

**NECTAR PREFERENCES OF SPECIALIST  
AND OCCASIONAL AVIAN  
NECTARIVORES, AND THEIR ROLE IN  
THE EVOLUTION OF FLORAL TRAITS**

MARK BROWN

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Faculty of Science and Agriculture  
University of KwaZulu-Natal

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## ABSTRACT

Our understanding of bird pollination systems has changed dramatically in the last few years. A long-standing paradigm was that hummingbirds and passerine birds select for different nectar properties in flowers (phylogenetic hypothesis). However, specialist passerines, such as sunbirds, have similar nectar preferences to hummingbirds and nectar in plants pollinated by these two bird groups is strongly convergent. Thus, as an alternative to the existing paradigm, it has been argued that the most useful distinction that can be drawn is that between specialist and generalist avian nectarivores (feeding niche hypothesis). This was supported by phylogenetically-controlled analyses that show that nectar in plants pollinated by specialist avian nectarivores (whether hummingbird or passerine) tends to have a lower volume, higher concentration and higher sucrose content than that in plants pollinated by generalist avian nectarivores. The aim of this thesis was to determine if these trends can be explained by the nectar preferences of avian nectarivores, and to determine whether the generalist-specialist dichotomy can be applied to *Kniphofia*, a largely bird-pollinated African plant genus.

This thesis consists of two sections. In the first, I present data from equicaloric choice experiments to determine sugar preferences of both specialist and occasional nectar feeding birds across a range of concentrations. In addition, I determine apparent sugar assimilation efficiencies and concentration preferences for a range of occasional nectar feeding birds. Using Malachite Sunbirds (*Nectarinia famosa*), Dark-capped bulbuls (*Pycnonotus tricolor*), Speckled Mousebirds (*Colius striatus*) and Red-winged Starlings (*Onychognathus morio*) (plus Village Weavers (*Ploceus cucullatus*) in an appendix) as representatives of the major groups of nectar feeding birds in South Africa, I show that some differences occur between occasional nectar feeding birds and all specialist nectar feeding birds studied so far. Specialists show a switch from hexose preference at low (5%) concentrations, exhibit no preference at medium concentrations (10-20%) and prefer sucrose or show no preference at high concentrations (25%). However, specialists are unable to maintain energy balance at low concentrations, and always select the higher concentration when given choices within this range. Occasional nectar feeding birds prefer hexose solutions at low and medium concentrations (5 and 10%), but show varied preferences at higher concentrations. Occasional nectar feeding birds are able to maintain energy balance at low concentrations, and either show no concentration preference, or a

preference for lower concentrations when given a choice. Occasional nectar feeding birds show a range in ability to digest sucrose, although some species, like the Dark-capped Bulbul, are quite proficient at it. Surprisingly, no significant difference is found between specialist and occasional nectarivores sugar preferences when analysed globally, even when phylogeny is accounted for. Instead, I found a significant relationship between body size and bird sugar preference at different concentrations, from which I build the body size hypothesis, which I suggest is a better predictor to use than bird diet type (specialist or occasional nectarivores – feeding niche hypothesis).

In the second section of the thesis, I examine the associations between plant traits and nectarivore nectar preferences. This section focuses on flower morphology and nectar characteristics in *Kniphofia* species, and ecotypes within species, pollinated by specialist versus occasional avian nectarivores. I show that apart from sugar type, which appears to be phylogenetically constrained, flower morphology and nectar characteristics appear to diverge according to whether plants are pollinated by generalist or specialist nectar feeding birds.

## PREFACE

The data described in this thesis were collected in the Republic of South Africa from June 2007 to November 2009. Experimental work was carried out while registered at the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor Steve D. Johnson and co-supervision of Professor Colleen T. Downs.

This thesis, submitted for the degree of Doctor of Philosophy in the Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.



.....  
Mark Brown  
December 2009

I certify that the above statement is correct.



.....  
Professor Steve D. Johnson  
Supervisor  
December 2009



.....  
Professor Colleen T. Downs  
Co-supervisor  
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### DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis.

#### Publication 1

**M Brown, CT Downs and SD Johnson. 2008. Sugar preferences of nectar feeding birds - a comparison of experimental techniques. *Journal of Avian Biology* 39: 479-483.**

*Author contributions:*

MB conceived paper with SDJ and CTD. MB collected and analysed data, and wrote the paper. SDJ and CTD contributed valuable comments to the manuscript.

#### Publication 2

**M Brown, CT Downs and SD Johnson. 2010. Concentration-dependent sugar preferences of the Malachite Sunbird *Nectarinia famosa*. *The Auk* 127: 151-155.**

*Author contributions:*

MB conceived paper with SDJ and CTD. MB collected and analysed data, and wrote the paper. SDJ and CTD contributed valuable comments to the manuscript.

#### Publication 3

**M Brown, CT Downs and SD Johnson. 2010. Sugar preferences and digestive efficiency in an opportunistic avian nectarivore, the Dark-capped Bulbul. *Journal of Ornithology* 151: 637-643.**

*Author contributions:*

MB conceived paper with SDJ and CTD. MB collected and analysed data, and wrote the paper. SDJ and CTD contributed valuable comments to the manuscript.

Publication 4

**M Brown, CT Downs and SD Johnson. In Press. Sugar preferences of a generalist non-passerine flower visitor, the African Speckled Mousebird. *The Auk*.**

*Author contributions:*

MB conceived paper with SDJ and CTD. MB collected and analysed data, and wrote the paper. SDJ and CTD contributed valuable comments to the manuscript.

Publication 5

**M Brown, CT Downs and SD Johnson. in prep. African Red-Winged Starlings feed on nectar, but don't like it too sweet.**

*Author contributions:*

MB conceived paper with SDJ and CTD. MB collected and analysed data, and wrote the paper. SDJ and CTD contributed valuable comments to the manuscript.

Publication 6

**M Brown, CT Downs and SD Johnson. 2009. Pollination of the red-hot poker *Kniphofia caulescens* by short-billed opportunistic avian nectarivore. *South African Journal of Botany* 75: 707-712.**

*Author contributions:*

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Publication 7

**M Brown, CT Downs and SD Johnson. 2010. Pollination of the red-hot poker *Kniphofia laxiflora* (Asphodelaceae) by sunbirds. *South African Journal of Botany* 76: 460-464.**

*Author contributions:*

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Publication 8

**M Brown, CT Downs and SD Johnson. Submitted. Covariation of flower traits and bird pollinator assemblages among populations of *Kniphofia linearifolia* (Asphodelaceae). Plant Systematics and Evolution.**

*Author contributions:*

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Publication 9 – Appendix 1

**T Odendaal, M Brown, CT Downs and SD Johnson. 2010. Sugar preferences and digestive efficiency of the Village Weaver, a key generalist avian pollinator of African plants. Journal of Experimental Biology 213: 2531-2535.**

*Author contributions:*

TO and MB conceived paper with SDJ and CTD. TO and MB collected and analysed data, and wrote paper. SDJ and CTD contributed valuable comments to the manuscript.



Signed: .....

Mark Brown

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## **Chapter 1**

### **Introduction**

Ever since the pioneering work of Darwin (1859), pollination systems have been used as a model for the study of evolutionary patterns and processes (Harder & Johnson 2009). Advances in the field often depend on a multidisciplinary approach, such as the combination of botanical studies of floral traits and plant fitness with zoological studies of pollinator foraging behavior (e.g. Chittka & Thompson 2001; Herrera & Pellmyr 2002). This has been attempted in the present thesis which focuses on the role of avian foraging preferences and digestive constraints in the evolution of plant nectar properties.

Nectar is the most common reward that flowers use to entice animals to visit their flowers (Nicolson 2007; Brandenburg et al 2009; De la Barrera et al. 2009). The volume, sugar concentration, and sugar composition of nectar are highly variable properties among plant species (Nicolson 2007). Since the pioneering efforts of Herbert and Irene Baker, there is a long tradition of research that attempts to link nectar properties with particular classes of animal pollinators (Brown et al. 1978; Baker & Baker 1982; Baker et al. 1998; Nicolson & Fleming 2003; Fenster et al. 2004; Nicolson 2007). It has been argued that nectar traits reflect the foraging preferences of flower visitors that act as effective pollinators (Martinez del Rio 1990; Cruden 1997; Lotz & Schondube 2006; Nicolson 2007; Brandenburg et al. 2009). The chief consideration for an animal that is faced with a choice of flowers differing in nectar traits is energetic gain (Heyneman 1983; Lotz & Schondube 2006; Nicolson 2007). Energetic gain should depend on factors such as the rate at which nectar can be ingested, and assimilation efficiency. Rate of ingestion can depend on factors such as bill, tongue or proboscis structure (Inouye 1980; Harder 1983; Kingsolver & Daniel 1983; Schondube & Martinez del Rio 2003; Collins 2008), licking rates (Roberts 1995; Collins 2008), floral structure (both flower size and shape, and nectar composition: Kingsolver & Daniel 1983; Montgomerie 1984; Harder 1986; Mitchell & Paton 1990; Tremeles 1996; Collins 2008). In particular, nectar viscosity, which can limit uptake by animals such as butterflies, long-tongued flies (that have narrow tubular mouthparts -May 1985; Goldblatt & Manning 2000), and those of birds (Schondube 2003) is well known to affect nectar intake rate. Essentially, intake responses are shaped by structural,

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behavioral and physiological processes (Martinez del Rio et al. 2001; Schondube 2003). A more recent perspective on floral nectar is that it can reflect the fitness gained by manipulation of pollinator behaviour (Klinkhamer & de Jong 1993; Biernaskie et al 2002). Thus some models predict that flowers should offer small or variable nectar rewards or even no rewards at all (e.g. Jersáková & Johnson 2006) in order to avoid animals visiting a large number of flowers per plant and thereby increasing the rate of pollinator-mediated self-pollination (Pappers et al 1999; Martinez del Rio et al. 2001; Biernaskie & Carter 2004; Willmer & Stone 2004; Brandenburg et al. 2009). It has also been suggested that selection may favour secondary compounds in nectar that render it unpalatable to animals that are ineffective pollinators (e.g. Johnson et al. 2006).

*Nectar in bird-pollinated plants*

Our understanding of the adaptive significance of nectar traits in bird pollination systems has changed dramatically in the last few years. Baker and Baker (1982; 1983) made the first attempt at a global summary, suggesting that plants pollinated by non-passerines (sucrose rich nectars) and plants pollinated by passerines (hexose rich nectars) were distinctly different in terms of the sugar composition of their nectars, at least in the New World. This was originally confirmed by sugar preference research conducted on birds in the laboratory, where hummingbirds were shown to prefer sucrose sugars (Stiles 1976; Hainsworth & Wolf 1976; Martinez del Rio 1990; Martinez del Rio et al. 1992), and passerines to show sucrose avoidance, and thus exhibit a hexose preference (Martinez del Rio et al. 1988; 1992; Martinez del Rio & Stevens 1989; Martinez del Rio 1990; Brugger & Nelms 1991; Brugger et al. 1993). In addition, researchers discovered differences in sugar composition of nectar from plants these two groups pollinate, suggesting some formal link between bird sugar preference and plant nectar characteristics along passerine/non-passerine lines (Martinez del Rio et al 1992). However, research done in the early to mid 1990's in South Africa, by two independent research groups, showed that South African passerine specialist nectarivores (sugarbirds and sunbirds) preferred sucrose, as do hummingbirds (Downs & Perrin 1996; Lotz & Nicolson 1996; Jackson et al. 1998a, b). In addition, it was discovered that many plants pollinated by passerine birds in South Africa had sucrose dominant nectar (Barnes et al. 1995), while others had hexose dominated nectar (Nicolson & van Wyk 1998), suggesting the passerine/non-passerine dichotomy may not

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represent a global pattern. It was then realized that sucrose aversion in passerines was probably limited to the Sturnidae-Muscicapoidea lineage (Malcarney et al. 1994; Martinez del Rio and Stevens 1989; Gatica et al. 2006; Lotz & Schondube 2006) and the Furnariidae (Lotz & Schondube 2006). For several years this posed a dilemma for pollination biologists, who were unable to explain evolutionary effects on nectar composition at a global scale.

However, a seminal paper by Johnson & Nicolson (2008) proposed a new global view of bird pollination systems, separated into two groups – plants pollinated by specialist nectar feeding birds (which includes hummingbirds, sunbirds, sugarbirds, honeyeaters, lorikeets and others) and plants pollinated by occasional nectar feeding birds (South African examples include bulbuls, white-eyes, starlings, orioles, weavers, mousebirds, etc.). A lack of a clear definition for these two groups of birds leads to some confusion. Here I define specialist nectarivores as those species for which nectar is the dominant component of the diet, and for which anatomical (bill and tongue structure) and physiological (high sucrase activity, gut morphology, and renal capabilities) adaptations to nectar feeding are clear. Using this definition, hummingbirds, sunbirds, honeyeaters and some flowerpierces can be categorised as specialist nectarivores. We define occasional nectarivores as those species where nectar is not the dominant part of the diet (although it may be seasonally), but is rather used either opportunistically, or perhaps seasonally. Although the latter group of birds have been recorded feeding on nectar for several decades, in widespread places – The Americas (Emlen 1973; Fisk 1972; Fisk & Steen 1976; Martin 1977; Spofford & Fisk 1977; Martinez del Rio & Eguiarte 1987; Melo 2001), Australasia (Franklin 1999), Africa (Oatley 1964; Jacot Guillarmod et al. 1979; Hoffman 1988), Asia (Raju & Rao 2004) and Europe (Merino & Nogueras 2003), they were only rarely identified as important pollinators, most notably in the genus *Erythrina* (Toledo 1977; Jacot Guillarmod et al. 1979; Morton 1979; Toledo & Hernandez 1979; Gryj et al. 1990; Bruneau 1997). However, new research suggests that these occasional nectar feeding birds are important primary pollinators in many plants (Vicentini & Fischer 1999; Cotton 2001; Sazima et al. 2001; Ornelas et al. 2002; Kunitake et al 2004; Raju & Rao 2004; Ortega-Olivencia et al. 2005; Johnson et al. 2006; Micheneau et al. 2006; Raju & Rao 2007; Botes et al. 2008; Rodriguez-Rodriguez & Valido 2008; Brown et al. 2009; Symes et al. 2008; 2009). Using nectar property data for 534 bird-pollinated plant species in Africa and the Americas, Johnson & Nicolson (2008) showed that

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nectar characteristics are distinct between plants pollinated by specialist and occasional nectar feeding birds, even when plant phylogeny was accounted for. In summary, they found that plants pollinated by specialist nectarivores, whether hummingbirds or passerine sunbirds, are characterised by low (10-30  $\mu$ l) volumes of relatively concentrated (15-25% w/w) (in terms of bird nectars) sucrose rich (40-60% of total sugar) nectars (Johnson & Nicolson 2008). In comparison, nectars of plants pollinated by occasional bird pollinators are characterised by large volumes (40-100 $\mu$ l) of very dilute (8-12%) nectar, with low (0-5%) sucrose content (Johnson & Nicolson 2008). The only exception to this rule within their dataset was the genus *Aloe*, which did not show flexibility in sugar type, all species producing hexose rich nectars. This confirmed Nicolson & van Wyk's (1998) argument that plant phylogeny can account for some nectar characteristics.

### *Methodological considerations*

In addition to these findings in the botanical literature, researchers started questioning the relevance of results of choice test experiments to determine sugar preferences of nectar feeding birds (Fleming et al. 2004). Until then, preference tests had used mainly equiweight solutions (Lotz & Nicolson 1996; Jackson et al. 1998a; b; Johnson et al. 1999; Blem et al. 2000) or equimolar solutions (Downs & Perrin 1996; Downs 1997) in choice tests. Equimolar solutions are perhaps useful in determining whether taste plays a role in sugar preference (Downs 1997), as this implies that a similar number of molecules interact with the gustatory receptors for each of the sugar types. However, while little is known about avian taste receptors, perception of sweetness by humans is not a simple consequence of molarity: fructose is almost twice as sweet as sucrose, and sucrose 30% sweeter than glucose, on a per gram basis (Wardlaw 1999; Smolin & Grosvenor 2003; Whitney & Rolfes 2005), with a 1:1 glucose:fructose mix presumably having a similar or slightly higher sweetness than sucrose, on a per gram basis. Furthermore, equimolar trials obviously cannot control for energy effects, since sucrose yields almost twice the energy as an equimolar hexose solution (Downs 1997; Stiles 1976). Choice tests using solutions that are equivalent in terms of sugar weight (Stiles 1976; Martinez del Rio & Karasov 1990) suffer a similar problem because of the c. 5% higher energy value for sucrose over the equivalent weight of hexose sugar (Bumstead 1980; Fleming et al. 2004). It was therefore recognized that method

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might have an influence on trial outcome, and that, in terms of bird choice, standardizing solutions by energy content should be used in choice tests (Schondube & Martinez del Rio 2003; Fleming et al. 2004). The first paper in this thesis sets out to test the influence these three different methodologies can have on the sugar preference of a specialist nectarivore, the Malachite Sunbird *Nectarinia famosa* (Chapter 2). Indeed, this chapter shows that method profoundly affects the results of preference trials, and that standardization between studies is essential in terms of global comparative work.

*Concentration dependence of sugar preference*

Most studies on bird sugar preference have examined preferences at a single, or at most two or three concentrations, while recent research has shown that sugar preference may vary with concentration (Schondube & Martinez del Rio 2003; Fleming et al 2004; 2008). Using equicaloric methods, several recent papers show that both passerine and non-passerine specialist avian nectarivores show an intriguing switch from hexose preference at low (<10) concentrations, to no preference at intermediate (10-20%) concentrations, to sucrose preference at high (>20%) concentrations (Schondube & Martinez del Rio 2003; Fleming et al. 2004; 2008). In order to further test these findings, and in an attempt to relate biological significance to them, the second paper in this thesis confirms the generality of these results using the Malachite Sunbird as a model (Chapter 3). In addition, this chapter questions the biological significance of hexose preference at low concentrations, by showing that all specialist nectarivores tested so far are unable to maintain energy balance at these low concentrations, suggesting these birds would not play a selective role for low concentration hexose nectars on the plants they feed on. No plants where specialist nectar feeding birds are the primary pollinator contains such low concentration hexose nectars, corroborating this conclusion. I also test for concentration preferences in the Malachite Sunbird over a range of concentrations reflective of bird pollinated plant nectars in southern Africa. Such tests are still needed to help explain concentration differences in plant nectars of bird pollinated systems (Leseigneur & Nicolson 2009).

*Sugar preferences and assimilation efficiencies of occasional nectar feeding birds*

In comparison to specialist nectarivores, relatively little work has been done on the sugar preferences and assimilation efficiencies of occasional nectar feeding birds. Unfortunately, what little work has been done has not standardized for energy value of test choice solutions, and by and large, has only tested preferences at one or at most two concentrations (Mata & Bosque 2004). I selected several species of occasional nectar feeding birds to redress this gap. Species selection for this work was based on two main points. Firstly, I chose representatives from frugivore, folivore, omnivore and granivore guilds, which represent the four main guilds of occasional nectar feeding birds in southern Africa. Secondly, I selected species within these guilds that are known to be common and widespread occasional nectar feeding birds in southern Africa. In addition, I chose the Red-winged Starling *Onychognathus morio* to examine whether the findings of sucrose intolerance in this superfamily elsewhere in the world extend to species based in Africa. I also used the Speckled Mousebird *Colius striatus* as a model occasional non-passerine nectar feeding bird, since apart from domestic chickens and a quail (Jukes 1938; Kare & Medway 1959; Harriman & Milner 1969), past research into non-passerine sugar preferences has been limited to specialist nectar feeding birds (hummingbirds and lorikeets - Hainsworth & Wolf 1976; Stiles 1976; Martinez del Rio 1990; Downs 1997; Schondube & Martinez del Rio 2003; Fleming et al. 2004; Fleming et al. 2008). Chapters 4, 5 and 6 (and appendix 1) of this thesis examine sugar preferences at five different concentrations for Dark-capped Bulbuls *Pycnonotus tricolor* (Chapter 4), Speckled Mousebirds (Chapter 5), Red-winged Starlings (Chapter 6) and Village Weavers *Ploceus cucullatus* (Appendix 1). In addition, these chapters examine apparent assimilation efficiency (at two different concentrations) and concentration preferences for these species. These studies are useful as a starting point to determine if occasional nectar feeding birds sugar preferences (mostly determined by bird physiology) potentially act as a selective force on the nectars of plants they pollinate (Fleming et al 2004; Lotz & Schondube 2006).

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### *Kniphofia as a model bird-pollination system*

In order to further test Johnson and Nicolson's (2008) generalist-specialist bird pollination hypothesis, the second half of the thesis presents the results of in-depth pollination ecology studies on a range of red-hot poker (*Kniphofia*: Asphodelaceae) species. The aim of this section of the thesis was to test if flower morphology and nectar characteristics would vary between species within a genus, or even within a species, reflecting the types of birds responsible for pollination. Although Johnson & Nicolson (2008) found this for two genera (*Erythrina* and *Aloe*), it remains to be tested if this pattern is widespread. The genus *Kniphofia* is surprisingly understudied in terms of its pollination ecology, considering the diversity of the genus (~ 70 species), with only one published study located during the course of the present work (Johnson et al. 2009). *Kniphofia*, along with *Aloe*, probably represent two of the more diverse groups of plants where the majority of species appear to be designed for bird pollination in Africa. Given the recent interest in studies on *Aloe* (Johnson et al. 2006; Botes et al. 2008; 2009; Symes & Nicolson 2008; Symes et al. 2008; 2009; Hargreaves et al. 2009), the opportunity to compare results with a closely related taxon was taken.

