984; Latscha 1990; Stradi 1998), and hence must be ingested by higher animals in order to be used in physiological processes and displayed in the integument. A major function of integumentary pigments is in signalling and given that carotenoids are important antioxidants and immunostimulants (reviewed in Møller *et al.* 2000; Surai 2002; Blount 2004), it is possible that their signalling function directly reflects physiological

properties of an individual's health or quality (Fletcher 1992; Lozano 1994; Shykoff & Widmer 1996; Olson & Owens 1998; Saino *et al.* 1999; von Schantz *et al.* 1999; Møller *et al.* 2000; Blount 2004).

The antioxidant and immunostimulant roles of carotenoids are of particular importance to developing embryos and young birds, which are subject to increased oxidative stress as a by-product of metabolism associated with rapid development (Blount *et al.* 2000; Blount 2004). Thus, carotenoids are a prominent component of egg yolk, giving it its characteristic yellow-orange colour (Blount *et al.* 2000). Research on domestic poultry has shown that supplementation with carotenoids can reduce oxidative damage and result in increased hatching success and an improved immune response in young birds (Haq *et al.* 1996; Surai & Speake 1998). Recent studies on wild birds have presented similar findings, with evidence of an increased propensity for

egg-laying, production of higher-quality eggs and links to the production of better quality nestlings (Blount *et al.* 2002, 2004; Saino *et al.* 2003). As such it is clear that parental ability to invest carotenoids into eggs may impact on the quality and survival of young. During offspring development, different carotenoids are selectively transferred from the yolk to specific tissues in the embryo, suggesting that they individually serve particular functions (Surai *et al.* 2001a). As Blount (2004) recently observed, this raises the question of whether there is a 'recipe' for a good egg and to what extent the concentration and balance of carotenoids deposited in the yolk varies among species.

Although eggs should provide a stable measure of species' typical carotenoid concentrations, in the few studies to have investigated these patterns there appears consistent evidence for a decline in carotenoid concentration over the laying sequence (Blount *et al.* 2001; Saino *et al.* 2002; Royle *et al.* 2003). This declining pattern might be expected if carotenoid reserves are depleted with each successive egg. However, given the potential advantages accrued to those eggs with relatively high carotenoid investment, it is interesting that breeding females appear not to regulate this deposition. If these results are widespread it raises questions associated with variable maternal investment to young within a clutch as well as more practical concerns associated with utilizing 'representative eggs' in studies of carotenoid investment and nestling fitness.

The importance of carotenoids to embryos, and young and adult birds means that decreased carotenoid availability could have substantial impacts on population health. Studies in birds and fish have found that changes in habitat, such as those associated with pollution or degradation, result in decreased signal brightness (Eeva *et al.* 1998; Camplani *et al.* 1999; Grether *et al.* 1999; Brawner *et al.* 2000; Hōrak *et al.* 2000). These results suggest not only that carotenoids may be limiting (Grether *et al.* 1999) but also that habitat modification, impacting on carotenoid availability and use, may have important fitness consequences (Eeva *et al.* 1998; Camplani *et al.* 1999; Brawner *et al.* 2000; Hōrak *et al.* 2000).

Here, we address the general paucity of information regarding interspecific variation in carotenoid concentrations among wild birds. We used eggs from naturally occurring clutches of eight common species of introduced European passerines in New Zealand to address whether variation in carotenoid concentration is related to phylogeny, integument coloration, native *vs* introduced distribution, breeding habitat and laying sequence.

First, we considered how the concentration and balance of carotenoids deposited in yolks varied across species and if it showed any phylogenetic pattern. Second, we assessed the relationship between the extent of carotenoid-based integument pigmentation in a species and the concentration of total carotenoids invested in egg yolk. Hōrak *et al.* (2000), Blount *et al.* (2003) and Koutsos *et al.* (2003) have all recently

shown that early impairment of neonatal nutrition can result in long-term impairment of carotenoid assimilation even when newly hatched offspring are reared in a carotenoid-rich environment. Thus, if there are species-typical mechanisms for the uptake and use of carotenoids from the environment we predict that across species there will be a positive relationship between egg carotenoid concentration and carotenoid-based integument pigmentation.

