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Pollen and the Nitrogen Requirements of the Lesser Double-collared Sunbird

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Nectarivorous birds typically are small, often weighing less than 10 g, and have high basal metabolic rates. To meet their high energy needs, many nectarivorous species spend a large proportion of their time foraging on nectar. Like all birds, they also require nutrients such as protein, vitamins, essential fatty acids, minerals, and trace elements. Amino acids occur in floral nectar, and their concentrations are thought to be related to pollinator type, being low in nectar of bird-pollinated plants (Baker and Baker 1982). However, the correlation between amino acid concentration and pollinator type has been disputed by Gottsberger et al. (1984), who suggested that most of the variation in nectar amino acids results from flower damage or contamination with pollen. In any event, the amounts are too low to satisfy the protein requirements of nectarivorous birds (Martínez del Río 1994). Other nitrogen sources are insects or pollen (Richardson and Wooller 1990, Brice 1992), both of which present birds with a number of potential problems.

In insects, a large proportion of the nitrogen is in the form of chitin, a polymer that may be difficult for a small nectarivorous bird to digest. Although some seabirds are capable of producing chitinases (Jackson et al. 1992), no nectarivorous birds are known to do so. Accordingly, the major nitrogen sources obtained from insects are likely to be the proteins, peptides, and free amino acids found in their tissues. However, the amino acid composition of insects varies greatly and often is dominated by nonessential amino acids such as proline (Tomlin et al. 1993). As a result, insects are not always as good a source of protein as is widely believed. Furthermore, it may be energetically very expensive for a small nectarivorous bird to catch enough insects to meet its nitrogen requirements.

Pollen has long been recognized as a major source of nitrogen for various insect pollinators, most notably honeybees (*Apis mellifera*; Schmidt and Buchmann 1985, Dobson and Peng 1997), and it has recently become clear that pollen also is an important element in the diet of vertebrate pollinators (Howell 1974, Richardson and Wooller 1990, Law 1992, van

Tets and Whelan 1997, van Tets 1998). However, the amino acid composition of pollen varies among plant species, and many hummingbird-pollinated plants in North America produce pollens that are deficient in two essential amino acids: methionine and lysine (Martínez del Río 1994).

The relative importance of insects and pollen in the diets of nectarivorous birds varies considerably. Anna's Hummingbirds (*Calypte anna*) appear to depend on insects for nitrogen. They thrive in captivity on a diet of fruit flies and nectar (Brice 1992), but when fed pollen they digest only 5% of the pollen grains (Brice et al. 1989). In contrast, Purple-crowned Lorikeets (*Glossopsitta porphyrocephala*) feed extensively on pollen and digest up to 50% of the pollen grains they ingest ($\bar{x} = 37\%$; Wooller et al. 1988, Richardson and Wooller 1990). New Holland Honeyeaters (*Phylidonyris novaehollandiae*) fall somewhere between these two extremes. The role of pollen in their diet has been studied in two sites with strongly conflicting results (Paton 1981, 1982; Wooller et al. 1988). It is important to note that all of these studies are based primarily on measures of full and empty pollen grains under a light microscope. Although these measurements provide an estimate of a bird's digestive capabilities, they are not accurate and can seriously underestimate pollen digestibility (van Tets and Hulbert 1999).

Lesser Double-collared Sunbirds (*Nectarinia chalybea*) are common in much of South Africa. They are important pollinators of many plants in the Cape Floral Kingdom of the southwestern Cape, a region with a high floral diversity characterized by the families Proteaceae, Ericaceae, and Restionaceae. In addition to the indigenous flora, *N. chalybea* feeds extensively on introduced species of *Eucalyptus*, which may have led to increases in the numbers and range of this group (Fraser and Crowe 1990, Parker 1994). In this study, we fed *Eucalyptus* pollen to Lesser Double-collared Sunbirds to determine whether it was a viable source of nitrogen for this species.