Documenting interactions between plants and occasional nectar feeding birds is of primary importance to further examine the relationship between these two groups. Although there is ample evidence of such systems in the genus *Erythrina* (Toledo 1977; Guillarmod et al. 1979; Morton 1979; Toledo & Hernandez 1979; Gryj et al. 1990; Bruneau 1997; Cotton 2001; Raju & Rau 2004; 2007), very few other examples have been well documented. However, the range of plant groups where it has been found (Vicentini & Fischer 1999; Sazima et al. 2001; Ornelas et al. 2002; Kunitake et al. 2004; Ortega-Olicencia et al. 2005; Johnson et al. 2006; Micheneau et al. 2006; Botes et al. 2008; Rodriguez-Rodriguez & Valido 2008; Brown et al. 2009; Symes et al. 2008) suggests it is a widespread phenomenon. Chapter 7 of this thesis documents how a group of occasional nectar feeding birds are the primary pollinators of *K. caulescens*, with a specialist nectar feeder (Malachite Sunbird) being primarily a nectar robber on this species.

In order to show pollinator mediated shifts within a group of bird pollinated plants, one needs to examine a range of species, each with different functional pollinator groups. Chapter 8 documents how specialist passerine nectarivores (Amethyst and Malachite Sunbirds) are the

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primary pollinators in two populations of *K. laxiflora*. This chapter, when compared to chapter 7, allows a simple comparison of nectar and flower morphology with bird pollinator type between two species in the same family.

While studying closely related species within a family is useful in terms of implying pollinator mediated selection on floral traits (Johnston 1991; Bruneau 1997; Cruden 1997; Ford & Johnson 2008), studies of ecotype variation within a species, in terms of both floral characteristics and pollinator types are perhaps even more useful, as they control for underlying phylogenetic effects. Chapter 9 documents geographical variation in bird pollinators in *K. linearifolia*, where some populations are shown to be specialist nectarivore pollinated, while others are pollinated by both specialist and occasional nectar feeding birds. Ecotype variation along various gradients, implying pollinator mediated selection, has been found in the past, particularly in terms of shifts in insect pollinators (Galen 1989; Robertson & Wyatt 1990; Johnson 1997; Herrera 2005) and between bird species within the specialist nectarivore guild (Boyd 2002; 2004; Nattero & Cocucci 2007). However, Chapter 9 appears to be the first to document the potential shift between a specialist nectarivore system and a mixed nectarivore system within a single plant species.

*Overview of the thesis*

The results of the first five chapters of the thesis suggest that bird sugar preference, apparent assimilation efficiency and, to some extent, concentration preference may explain the nectar characteristics of both specialist and occasional nectar feeding bird pollinated plants. While specialist nectar feeding birds show a concentration dependant switch from hexose preference at low concentrations to sucrose preference at high concentrations, occasional nectar feeding birds generally all show hexose preference at low concentrations, and either show hexose preference (Bulbul) or no preference (weavers, starlings) at intermediate and/or high concentrations. The exception is the non-passerine mousebird, which showed a pattern of nectar preference similar to that of specialist nectar feeding birds. In addition to this, all occasional nectar feeding passerines studied were able to maintain energy balance at low concentrations, appeared to show preference for low concentration solutions when given a choice, and were

slightly more efficient at assimilating energy from low concentration diets. In all three chapters examining case studies within *Kniphofia*, flower morphologies and nectar characteristics (with the exception of nectar sugar type) reflect the bird species responsible for pollination, indicating that pollinator mediated selection may have occurred within the genus. Combined with the results from previous chapters, this thesis suggests that plant nectar characteristics may reflect bird sugar preferences, that these can be partitioned into specialist and occasional nectar feeding groups, and that these birds may place pollinator mediated selection on the plants they feed on.

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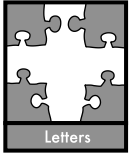
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## Sugar preferences of nectar feeding birds – a comparison of experimental techniques

Mark Brown, Colleen T. Downs and Steven D. Johnson

M. Brown (correspondence), C. T. Downs and S. D. Johnson, School of Biological & Conservation Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, 3201, South Africa. E-mail: brownma@ukzn.ac.za

Experiments to determine sugar preferences of nectarivorous animals have been conducted using a wide variety of experimental procedures, all of which aim at ensuring that the solutions offered in choices are “equivalent”. Each method used historically has controlled for a particular variable, such as number of molecules in solution, weight of sugar in solution, or amount of energy in solution, depending on what question the researchers have tried to answer. Biologists interpreting these results in terms of bird sugar preference have seldom taken these differences into account. The consequences of using different experimental procedures for sugar preferences exhibited by a nectarivorous bird, the malachite sunbird *Nectarinia famosa*, were examined using paired sucrose and hexose sugar solutions made up to be either equimolar, equiweight or equicaloric. We found the effect of methodology on bird sugar preference to be quite distinct, especially at low concentrations, where malachite sunbirds showed either sucrose preference, no preference, or hexose preference, depending on the method used. This study highlights the need for researchers to consider methodology when interpreting, or comparing among, results from previous studies.

It has often been claimed that nectarivorous animals, such as birds, bats and insects, exhibit preferences for particular sugars in choice tests (Martinez del Rio 1990, Downs 1997a, 2000, Jackson et al. 1998a, b, Johnson et al. 1999). However, the results of choice tests may be highly dependent on experimental procedure. Previous tests have involved solutions that, in terms of sugars, are equimolar (Downs 1997a, b, 2000), equivalent by weight (hereafter termed equiweight; Lotz and Nicolson 1996, Jackson et al. 1998a,b, Johnson et al. 1999, Blem et al. 2000) or equicaloric (Fleming et al. 2004).

Choice tests using equimolar solutions (Downs 1997a,b) have standardised solutions by offering equal numbers of molecules per solution, but have the obvious problem that a given sucrose solution has approximately twice the energy of an equimolar hexose solution (Downs 1997a, Stiles 1976). Choice tests using solutions that are equivalent in terms of sugar weight (Stiles 1976, Martinez del Rio and Karasov 1990) suffer a similar problem because of the c. 5% higher energy value for sucrose over the equivalent weight of hexose sugar (Bumstead 1980, Fleming et al. 2004). For these reasons, recent authors have advocated that choice tests be conducted between solutions that are potentially equivalent in energetic terms for the animal (assuming complete or similar digestion efficiency of the two choices), since the results of previous studies suggest that energy and concentration appear to be the most important features determining preference in specialist nectarivores (Fleming et al. 2004).

None of these experimental procedures are ideal from a botanical perspective (indeed, it could be argued that experiments designed to explain why nectar sugar composition varies among plants should actually involve solutions that are equivalent in terms of the energy invested into nectar by plants, as this would be the most biologically realistic null model), however from an ornithocentric view it becomes necessary to standardise various variables. Standardising energy makes sense in terms of optimal foraging theory (Hixon and Carpenter 1988) and optimal diet theory (Schaefer et al. 2003), which has shown that nectarivorous birds, for the most part, discriminate among food items according to their energetic value.

Using equicaloric solutions, Fleming et al. (2004) found that broadtailed hummingbirds *Selasphorus platycercus* and whitebellied sunbirds *Nectarinia talatala* showed very little discrimination between sucrose and hexose solutions at most concentrations, except at 0.1 mol/l sucrose equivalent (SE) concentrations where the sunbirds preferred hexose solutions. These results seem intuitive, and were predicted by Martinez del Rio and Karasov's (1990) hummingbird model for situations where hexose hydrolysis is limiting. Unless there is an underlying physiological reason (Martinez del Rio and Karasov 1990, Lotz and Schondube 2006), or a taste issue (Mata and Bosque 2004), birds should show no preference when presented with equicaloric solutions at most concentrations (Martinez del Rio and Karasov 1990). Based on their results, Fleming et al. (2004c) called for renewed research on specialist nectarivores using equicaloric

solutions in order to test the validity of long-standing assumptions (e.g. that all hummingbirds prefer sucrose). Interestingly, some animals still exhibit a sucrose preference even when equicaloric solutions are presented. For example, three species of fruit and nectar feeding bats exhibited sucrose preference, even though the plants they feed on have hexose dominant fruit and nectars (Herrera 1999).

The current study aims to quantify the effects of three experimental procedures (equimolar, equiweight and equicaloric) on the results of sugar preference tests using a specialized avian nectarivore. We used the malachite sunbird *Nectarinia famosa* as the study organism as it has previously been reported to exhibit sucrose preference in an experiment using equimolar solutions (Downs and Perrin 1996) and is a particularly important nectarivore in southern Africa.

## Materials and methods

Eight malachite sunbirds were captured under permit using mistnets in Underberg (29° 48'S 29° 30'E) and Himeville (29° 40'S 29° 32'E), KwaZulu-Natal Midlands, South Africa between December 2006 and March 2007. Birds were housed at the Animal House, University of KwaZulu-Natal, Pietermaritzburg in small cages (900mm × 30mm × 45mm) in a constant environment room at 25°C with 12:12 light:dark cycle (06:00 to 18:00). All birds were non-moulting, post breeding adults. Birds were fed a maintenance diet composed of approximately 20% sucrose, glucose, fructose solution with nutrient supplement (Ensure) and adlib fruit flies and water.

Three experiments were conducted using equimolar, equiweight and equicaloric solutions, respectively. Each experiment consisted of three trials, involving choices between a standardized sucrose solution (5, 10 and 25% sucrose by weight) and an equivalent equimolar, equiweight or equicaloric hexose solution. The experiments with equimolar solutions involved the following three trials: a) a choice of 5% (weight:weight) sucrose and 2.63% hexose (1:1 glucose:fructose mix – hereafter referred to as hexose mix), b) a choice of 10% sucrose and 5.26% hexose mix and c) a choice of 25% sucrose and 13.15% hexose mix. The experiment with equiweight solutions involved the following three trials: a) a choice of 5% sucrose and 5% hexose mix, b) a choice of 10% sucrose and 10% hexose mix, and c) a choice of 25% sucrose and 25% hexose mix. The experiment with equicaloric solutions involved the following trials: a) a choice of 5% sucrose and 5.26% hexose mix, b) a choice of 10% sucrose and 10.52% hexose mix, and c) a choice of 25% sucrose and 26.31% hexose mix.

The composition of equicaloric solutions of sucrose and hexose mixes and their energy values for a range of different solutions, is presented in Appendix 1. This indicates the correction factor necessary to ensure solutions mixed on a weight:weight basis are equicaloric. Essentially, equicaloric solutions of sucrose are half the molarity of hexose solutions, and contain 5.02% less sugar by weight.

Trials were conducted from 06:00 to 18:00, with birds weighed before and after each trial. The volume of each nectar type consumed was recorded each hour between 7:00

and 18:00. Artificial nectars were provided in modified 50ml glass burette tubes calibrated at 0.1ml intervals, with feeder position alternated at midday to avoid positional or side bias (Jackson et al. 1998b, Fleming et al. 2004). Preference for one solution over another was calculated as the proportion of sucrose nectar consumption/total nectar consumption (Martinez del Rio 1990, Mata and Bosque 2004). Trials were run on Tuesdays and Thursdays, with birds being fed maintenance diet on every other day.

To determine the effect of methodology on sugar preferences of birds, we analysed the effect of experimental procedure on the volumetric sucrose preference value (calculated as the volume of sucrose solution ingested divided by the total volume sugar solution ingested). Presenting the data as energy consumed is useful in terms of revealing the contributions of each sugar to total energy budget, however the volumetric sucrose preference data reveal actual choices made by birds and how these are affected by differences in concentrations between trials. We used repeated measures analysis of variance (RMANOVA), with individual birds being the units on which repeated measures were made, with post-hoc Tukey HSD tests, to determine the effects of experimental method and sucrose concentrations (independent variables) on volumetric sucrose preference values. The two predictor variables in this analysis were sugar concentration (5%, 10% and 25%) and method (equicaloric, equiweight and equimolar). Mean volumetric sucrose preference values in each trial were then compared to 0.5 (no preference) using one-sample *t*-tests (Martinez del Rio 1990, Fleming et al. 2004). We conducted these analyses for each of the three concentrations presented to the birds, i.e. 5%, 10% and 25% SE, in each of the three experiments. Volumetric sucrose preference values are proportions and were thus arcsine-square root transformed prior to all statistical analyses.

The effect of methodology on total energy intake of birds per day was determined using separate RMANOVAs for each set of trials linked to a particular sucrose concentration. We also determined the effect of methodology used (equicaloric, equiweight and equimolar) on sucrose intake using separate RMANOVAs for each set of trials linked to a particular sucrose concentration. In these analyses, post-hoc Tukey HSD tests were used to determine where significant interactions occurred. All RMANOVA analyses assumed compound symmetry covariance structures. All analyses were conducted using Statistica (Statsoft, Tulsa USA).

## Results

There was a significant overall interaction between experimental method and sucrose concentration on the volumetric sucrose preference values (RMANOVA:  $F_{4,28} = 15.885$ ,  $P < 0.001$ ).

### Choices relative to 5% sucrose solutions

We found no significant difference in total energy intake between the three different methods (RMANOVA:  $F_{2,14} = 0.017$ ,  $P = 0.998$ ), indicating that birds consumed

similar amounts of energy regardless of method. Differences found between methods at the sugar type level were therefore not affected by total energy intake. Since energy obtainable from sucrose in each trial was equal, we used a comparison of sucrose energy intake across the three trials to determine the full effect of method used on sucrose intake. This was significant (RMANOVA:  $F_{(2, 14)} = 40.753$ ,  $P < 0.001$ ), with birds consuming more energy as sucrose on the equimolar trial compared to the equicaloric (Tukey;  $P < 0.001$ ) or equiweight (Tukey;  $P < 0.05$ ) trials (Fig. 1).

When analysed as volumetric proportions, the data indicated that birds significantly preferred sucrose in the equimolar trials (one-sample  $t$  test:  $t = 8.643$ ,  $P < 0.001$ ), preferred hexose in the equicaloric trial (one-sample  $t$  test:  $t = -2.742$ ,  $P < 0.05$ ) and showed no preference in the

equiweight trial (one-sample  $t$  test:  $t = -2.315$ ,  $P = 0.054$ ) trials (Fig. 2a).

### Choices relative to 10% sucrose solutions

We found no difference in total energy intake between the three different methods (RMANOVA:  $F_{2, 14} = 2.126$ ,  $P = 0.156$ ), indicating that birds consumed similar amounts of energy regardless of method. Since energy obtainable from sucrose in each trial was equal, we used a comparison between sucrose energy intake across the three trials to

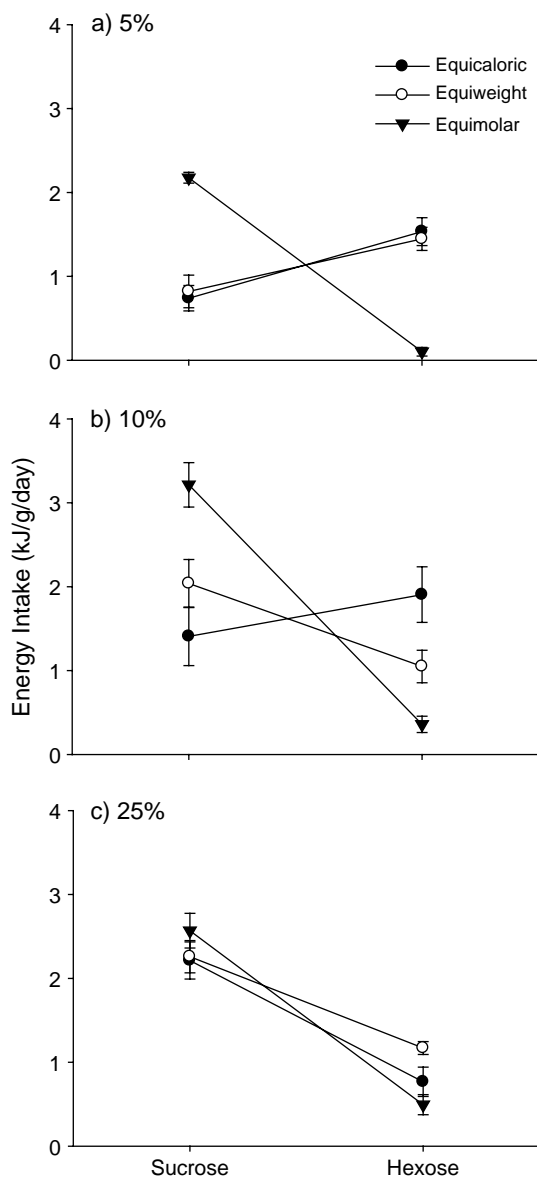


Figure 1. Energy consumed by malachite sunbirds as sucrose and hexose during pair-wise choice tests at: a) 5%, b) 10%, and c) 25% concentrations for all three methods.

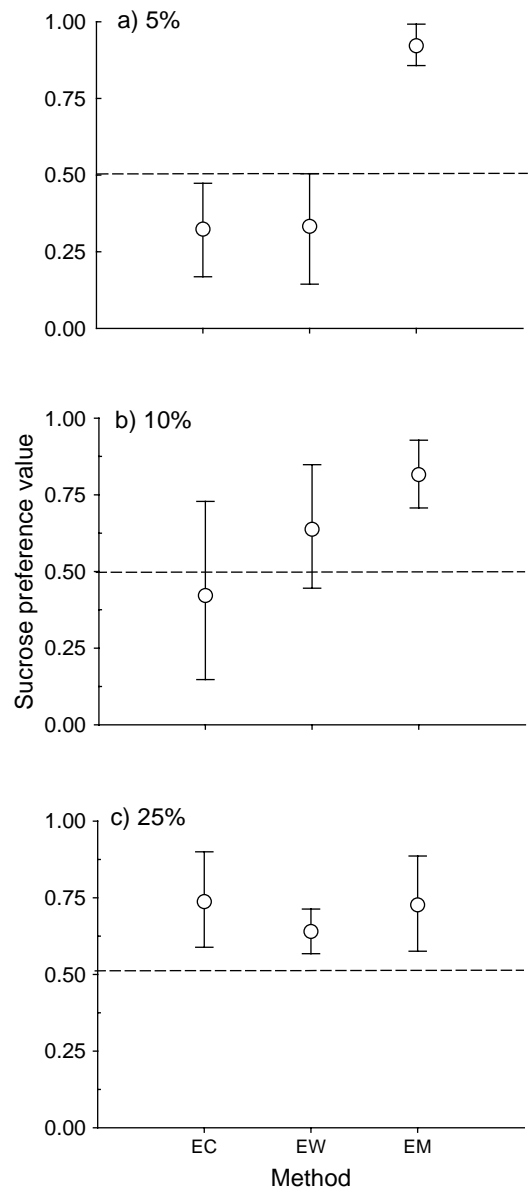


Figure 2. Preference values (PV), calculated as the volume of sucrose solution ingested divided by the total volume of solution ingested for the day, for all three methods at: a) 5%, b) 10%, and c) 25% concentrations. Values presented are backtransformed means and 95% confidence intervals obtained from arcsine square-root transformation of the original proportion data. If 0.5 (no preference) falls outside the 95% confidence intervals, the mean preference value is considered significant. EC = equicaloric, EW = equiweight, EM = equimolar.

determine the full effect of method used on sucrose intake. This was significant (RMANOVA:  $F_{2,14} = 11.878$ ,  $P < 0.001$ ), with birds consuming more energy as sucrose on the equimolar trial compared to the equicaloric (Tukey;  $P < 0.001$ ) or equiweight (Tukey;  $P < 0.05$ ) trials.

When analysed as volumetric proportions, the data indicated that birds significantly preferred sucrose in the equimolar trials (one-sample  $t$  test:  $t = 5.724$ ,  $P < 0.001$ ), but showed no preference in both the equicaloric trial (one-sample  $t$  test:  $t = -0.580$ ,  $P = 0.580$ ) and the equiweight trial (one-sample  $t$  test:  $t = 1.775$ ,  $P = 0.119$ ; Fig. 2b).

### Choices relative to 25% sucrose solutions

Again we found no difference in total energy intake between the three different methods (RMANOVA:  $F_{2,14} = 3.118$ ,  $P = 0.076$ ), indicating that birds consume similar amounts of energy regardless of method (although, given that  $p$  value is marginally non-significant, we cannot exclude the possibility that an effect would be detected if the sample size was larger).

When analysed as volumetric proportions, the data indicated that birds significantly preferred sucrose in the equimolar trial (one-sample  $t$  test:  $t = 3.348$ ,  $P < 0.05$ ), the equicaloric trial (one-sample  $t$  test:  $t = 3.490$ ,  $P < 0.001$ ) and the equiweight trial (one-sample  $t$  test:  $t = 4.465$ ,  $P < 0.005$ ; Fig. 2c).