Third, we investigated differences in carotenoid deposition in egg yolk among contrasting agricultural habitats within an introduced range, and between this introduced range and samples collected from the species natural range. In Europe, a decline in the diversity of seed plants utilized by, among others, cardueline finches, and a general reduction in invertebrate species linked to habitat loss and increased pesticide use, are noted effects of changing land management (Wilson *et al.* 1999). However, the relative importance of these various changes, and hence the direction of any difference in carotenoid profiles between Europe and New Zealand, is *a priori* unpredictable. Within New Zealand, we predict that carotenoid availability will be greater in farmland matrices compared with horticultural orchards. This prediction is based on the observation that within orchards the relative monoculture (*Vaccinium* sp.) and common insecticide use (fixed copper, oil, azinphos-methyl, lime sulphur) may lead to the farmland areas (which are characterized by large numbers of native bush edges and diverse hedgerows) having more abundant insect fauna and diverse seed types. We assume that these differences will be important for carotenoid availability.

Finally, we compared patterns of carotenoid investment within clutches. We test the previous finding that within a clutch carotenoid concentration declines over the laying sequence. We also explore the variability of carotenoid investment within and between clutches of the same species.

Methods

Eggs were collected from naturally occurring wild nests in two agricultural habitats around Benneydale (175°22' E, 38°32' S) in the central North Island, New Zealand, during the breeding season of 2003–04. The habitats consisted of either (a) open farmland matrix of pastures, exotic hedgerows, native bush patches (< 0.01 ha) and associated farm buildings, or (b) exotic horticultural fruit (*Vaccinium* sp.) orchards, surrounding windbreaks and associated orchard buildings. All eggs were collected on private land with the express permission of the landowners. Nests were sampled only if they were located during the laying sequence, i.e. before incubation and the early development of embryos. Where possible eggs were marked and the clutch left *in situ* for the female to complete the clutch. In a number of cases this was not possible because nests were located late in the sequence, or because risk of predation of the nest following its initial location was

high. Clutches were lost through predation from 40% of the nests that were marked during the laying sequence ($n = 22$).

In total 350 eggs were collected from 148 clutches of eight passerine species (*Turdus philomelos*, $n = 38$; *T. merula*, $n = 32$; *Sturnus vulgaris*, $n = 29$; *Carduelis chloris*, $n = 9$; *C. carduelis*, $n = 11$; *Fringilla coelebs*, $n = 11$; *Emberiza citrinella*, $n = 3$; *Passer domesticus*, $n = 15$). Eggs were measured using vernier callipers and precision electronic scales and their width, length and mass recorded. Eggs were split equatorially so that the yolk and albumen could be separated into 50 mm × 100 mm zip lock bags before freezing. Any eggs that could not be separated were not used in further statistical analyses.

European samples from clutches of five of the eight species (*T. philomelos*, $n = 5$; *C. carduelis*, $n = 8$; *C. chloris*, $n = 5$; *E. citrinella*, $n = 4$; and *P. domesticus*, $n = 4$) were collected in April–May 2001 on farmland in Spain and Italy (Biard 2004). No European samples were available for *F. coelebs*, *T. merula* or *S. vulgaris*.

Yolk samples were homogenized with 0.7 ml NaCl 5% and 1 ml ethanol, and carotenoids were extracted adding 2 ml hexane and further homogenization, centrifugation and collection of the hexane phase (extractions were repeated twice). Hexane extracts were pooled and evaporated at 60–65 °C under nitrogen flow, and the residue was dissolved in 0.1 ml dichloromethane and 0.1 ml methanol. Carotenoid composition and concentration were determined following previously published procedures (Surai *et al.* 2001a,b,c). Individual carotenoids were identified, and their quantities in the samples determined using high-performance liquid chromatography (HPLC) with a Spherisorb type ODS2 5 µ C18 reverse-phase column, 25 cm × 4.6 mm (Phase Separation, Clwyd, UK) with a mobile phase of acetonitrile–methanol (85 : 15) and acetonitrile–dichloromethane–methanol (70:20:10) at a flow rate of 2 ml min⁻¹, using detection by absorbance at 445 nm. Total carotenoid concentration was determined by HPLC with a Spherisorb type S5NH₂ reverse-phase column 25 cm × 4.6 mm (Phase Separation) with a mobile phase of methanol–distilled water (97:3), at a flow rate of 1.5 ml min⁻¹ as described by Hōrak *et al.* (2002). Lutein was used to calibrate samples (Sigma, Poole, UK). Individual carotenoid composition profiles were analysed for a single randomly chosen egg from at least six clutches (with the exception of *E. citrinella* where $n = 3$).