Methods.—We captured eight sunbirds (seven males and one female) in Kirstenbosch Botanic Gardens in February 1998 and housed them separately in holding cages (70 × 80 × 40 cm) in the University of Cape Town Animal House. Except during experiments, birds were fed a mixture of 0.7 M sucrose and Ensure® (3 g per 100 mL solution) presented in commercially manufactured feeders and renewed twice daily. The sunbirds maintained body mass and condition on this diet.

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TABLE 1. Percentage of intact and empty pollen grains in *Eucalyptus calophylla* pollen ($n = 8$), in the experimental diet ($n = 15$ batches, 5 each with low, medium, and high pollen density), and in the cloacal fluid of *Nectarinia chalybea* ($n = 16$). Values are $\bar{x} \pm SE$.

Pollen state	<i>E. calophylla</i> pollen	Experimental diet	Cloacal fluid
Intact	88 \pm 1.5	85.2 \pm 1.0	68.6 \pm 2.9
Empty	12 \pm 1.5	10.9 \pm 0.9	29.8 \pm 2.7
Partially empty	0	0.3 \pm 0.1	1.7 \pm 0.3
Germinating	0	3.6 \pm 0.9	0

During the feeding trials, birds were kept in cylindrical cages (diameter 36 cm, height 50 cm) in a constant-environment room at 20°C, 50% relative humidity, and a 12L:12D light cycle. Birds were fed from glass feeders constructed from modified burettes with the bottom end curved upward to prevent spillage and the opening enlarged to a diameter of 3 to 4 mm. We recorded body mass of each bird at the beginning and end of each feeding trial.

For the first 48 h of each trial, birds were fed *ad libitum* a maintenance diet of 1.2 M sucrose supplemented with 3 g of Ensure per 100 mL solution. For the next 48 h, they were fed one of three diets: 1 g, 3 g, or 5 g of bee-collected pollen suspended in 100 mL of 1.2 M sucrose solution. We used a relatively high concentration to minimize settling of the pollen; *N. chalybea* readily feeds on sugar solutions of this concentration (Lotz and Nicolson 1999). The pollen was obtained commercially (Ridley Bee Products, Western Australia) and was collected by bees kept in an *Eucalyptus calophylla* plantation. The pollen contained 95% *Eucalyptus* pollen (measured using light microscopy), almost all of which was from a single species, presumably *E. calophylla*. A single drop of a commercial multivitamin supplement (Abbott Laboratories) was added to each 100 mL of sucrose solution. The mixture was kept at 4°C and a fresh solution made every 24 h.

To minimize the amount of pollen that settled out, the experimental diet available to each bird did not exceed 2 mL. Small volumes of fresh food were provided at intervals of 2 to 3 h throughout the day, and the added volumes recorded. At the end of each trial, the remaining solution was filtered and the dry mass of pollen determined.

During the second 48 h of the trial, trays of liquid paraffin were placed beneath the cages to collect the cloacal fluid. Cloacal fluid was then removed from the trays using Pasteur pipettes. During the second and third feeding trials, three subsamples, each of less than 0.1 mL, were removed from the cloacal fluid of each bird and placed on microscope slides. From these subsamples we measured the mean percentage of full, empty, germinating, and partially empty pollen grains. The remainder of the cloacal fluid was frozen. We also measured the percentage of full,

empty, partially empty, and germinating pollen grains in pollen that had been in the diet solutions for 24 h. The delay ensured that any effects on the pollen of immersion in sucrose solution, such as induced germination, were taken into account. Similar measurements were also made using fresh pollen.

We conducted three trials, with a 72-h rest period between each trial, so that each bird was tested on each diet. The diet given to an individual during a trial was determined randomly, with the sole proviso that no bird was given the same diet twice. When all three trials were completed, the samples of cloacal fluid were thawed and diluted to 50 mL with distilled water. The level of organic nitrogen in each was then determined using Kjeldahl analysis. The nitrogen content of all three experimental diets was determined at the same time. Using these values, we determined nitrogen intake and nitrogen output of each bird. By regressing nitrogen balance (the difference between intake and output) against nitrogen intake, we determined the dietary maintenance nitrogen requirements of *N. chalybea* on *E. calophylla* pollen. We also calculated the standard error of the dietary maintenance nitrogen requirement.