## Discussion

This study shows that results obtained in trials to test nectarivore sugar preferences are highly affected by experimental procedures. Only at high concentrations (choices relative to 25% sucrose solution) did the method used not affect sugar preference in this study. In trials involving 10% sucrose solutions, birds significantly preferred the sucrose solution over an equimolar hexose solution, but did not discriminate among the sucrose solution and equicaloric or equiweight hexose solutions. In trials involving 5% sucrose solutions, birds showed a marked preference for the sucrose solution over an equimolar hexose solution, exhibited no preference for either sugar type when equiweight solutions were used, and exhibited a significant preference for hexose sugars when alternative sugars were presented in equicaloric solutions.

Sucrose concentrations were kept constant across each of the three methods used, allowing us to directly compare the effect of method on sucrose preference, in terms of energy consumed or volumetric proportions. At high concentrations this had no effect, but at lower concentrations the effect of method was pronounced (Figs. 1 and 2). Birds consumed less of the 5% sucrose solution in the equicaloric experiments than in the equiweight experiments, indicating that hexose preference only becomes evident when birds are presented with equicaloric low sugar concentration solutions. These results indicate that the methodological concerns raised by Fleming et al. (2004) are indeed significant, and will need to be addressed immediately in current and new research projects. It also questions the

conclusions regarding species sugar preferences based on previous research using either equimolar or equiweight solutions, and highlights the need to repeat some of the early work done to test whether the general assumption of sucrose preference for most specialist nectarivorous birds holds true.

One of the key issues is to identify what a specific experiment is trying to test. If one is trying to determine whether taste overrides energy considerations, then presenting equimolar solutions is logical as it implies that a similar number of molecules interact with the gustatory receptors for each of the sugar types (Downs 1997a). However, in these equimolar experiments both sugar type and energy varies between solutions offered, making it impossible to establish if preference for sucrose observed is due to taste or energy considerations. If the main emphasis is whether birds select one sugar type over another based on factors other than the potential energy value of solutions, then it is essential that solutions are presented using the equicaloric technique. This has obvious consequences for birds that can detect differences as low as 1% between solutions (e.g. rufous hummingbirds *Selasphorus rufus*, Blem et al. 2000), where the 5.02% energy difference between equiweight solutions (1g sucrose yields 16.5 kJ, whereas 1g glucose or fructose yields 15.6 kJ, Bumstead, 1980) may mask real preference trends. Some authors in the past have mistakenly called equiweight solutions equicaloric (Martinez del Rio 1990), or incorrectly used a 9.1% correction factor between sucrose and glucose solutions (Macdonald et al. 1981). It therefore becomes crucial when interpreting results from preference trials to determine the variables in need of control.

Finally, it is useful to note that sugar preference experiments of the type carried out in this study can only partly explain the evolution of sugar composition of nectar in bird-pollinated flowers. Plants use nectar to manipulate the behaviour of pollinators, and in some cases, this is achieved by offering rewards that are less than optimally attractive, as illustrated by the many plants that do not offer any nectar at all in order to maximize cross-pollination (Johnson et al. 2004). In addition, nectar is costly to plants (between 3% and 35% dry mass – see De la Barrera and Nobel 2004) and these costs may vary according to the sugar composition of nectar and thus have an important influence on the evolution of nectar offerings. Nevertheless, the sugar preferences of avian nectarivores will be an important part of the sought-after evolutionary equation that will explain why bird-pollinated plants vary in nectar sugar composition (Johnson and Nicolson 2008), which is why it is so important to optimize and standardize the methodology used in experiments to study these preferences.

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Appendix 1. Comparison of molarity (moles/l) and weight of sucrose and glucose solutions to ensure equicalority using common concentration values from the literature.

Sucrose mol/l	%w:w	Hexose mol/l	%w:w	kJ/g
0.073	2.5	0.146	2.63	0.413
0.146	5	0.292	5.26	0.825
0.292	10	0.584	10.52	1.65
0.438	15	0.876	15.79	2.475
0.73	25	1.46	26.31	4.125
1.022	35	2.044	36.85	5.775
1.461	50	2.922	52.64	8.25
2.337	80	4.674	84.23	13.2



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## CONCENTRATION-DEPENDENT SUGAR PREFERENCES OF THE MALACHITE SUNBIRD (*NECTARINIA FAMOSA*)

MARK BROWN,<sup>1</sup> COLLEEN T. DOWNS, AND STEVEN D. JOHNSON

*School of Biological and Conservation Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville 3201, South Africa*

**ABSTRACT.**—Sugar-type preferences of nectarivores may be an important selective factor in the evolution of sugar composition in floral nectar. We investigated sugar preferences of the Malachite Sunbird (*Nectarinia famosa*), using experiments in which birds were offered paired choices between energetically equivalent solutions over a range of sugar concentrations. The birds preferred hexose at low (5%) concentration and sucrose at high (25%) concentration; they showed no preference at 10%, 15%, and 20% concentrations. The birds regulated energy intake by adjusting volumetric consumption, except on a 5% concentration diet, where they failed to maintain energy balance. They also exhibited a strong preference for concentrated solutions, given a choice between 10%, 15%, 20%, and 25% sucrose solutions. We discuss the significance of these results in terms of the nectar composition of sunbird-pollinated plants. *Received 5 August 2008, accepted 29 July 2009.*

**Key words:** bird pollination, choice experiment, floral reward, Malachite Sunbird, nectar, *Nectarinia famosa*, nectarivore, pollination, sugar composition.

### Les préférences en sucres dépendent de la concentration chez *Nectarinia famosa*

**RÉSUMÉ.**—Les préférences quant au type de sucre chez les nectarivores peuvent constituer un facteur de sélection important dans l'évolution de la composition en sucres du nectar floral. Nous avons étudié les préférences en sucre chez *Nectarinia famosa* à partir d'expériences au cours desquelles les oiseaux avaient le choix entre des solutions équivalentes du point de vue énergétique, pour une gamme de concentrations en sucre. Les oiseaux ont préféré l'hexose à une concentration faible (5%) et le sucrose à une concentration élevée (25%); ils n'ont présenté aucune préférence à des concentrations de 10%, 15% et 20%. Les oiseaux régulaient l'apport énergétique en ajustant le volume consommé, sauf pour le régime à 5% de concentration en sucre, pour lequel ils n'ont pu maintenir une balance énergétique. Ils ont aussi montré une forte préférence pour les solutions concentrées, lorsqu'ils avaient le choix entre des solutions de sucrose à 10%, 15%, 20% et 25%. Nous discutons le sens de ces résultats en termes de composition du nectar des plantes pollinisées par les souïmangas.

THE EXTENT TO which the sugar composition of floral nectar reflects selection by avian nectarivores, as opposed to plant phylogeny, is debated (Van Wyk et al. 1993, Ornelas et al. 2007, Schmidt-Lebuhn et al. 2007, Johnson and Nicolson 2008). Johnson and Nicolson (2008) found a strong dichotomy in nectar traits (volume, concentration, and sugar type) between plants pollinated by specialist avian nectarivores (e.g., sunbirds, honeyeaters, and hummingbirds) and those pollinated by generalist ones (e.g., bulbuls and starlings), even when plant phylogeny was accounted for. This suggests that bird pollinators have imposed selection on the nectar characteristics of the plants they pollinate. Hence, it is important to understand the sugar-type preferences of a range of specialist and generalist nectarivorous bird species across passerine and nonpasserine lineages.

It was recently pointed out that the long-standing paradigm that specialist avian nectarivores have a preference for sucrose over

hexose sugars was based largely on studies that used flawed experimental procedures (Fleming et al. 2004). In particular, many earlier investigators did not ensure that sugar solutions offered in choices were energetically equivalent, nor did they test how preferences might vary across a range of sugar concentrations. Fleming et al. (2004) called for past work to be re-examined to test assumptions about sugar preferences in birds. In a recent empirical paper comparing methodologies for determining sugar preferences, Brown et al. (2008) showed that diametrically opposite conclusions can be reached when the same bird species is subjected to different methodologies. In a recent study involving choices between energetically equivalent solutions (the "equicaloric method"), White-bellied Sunbirds (*Cinnyris talatala*) preferred hexose sugars at low concentrations and sucrose at high concentrations (Fleming et al. 2004). The generality of this interesting concentration-dependence of preference is not yet known because the sugar preferences of

<sup>1</sup>E-mail: [brownma@ukzn.ac.za](mailto:brownma@ukzn.ac.za)

birds across a wide range of sugar concentrations have been tested in only a few species (Schondube and Martínez del Rio 2003, Lotz and Schondube 2006, Fleming et al. 2008).

In addition, more data are needed to describe differences among birds in their preferences for nectar of different concentrations. It is already known that avian nectarivores tend to prefer solutions of high concentrations over those of lower concentrations (Hainsworth and Wolf 1976, Tamm and Gass 1986, Blem et al. 1997), but how this preference varies among species is unknown. Few studies have documented sugar-concentration preferences of sunbirds, and these have used low sample sizes (Lloyd 1989) or only two concentrations, which makes estimating the shape of the relationship impossible (Lotz and Nicolson 1996). Examining concentration preferences of nectarivorous birds is important for understanding whether they have had a selective influence on floral nectar concentrations (Johnson and Nicolson 2008).

We re-examined sugar-type preferences of the Malachite Sunbird (*Nectarinia famosa*) using paired equicaloric hexose and sucrose solutions across a range of concentrations. The aim was to determine whether birds exhibit a fixed preference for a particular sugar type or whether preference varies with sugar concentration. In addition, we wanted to establish the strength and shape of the relationship between concentrations of solutions and their attractiveness to birds.

#### METHODS

Eight Malachite Sunbirds (body mass  $\bar{x} = 16.6 \pm 0.63$  g) were captured using mist nets in Underberg (29°48'S, 29°30'E) and Himeville (29°40'S, 29°32'E) in the KwaZulu-Natal Midlands, South Africa, between December 2006 and March 2007. These birds were kept at the Animal House, University of KwaZulu-Natal, Pietermaritzburg, in small cages (900 × 300 × 450 mm) in a constant-environment room at 25°C with a 12:12 h light:dark cycle. They were fed a maintenance diet composed of ~20% sucrose, glucose, fructose solution (2:1:1) with Ensure nutrient supplement (Abbott Laboratories, Hoofddorp, The Netherlands) and *ad libitum* fruit flies. To establish sugar preferences, pairwise choice tests were conducted using the following equicaloric pairs of solutions: (1) 5% (0.146 mol<sup>-1</sup>) sucrose and 5.26% hexose (0.292 mol<sup>-1</sup>) mix (1:1 glucose:fructose), (2) 10% (0.292 mol<sup>-1</sup>) sucrose and 10.52% hexose (0.584 mol<sup>-1</sup>) mix, (3) 15% (0.438 mol<sup>-1</sup>) sucrose and 15.79% hexose (0.88 mol<sup>-1</sup>) mix, (4) 20% (0.584 mol<sup>-1</sup>) sucrose and 21.06% hexose (1.17 mol<sup>-1</sup>) mix, and (5) 25% (0.73 mol<sup>-1</sup>) sucrose and 26.31% hexose (1.46 mol<sup>-1</sup>) mix.

Artificial nectars were provided in modified 50-mL burette tubes calibrated at 0.1-mL intervals, with positions alternated at midday to avoid positional or side bias (Jackson et al. 1998, Fleming et al. 2004). Trials were conducted from 0600 to 1800 hours, and birds were weighed before and after each trial. The volume of each nectar type consumed was recorded each hour between 0600 and 1800 hours. Preference for one solution over another was calculated as the proportion of a single sugar type consumed in relation to total nectar consumption (Martínez del Rio 1990, Mata and Bosque 2004). We also converted the total volume of each solution consumed into energy consumed per gram of body mass per day, expressed as kJ g<sup>-1</sup> day<sup>-1</sup>. Data for 5%, 10%, and 25% concentration were taken from Brown et al. (2008).

In a separate trial, birds were presented with randomly positioned feeders that contained solutions of 4 concentrations, namely 10%, 15%, 20%, and 25% sucrose, for a period of 5.5 h (from 0600 to 1130 hours). The volume of each solution consumed was converted into energy consumed.

We tested the effect of diet concentration on the energy balance of birds by comparing body mass measured before and after each trial using repeated-measures analysis of variance (RMANOVA), with birds as the units of repeated measure and both diet concentration and time (before and after trial) as the independent variables. As a second test of energy balance, we used RMANOVA with post hoc Tukey's HSD tests to compare the total energy ingested by each individual on each trial day, using concentration as the independent variable and total daily energy intake as the dependant variable.

We also used RMANOVA to determine whether there was an overall significant relationship between sugar type and concentration (independent variables) and the preference of birds for sucrose solutions in terms of volumetric intake. Mean volumetric sucrose-preference values in each trial were then compared with 0.5 (no preference) using one-sample *t*-tests (Martínez del Rio 1990, Fleming et al. 2004). We conducted these analyses for each of the 5 concentrations presented to the birds (i.e., 5%, 10%, 15%, 20%, and 25% sucrose equivalent). Volumetric sucrose-preference values are proportions and were arcsine square-root transformed before statistical analyses.

We compared sucrose intake (kJ) from each of the 4 sucrose concentrations presented simultaneously to birds in the concentration trial using RMANOVA with post hoc Tukey's HSD tests. All analyses were conducted using STATISTICA (Statsoft, Tulsa, Oklahoma).

#### RESULTS

There was a significant overall effect of the concentration of solutions and time (before and after each trial) on body mass over the trials (RMANOVA:  $F = 8.293$ ,  $df = 4$  and  $28$ ,  $P < 0.001$ ). However, post hoc tests revealed that birds lost mass only after a day of feeding on the 5% solutions (Tukey's HSD,  $P = 0.007$ ); on average, birds on this diet lost 6.04% body mass in 1 day. In all other trial days, body mass was not affected (Tukey's HSD, 10%:  $P = 0.69$ ; 15%:  $P = 1$ ; 20%:  $P = 0.14$ ; 25%:  $P = 0.52$ ). The energetic value of nectar consumed varied among trials of different concentrations (RMANOVA:  $F = 13.785$ ,  $df = 4$  and  $28$ ,  $P < 0.001$ ). Post hoc Tukey's HSD tests revealed that this was because the energetic value of the 5% solutions consumed was significantly lower than in the other trials.

There was a highly significant combined effect of concentration and sugar type on birds' sugar preference, when examined as volumetric proportion of sucrose solution ingested (RMANOVA:  $F = 5.257$ ,  $df = 4$  and  $28$ ,  $P = 0.003$ ; Fig. 1). The birds showed a significant preference for hexose sugars when offered solutions of 5% concentration (one-sample *t*-test:  $t = -2.472$ ,  $P = 0.029$ ) and a significant preference for sucrose when offered solutions of 25% concentration ( $t = 3.490$ ,  $P = 0.010$ ). They showed no significant preferences when offered solutions at concentrations of 10% (one-sample *t*-test:  $t = -0.580$ ,  $P = 0.580$ ), 15% ( $t = 0.210$ ,  $P = 0.840$ ), or 20% ( $t = 1.351$ ,  $P = 0.218$ ) (Fig. 1). As expected, the birds adjusted volumetric intake at different concentrations to regulate daily

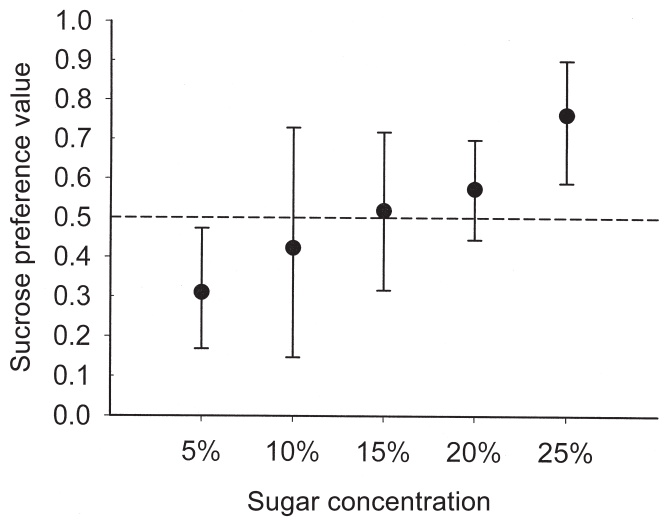


FIG. 1. Preference values, calculated as the volume of sucrose solution ingested by captive Malachite Sunbirds, divided by the total volume of solution ingested per day, for paired choice tests at 5 concentrations. Values presented are back-transformed means and 95% confidence intervals obtained from arcsine square-root transformation of the original proportion data. If 0.5 (no preference) falls outside the 95% confidence intervals, the mean preference value is considered significant. Data for 5%, 10%, and 25% concentrations are extracted from Brown et al. (2008).

energy intake (Fig. 2). However, intake at 5% was insufficient to maintain energy balance.

There was a significant effect of sugar concentration on the energy consumed from sugar solutions when birds were offered a choice of 4 different sucrose concentrations (RMANOVA:  $F = 4.566$ ,  $df = 3$  and  $21$ ,  $P = 0.013$ ; Fig. 3). The birds consumed significantly more 25% sucrose solution than 10% (Tukey's HSD,  $P = 0.012$ ) or 15% ( $P < 0.046$ ) solutions, but they did not consume significantly more 25% solution than 20% solution ( $P = 0.114$ ).

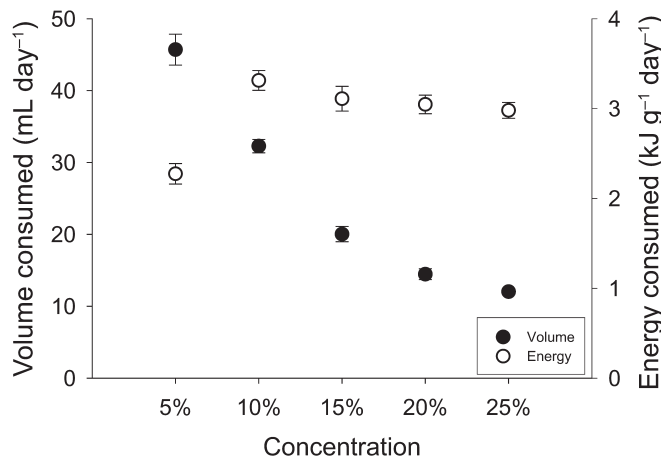


FIG. 2. Adjustment of volumetric intake by captive Malachite Sunbirds resulted in similar energy intake across 4 of 5 concentrations. At 5%, energy balance was not maintained. Values presented are means ± SE.

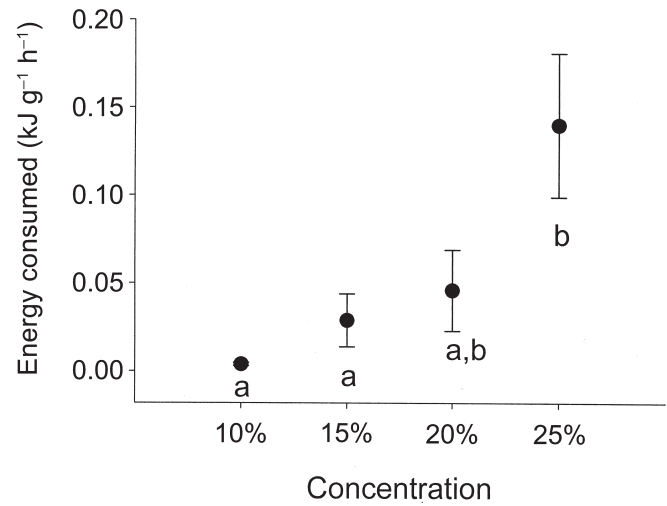


FIG. 3. The energetic value of total volume consumed on each of 4 sucrose concentrations simultaneously offered to captive Malachite Sunbirds. Values presented are means ± SE. Means that share the same letter are not significantly different.

### DISCUSSION

Our results show that sugar-type preference in Malachite Sunbirds was significantly influenced by the concentration of solutions. The birds switched from preferring hexose sugars at 5% concentrations to preferring sucrose at 25% concentrations, with no sugar preference exhibited at intermediate concentrations. Few authors have considered the possibility that sugar-type preferences change with concentration (Downs and Perrin 1996; Avery et al. 1999; Schondube and Martínez del Rio 2003; Fleming et al. 2004, 2008; Lotz and Schondube 2006). Our results are similar to those obtained with Magnificent Hummingbirds (*Eugenes fulgens*) and Cinnamon-bellied Flowerpiercers (*Diglossa baritula*) (Schondube and Martínez del Rio 2003); New Holland Honeyeaters (*Phylidonyris novaehollandiae*), Red Wattlebirds (*Anthochaera carunculata*), and Rainbow Lorikeets (*Trichoglossus haematotus*) (Fleming et al. 2008); and White-bellied Sunbirds (Fleming et al. 2004). Thus, this relationship is found in all specialist nectarivore taxonomic groups and in several species within each group.