Adult body carotenoid cover among the eight species was scored from specimens according to previously recognized ‘biologically significant’ body regions (Olson 2002). The 17 body regions are: crown, supercilium, eye, lore, bill, auricular, nape, mantle, wing, rump/uppertail coverts, tail, chin/throat, breast, flank, belly, undertail coverts and legs/feet. For the eight species, 15 body regions contain plumage (including the feathering around the eye), and 3 do not (bill, eye when no feathering surrounds it, and legs).

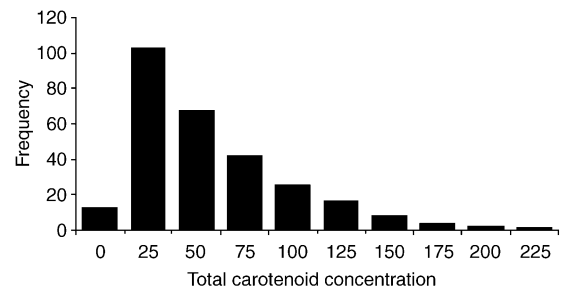


Fig. 1. The frequency distribution of total egg carotenoid concentration sampled among eight exotic passerine bird species introduced to New Zealand and subsequently used for analysis of differences between species, clutches within species, habitat types and region.

Differences in total carotenoid concentrations among eggs were analysed in SAS version 8.02 using generalized linear mixed models (Littell *et al.* 1996) that accounted for the random effects between species and between clutches within species. We controlled for the possible association between carotenoid concentration and volume by including fresh egg mass as a fixed effect in the linear models. For all species, the distributions of total carotenoid concentration were visibly right-skewed with an average skewness of 1.41. Across all species the distribution has a modal frequency of 25 µg g⁻¹, a median of 41.54 and range of 220–0.02 (Fig. 1). We subsequently log-transformed total carotenoid concentration and modelled the response specifying a Poisson error distribution with a log-link function (Littell *et al.* 1996). Phylogenetic regression of egg carotenoid concentration and plumage carotenoid measures was performed in CAIC version 2.6.7 (Purvis & Rambaut 1995). Taxonomic relatedness was determined following Sibley & Ahlquist (1990) and Sibley & Monroe (1990) and assumed that all branches in the phylogeny are of equal length. We note that the relatedness of our particular species is not contentious and our phylogenetic hypothesis is supported by the most recent available analysis (Barker *et al.* 2004). Our results are not affected by either the evolutionary assumption of the independent method (that evolution proceeds according to a random walk process), or the statistical assumption of homogeneity of variance (Purvis & Rambaut 1995).

Results

Different species allocated significantly different total carotenoid concentrations to their eggs (Type III $F_{7,277} = 67.83$, $P < 0.001$), and within species, significantly different total carotenoid concentrations to their clutches (Type III $F_{84,200} = 19.19$, $P < 0.001$). We identified a group of species (*C. carduelis*, *C. chloris*, *E. citrinella*) that had significantly greater carotenoid concentrations than all other species (Fig. 2a). Controlling for egg size considerably reduced the variability in egg carotenoid concentrations across species with three significantly

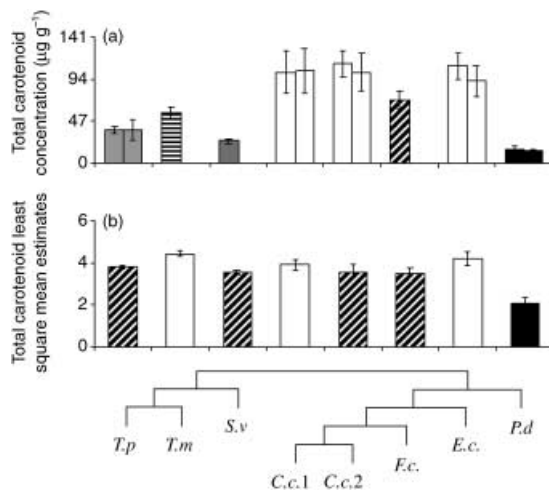


Fig. 2. (a) The average maternally derived yolk carotenoid concentration for eight exotic passerine bird species introduced to New Zealand. Species are *Turdus philomelos* (*T.p.*), *T. merula* (*T.m.*), *Sturnus vulgaris* (*S.v.*), *Carduelis chloris* (*C.c.1*), *C. carduelis* (*C.c.2*), *Fringilla coelebs* (*F.c.*), *Emberiza citrinella* (*E.c.*) and *Passer domesticus* (*P.d.*). For each species the first column is the estimate (and 95% confidence interval) for clutches in New Zealand followed (where available) by the estimate for a European population (Biard 2004). In each of these cases the differences between New Zealand and Europe are non-significant ($\alpha = 0.05$). (b) The least square mean estimates for total carotenoid concentrations controlling for fresh egg size. Differences in histogram pattern between groups of species highlight statistically significant least square mean differences (all P -values < 0.01). The bifurcating phylogenetic hypothesis is described in the Methods section.