Results.—All eight birds maintained body mass and condition during the three feeding trials. At the start of the three trials, mean body mass was 8.0 \pm SE of 0.2 g, 8.0 \pm 0.2 g, and 8.2 \pm 0.1 g, respectively; at the end of the trials, body mass averaged 8.1 \pm 0.2 g, 7.9 \pm 0.1 g, and 8.0 \pm 0.2 g.

In the pollen-sucrose mixtures fed to the birds, 10.9% of the pollen grains were empty and 3.6% had begun to germinate after 24 h (Table 1). In the cloacal fluid, 29.8% of the pollen grains were empty and none was germinating (Table 1). This suggests that 18.9% of the pollen grains had been emptied in transit, as well as all those that had begun to germinate. Because the latter were less than 4% of the total, and because most pollen was eaten in less than 24 h, 19% is a reasonable estimate of the ability of *N. chalybea* to digest *E. calophylla* pollen grains.

Values for nitrogen balance versus dietary nitrogen intake are shown in Figure 1. The maintenance nitrogen requirement of a bird is its nitrogen intake when nitrogen balance equals zero. In this case, the dietary maintenance nitrogen requirement of *N. chal-*

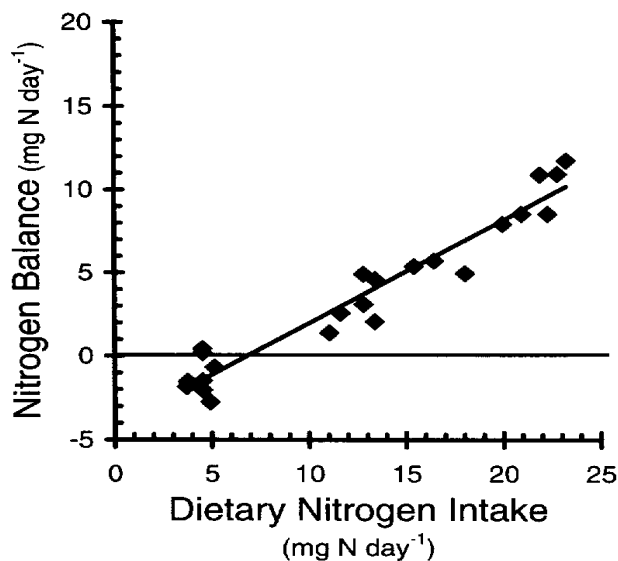


FIG. 1. Relationship between nitrogen balance (NB, the difference between dietary nitrogen intake and nitrogen output) and dietary nitrogen intake (NI) for *Nectarinia chalybea* on a diet of *Eucalyptus calophylla* pollen. $NB = 0.62 NI - 4.2$ ($R^2 = 0.94$).

ybea when fed on *E. calophylla* pollen was 6.8 ± 0.45 mg N day⁻¹, which is equivalent to 57 ± 4 mg of pollen per day. A very strong linear relationship existed between nitrogen balance and intake ($R^2 = 0.94$).

Discussion.—The proportion of empty pollen grains in the excreta and the low dietary maintenance nitrogen requirements suggest that *N. chalybea* is capable of meeting its nitrogen requirements solely from pollen. However, the extent of pollen ingestion in the field is unknown. Some mammalian pollinators are capable of gathering similar or larger quantities of pollen (Law 1992, van Tets 1998), but the systems are so different that it is impossible to make valid comparisons.