Preference for a specific sugar type when a bird is presented with equicaloric solutions suggests that some underlying physiological mechanism is driving such preferences (Martínez del Rio and Karasov 1990), particularly when assimilation efficiency for both sugar types is not measurably different; for Malachite Sunbirds, it is >99% for both sucrose and hexose sugars (Downs 1997). Intriguingly, the switch from hexose preference at 5% solutions to sucrose preference at 25% solutions suggests physiological constraints that are concentration dependent. Hexose preference is often explained by sucrose hydrolysis limitations, especially at low concentrations (Schondube and Martínez del Rio 2003), or by the greater osmolality of hexose solutions (Nicolson and Fleming 2003b, Fleming et al. 2008). Although sucrose preference has often puzzled researchers (Martínez del Rio and Karasov 1990), Schondube and Martínez del Rio (2003) suggested that at high

concentrations a preference for sucrose solutions would be explained by their lower osmotic potential compared with hexose solutions, which would speed up the rate at which food can be delivered from the crop or stomach to the intestine. In practice, however, this has been shown to not necessarily be the case (Martínez del Río 1990). Alternatively, sucrose preferences at high concentrations could be linked to the equicaloric hexose solution having about twice the osmolality of sucrose solutions and, thus, being more likely to dehydrate the gut (Fleming et al. 2008). The molar difference between equicaloric hexose and sucrose solutions may facilitate the proximate discrimination of these solutions by birds through taste (Fleming et al. 2008). A third alternative presented by Fleming et al. (2008) is that birds' preferences indicate a learned response to the nectars available in the plants they feed on.

Malachite Sunbirds preferred higher concentrations when offered a choice of 4, which was not unexpected. Hummingbirds have long been known to show preference for higher concentrations when offered a choice (Bene 1945, Colias and Colias 1968, Stiles 1976, Stromberg and Johnsen 1990, Blem et al. 2000). Although some research has shown that sunbirds do the same (Lloyd 1989, Lotz and Nicolson 1996), our data appear to be the first to show this over a range of 4 concentrations (10–25%) that accurately represent nectar concentrations that sunbirds can be expected to encounter in the wild (Johnson and Nicolson 2008).

The Malachite Sunbirds in our study adjusted volumetric intake at concentrations between 10% and 25% and thereby regulated their total daily energy intake. However, they were unable to increase volumetric intake enough at 5%, which resulted in an energy deficit that over the course of a single day led to a decrease in body mass of 6.04%. Similar negative energy budgets on low-concentration nectars occur in other sunbirds (Nicolson and Fleming 2003a, Fleming et al. 2004), in hummingbirds (McWhorter and Lopez-Calleja 2000, McWhorter and Martínez del Río 2000, Martínez del Río et al. 2001, Fleming et al. 2004), and in honeyeaters and lorikeets (Fleming et al. 2008). For most specialist avian nectarivores tested so far, concentrations below  $0.25 \text{ mol L}^{-1}$  (8.56%) resulted in a negative energy balance or signs of nutritional stress (Lloyd 1991). This suggests a physiological constraint on intake rate, with birds failing to maintain energy balance on these dilute diets (McWhorter and Martínez del Río 2000; Martínez del Río et al. 2001; Nicolson and Fleming 2003a, b). Thus, experiments that deal with sugar-type preferences of specialist nectarivores at very low sugar concentrations (e.g., 2.8%; Fleming and Nicolson 2008) may have little biological relevance for explaining the evolution of nectar in bird-pollinated plants. However, the finding in these previous studies and in ours that sunbirds did not maintain weight on solutions with concentrations <10% partly explains Johnson and Nicolson's (2008) finding that bird-adapted plants with very dilute nectar (<10%) were seldom pollinated by sunbirds (Johnson and Nicolson 2008).

Recent research has shown that opportunistic avian nectarivores are the primary pollinators of ornithophilous plants with very dilute nectar (<10%; Johnson et al. 2006, Botes et al. 2008, Johnson and Nicolson 2008, Symes et al. 2008, Brown et al. 2009). This suggests that the hexose preference demonstrated by several specialist nectarivores feeding on solutions of very low sugar concentration, although interesting, probably is not often a selective factor in the evolution of nectar composition. Rather, we suggest

that selection for sugar composition by sunbirds will typically be neutral (at intermediate sugar concentration) or favor sucrose (at higher concentrations). We speculate, as did Lotz and Shon-dube (2006), that in cases where bird-pollinated plants have nectar dominated by hexose sugars, the explanation will be found in plant phylogenetic constraints or in selection imposed by generalist avian nectarivores.

#### ACKNOWLEDGMENTS

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## Sugar preferences and digestive efficiency in an opportunistic avian nectarivore, the Dark-capped Bulbul *Pycnonotus tricolor*

Mark Brown · Colleen T. Downs · Steven D. Johnson

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**Abstract** It has recently been recognized that flowers pollinated by generalist opportunistic nectarivores tend to have different nectar properties to those pollinated by specialist nectarivores (including both hummingbirds and specialist passerines). While renewed interest in specialist avian nectarivore sugar preferences and digestive physiology has helped explain the concentrated sucrose-dominated nectar of plants they feed on, there has been little progress in understanding why generalist or occasional nectar-feeding birds tend to be associated with flowers that have dilute hexose-dominated nectar. We examined sugar preferences and assimilation efficiencies over a range of concentrations, and concentration preferences, in Dark-capped Bulbuls *Pycnonotus tricolor*, one of the more common occasional avian nectarivores in southern Africa. Dark-capped Bulbuls showed significant preference for hexose sugar solutions, irrespective of concentration, when given a choice between hexose and sucrose solutions in equicaloric pair-wise choice tests conducted at five different concentrations (5–25%). This contrasts with results from specialist nectarivore groups which generally show a significant concentration-dependant switch in preference from hexose at low concentrations to sucrose at high concentrations for equicaloric solutions. In addition, Dark-capped Bulbuls showed an unusual lack of preference for solutions of higher sugar concentration when simultaneously offered four solutions varying in concentration from 10 to 25%.

Dark-capped bulbuls also showed a unique effect of concentration on sugar assimilation efficiency, assimilating relatively more energy on 5% diets than on 25% diets. Although able to assimilate sucrose effectively, assimilation rates of hexose sugars were marginally higher. These results shed new light on pollination systems involving occasional nectarivores and, in particular, help to explain the prevalence of low concentration hexose-dominated nectars in flowers pollinated by these birds.

**Keywords** Assimilation efficiency · Bird pollination · Nectarivore · Sugar composition

### Introduction

While flower visitation by generalist avian nectarivores was noted in the past (Cruden and Toledo 1977; Toledo 1977), floral adaptation for pollination by these birds has only recently been well documented (Vicentini and Fischer 1999; Mendonca and Anjos 2006; Rocca et al. 2006; Schmidt-Lebuhna et al. 2007; Botes et al. 2008; Johnson and Nicolson 2008; Rocca and Sazima 2008; Rodriguez-Rodriguez and Valido 2008; Symes et al. 2008; Brown et al. 2009). Studies in the past tended to lump both generalist and specialist passerine nectarivores into a single group that was contrasted with hummingbirds. The fallacy of this hummingbird–passerine dichotomy was pointed out by Johnson and Nicolson (2008) who presented data showing that flowers pollinated by specialist passerines are strongly convergent with those pollinated by hummingbirds, and that the most significant dichotomy in bird pollination systems is between specialist and generalist nectarivores, regardless of their phylogenetic affinity. In particular, flowers of plants pollinated by specialist avian

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M. Brown (✉) · C. T. Downs · S. D. Johnson  
School of Biological and Conservation Sciences,  
University of KwaZulu-Natal, Private Bag X01, Scottsville,  
Pietermaritzburg 3201, South Africa  
e-mail: brownma@ukzn.ac.za

nectarivores tend to have small volumes of relatively concentrated (c. 20%) sucrose-rich nectar, while those of plants pollinated by generalist avian nectarivores tend to have copious volumes of low concentration (c. 10%) hexose-rich nectar (Johnson and Nicolson 2008). A remaining challenge is thus to determine whether these differences in nectar properties can be linked to differences in the digestive physiology of generalist and specialist avian nectarivores.

Methods for investigating the sugar preferences of avian nectarivores are receiving a critical re-examination (Fleming et al. 2004, 2008; Brown et al. 2008). This is because choice of methodology can affect the results of choice experiments (Brown et al. 2008), which in turn biases theoretical understanding of the selective pressures that avian pollinators impose on plant nectar characteristics. While new research is addressing these issues for specialist nectarivore species, most notably sunbirds, hummingbirds, honeyeaters and lorikeets (Schondube and Martinez Del Rio 2003; Fleming et al. 2004, 2008), there has been little work done on generalist avian nectar feeders.

In this study, we examined the sugar preferences of Dark-capped Bulbuls *Pycnonotus tricolor* at five different concentrations, reflective of the concentration range of southern African ornithophilous plants (Johnson and Nicolson 2008). We also examined whether bulbuls show a concentration preference, as this may be important in terms of explaining the low concentration nectars predominantly found in generalist pollinated plants (Johnson and Nicolson 2008). In addition, we determined apparent assimilation efficiency of bulbuls for both low and high concentration hexose and sucrose sugars, to determine if differences in this parameter affect their sugar preference. We also tested Lotz and Schondube's (2006) prediction that passerines (sunbirds excluded) should have lower intake rates when feeding on sucrose than on hexose of equicaloric concentration, because hexose sugars are more readily assimilated than sucrose. We predicted that Dark-capped Bulbuls would exhibit a hexose preference, particularly at low concentrations, and that this might be linked to a lower apparent assimilation efficiency for sucrose than for hexose sugars.

## Methods

### Study species

Although classified as a frugivore, the Dark-capped Bulbul includes insects, other arthropods, flower buds and nectar in its diet (Lloyd 2005). Species in the genus *Pycnonotus* are amongst the more ubiquitous generalist nectar feeders and pollinators in southern Africa (Johnson et al. 2006; Symes et al. 2008).

### Animal husbandry

We captured nine Dark-capped Bulbuls ( $37.47 \pm 0.98$  g) on the campus of the University of KwaZulu-Natal ( $29^{\circ}38'S$ ,  $30^{\circ}24'E$ ), using a combination of mistnets and cage traps baited with fruit during September 2007. Birds were initially housed together in outdoor aviaries ( $2 \times 3 \times 2$  m) and fed a maintenance diet of fresh fruit (apples, bananas, pawpaw, banana, pear, oranges, grapes) supplemented with Aviplus Softbill Mynah Pellets (Avi-products, Durban, RSA), mealworms, bonemeal, a 20% sucrose/glucose/fructose mix supplemented with a multi-vitamin (Avi-Sup Soluble Multivitamins; Avi-products) and water ad lib. After an initial acclimation period of 2 weeks to captivity, birds were transferred into a constant environment room ( $25^{\circ}C$ , 12:12 light:dark cycle) where they were individually housed in small, double breeder cages ( $900 \times 300 \times 450$  mm). Birds were acclimated on the maintenance diet for another 2 weeks.

### Sugar type preference trials

Pair-wise choice tests were conducted between the following w:w equicaloric pairs of solutions: (1) 5% (0.146 M) sucrose and 5.26% hexose (0.292 M); (2) 10% (0.292 M) sucrose and 10.52% hexose (0.584 M); (3) 15% (0.438 M) sucrose and 15.79% hexose (0.88 M); (4) 20% (0.584 M) sucrose and 21.06% hexose (1.17 M); and (5) 25% (0.73 M) sucrose and 26.31% hexose (1.46 M). All hexose solutions were made up of equal parts glucose and fructose sugars, as this is the usual combination of hexose sugars in many flower nectars (Freeman et al. 1991; De la Barrera and Nobel 2004). For a discussion on the use of equicaloric solutions in choice tests, see Fleming et al. (2004) and Brown et al. (2008). Artificial nectars were provided in modified 50-ml burette tubes calibrated at 0.1-ml intervals, whose position was alternated at midday to avoid positional or side bias (Jackson et al. 1998b; Fleming et al. 2004). Trials were conducted from 0600 to 1800 hours, with birds weighed before and after each trial. We measured hourly consumption of each sugar solution between 0700 and 1800 hours. Preference for one solution over another was calculated as the proportion of single sugar type consumption/total nectar consumption (Martinez del Rio 1990; Mata and Bosque 2004). We also converted the total volume consumed of each solution into energy consumed, expressed as  $\text{kJ g}^{-1} \text{day}^{-1}$  (Brown et al. 2008).

We analysed total energy intake ( $\text{kJ g}^{-1} \text{day}^{-1}$ ), using repeated measures analysis of variance (RMANOVA) with post-hoc Tukey HSD tests to determine differences in total energy intake (i.e. energy regulation) between the five different concentration trials. We conducted these analyses

for each of the five concentrations presented to the birds, i.e. 5, 10, 15, 20 and 25%. In order to determine the effect energy intake on each concentration had on bird energy balance, we compared bird body mass before and after each trial using RMANOVA with post-hoc Tukey HSD tests.

We used RMANOVA, with concentration as the independent variables in order to determine the overall effect of concentration on birds' sucrose preference. We then compared volumetric sucrose preference values (calculated as the volume of sucrose solution ingested divided by the total volume sugar solution ingested) to 0.5 (no preference), using one-sample *t* tests (Martinez del Rio 1990; Fleming et al. 2004). Since volumetric sucrose preference values are proportions, they were arcsine-square root transformed prior to all statistical analyses.

#### Concentration preference trials

In order to determine if Dark-capped Bulbuls exhibit a concentration preference, we did a further trial whereby each bird was given a choice of four randomly positioned concentrations of hexose solutions (10, 15, 20 and 25%) for a 12-h period. The volume of each solution drunk was converted into energy consumed per g per day (Brown et al. 2008). While some authors have equated volumetric proportions of nectars consumed in concentration trials as indicating preference (Mata and Bosque 2004), we feel this is erroneous as one cannot separate out the effects of concentration and volume drunk. Birds drinking a greater volume of a dilute solution may in fact be ingesting less energy when compared to the lower amount drunk of the more concentrated solution. We independently compared hexose intake as energy ( $\text{kJ g}^{-1} \text{day}^{-1}$ ) and as volume ( $\text{ml day}^{-1}$ ) from each of the four hexose concentrations presented simultaneously to birds in the concentration trial using RMANOVA followed by post-hoc tests to determine if a concentration preference was evident.

#### Assimilation efficiency

We determined apparent assimilation efficiencies (AE) for sucrose and hexose diets at two different concentrations (5 and 25%) respectively. For each experimental day, birds were placed in wire-bottomed cages placed over a tray of liquid paraffin (used to collect liquid faeces without evaporation loss) and fed a single sugar solution for a 12-h period (0600–1800 hours). While on a liquid diet, Dark-capped Bulbuls excrete liquid-only faeces, which they excrete while perching, ensuring that all faeces drop directly into the collecting tray. Birds were then deprived of food overnight during the scotophase to ensure all

consumed food was processed and voided, and removed from their tray to maintenance cages by 0800 hours the following morning. Volume of nectar consumed was recorded for the duration of the day (this was later used to compare intake rates on different sugars). All liquid excreta were collected the following day by syringe from the liquid paraffin, and weighed to determine total excreta weight. Samples were then centrifuged at 1,300g for 3 min. Samples were cleaned by crystallisation, and then reconstituted into 2 ml of ultrapure water. We analysed excreta sugar content using a Shimadzu (LC-20AT) high-performance liquid chromatograph (HPLC). Detection was by refractive index (RID-10A) with a Phenomenex column (Rezex RCM-Monosaccharide,  $200 \times 780$  mm, 8 micron). Isocratic separation was accomplished using ultrapure water as the mobile phase. We compared sample spikes to known standards to quantify sugar composition. AE was determined using the following equation:

$$AE = 100 \times [(mg \text{ sugar}_{in}) - (mg \text{ sugar}_{out}) / (mg \text{ sugar}_{in})],$$

where  $(mg \text{ sugar}_{in})$  was a function of molar concentration and volume of sugar solution consumed, and  $(mg \text{ sugar}_{out})$  was a function of excreta volume and concentration of sugar in the excreta (mg/ml).

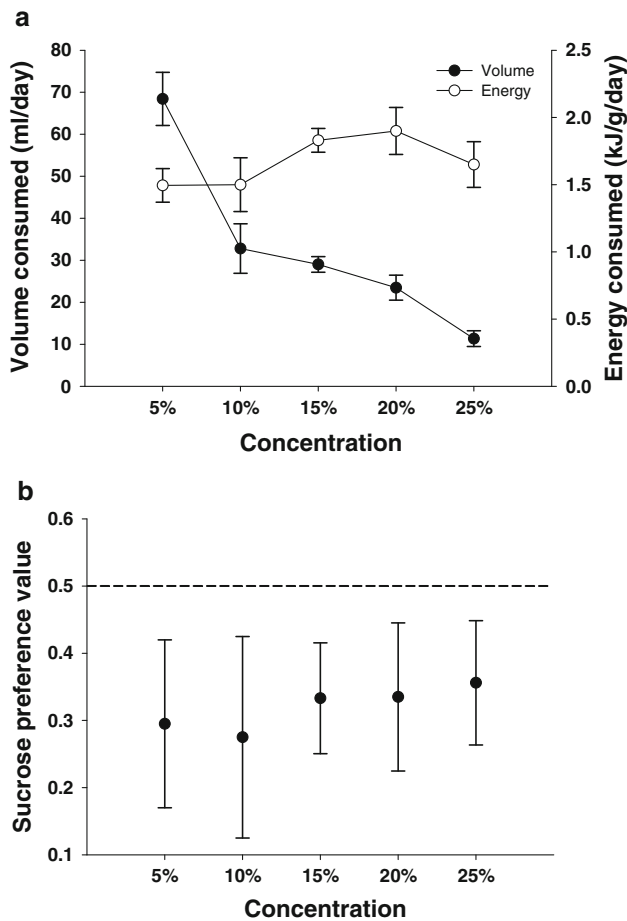
To determine if there was any effect of sugar type or concentration on sugar solution assimilation efficiency, we used RMANOVA on arcsine-transformed AE data, with post-hoc Tukey HSD tests used to pinpoint significant differences. Finally, we compared volumetric intake ( $\text{ml day}^{-1}$ ) of sucrose and hexose fed exclusively to birds at both 5 and 25% concentrations, using RMANOVA. All statistical analyses were conducted using Statistica (Statsoft USA).

## Results

#### Sugar type preference trials

Dark-capped Bulbuls consumed similar amounts of energy per day at all five concentrations (RMANOVA:  $F_{4,28} = 1.186$ ,  $P = 0.337$ ), and adjusted volumetric intake to meet energy demands, with decreased intake at higher concentrations (Fig. 1a). Bird body mass did not differ significantly between any of the trials (RMANOVA:  $F_{4,28} = 2.116$ ,  $P > 0.1$ ).

There was no overall effect of concentration on bird sugar preference, in terms of volumetric sucrose intake (RMANOVA:  $F_{4,28} = 1.049$ ,  $P = 0.400$ ; Fig. 1b). Dark-capped Bulbuls ingested significantly more hexose than sucrose at 5% (one-sample *t* test:  $t = 3.640$ ,  $P < 0.05$ ), at 10% ( $t = 3.380$ ,  $P < 0.05$ ), at 15% ( $t = 4.464$ ,  $P < 0.05$ ), at 20% ( $t = 3.581$ ,  $P < 0.05$ ) and at 25% ( $t = 3.300$ ,  $P < 0.05$ ).



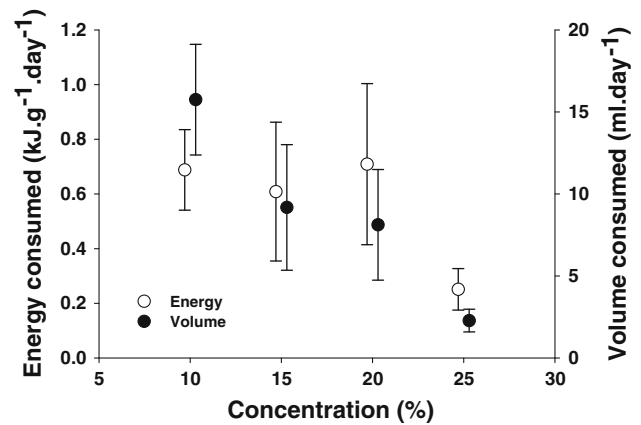
**Fig. 1** **a** The relationship between volumetric intake and diet concentration across five different concentrations in Dark-capped Bulbuls *Pycnonotus tricolor*. Values represent means  $\pm$  standard error. **b** Preference values (PV), calculated as the volume of sucrose solution ingested divided by the total volume of solution ingested for the day, for paired choice tests at five different concentrations. Values presented are back-transformed means and 95% confidence intervals obtained from arcsine square-root transformation of the original proportion data. If 0.5 (no preference) falls outside the 95% confidence intervals, the mean preference value is considered significant

#### Concentration preference trials

Dark-capped Bulbuls showed no overall interaction of concentration on preference for any of the four concentrations of hexose solutions simultaneously presented to them, in terms of energy consumption (RMANOVA:  $F_{3,15} = 0.567$ ,  $P = 0.645$ ; Fig. 2), or volumetric intake (RMANOVA:  $F_{3,15} = 1.696$ ,  $P = 0.211$ ; Fig. 3). However, a post-hoc Fisher LSD test indicated that volumetric intake at 10% was significantly higher than at 25% ( $P < 0.05$ ).