different species groups remaining, independent of phylogeny (Fig. 2b). Across all eight species, four individual carotenoids (lutein, zeaxanthin, *cis*-lutein and β -carotene) consistently made up over 88% (and as much as 99.8%) of the total concentration of carotenoids deposited in egg yolks (Table 1). For each of the individual carotenoids, and controlling for phylogenetic relatedness among genera and families, the proportions deposited in the eggs varied significantly among the species (Table 1).

Controlling for phylogenetic independence, the total number of carotenoid pigmented body regions in the eight species was positively correlated between males and females (slope = 0.65 ± 0.24 , $F_{1,6} = 7.37$, $R^2 = 0.55$, $P = 0.035$). Across species, total egg carotenoid concentrations were positively associated with the total number of carotenoid pigmented body regions in both males (slope = 7.40 ± 2.18 , $F_{1,6} = 11.54$, $R^2 = 0.60$, $P = 0.015$) and females (slope = 6.71 ± 2.61 , $F_{1,6} = 6.60$, $R^2 = 0.44$, $P = 0.042$). However, these species differ in the size of their eggs, while egg size (mass or volume) and carotenoid concentration may be negatively related. Controlling for fresh egg mass did indeed markedly reduce the interspecific species relationship between egg carotenoid concentration and number of pigmented body regions for both sexes (males: slope = 0.07 ± 0.05 , $F_{1,6} = 2.23$, $R^2 = 0.27$, $P = 0.186$; females: slope = 0.06 ± 0.05 , $F_{1,6} = 1.45$, $R^2 = 0.19$, $P = 0.275$). However, phylogenetic regression revealed that the relationship between total egg carotenoid concentrations and the total number of carotenoid pigmented body regions, controlling for fresh egg mass as well as relatedness among species, was significant among males (slope = 0.14 ± 0.05 , $F_{1,6} = 9.35$, $R^2 = 0.61$, $P = 0.022$) but not among females (slope = 0.09 ± 0.05 , $F_{1,6} = 2.99$, $R^2 = 0.33$, $P = 0.134$).

In a generalized linear mixed model that controlled for differences of the random effects among species, and among clutches within species, total carotenoid concentrations were not detectably different among species between agricultural habitats in New Zealand and Europe (Table 2). In contrast, there was a consistent significant difference across species between two New Zealand agricultural habitats (open farmland compared with exotic orchard; Table 2). In all cases species with populations in both habitats had higher carotenoid egg concentrations in open farmland.

Among species the within-clutch variability in carotenoid concentration for sampled nests, containing at least three eggs, ranged from 43% (*C. carduelis*) to 27% (*S. vulgaris*). In a generalized linear mixed model that

Table 1. Composition of the four major (and sum remaining other) individual carotenoids deposited in yolk. The 'total' column refers to the total average carotenoid concentration (see Fig. 2) whereas all other numbers are average percentages. Percentages grouped in bold reflect species proportional depositions significantly greater than non-bold species, controlling for phylogenetic relatedness within genera and families ($\alpha = 0.05$). Type III results for these tests are presented for each individual carotenoid (degrees of freedom for all tests were $F_{7,39}$)

	Lutein	Zeaxanthin	<i>cis</i> -Lutein	β -Carotene	Other	Total
<i>Turdus philomelos</i>	62.4	8.0	5.3	15.5	8.8	36.2
<i>T. merula</i>	49.3	8.9	12.7	18.0	11.1	56.2
<i>Sturnus vulgaris</i>	78.8	11.6	5.1	4.0	0.5	24.5
<i>Carduelis chloris</i>	72.0	18.8	7.2	1.8	0.3	101.9
<i>C. carduelis</i>	68.6	23.9	6.1	1.2	0.3	111.5
<i>Fringilla coelebs</i>	78.3	12.2	8.3	0.9	0.3	70.3
<i>Emberiza citrinella</i>	73.1	13.6	7.0	4.6	1.6	109.1
<i>Passer domesticus</i>	75.8	12.5	7.2	3.8	0.7	15.6
<i>F</i> -value	12.18***	25.31***	4.32***	5.48***	9.27***	