The percentage of empty pollen grains (19%) fell between the earlier values for lorikeets (37%; Wooller et al. 1988) and hummingbirds (5%; Brice et al. 1989). Interestingly, Cockatiels (*Nymphicus hollandicus*) fed pollen from the same species of *Eucalyptus* achieved a nearly identical result (18%; Brice et al. 1989). These values are much lower than those for mammals that feed on flowers (50 to 70%; van Tets 1997, van Tets and Whelan 1997), but 19% is high enough to suggest that pollen is a potentially useful nitrogen source for these birds. Although in our study the number of germinating pollen grains was not enough to have a major effect on digestion, it was interesting that germination occurred in the sucrose solution. Nectarivorous birds often ingest pollen that has fallen into the nectar. If a large proportion of

such pollen has begun to germinate, it would be easier for the birds to digest.

The nitrogen requirement of *N. chalybea*, 6.8 mg N day⁻¹, is exceptionally low. Costa's Hummingbird (*Calypte costae*) has lower requirements when maintained on an artificial diet (4.5 mg N day⁻¹; Brice and Grau 1991) but is only half the size of *N. chalybea*. When allometric effects are taken into account, the two values are very similar (using a mass exponent of 0.75, and with body masses of 8 g and 4 g respectively, *N. chalybea* has a requirement of 254 mg N kg^{-0.75} day⁻¹ and *C. costae* 283 mg N kg^{-0.75} day⁻¹). Because the low nitrogen requirement takes into account the low digestibility of *E. calophylla* pollen to *N. chalybea*, it is clear that the nitrogen that is absorbed from pollen is used efficiently. This is almost certainly due to the amino acid composition of the pollen. Levels of methionine and lysine (1.7% and 5.5% of total amino acid composition, respectively; van Tets and Hulbert 1999) are typical of those of good protein sources (Moir 1994), and the birds should retain the amino acids they absorbed during digestion.

It is possible that avian nectarivores as a group have unusually low nitrogen requirements. Anna's Hummingbirds can survive for 10 days on a zero nitrogen diet of 22% sucrose (Brice 1992). Recent work on Orange-tufted Sunbirds (*Nectarinia osea*) in Israel shows that this species (mass = 6.9 g) requires only 2.8 mg N day⁻¹ to maintain nitrogen balance on a diet of *Drosophila* (L. Roxburgh pers. comm.). Given the potential difficulty for small nectarivores to simultaneously gather nitrogen and sufficient nectar to meet their energy needs, it is easy to imagine that strong selective pressure exists in favor of birds with low nitrogen needs. Unfortunately, the data set on avian nitrogen requirements is too small to make detailed comparisons.

It is particularly interesting to note that the regression of nitrogen balance against nitrogen intake for *N. chalybea* was strongly linear (Fig. 1), indicating that as more nitrogen was consumed, more was retained. In theory, this should not occur, because the birds were neither growing nor reproducing (Brice and Grau 1991). In practice, however, it is a very common result that for some reason is consistently ignored (e.g. Smith and Green 1987, Brice and Grau 1991, Witmer 1998, van Tets and Hulbert 1999). Although it is possible that some form of unmeasured nitrogen excretion is taking place, it seems more likely that vertebrates are capable of some degree of nitrogen retention. Determining whether and how they do so should be an interesting avenue of future research.

Although our results show that pollen is a viable food source for *N. chalybea*, the results do not preclude the use of insects. Cloacal fluid produced by freshly caught *N. chalybea* contains pollen and insect fragments. The relative importance of the two nitro-

gen sources may vary in different times and places. The Cape Floral Kingdom includes a large number of plants that flower in winter, many of which have large inflorescences that enable efficient pollen harvesting. Because insect availability is likely to be low during the cold winters typical of the area, whereas pollen availability is likely to be high, pollen may be an important seasonal source of nitrogen for *N. chalybea*. The same may apply to the honeyeaters of Australia's coastal temperate regions for similar reasons. In tropical areas such as Central America, it is less likely that insect activity drops during winter.

The amino acid composition of pollen and insects will play a major role in determining the extent to which either food type serves as a nitrogen source for nectarivorous birds. If insects or pollen are deficient in one or more amino acids, then birds will require access to those amino acids from another source. Because feeding on pollen versus insects involves very different strategies, it is important that the relative merits of the two nitrogen sources are taken into account in studies of the foraging behavior of small nectarivorous birds.

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