#### Assimilation efficiency

There was no significant overall interaction between sugar type and concentration on apparent assimilation efficiency



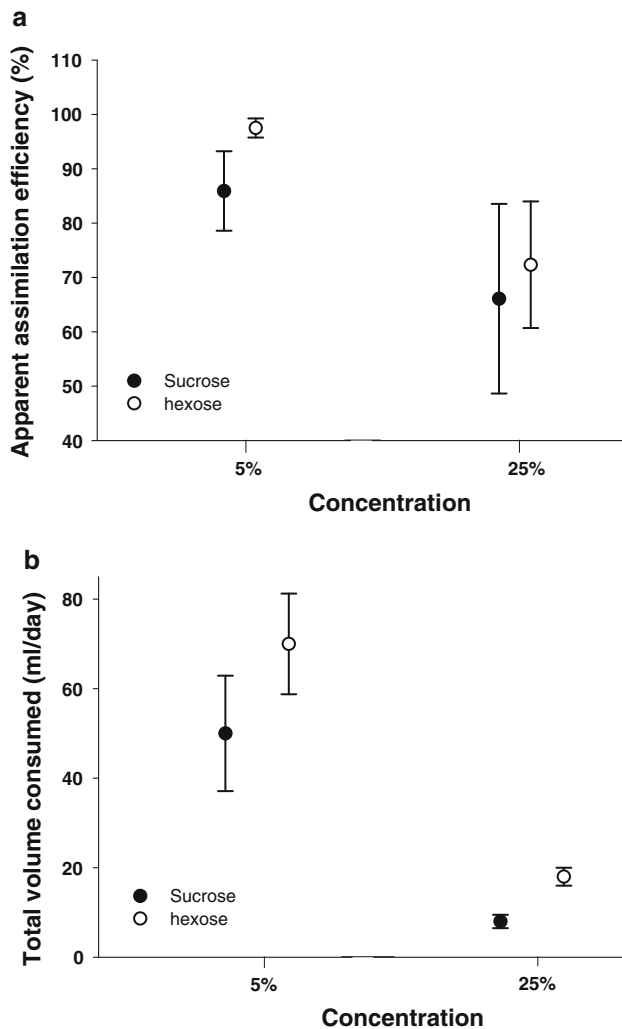
**Fig. 2** Energy and volume consumed from each of four concentrations of hexose sugar solutions simultaneously presented to Dark-capped Bulbuls. Values represent means  $\pm$  standard error

of bulbuls (RMANOVA:  $F_{1,5} = 1.970$ ,  $P = 0.233$ ; Fig. 3a). Sugar type had no independent effect on apparent assimilation efficiency (RMANOVA:  $F_{1,5} = 0.528$ ,  $P = 0.508$ ). There was, however, a significant independent effect of concentration on the apparent assimilation efficiency of birds (RMANOVA:  $F_{1,5} = 10.70$ ,  $P < 0.05$ ), with them extracting energy on average 22.49% more efficiently from 5% solutions than from 25% solutions (Fig. 3a). Post-hoc Tukey tests revealed that birds were more efficient at extracting sugars from 5% hexose solutions than from 25% sucrose or 25% hexose solutions (Fig. 3a).

There was a significant interaction between sugar type and concentration in their effect on total volumetric intake (RMANOVA:  $F_{1,5} = 12.009$ ,  $P < 0.05$ ). Birds ingested significantly more hexose sugars than sucrose at both 5% (Tukey;  $P < 0.05$ ) and 25% (Tukey;  $P < 0.05$ ) concentrations (Fig. 3b).

#### Discussion

Our results show that Dark-capped Bulbuls show consistent preferences for hexose solutions when given a choice between equicaloric hexose and sucrose solutions at five different concentrations ranging from 5 to 25%. This is in stark contrast to results from equicaloric studies of all the main groups of specialist nectar feeding birds studied so far, which all generally show a switch from hexose preference at very low concentrations, to no preference at medium concentrations, to sucrose preference at high concentrations (Schondube and Martinez Del Rio 2003; Fleming et al. 2004, 2008). In addition, Dark-capped Bulbuls exhibited moderate preference for the 10% over the 25% solution when given a choice of four different hexose solutions, unlike specialist avian nectarivores which



**Fig. 3** **a** Apparent assimilation efficiency of *sucrose* and *hexose* solutions at low and high concentrations by Dark-capped Bulbuls. **b** Total daily volumetric intake for hexose and sucrose, presented independently for a whole day at 5 and 25% concentrations. Symbols Mean  $\pm$  SE

generally exhibit preference for the higher concentrations (Stromberg and Johnsen 1990; Lotz and Nicolson 1996; Blem et al. 2000; Brown et al. 2010).

Distinct hexose preference has been found in other occasional nectar feeders, but only those in the Sturnidae–Muscicapoidea lineage (Malcarney et al. 1994; Martinez del Rio and Stevens 1989; Gatica et al. 2006). However, these species show sucrose aversion due to a lack of the sucrase enzyme, rendering them incapable of breaking down sucrose into its monosaccharide constituents (Martinez del Rio and Stevens 1989). Dark-capped Bulbuls, however, show relatively high apparent assimilation efficiencies for both hexose and sucrose sugars at low concentrations, suggesting that sucrase activity is high within this species. Another generalist bird, the Cape White-eye

*Zosterops virens*, has similar apparent assimilation efficiencies for sucrose in nectar and fruit (88.6%, Brown and Downs 2003; 92–94%, Wellmann and Downs 2009; >99%, Franke et al. 1998—differences may be due to concentrations tested, see below, or methodology). Even so, Dark-capped Bulbuls were slightly more efficient at extracting energy from hexose than sucrose nectars (11.60% difference for 5% solutions and 6.25% difference for 25% solutions). Differences like this may explain the hexose preferences exhibited by Dark-capped Bulbuls at all concentrations, given that hummingbirds are able to discriminate differences in concentrations as low as 1% on 25% diets (Blem and Blem 2000). Lotz and Schondube (2006) also suggest that birds with intermediate sucrase activity levels potentially act as selective agents for hexose-rich nectar and fruit, even if they have high assimilation efficiency for this sugar, due to different intake rates between different sugar types (see below). Of particular interest, however, is the significant difference in apparent assimilation efficiencies at different concentrations (Fig. 3). Generally, specialist avian nectarivores show no effect of concentration on apparent assimilation efficiency, all exhibiting very high efficiencies (Downs 1997b; Markman et al. 2006; McWhorter and Lopez-Calleja 2000). Dark-capped Bulbuls, however, are more efficient at energy extraction at 5% than at 25% (by 25.16% on hexose nectar and 19.81% on sucrose nectar), suggesting that energy optimisation of nectar diets occurs at lower concentrations for this generalist nectarivore.

It is also of interest that Dark-capped Bulbuls conformed to Lotz and Schondube's (2006) prediction that intake rate would be higher for hexose than equicaloric sucrose diets, allowing higher energy intake rates. This may be especially important at low concentrations where birds are more constrained to meet daily energy balance.

Dark-capped Bulbuls were able to maintain energy balance on all diets at all concentrations. This finding contrasts with specialist avian nectarivores which are unable to maintain energy balance on solutions below 8.56% (0.25 M). This applies to sunbirds (Nicolson and Fleming 2003; Fleming et al. 2004; Brown et al. 2010), hummingbirds (Fleming et al. 2004), honeyeaters (Fleming et al. 2008) and lorikeets (Fleming et al. 2008). In a recent study, honeyeaters and sunbirds displayed significantly lower non-mediated (paracellular) glucose uptake on 8.56% diet compared with 34.23% diet (Napier et al. 2008). Napier et al. (2008) concluded that non-mediated glucose uptake is positively correlated with diet concentration, and is influenced by the positive correlation between digesta retention time and diet energy density. Since apparent assimilation efficiency is not affected by concentration in specialist nectarivores (see above), such results may be one factor why specialists prefer higher

concentration diets when given a choice (Hainsworth and Wolf 1976; Tamm and Gass 1986; Blem et al. 1997; Brown et al. 2010). However, our current research shows that at least some generalist nectarivores exhibit changes in apparent assimilation efficiency that are concentration dependant, suggesting that the interplay between mediated and non-mediated glucose uptake may function differently for these birds than for specialist nectarivores, thereby affecting not only their sugar preferences but also their concentration preferences.

Our results suggest that the presence of large volume, low concentration, hexose-dominant nectars in plants pollinated by generalist or occasional nectarivores can be accounted for by the sugar preferences, concentration-dependant assimilation efficiencies, higher hexose-solution intake rates on equicaloric solutions, and the apparent preference for low concentration solutions of these birds. These are perhaps the first data that show this, and represent some of the missing pieces in unravelling the evolution of nectar rewards in pollination systems involving occasional avian nectarivores.

## Zusammenfassung

Zuckerpräferenz und Verdauungseffizienz eines opportun nectarivoren Vogels, dem Graubülbül *Pycnonotus tricolor*

In letzter Zeit wurde deutlich, dass der Nektar von Blumen, die von generalistischen, opportunen nectarivoren Vögeln bestäubt werden, oft andere Eigenschaften hat als solcher von Blumen, die von spezialisierten Nektarivoren (Kolibris und andere spezialisierte Singvögel) befruchtet werden. Während dieses neue Interesse an Zuckerpräferenzen und Verdauungseffizienzen von nectarivoren Spezialisten unter den Vögeln dazu beigetragen hat, den konzentrierten, Saccharose-dominierten Nektar von Futterpflanzen zu erklären, wissen wir trotzdem relativ wenig darüber, warum Generalisten oder Vogelarten, die nur gelegentlich Nektar aufnehmen, zur Assoziation mit Blumen mit verdünnten Hexose-dominierten Nektar neigen. Wir untersuchen Zuckerpräferenzen und Verdauungseffizienz über einen Gradienten von Zuckerkonzentrationen und Konzentrationspräferenzen beim Graubülbül, einer häufig vorkommenden, gelegheitsnectarivoren Vogelart in Südafrika. Bei einem Angebot von equikalorischer Hexose- und Saccharoselösung präferierten Graubülbüls signifikant Zuckerlösungen aus Hexose, unabhängig von der Konzentration (5–25%). Dies widerspricht Ergebnissen von Studien an spezialisierten nectarivoren Arten, welche generell in Abhängigkeit der Zuckerkonzentration einen signifikanten Wechsel der Präferenz von Hexose in

niedrigen Konzentrationen zu Saccharose in hohen Konzentrationen zeigen, bei equikalorischen Lösungen. Zusätzlich präferierten Graubülbüls nicht, wie üblich, höher konzentrierte Zuckerlösungen, wenn ihnen gleichzeitig vier Lösungen in verschiedenen Konzentrationen (10–25%) zur Auswahl gegeben wurden. Außerdem fanden wir in Graubülbüls einen ungewöhnlichen Effekt der Zuckerkonzentration auf die Verdauungseffizienz,—bei einer Ernährung von 5% wurde relativ mehr Energie assimiliert als bei einer 25% Ernährung. Obwohl sie die Fähigkeit besitzen, Saccharose wirksam zu assimilieren, waren die Verdauungsraten von Hexose nur geringfügig höher. Dies wirft neues Licht auf die Befruchtungssysteme, die gelegentliche Nektarivoren einschließen, und hilft das Vorkommen von niedrig konzentriertem, Hexose-dominiertem Nektar in Blumen, die von Vögeln bestäubt werden, zu erklären.

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# **SUGAR PREFERENCES OF A GENERALIST NON-PASSERINE FLOWER VISITOR, THE AFRICAN SPECKLED MOUSEBIRD**

Mark Brown<sup>1\*</sup>, Colleen T. Downs<sup>1</sup> & Steven D. Johnson<sup>1</sup>

School of Biological & Conservation Sciences, University of KwaZulu-Natal, Private Bag X01,  
Scottsville, Pietermaritzburg, 3209, South Africa

In Press *Auk*

\*Corresponding author:

Mark Brown

<sup>1</sup>School of Biological & Conservation Sciences, University of KwaZulu-Natal, Private Bag X01,  
Scottsville, Pietermaritzburg, 3209, South Africa, brownma@ukzn.ac.za

## **Abstract**

The preferences bird species exhibit for different sugar types may play a role in pollinator mediated selection on plant nectar composition. How bird sugar type preferences are affected by concentration is a fundamental question for research that aims to use bird sugar preferences to explain plant nectar composition. Such research has been done for several specialist avian nectarivores, and for a few passerine occasional nectarivores, but not apparently for any non-passerine occasional nectarivore. In this study we examined the effect of concentration of sugar solutions (equicaloric hexose and sucrose) on sugar type preferences of Speckled Mousebirds *Colius striatus*, a common African non-passerine that occasionally feeds on floral nectar.

Surprisingly, mousebirds exhibited a concentration-dependent switch in sugar preference, from hexose sugars at low concentration to sucrose at high concentrations, that was remarkably similar to that shown by specialist avian nectarivores, and which contrasts to results reported for passerine occasional nectarivores. During these experiments, mousebirds were unable to meet energy demands on low concentration diets, and lost significant body mass when fed 5% and 10% solutions. Speckled Mousebirds showed no preference when simultaneously presented with four different hexose solutions representing the range of concentrations (10-25%) found in bird-pollinated flowers. Speckled Mousebirds showed no difference in ability to digest sucrose and hexose sugars. Our results suggest that Speckled Mousebirds exhibit sugar preferences traits which are similar to those of specialist avian nectarivores, while their apparent lack of concentration preference is similar to that found in passerine occasional nectarivores.

**Key words:** Speckled Mousebird, Occasional Nectarivore, Sucrose, Sugar preference

## **Introduction**

There are several recent advances in our understanding of bird pollination systems, in terms of plant nectar composition and concentration (Johnson and Nicolson 2008), bird choice-test methodology (Brown et al. 2008) and specialist avian nectarivore sugar preferences across a range of concentrations (Fleming et al. 2004, 2008; Brown et al. 2010). This has led to a growing interest in the sugar preferences and apparent digestive efficiency of both passerine and non-passerine occasional avian nectarivores (Lotz and Schondube 2006; Brown et al. 2008; Brown et al. 2010). In particular, recent research has focused on three interlinked factors that determine selective pressure by birds on nectar characteristics. These are ability to detect differences between different kinds and concentrations of sugar solutions, sugar preferences exhibited at different concentrations, and the assimilation efficiencies of different sugars in the digestive system.

Occasional nectarivores (birds that include nectar as a small component of their diet) are now known to be important primary pollinators for many plants (Ortega-Olivencia et al. 2005; Botes et al 2008; Johnson and Nicolson 2008; Rodriguez-Rodriguez and Valido 2008; Symes et al. 2008; Brown et al. 2009), and it is thus of interest to examine the role, if any, they play in

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selection on nectar characteristics in the plants they feed on. In particular, there is a need to understand their preferences among solutions that differ in sugar composition and concentration, and to establish how these preferences relate to assimilation efficiency (Brown et al. 2010). Occasional nectarivores are found across a wide range of taxa, and include both passerine and non-passerine species. Common African representatives include the starlings, weavers, white-eyes, bulbuls, mousebirds, barbets, orioles, and drongos (Oatley 1964; Symes et al. 2008).

Preliminary research on sugar type preferences in passerine occasional nectarivores has revealed consistent preferences for hexose sugars at low concentrations, and either hexose preference, or no preference, at high concentrations (Brown et al. submitted; in press; Odendaal et al. in press). These results are very different to the concentration dependent switch in sugar preferences from hexose to sucrose sugars in specialist nectarivores (Fleming et al. 2004; 2008; Brown et al. 2010). Rather surprisingly, given the incredibly high phylogenetic diversity found amongst non-passerine birds, very little is known about sugar preferences within this group, with the exception of the Trochilidae (hummingbirds, Hainsworth and Wolf 1976, Stiles 1976, Martínez del Río 1990, Schondube and Martínez del Río 2003, Fleming et al. 2004). Research on non-hummingbird lineages has so far been mostly limited to two species of fowl (chickens, *G. gallus*; and Japanese quail, *Coturnix coturnix japonica*; family Phasianidae; Jukes 1938; Kare and Medway 1959; Harriman and Milner 1969), and two lorikeets (Red Lory, *Eos bornea*; Downs 1997; and Rainbow Lorikeet, *Trichoglossus haematonotus*; Fleming et al. 2008). Most of this research was conducted using a single concentration, and equiweight or equimolar solutions (see Brown et al. 2008). Recent work, covering a range of concentrations and using equicaloric solutions, has been limited to hummingbirds and the Rainbow Lorikeet, both specialist nectarivores (Fleming et al. 2004; 2008). Whether different responses are found in non-passerine occasional nectarivores remains to be tested (Lotz & Schondube 2006). Such research allows direct testing of whether phylogeny has any bearing on the evolution of sugar and concentration preferences in birds, and whether these in turn affect plant floral nectar characteristics.

One of the strong patterns to emerge from the study by Johnson and Nicolson (2008) was that while flowers pollinated by specialist avian nectarivores tend to have relatively concentrated nectar (>20% sugar by weight), nectar in flowers pollinated by occasional avian nectarivores tends to be very dilute (<12%). As might be expected, specialist avian nectarivores show a

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preference for higher concentrations (within the range of concentrations reflective of most bird-pollinated plant nectars) when given a choice (Blem et al. 1997; Hainsworth and Wolf 1976; Tamm and Gass 1986, Leseigneur & Nicolson 2009, Brown et al. 2010). The little data available for passerine occasional avian nectarivores so far suggests that they show little or no concentration preference (Red-winged Starlings *Onychognathus morio* Brown et al. submitted; Dark-capped Bulbuls *Pycnonotus tricolor* Brown et al. in press; Village Weavers *Ploceus cucullatus* M. Brown unpub. data).

Sugar preference has been linked to digestive assimilation efficiency for different sugars in bird species of the Sturnidae-Muscicapoidea lineage (Malcarney et al. 1994; Martinez del Rio and Stevens 1989; Gatica et al. 2006; Lotz & Schondube 2006). While apparent assimilation efficiencies for both sucrose and hexose sugars in specialist nectarivores are high (> 95% Downs 1997; Jackson et al. 1998), most occasional nectarivores are expected to have lower assimilation efficiencies for different sugars due to their gut structure being less specialized, or adapted to different diets (Brown & Downs 2003). The interplay between bird physiology (assimilation efficiency) and behavior (sugar preference) is what determines selective pressure placed by birds on plant nectar characteristics (Brown et al in press). In terms of apparent assimilation efficiency of sucrose, Lotz and Schondube (2006) present a phylogenetic hypothesis whereby they suggest that all non-passerine nectarivorous birds should show high efficiency (90-100%). Lotz and Schondube (2006) suggested that a range of non-passerine groups, including the mousebirds (Coliidae), which are common occasional nectarivores in Africa, should be studied to further test this hypothesis.

The aims of this study were to determine the sugar preferences, concentration preferences, and apparent assimilation efficiency of Speckled Mousebirds (*Colius striatus*) over a range of concentrations reflective of bird-pollinated plant nectar concentrations in the field. Speckled Mousebirds are omnivores, and feed on fruit, nectar, insects and leaves (Downs et al. 2000). Mousebirds are frequently seen drinking nectar on *Aloe* (Hockey et al 2005), which appears to be phylogenetically constrained to produce hexose rich nectar (Johnson & Nicolson 2008), *Erythrina* (Hockey et al 2005) and *Schotia* (M. Brown unpublished data)

We hypothesized that mousebirds would exhibit sugar preferences and assimilation efficiencies that are convergent with those of passerine occasional nectarivores. We thus predicted that

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Speckled Mousebirds would exhibit hexose preference at low and intermediate concentrations, and either hexose preference or no preference at high concentrations. In addition, we predicted that Speckled Mousebirds would show either no concentration preference, or a preference for lower concentrations. Finally, we predicted that Speckled Mousebirds would have higher apparent assimilation efficiencies for hexose than for sucrose, and that concentration may affect this parameter.

### **Methods**

We captured ten Speckled Mousebirds using Ecotone mistnets and walk-in traps in Hilton, KwaZulu-Natal, South Africa (2930S 3018E) in February 2008. We acclimated birds initially to captivity in a large outdoor aviary (2 X 3 X 2m) in a single group on a maintenance diet of fresh fruit (apples, bananas, pawpaw, pear, oranges) supplemented with Aviplus Softbill Mynah Pellets (Aviproducts, Durban, RSA) and a 20% sucrose/glucose/fructose mix supplemented with a multivitamin (Avi-Sup Soluble Multivitamins; Aviproducts, Durban, RSA) for nine months. Thereafter, we transferred birds into a constant environment room (25° C, 12:12 L:D cycle) where they were housed individually in small cages (900 x 300 x 450mm). Birds were acclimated on the maintenance diet for another two weeks.

### *Sugar type preference trials*

We conducted pair-wise choice tests using the following w:w equicaloric pairs of solutions: a) 5% (0.146 mol l<sup>-1</sup>) sucrose and 5.26% hexose (0.292 mol l<sup>-1</sup>); b) 10% (0.292 mol l<sup>-1</sup>) sucrose and 10.52% hexose (0.584 mol l<sup>-1</sup>); c) 15% (0.438 mol l<sup>-1</sup>) sucrose and 15.79% hexose (0.88 mol l<sup>-1</sup>); d) 20% (0.584 mol l<sup>-1</sup>) sucrose and 21.06% hexose (1.17 mol l<sup>-1</sup>); and e) 25% (0.73 mol l<sup>-1</sup>) sucrose and 26.31% hexose (1.46 mol l<sup>-1</sup>). All hexose solutions were made up of equal parts glucose and fructose sugars. We used modified 50-ml burette tubes calibrated at 0.1-ml intervals, with initial position randomised and position alternated at midday to avoid side bias (Jackson et al. 1998; Fleming et al. 2004). Trials were conducted from 06h00 to 18h00, with birds weighed before and after each trial. We converted volumes drunk into a preference value for sucrose by

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using the proportion of sucrose consumption / total nectar consumption (Martínez del Rio 1990; Mata and Bosque 2004; Brown et al. 2008). We also converted the total volume consumed of each solution into energy consumed, expressed as  $\text{kJ g}^{-1} \text{day}^{-1}$  (Brown et al. 2008). Trials were run every second day, with birds being fed the maintenance diet on alternate days.