*** $P < 0.001$.

Table 2. Type III results from generalized linear mixed models between total yolk carotenoid concentration in two New Zealand agricultural habitats (open farmland compared with exotic orchard) and between New Zealand and European agricultural habitats. Only five species (*Turdus merula*, *T. philomelos*, *Sturnus vulgaris*, *Carduelis chloris*, *C. carduelis*) occurred within both New Zealand habitats in sufficient numbers (greater than three clutches) to be included in the analysis. Data from Europe were only available for five species (*Turdus philomelos*, *Carduelis carduelis*, *C. chloris*, *Emberiza citrinella* and *Passer domesticus*)

	Degrees of freedom	F-value
Between New Zealand and Europe		
Species	4,150	42.57***
Site	1,150	1.68
Species * Site (interaction)	4,150	0.49
Within New Zealand		
Species	4,252	27.53***
Site	1,252	4.11*
Species * Site (interaction)	4,252	1.53

* $P < 0.05$, *** $P < 0.001$.

Table 3. Estimates from a reduced minimum adequate generalized linear mixed model that controls for the random effects of species, and clutches within species. Both of the remaining variables explain significant variability in increasing concentrations of total egg carotenoids

	Estimate	SE	t-value
Intercept	4.51	0.33	
Log (fresh egg mass)	-1.38	0.67	-2.06*
Laying sequence ^a	-0.23	0.04	-5.78***

^aLaying sequence is the total carotenoid concentration of eggs laid late in the clutch relative to eggs laid earlier in the clutch.

* $P < 0.05$, *** $P < 0.001$.

controlled for differences of the random effects among species, and among clutches within species, total carotenoid concentrations were significantly higher in eggs with lower fresh mass (log-transformed) and in eggs laid earlier in the laying sequence (Table 3). Controlling for the differences among species, and among clutches within species, the association between egg mass and laying sequence was not significant (Type III $F_{1,66} = 0.44$, $P = 0.51$).

Discussion

The concentration and balance of carotenoids deposited in yolks varied significantly among species. Much of the variability in carotenoid concentration was found to be due to differences in fresh egg size. After controlling for fresh egg size, three species groups were identified whose eggs differ significantly in carotenoid concentration, with these differences being independent of phylogenetic relatedness among the species (Fig. 2b).

It is well known that species exploit different ecological niches within the same habitats (e.g. Lack 1971; von Euler 1999). Our results strongly suggest that factors other than those shared through common descent (as indicated by phylogenetic relatedness) are associated with interspecific variability in egg carotenoid concentrations. We suggest that ecological factors may thus be important in determining egg carotenoid concentrations, although firmer conclusions clearly await future studies that can determine carotenoid content of shared food items of these species. All species relied heavily on four individual yolk carotenoids; however, variability in their individual ratios was significantly different among the species. Notably, these ratios appear to reflect a much stronger phylogenetic association than total carotenoid concentrations (e.g. compare the zeaxanthin proportions within the *Carduelis* species with the β -carotene and remaining 'other' (largely *cis*- β -carotene) proportions within the *Turdus* species). Clearly these patterns require further study, but if they are robust they imply that the relative importance of ecological and phylogenetic factors differs, respectively, between the concentration and balance of carotenoids deposited in the egg yolk.

Across species, egg yolk carotenoid concentrations were highly correlated with the extent of carotenoid-based plumage coloration in males. This result complements a recent comparative study by Tella *et al.* (2004) that found species with higher carotenoid plumage scores were also those that had higher plasma carotenoid concentration. Earlier research has identified a similar relationship with higher plasma carotenoids in Scarlet Ibis, *Eudocimus ruber*, vs White Ibis, *E. albus* (Trams 1969), and in the plasma hue of 14 common bird species (Hill 1995). Together, these data suggest that (i) dietary carotenoid availability is higher for some species, thus allowing greater species-typical carotenoid pigmentation, and/or (ii) there are species-typical mechanisms for the uptake and use of carotenoids from the environment. This latter scenario may reflect differences in life history and the importance of signalling driving the evolution of both display and absorption/metabolism mechanisms. As noted by Tella *et al.* (2004) the comparative approach does not allow for the distinction of these processes and teasing them apart certainly warrants further attention.