We analysed total daily energy intake ( $\text{kJ g}^{-1} \text{day}^{-1}$ ) at each concentration using GLM Repeated Measures Analysis of Variance (RMANOVA) with post-hoc Tukey HSD tests to determine differences in total energy intake (i.e. daily energy regulation). To determine the effect that energy intake from each concentration had on bird energy balance, we compared bird body mass before and after each trial using RMANOVA with post hoc Tukey HSD tests.

The overall effect of concentration on birds' sugar preference was determined using RMANOVA, with concentration as the independent variable. Thereafter, we compared arcsine-square root transformed volumetric sucrose preference values to 0.785 (no preference value for arcsin-squareroot transformed data), using one-sample *t*-tests (Martínez del Rio 1990; Fleming et al. 2004; Brown et al. 2010).

#### *Concentration preference trials*

In a separate trial, we gave Speckled Mousebirds a choice of four randomly-positioned hexose solutions of different concentrations (equicaloric 10, 15, 20 and 25% as above) for 12h. Volume of each solution drunk was recorded to determine a daily intake and converted into energy consumed per g per day (Brown et al. 2008). We compared energy intake ( $\text{kJg}^{-1}\text{day}^{-1}$ ) and volume consumed ( $\text{ml}\text{day}^{-1}$ ) on each of the four hexose concentrations presented simultaneously to birds in the concentration trial using RMANOVA to determine if a concentration preference was evident (Brown et al. in press).

#### *Apparent assimilation efficiency*

We determined apparent assimilation efficiencies (AE) for sucrose and hexose diets at two different concentrations (equicaloric 5% and 25% as above) respectively. Birds were placed in

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wire bottomed cages placed over a tray of liquid paraffin (used to collect liquid faeces without evaporation loss) and fed a single sugar solution for 4h (08h00 to 12h00). Birds were then deprived of food for a further 2h to ensure all consumed food was processed and voided, and then removed and taken to maintenance cages thereafter. Total nectar volume consumed over the 4h was recorded. All liquid excreta were then collected by syringe from the liquid paraffin, and weighed to determine total excreta weight. Samples were then centrifuged at 1300rpm for three min. We analysed excreta sugar content using a Shimadzu (LC-20AT) high-performance liquid chromatograph (HPLC). Detection was by refractive index (RID-10A) with a Phenomenex column (Rezex RCM-Monosaccharide, 200 x 780mm 8micron). Isocratic separation was accomplished using ultrapure water as the mobile phase. AE was corrected for volume consumed and excreted as per Jackson et al. (1998). We used RMANOVA on arcsine transformed AE data, to determine if there was any effect of sugar type or concentration (independent variables) on sugar AE. All statistical analyses were conducted using Statistica (Statsoft, version 7, Tulsa, USA).

Approval for this project was received from the Animal Ethics sub-committee of the University of KwaZulu-Natal.

**Results***Sugar type preference trials*

Speckled Mousebirds did not consume the same amount of energy per day at the respective five concentrations (RMANOVA:  $F_{4, 36} = 22.015$ ,  $p < 0.001$ ; Fig. 1). Total daily energy intake ( $\text{kJ g}^{-1} \text{hr}^{-1}$ ) was significantly lower at 5%, 10% and 25% than at 15% and 20% concentrations (Post-hoc Tukey:  $p < 0.05$ ). Bird body mass overall was significantly influenced by the interaction of time and concentration (RMANOVA:  $F_{4, 36} = 4.000$ ,  $p < 0.01$ ; Fig. 2). Post-hoc Tukey tests revealed that birds lost body mass significantly after feeding on 5% and 10% concentrations respectively ( $p < 0.05$ ) but maintained body mass on 15% ( $p = 0.84$ ), 20% ( $p = 0.66$ ) and 25% ( $p = 1.00$ ) concentrations. Average body mass loss was 2.9% at both 5% and 10% respectively.

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There was a significant effect of concentration on mousebird sugar preference, in terms of volumetric sucrose intake (RMANOVA:  $F_{4,36} = 3.188$ ,  $p < 0.05$ ; Fig. 3). Speckled Mousebirds significantly preferred hexose over sucrose at 5% (one-sample  $t$  test:  $t = 3.162$ ,  $p < 0.05$ ), and at 10% (one-sample  $t$  test:  $t = 3.263$ ,  $p < 0.01$ ), showed no preference at 15% (one-sample  $t$  test:  $t = 0.179$ ,  $p = 0.862$ ) or 20% (one-sample  $t$  test:  $t = 0.160$ ,  $p = 0.877$ ), but showed a significant preference for sucrose at 25% (one-sample  $t$  test:  $t = 2.878$ ,  $p < 0.05$ ). Birds did attempt to adjust volumetric intake to meet energy demands, but were unable to do so sufficiently at 5% and 10% (Fig. 1).

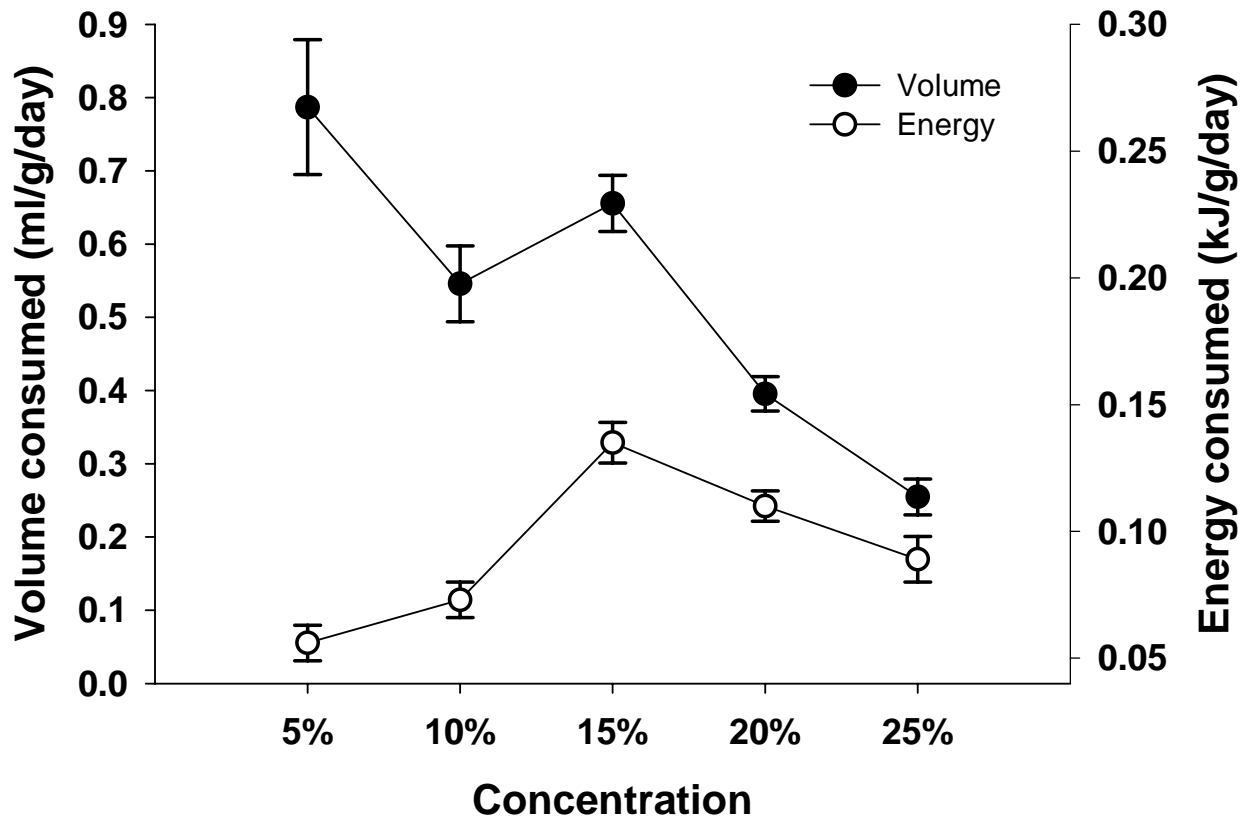


Figure 1: Adjustment of volumetric intake by Speckled Mousebirds when presented with equicaloric choices between different sugar solutions across a range of five different concentrations. Values represent means  $\pm$  standard error.

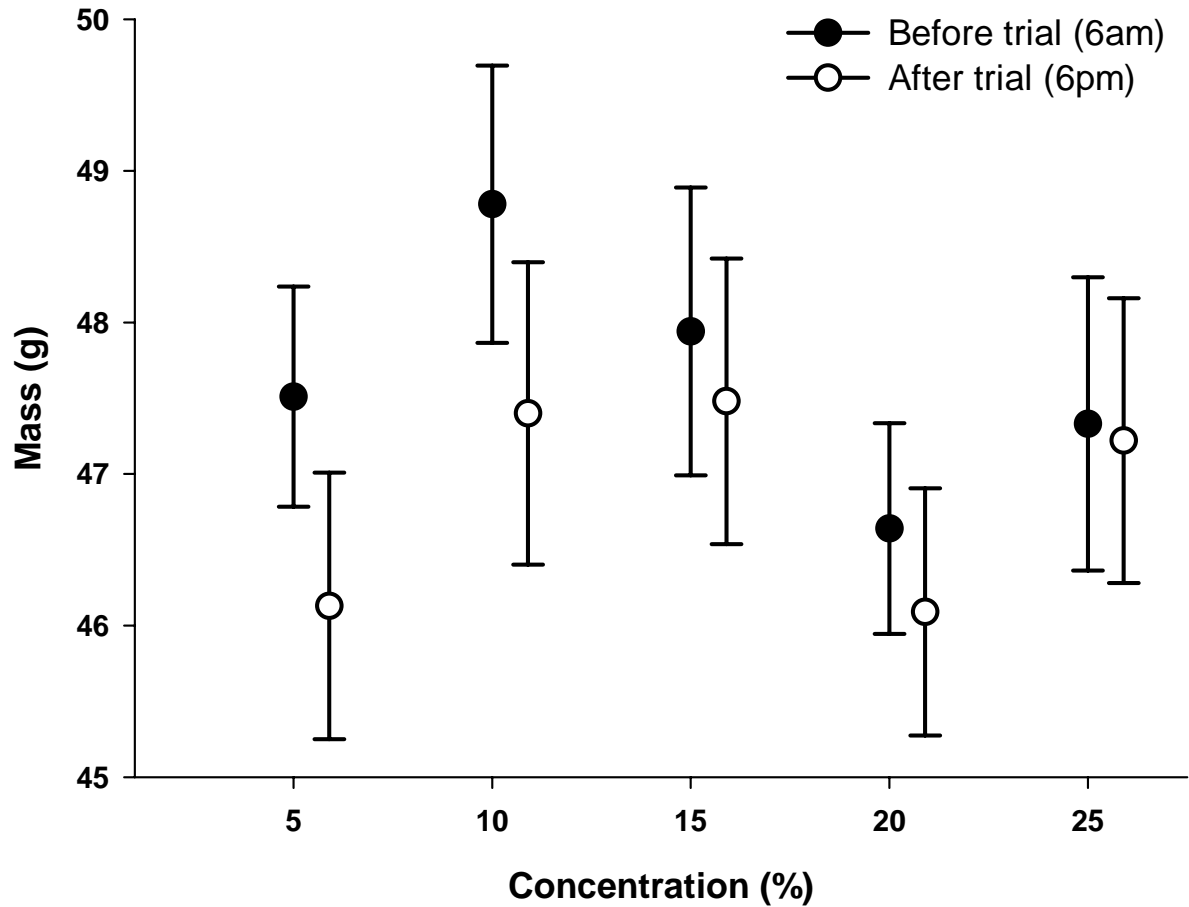


Figure 2: Body mass (g) of Speckled Mousebirds before and after pair-wise choice trials at five different concentrations of sugar solutions. Values represent means  $\pm$  standard error.

#### *Concentration preference trials*

Speckled Mousebirds did not exhibit a significant difference in terms of energy intake (RMANOVA:  $F_{3, 21} = 1.783$ ,  $p = 0.181$ ; Fig. 4) or total volume consumed (RMANOVA:  $F_{3, 21} = 1.651$ ,  $p = 0.347$ ; Fig 4) when given a choice of four different concentrations of hexose solutions simultaneously.

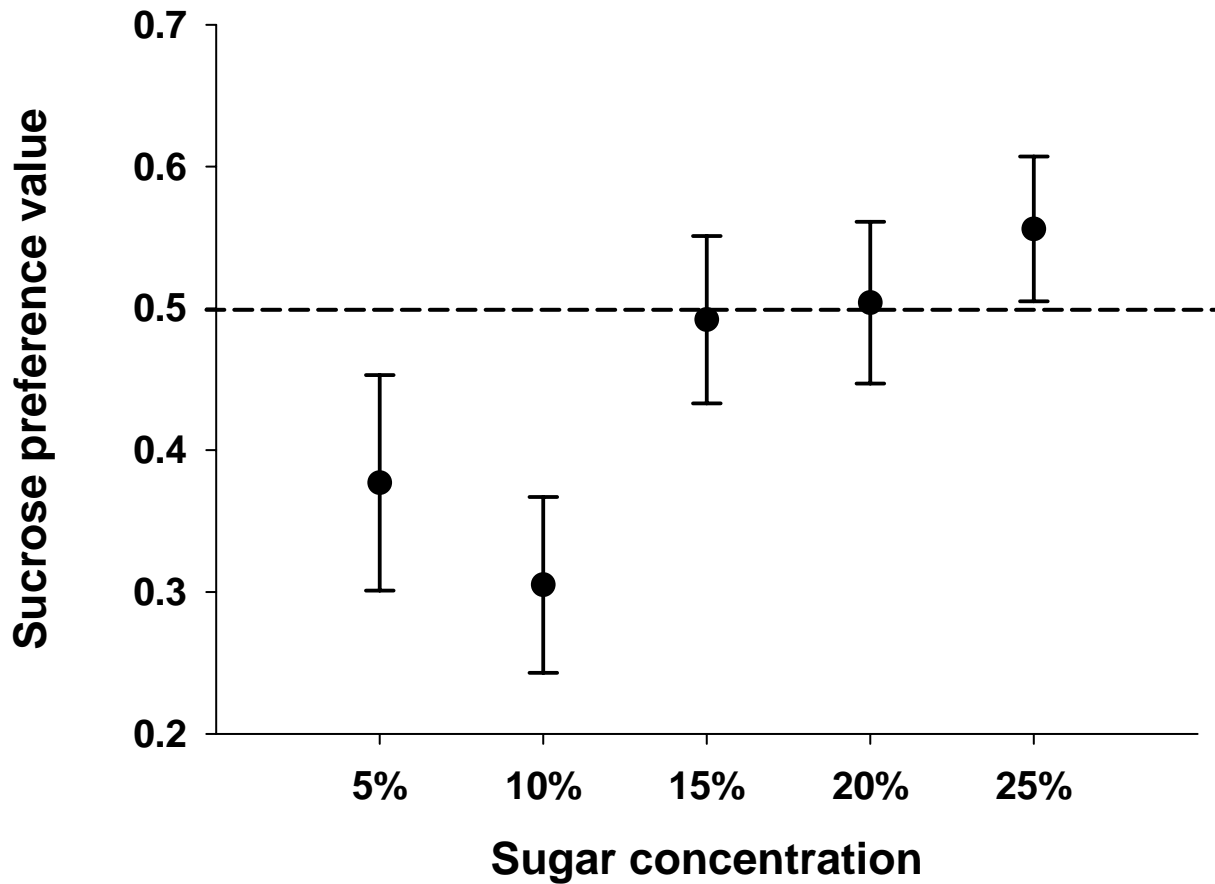


Figure 3: Preference values (PV), calculated as the volume of sucrose solution ingested divided by the total volume of solution ingested for the day, for paired choice tests at five different concentrations. Values presented are back-transformed means and 95% confidence intervals obtained from arcsine square-root transformation of the original proportion data. If 0.5 (no preference) falls outside the 95% confidence intervals, the mean preference value is significant (one-sample t-tests, see text).

#### *Apparent Assimilation efficiency*

Speckled Mousebirds assimilated sucrose and hexose sugars efficiently at both concentrations (Fig. 5). Apparent assimilation efficiency in Speckled Mousebirds was not affected significantly by concentration (RMANOVA:  $F_{1,7} = 0.841$ ,  $p = 0.78$ ). Apparent assimilation efficiencies were

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similar across concentrations for both sucrose (paired sample t-test  $t = 0.33$ ,  $p = 0.75$ ) and hexose (paired sample t-test  $t = 0.14$ ,  $p = 0.89$ ) solutions. Apparent assimilation efficiency was not affected by sugar type either (RMANOVA:  $F_{1, 7} = 2.290$ ,  $p = 0.017$ ). AE's were similar for sucrose and hexose solutions compared at both 5% (paired sample t-test  $t = 0.73$ ,  $p = 0.48$ ) and 25% (paired sample t-test  $t = 0.66$ ,  $p = 0.52$ ) concentrations.

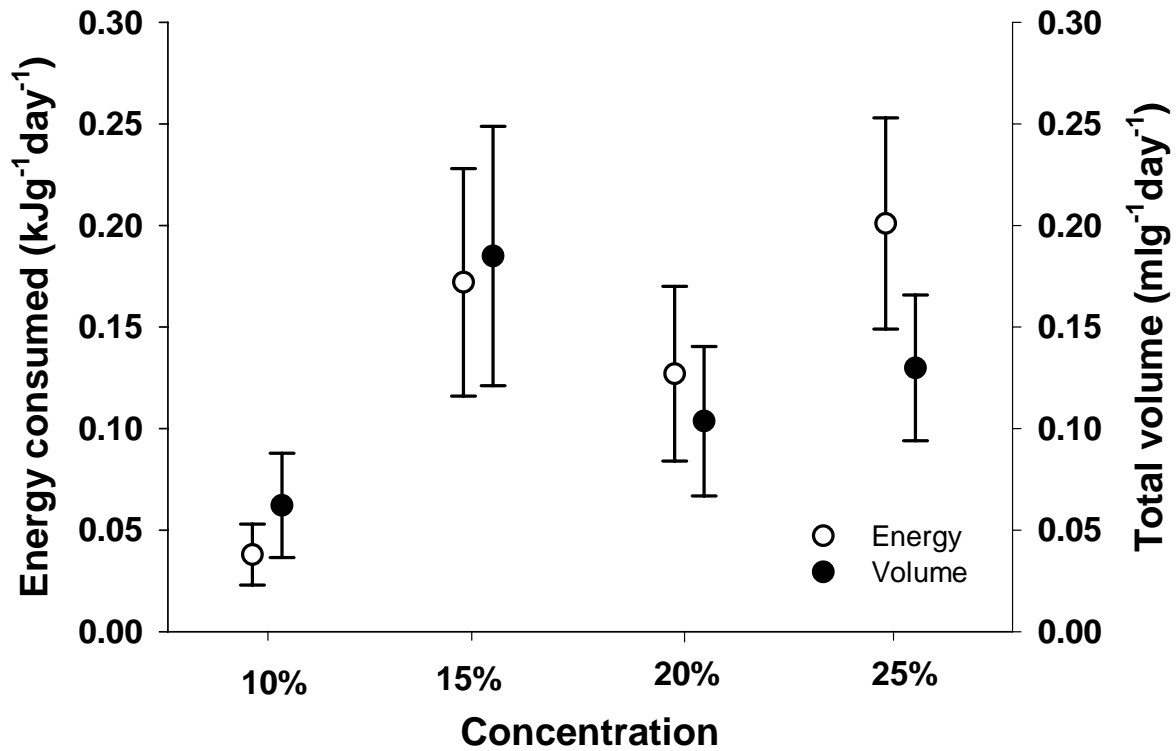


Figure 4: Energy and volume consumed for each of four concentrations of hexose sugar solutions simultaneously presented to Speckled Mousebirds. Values represent means  $\pm$  standard error.

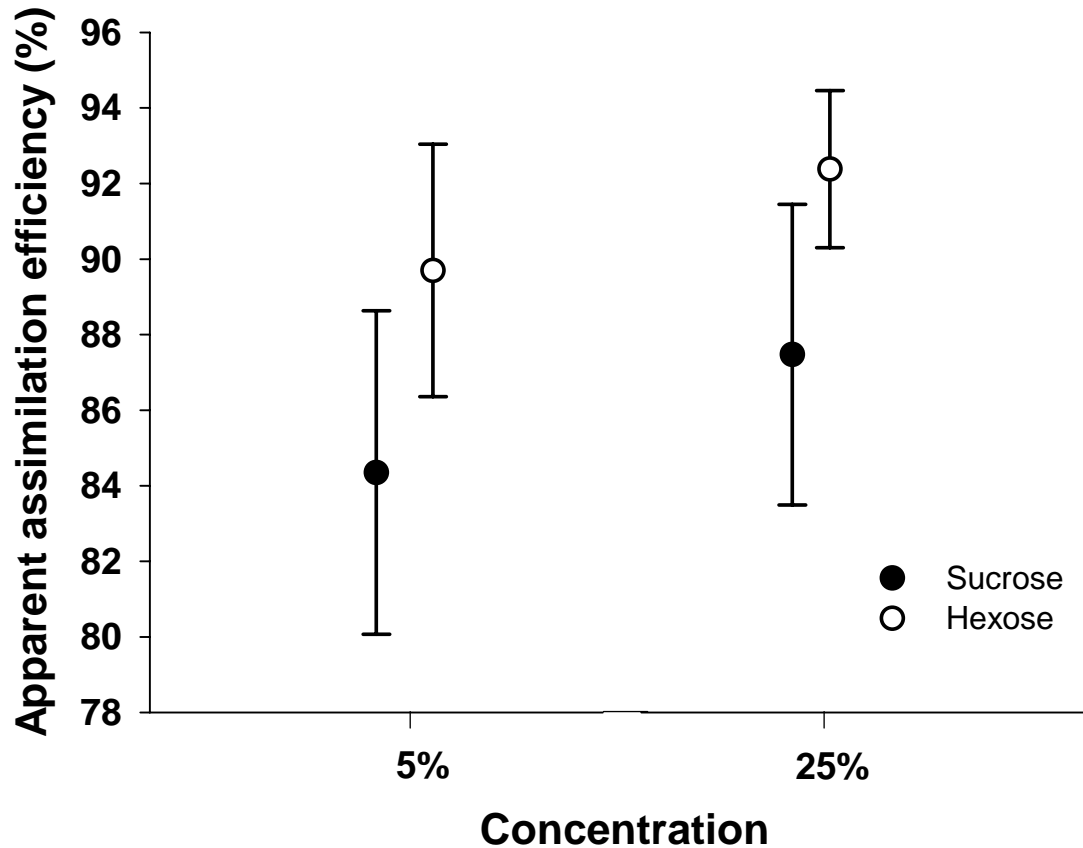


Figure 5: Apparent assimilation efficiency of Speckled Mousebirds when fed low (5%) and high (25%) concentration sucrose and hexose solutions respectively (n=8).

## Discussion

The results of this study are not consistent with our initial hypothesis that sugar preferences and assimilation efficiencies of mousebirds would be similar to those of passerine occasional nectarivores. Speckled Mousebirds exhibited a significant preference for hexose solutions at 5% and 10%, no preference at 15% and 20%, and a sucrose preference at 25% (Fig. 3). This contrasts to results obtained for passerine occasional nectarivorous birds which generally show either hexose preferences at all concentrations (Brown et al. in press), or hexose preference at low concentrations with no preference at high concentrations (Brown et al submitted; Odendaal et al. in press). Instead, the pattern of sugar preference across different concentrations in

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Speckled Mousebirds mirrors that of both passerine and non-passerine specialist nectarivorous birds (Fleming et al. 2004; 2008; Brown et al. 2010).

Contrary to expectations, Speckled Mousebirds were unable to increase volumetric intake sufficiently at low concentrations (5% and 10%) to maintain energy balance (Fig. 1), and consequently lost body mass (Fig. 2). Speckled Mousebirds efficiently assimilated both sugar types when fed low and high concentration solutions, which is similar to specialist nectar feeding birds (Markman et al. 2006; McWhorter and Lopez-Calleja 2000). As expected, Speckled Mousebirds showed no concentration preference when given a choice among four different hexose solutions (Fig. 4). This is similar to results found in other occasional avian nectarivorous species (Brown et al. in press; submitted; Odendaal et al. in press).

Despite the lack of data on non-passerines, Lotz and Schondube (2006) assumed that high sucrose assimilation efficiency is present throughout all non-passerines, and probably represents the ancestral digestive condition for passerines. Our data on Speckled Mousebirds, the first non-passerine occasional nectarivore studied to date, supports their assessment. Rather surprisingly, Speckled Mousebirds showed a pattern of sugar preference across a range of concentrations that was similar to all specialist nectarivores studied so far, both passerine and non-passerine (Fleming et al. 2004, 2008; Brown et al. 2010). In addition, Speckled Mousebirds were unable to meet energy requirements at low concentrations, which again was similar to specialist nectarivores (McWhorter and Lopez-Calleja 2000; McWhorter and Martínez del Rio 2000; Martínez del Rio et al. 2001; Nicolson and Fleming 2003a; Fleming et al. 2004; Fleming et al. 2008; Brown et al. 2010). In contrast, passerine occasional nectar feeding birds studied so far show varying sugar preference patterns (at a range of concentrations), arguably linked to varying differences in apparent assimilation efficiencies of sucrose solutions, and ability to increase volumetric intake to meet daily energy intake at low concentrations (Brown et al. in press; Odendaal et al. in press).

Research done on passerines so far suggests that adaptation, rather than phylogeny, accounts for differences in sugar preference, concentration preference, and apparent assimilation efficiency between specialist and occasional nectarivores (Fleming et al 2004, 2008; Brown et al. 2010, in press). The results of the present study, by contrast, suggests that non-passerines as a group might be relatively phylogenetically constrained in terms of sugar preferences and

apparent assimilation efficiency. The underlying reasons for this pattern are unclear at this stage, but warrant further research.

### Acknowledgments

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# **African Red-winged Starlings feed on nectar, but don't like it too sweet**

Mark Brown\*, Colleen T. Downs & Steven D. Johnson

School of Biological & Conservation Sciences, University of KwaZulu-Natal, Private Bag X01,  
Scottsville, Pietermaritzburg, 3209, South Africa

\*Corresponding author:

Mark Brown

brownma@ukzn.ac.za, +27 33 2605661, fax +27 86 5152114

## **Summary**

1. Globally, there are two bird pollinated systems recognised – plants pollinated by specialist nectar feeding birds, and plants pollinated by occasional nectar feeding birds. Whether pollinators place selective pressure on the plants they feed on, in terms of flower morphology and nectar traits, is still debated.

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2. While considerable research exists that examines the sugar preferences, concentration preference and digestive ability of specialist nectar feeding birds, there is little known about these parameters for occasional nectar feeding birds, especially without experimental bias.
3. Previous research shows that birds of the Sturnidae-Muscicapoidea lineage lack sucrase and are intolerant of solutions or fruit above 11-15% sucrose content. African Red-winged Starlings *Onychognathus morio* are occasional nectarivores that play a role as pollinators of several plant groups, particularly aloes, which produce large amounts of dilute floral nectar.
4. We confirmed that these birds, like other members of their lineage studied outside of Africa, are unable to digest sucrose. In laboratory trials, they strongly preferred hexose over sucrose solutions at a range of concentrations (5-20%). However, they were unable to meet daily energy requirements when given a choice between sucrose and hexose solutions at the highest concentration (25%) and showed no sugar preference at this level. Starlings prefer lower concentration solutions when offered hexose solutions of varying concentrations (10-25%).
5. These results suggest that starlings impose pollinator mediated pressure for low concentration, hexose-rich nectar in the plants they pollinate.

**Key words:** Red-winged Starlings *Onychognathus morio*, sugar preferences, occasional nectarivore

**Introduction**

The role opportunistic avian nectarivores play as important pollinators is now widely accepted (Ortega-Olicencia, Rodriguez-Riano, Valtuena, Lopez & Devesa 2005; Johnson, Hargreaves &

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Brown 2006; Brown, Downs & Johnson 2009; Botes, Johnson & Cowling 2008; Rodriguez-Rodriguez & Valido 2008; Symes & Nicolson 2008; Symes, Nicolson & McKechnie 2008; Symes, Human & Nicolson 2009). Plants pollinated by these birds possess a set of floral traits (flower morphology, nectar volume and concentration, and sugar type) which is distinct from those of plants pollinated by specialist avian nectarivores (Botes et al. 2008; Johnson & Nicolson 2008; Symes & Nicolson 2008; Brown et al. 2009). Typically, flowers pollinated by occasional nectarivorous birds have high volumes (40-100 $\mu$ l) of low concentration (8-12%) nectar, with low (0-5%) sucrose content (Johnson & Nicolson 2008).

It is still debated whether nectar characteristics of plant species reflect selective pressure imposed through the foraging preferences of primary pollinators or phylogenetic constraints in plants (van Wyk, Whitehead, Glen, Hardy, Van Jaarsveld & Smith 1993; Ornelas, Ordano, De-Nova, Quintero & Garland 2007; Schmidt-Lebuhn, Schwerdtfeger, Kessler & Lohaus 2007). Johnson & Nicolson's (2008) phylogenetically-controlled analysis of the relationships between nectar characteristics and bird pollinator type strongly supported the idea that nectar characteristics are adaptive to pollinator preferences. To identify the selective pressures imposed by specialist and occasional avian nectarivores on plant nectar characteristics, it is necessary to determine their food preferences and digestive ability, and to determine whether these behavioural and physiological responses correlate with the nectar characteristics of the plants they pollinate.

Although choice experiments have been performed on specialist avian nectarivores for several decades, differences in experimental design have often led to confusing or conflicting ideas about these birds' sugar preferences (Brown, Downs & Johnson 2008). More recent research has shown that all specialist avian nectarivores tested so far (from all major groups)

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show a hexose preference at low (e.g. <10% sucrose equivalent (SE)) concentrations, often with no preference at intermediate (10-20% SE) concentrations, and a switch to sucrose preference (or no preference) at high (e.g. 25% SE) concentrations (Schondube & Martínez del Rio 2003; Fleming, Bakken, Lotz & Nicolson 2004; Fleming, Xie, Napier, McWhorter & Nicolson 2008; Brown, Downs & Johnson 2010) when tested using equicaloric solutions. To date, few equicaloric choice experiments on occasional avian nectarivores have been undertaken. Early work on the Sturnidae-Muscicapoidea lineage (Malcarney, Martínez del Rio & Apanius 1994; Martínez del Rio & Stevens 1989; Gatica, Gonzalez, Vasquez & Sabat 2006) led to the idea that all passerine nectarivores would show sucrose aversion, primarily due to a lack of the enzyme sucrase. However, we now know that this sucrase dependent sucrose aversion is seemingly limited to this lineage, and that both specialist (Lotz & Nicolson 1996; Downs 1997; Jackson, Nicolson & Van Wyk 1998a) and occasional passerine nectarivores (Lane 1997; Franke, Jackson & Nicolson 1998; Brown & Downs 2003; Wellman & Downs 2009; Brown, Downs & Johnson in press; Odendaal, Brown, Downs & Johnson in press) not only have relatively high sucrose assimilation rates, but maintain energy balance on sucrose-only diets and can show concentration-dependant sugar preferences. However, if we exclude specialist passerine nectarivores (who generally have assimilation efficiencies for sucrose of >95%) and passerines from the Sturnidae-Muscicapoidea lineage (which lack sucrase), we find that assimilation efficiency of sucrose is generally lower than for hexose sugars in passerines (Lane 1997; Brown et al. in press; Odendaal et al. in press; Wellmann & Downs 2009). Lotz & Schondube (2006) suggest that such birds could potentially act as selective agents for hexose-rich nectar and fruit, despite sometimes having relatively high sucrose activity.

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Although the Red-winged Starling *Onychognathus morio* has been reported to have a sucrose assimilation efficiency of 0%, this is based on a personal communication, with no empirical data having been presented (Lotz & Schondube 2006). Red-winged Starlings are regular visitors to flowers which produce copious amounts of dilute nectar, such as those of various aloe and red-hot poker species (Hoffman 1988; Symes et al. 2008; Brown et al. 2009), and Proteaceae (Fraser 1990). In this study, we examined the sugar preference, concentration preference and assimilation efficiency of Red-winged Starlings over a range of concentrations (5-25%) reflective of nectar concentrations in bird pollinated plants (Johnson & Nicolson 2008). We predicted that Red-winged Starlings would prefer hexoses over sucrose irrespective of diet concentration, would prefer higher concentrated solutions when given a choice among different hexose solutions, and would have high hexose, but low sucrose apparent assimilation efficiencies.

**Materials & Methods**

We captured nine Red-winged Starlings using Ecotone mistnets in Waterfall, KwaZulu-Natal (2944S 3049E) in February 2008. Birds were acclimated after capture in a large outdoor group aviary (4 x 3 x 2m) and fed a maintenance diet of fresh fruit (apples, bananas, pawpaw, banana, pear, oranges, grapes) supplemented with Aviplus Softbill Mynah Pellets (Aviproducts, Durban, RSA), mealworms, bonemeal, and a 20% sucrose/glucose/fructose mix supplemented with a multivitamin (Avi-Sup Soluble Multivitamins; Aviproducts, Durban, RSA) for two weeks. After this initial outdoor acclimation period, birds were transferred into a constant environment room

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(25° C, 12:12 L:D cycle) where they were individually housed in small cages (600 x 450 x 450mm). Birds were acclimated on the maintenance diet for another two weeks.

*1.1 Sugar type preference trials*

We conducted pair wise choice tests using the following w:w equicaloric pairs of solutions: a) 5% (0.146 M) sucrose and 5.26% hexose (0.292 M); b) 10% (0.292 M) sucrose and 10.52% hexose (0.584 M); c) 15% (0.438 M) sucrose and 15.79% hexose (0.88 M); d) 20% (0.584 M) sucrose and 21.06% hexose (1.17 M); and e) 25% (0.73 M) sucrose and 26.31% hexose (1.46 M). All hexose solutions were made up of equal parts glucose and fructose sugars. For a discussion on the use of equicaloric solutions in choice tests, see Fleming et al. (2004) and Brown et al. (2008). Solutions were provided in modified 50-ml burette tubes calibrated at 0.1-ml intervals, with position alternated at midday to avoid side bias (Jackson, Nicolson & Lotz 1998b; Fleming et al. 2004). Trials were conducted from 06:00 to 18:00, with birds weighed before and after each trial. We measured hourly consumption of each sugar solution between 7:00 and 18:00. We converted volumes drunk into a preference value for sucrose by using the proportion of single sugar type consumption / total nectar consumption (Martínez del Rio 1990; Mata & Bosque 2004; Brown et al. 2008). We also converted the total volume consumed of each solution into energy consumed per gram body mass per day, expressed as  $\text{kJg}^{-1}\text{day}^{-1}$  (Brown et al. 2008).

We determined differences in total energy intake (i.e. daily energy regulation) by analysing total daily energy intake ( $\text{kJg}^{-1}\text{day}^{-1}$ ) at each concentration (by combining energy intake from each of the two solutions), using Repeated Measures Analysis of Variance

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(RMANOVA) with post-hoc Tukey HSD tests. In order to determine the effect of energy intake at each concentration on bird energy balance we compared bird body mass before and after each trial using RMANOVA with post-hoc Tukey HSD tests.

We determined the overall effect of concentration on birds' sugar preference using RMANOVA, with concentration as the independent variables. We then compared sucrose preference values (calculated as the volume of sucrose solution ingested divided by the total volume sugar solution ingested) to 0.5 (no preference), using one-sample *t*-tests (Martínez del Rio 1990; Fleming et al. 2004; Brown et al. 2010). Since volumetric sucrose preference values are proportions, they were arcsine-square root transformed prior to all statistical analyses.

*1.2 Concentration preference trials*

In a further trial, we examined whether Red-winged Starlings exhibited a concentration preference. Each bird was given a choice of four randomly positioned hexose solutions of different concentrations (10, 15, 20 and 25%) for a twelve hour period. The volume of each solution drunk was converted into energy consumed per gram body mass per day (Brown et al. 2010). We compared energy intake ( $\text{kJg}^{-1}\text{day}^{-1}$ ) and volumetric intake (ml/day) from each of the four hexose concentrations presented simultaneously to birds in the concentration trial using RMANOVA to determine if a concentration preference was evident.

*Chap. 6 Sugar Preferences of Starlings**1.3 Apparent Assimilation efficiency*

We determined apparent assimilation efficiencies (AE) for sucrose and hexose diets at two different concentrations (5% and 25%) respectively. For each experimental day birds were placed in wire bottomed cages placed over a tray of liquid paraffin (used to collect liquid faeces without evaporation loss) and fed a single sugar solution for a 4h period (08:00 to 12:00). Birds were then deprived of food for a further 2h to ensure all consumed food was processed and voided, and then placed on the maintenance diet. Volume of sugar solution consumed was recorded for the duration of the day. All liquid excreta were then collected by syringe from the liquid paraffin, and weighed to determine total excreta weight. Samples were then centrifuged at 1300rpm for three min. We analysed excreta sugar content using a Shimadzu (LC-20AT) high-performance liquid chromatograph (HPLC). Detection was by refractive index (RID-10A) with a Phenomenex column (Rezex RCM-Monosaccharide, 200 x 780mm 8micron). Isocratic separation was accomplished using ultrapure water as the mobile phase. AE was corrected for volume consumed and excreted as per Jackson et al. (1998a). To determine if there was any effect of sugar type or concentration on nectar assimilation efficiency, we used RMANOVA on arcsine transformed AE data, with post-hoc Tukey HSD tests used to pinpoint significant differences. All statistical analyses were conducted using Statistica (Statsoft, Tulsa, OK, USA).

Approval for this project was received from the Animal Ethics sub-committee of the University of KwaZulu-Natal.

**Results***1.1 Sugar type preference trials*

Red-winged Starlings did not consume similar amounts of energy per day when presented a choice of sucrose and hexose sugars at all five concentrations (RMANOVA:  $F_{4, 32} = 9.601$ ,  $p < 0.001$ ; Figure 1). Post-hoc Tukey tests revealed that energy intake ( $\text{kJg}^{-1}\text{hr}^{-1}$ ) was significantly lower ( $\pm$  half) when birds were fed 25% solutions compared with when they fed on 5, 10, 15 or 20% solutions (Figure 1). Bird body mass differed significantly overall between trials and after trials (RMANOVA:  $F_{4, 32} = 16.528$ ,  $p < 0.001$ ), but this was only because of significant body mass loss after the 25% trial (Post-hoc Tukey:  $p = 0.02$ ; Figure 2). On average, birds lost 9% body mass when fed 25% solutions for 4 hours. As expected, birds adjusted volumetric intake to meet energy demands, with decreased intake at higher concentrations (Figure 1), although they could not meet energy demands at 25% (see above).

There was a significant overall effect of concentration on bird sugar preference, in terms of volumetric sucrose intake (RMANOVA:  $F_{4, 32} = 3.327$ ,  $p = 0.022$ ; Figure 3). Red-winged Starlings ingested significantly more hexose than sucrose at 5% (one-sample  $t$  test:  $t = 8.708$ ,  $p < 0.001$ ), at 10% ( $t = 2.478$ ,  $p < 0.05$ ), at 15% ( $t = 5.856$ ,  $p < 0.001$ ), and at 20% ( $t = 2.631$ ,  $p < 0.05$ ). However, Red-winged Starlings showed no preference for either hexose or sucrose solutions at 25% ( $t = 0.246$ ,  $p = 0.812$ ).

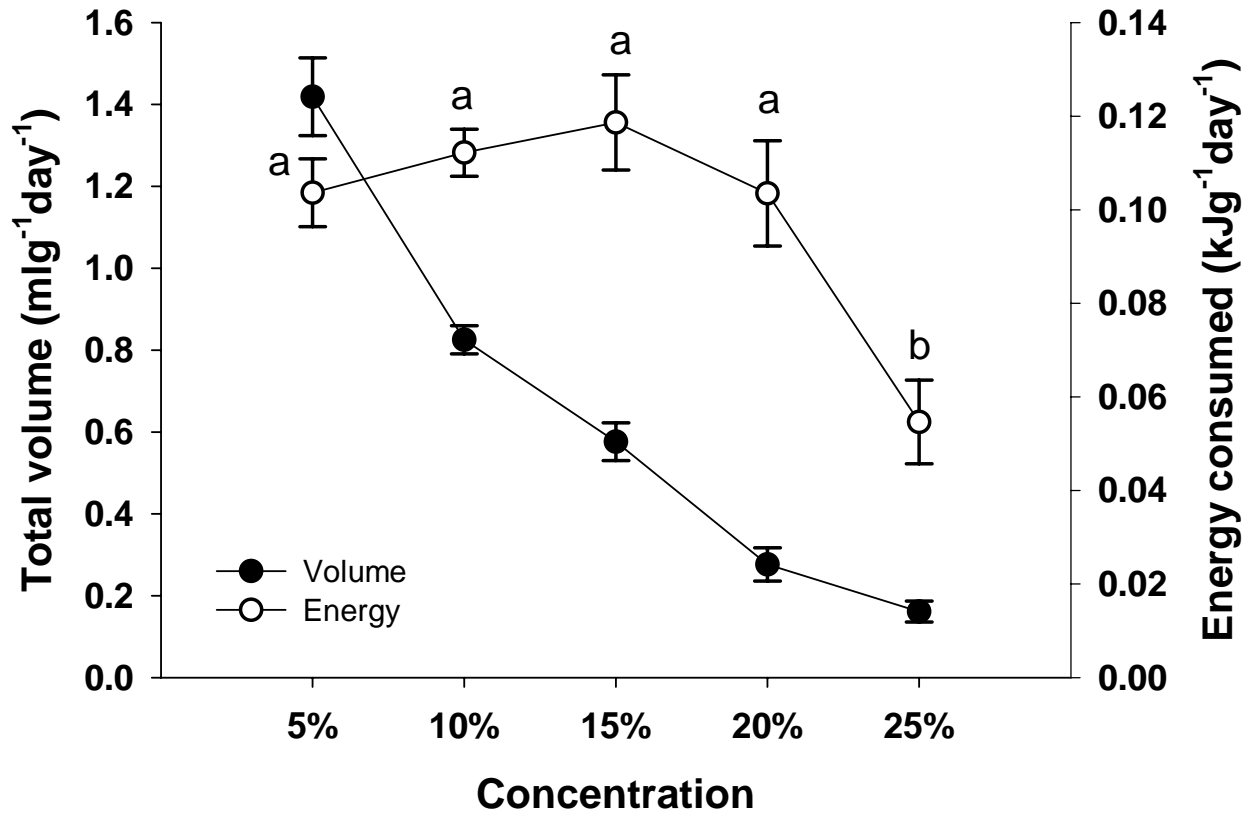


Figure 1: Adjustment of volumetric intake by Red-winged Starlings across five different concentrations (when presented equicaloric solutions of sucrose and hexose sugars in pair-wise choice tests) in order to maintain energy balance. Values represent means  $\pm$  standard error. Letters indicate significant differences in energy intake.