It has been hypothesized that females may advertise their egg-laying capacity to prospective males through the expression of carotenoid display (Blount *et al.* 2000). Although carotenoid-based plumage was positively correlated between the sexes, egg carotenoid concentration was associated only with the extent of male carotenoid-based plumage when controlling for both phylogeny and egg size. It is tempting to infer that the correlation in plumage between sexes may simply reflect the selection of generally 'colourful' species for acclimatization in New Zealand (e.g. see Cassey *et al.* 2004). Consequently, we compared our result with previously collected data (Olson 2002) from 235 passerine

species (14 families). Controlling for phylogeny, the degree of carotenoid-based plumage was still significantly correlated among males and females ($r = 0.62$, $n = 92$, $P < 0.01$). Therefore, although males have more extensive carotenoid pigmentation than females ($F_{13,221} = 5.46$, $P < 0.01$), an association between generally colourful males and females appears to reflect a genuine trend among passerine species. Given that males may accumulate much higher concentrations of carotenoids than females (Hill *et al.* 1994; Bortolotti *et al.* 1996; Gray 1996; Figuerola & Gutiérrez 1998), it is interesting that the interspecific relationship between plumage and egg carotenoids was significant only among males and not females. It is likely that males and females differ considerably in their carotenoid needs (see Blount *et al.* 2003). For example, sexual dichromatism is largely male biased, whereas females invest heavily in the cost of reproduction. These different strategies, in relation to antioxidant use, may explain the differences in the associations we observe.

Habitat perturbation can impact on carotenoid availability and use. This has been shown in numerous studies of both birds and fish, leading to reduced expression of carotenoid signals, often linked with reduced fitness (Linville & Breitwisch 1997; Eeva *et al.* 1998; Camplani *et al.* 1999; Grether *et al.* 1999; Brawner *et al.* 2000; Hõrak *et al.* 2000), and even the occurrence of aberrant or novel colour display (Witmer 1996). However, contrary to our *a priori* predictions, there was no detectable difference in total carotenoid concentrations in eggs between samples from agricultural habitats in the native European ranges and the introduced New Zealand ranges. Indeed, Fig. 2 clearly shows, from the similarity of average species concentrations and overlapping confidence intervals, that despite the small number of available clutches from Europe, it is evident that yolk carotenoid concentrations have remained conserved across divergent (native and introduced) populations of these species.

Although differences in total carotenoid concentrations in eggs were not detectable between the introduced and native range, they were apparent between different habitats within the introduced range. This shows that while total carotenoid concentrations remain conserved within species, considerably larger samples can still detect local scale differences that we assume occur when ecological factors are broadly different. Unfortunately, we cannot establish from this study what roles individual factors have in determining these differences. However, we note that qualitative differences of diets, pesticide application and predation pressure have been observed between the habitats and future study of these and related ecological variables will be highly informative.

Given that the antioxidant and immunostimulant roles of carotenoids are of particular importance to developing embryos (and young birds) there should be a premium on a female's ability to transfer carotenoids to the egg yolk. However, available data suggest that females do not invest carotenoids equally across the

laying sequence. For example, Blount *et al.* (2002) found a decline in carotenoid concentration across the clutch in Lesser Black-Backed Gulls, *Larus fuscus*, and showed that egg-laying capacity was limited by carotenoid availability (Blount *et al.* 2004). Similar patterns have been reported in the Great Tit, *Parus major* (Hõrak *et al.* 2002), and Zebra Finch, *Taeniopygia guttata* (Royle *et al.* 2003). Another study detected a more complex quadratic relationship of carotenoid investment across the laying sequence in Barn Swallows, *Hirundo rustica* (Saino *et al.* 2002). Results presented here also reveal a consistent decline in carotenoid investment across the laying sequence within the species introduced to New Zealand. We note that this decline is fitted better by a linear than a quadratic term. These findings suggest either that females do not regulate carotenoid deposition across the laying sequence, or that they bias deposition to earlier laid eggs.

During our study, species invested significantly different total carotenoid concentrations in their eggs, and within species significantly different concentrations of total carotenoids in their clutches. Within clutches, the variability among carotenoid concentrations was significantly influenced by egg size and laying order. Controlling for egg size, carotenoid concentrations were conserved across species and were not as phylogenetically labile as sexually selected traits. Nevertheless, the range in concentrations of maternally derived yolk carotenoids across species is still arresting especially considering their purported importance for protecting yolk lipids from peroxidation during development. We suggest that the relationship between male carotenoid pigmentation and yolk carotenoid concentration supports the growing evidence that shows young birds need their carotenoid absorption machinery 'kick-started' in order to develop efficient absorption later in life (Hõrak *et al.* 2000; Blount *et al.* 2003; Koutsos *et al.* 2003; Blount 2004). We emphasize that further studies of the potential effects of variability in carotenoid concentrations are needed at both the intra- and inter-specific levels.

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