### 1.2 Concentration preference trials

Red-winged Starlings showed no significant preference, in terms of energy consumption (RMANOVA:  $F_{3, 24} = 1.509$ ,  $p = 0.238$ ), for any of the four concentrations of hexose solutions simultaneously presented to them, but did show a significant interaction of concentration on

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volumetric intake (RMANOVA:  $F_{3, 24} = 6.120$ ,  $p < 0.05$ ). Starlings consumed more of the 10 and 15% solutions than the 20 and 25% solutions (Tukey:  $p < 0.05$ ; Figure 4).

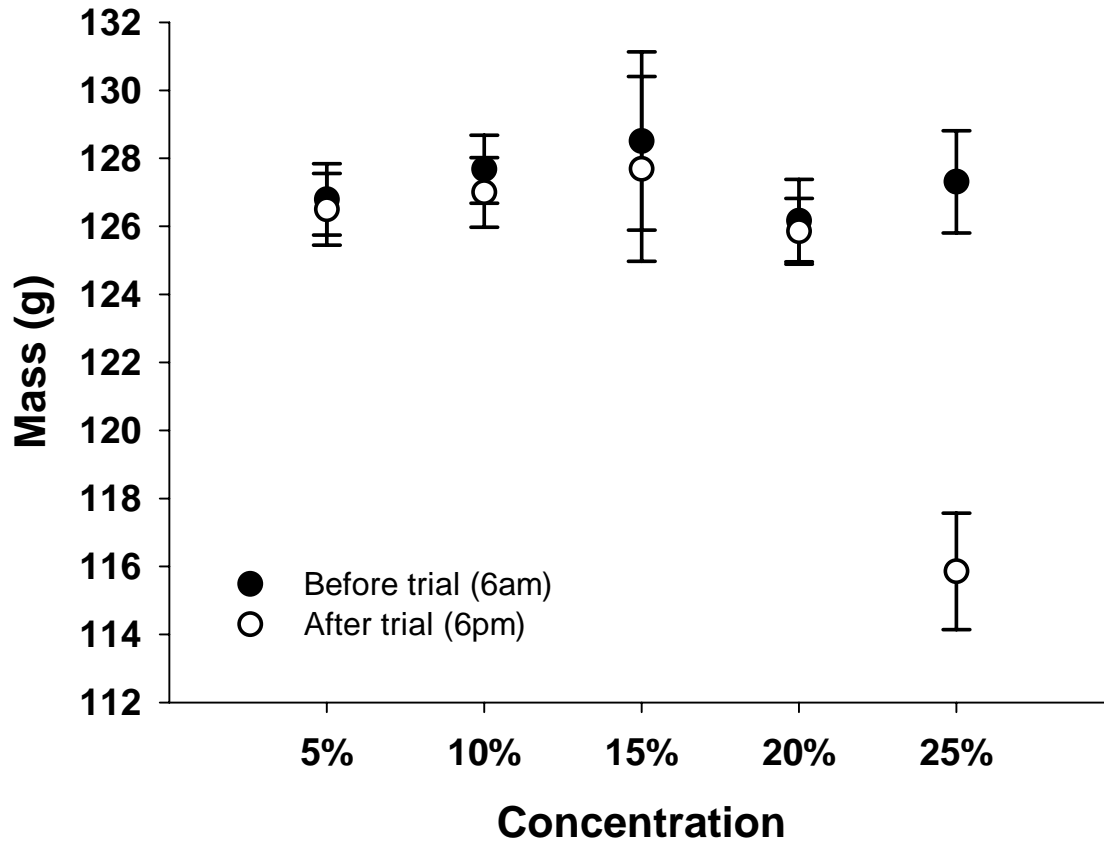


Figure 2: Body mass (g) of Red-winged Starlings before and after pair-wise choice trials at five different concentrations of equicaloric sucrose and hexose solutions. Values represent means  $\pm$  standard error.

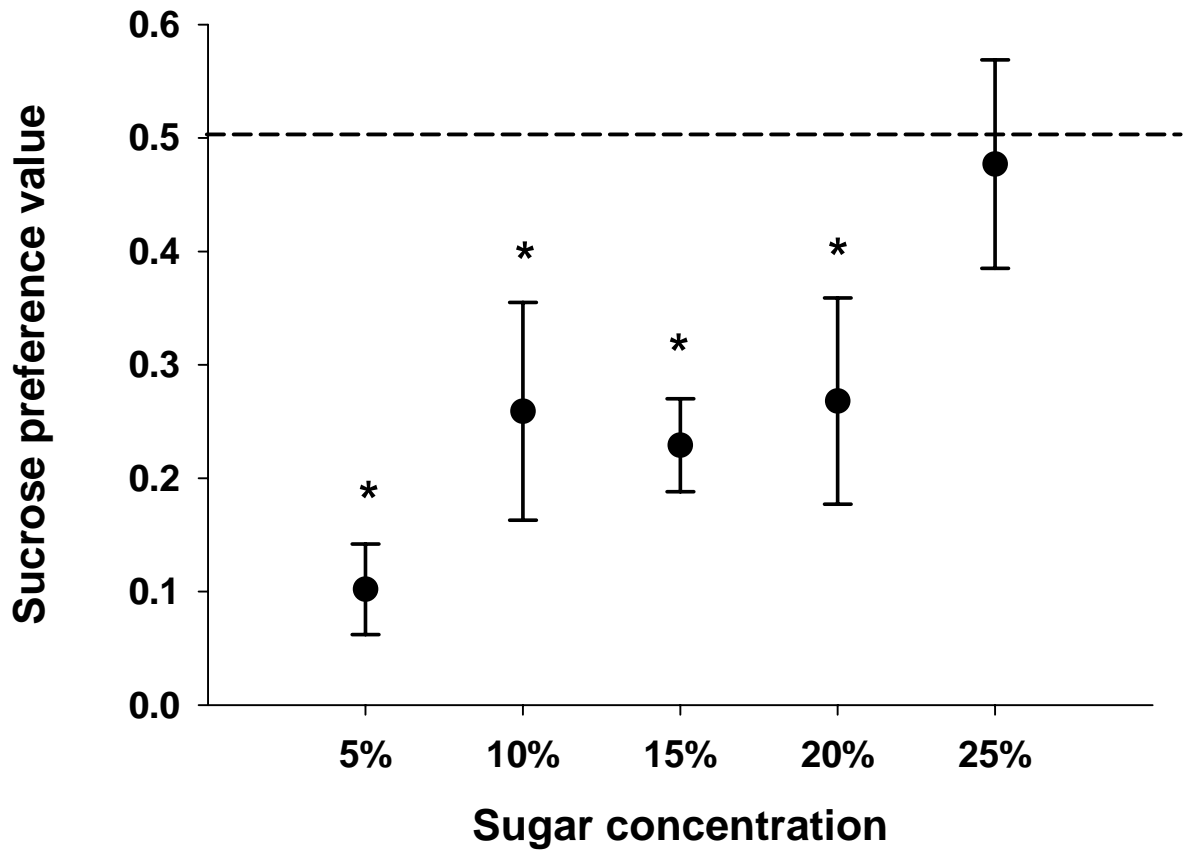


Figure 3: Preference values (PV), calculated as the volume of sucrose solution ingested divided by the total volume of solution ingested for the day, for paired choice tests at five different concentrations. Values presented are back-transformed means and 95% confidence intervals obtained from arcsine square-root transformation of the original proportion data. \* indicates a significant preference.

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1.3 Apparent Assimilation efficiency

Assimilation efficiency was significantly affected by sugar type (RMANOVA:  $F_{1,8} = 338.36$ ,  $p < 0.001$ ) but not by concentration (RMANOVA:  $F_{1,8} = 0.63$ ,  $p = 0.452$ ). Red-winged Starlings were unable to digest sucrose, but were relatively efficient at digesting hexose sugars (Figure 5).

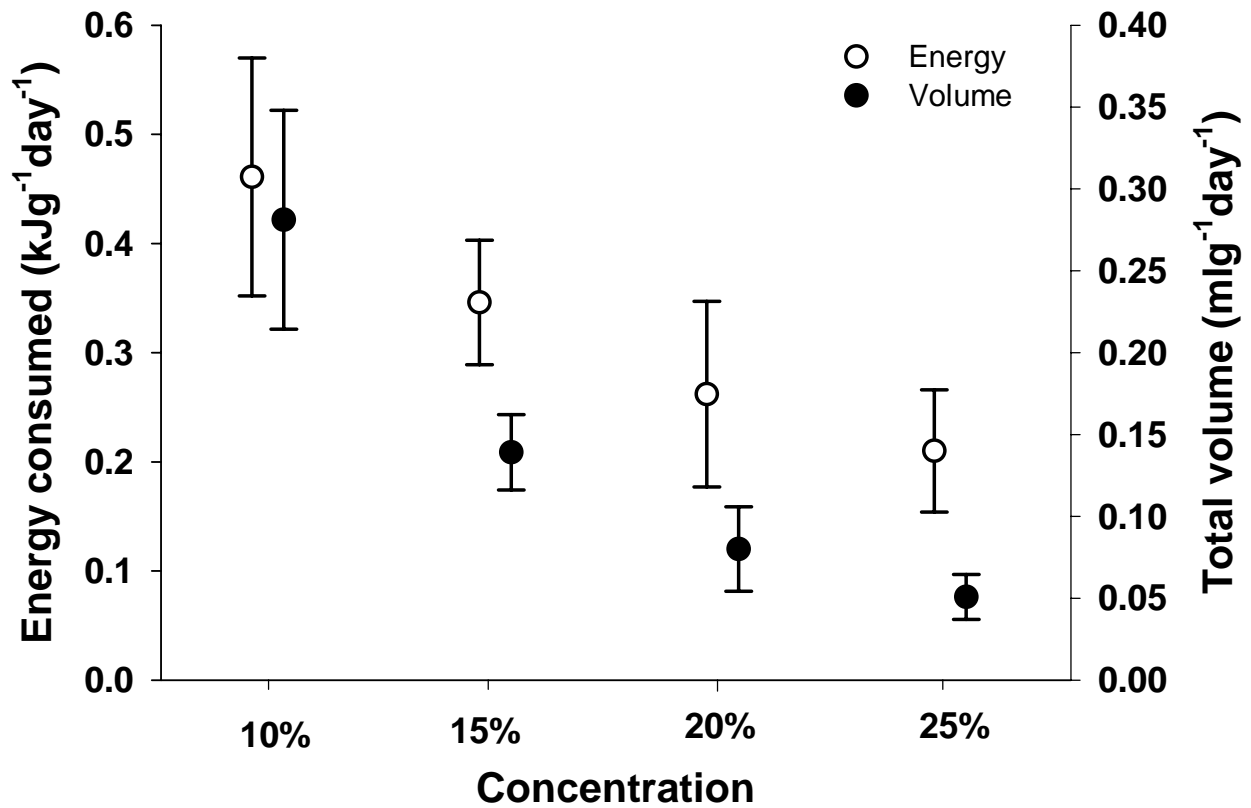


Figure 4: Energy value, and volume, of consumed hexose sugar solutions of four concentrations simultaneously presented to Red-winged Starlings. Values represent means  $\pm$  standard error.

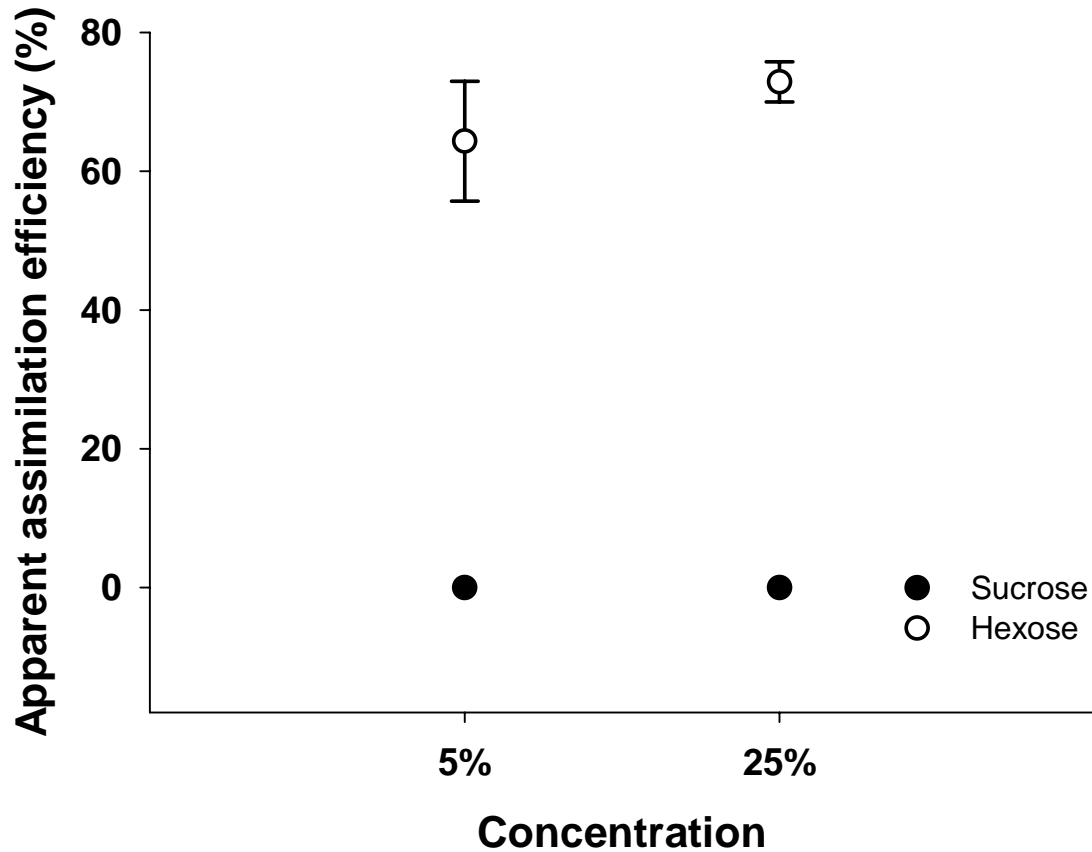


Figure 5: Apparent assimilation efficiency of Red-winged Starlings when fed 5% and 25% sucrose and hexose diets respectively.

## Discussion

Red-winged Starlings showed distinct hexose preference when presented equicaloric hexose and sucrose solutions at 5, 10, 15 and 20% concentrations. Red-winged Starlings, as expected, are unable to digest sucrose, which suggests a lack of the enzyme sucrase. This is not surprising, given the vast amount of work already done on this lineage elsewhere (Martinez del Rio et al.

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1988; 1992; Martinez del Rio & Stevens 1989; Martinez del Rio 1990; Brugger & Nelms 1991; Brugger, Nol & Phillips 1993)

Contrary to our predictions, Red-winged Starlings showed no preference for sugar type at a 25% concentration. Interestingly, starlings were unable to maintain energy balance at this concentration, losing on average 9% body mass over the course of the day. We suggest that two interlinking processes explain these results. Firstly, it appears as if Red-winged Starlings are unable to cope with such high concentration solutions. We speculate that retention time at this concentration is high, and that birds may suffer from dehydration effects, irrespective of sugar type. Secondly, we suggest that starlings, when under such constraints, become less discriminatory and ingest both sucrose and hexose sugar solutions in an attempt to regulate energy intake, or perhaps to reduce osmotic stress. Lotz & Schondube (2006) argue that increasing sucrose intake at high concentrations reduces osmotic concentration of intake, thereby reducing osmotic stress. However, members of the Sturnidae-Muscicapoidea lineage become intolerant when sucrose levels in fruit rise higher than 11.25-15% (Brugger 1992; Brugger et al. 1993; Martinez del Rio, Avery & Brugger 1995), suggesting that the behavioural response exhibited by Red-winged Starlings to high concentration solutions is maladaptive.

A preference for hexose solutions over sucrose solutions is not unexpected, and indeed has been found for several passerine occasional avian nectarivores, not just those from the Sturnidae-Muscicapoidea lineage. The concentration dependent relationship of this preference, however, seems to vary between different species. Dark-capped Bulbuls *Pycnonotus tricolor* show hexose preference at all five concentrations tested (5-25%; Brown et al. in press), whilst Village Weavers *Ploceus cucullatus* exhibit hexose preference at 5, 10 and 15% concentrations, but show no preference at 20 and 25% concentrations (Odendaal et al. in press). Speckled

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Mousebirds *Colius striatus*, a non-passerine occasional nectarivores, show hexose preference at 5 and 10%, no preference at 15 and 20% but sucrose preference at 25% (Brown et al. provisionally accepted). Bananaquits *Coereba flaveola* show no preference, but were only tested at 25% (Mata & Bosque 2004). Speckled Mousebirds (*Colias striatus*), the only non-passerine occasional nectarivore studied so far, shows remarkably similar preferences responses (to concentration) to specialist nectarivores (Brown, Downs & Johnson provisionally accepted). Unfortunately, at this stage no other occasional avian nectarivores have been tested with equicaloric solutions, and we feel it would be speculative to report results from equimolar or equiweight trials (see Brown et al. 2008). At this stage, it does appear that, irrespective of sucrose assimilation efficiency, passerine occasional avian nectarivores may play a role in selection for hexose sugars, particularly at low concentrations, in the plants they feed on.

Specialist avian nectarivores seem to always select the most concentrated solution when given a choice (Bene 1945; Blem & Blem 2000; Colias & Colias 1968; Stiles 1976; Tamm & Gass 1986; Stromberg & Johnson 1990; Leseigneur & Nicolson 2009; Brown et al. 2010), except at concentrations of 1 M or more (Leseigneur & Nicolson 2009). However, occasional avian nectarivores tested so far appear to show no preference at all, or a preference for low concentration solutions (Village Weaver, M. Brown unpublished data; Dark-capped Bulbul, Brown et al. in press; Speckled Mousebirds, Brown et al. provisionally accepted; current study). Both Dark-capped Bulbuls (Brown et al. in press) and Red-winged Starlings drank less of the 25% solution (in terms of volume) when given a choice between four different concentrations, and most surprisingly Red-winged Starlings were unable to maintain energy balance (and indeed significantly lost body mass) at this high concentration. These results suggest that high concentration solutions may place physiological stress on some occasional avian nectarivores,

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which may lead to them placing selective pressure on plants to produce more dilute nectars. The physiological parameters leading to this inability to cope with high concentration solutions in these birds requires further study. Of additional interest, Red-winged Starlings, and indeed most occasional nectarivores, have not been recorded frequently feeding on any plant containing high concentration sucrose rich nectars.

The results of this study, and those of other similar studies on passerine occasional avian nectarivores (Brown et al. in press; Odendaal et al. in press) are beginning to assist evolutionary biologists explain the occurrence of low concentration hexose dominant nectar in the plants they pollinate. Indeed, it would appear that the behavioural and physiological mechanisms determining sugar preference at different concentrations in these birds have placed selective pressure on these plants that rely on them for pollination.

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## Pollination of the red hot poker *Kniphofia caulescens* by short-billed opportunistic avian nectarivores

M. Brown<sup>\*</sup>, C.T. Downs, S.D. Johnson

*School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3201, South Africa*

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### Abstract

Recent studies indicate that short-billed birds which visit flowers opportunistically for nectar are important pollinators of African plants and select for floral characteristics which are distinct from those found in sunbird-pollinated species. Here we report the existence of a pollination system involving opportunistic nectarivores in *Kniphofia caulescens*, a high altitude member of a genus previously considered to contain only sunbird- and insect-adapted pollination systems. Plants of *K. caulescens* set approximately twice as many fruits and produced more seeds per fruit when exposed to both bird and insect pollination than to just insect pollination. Controlled pollination experiments showed that the species is genetically self-incompatible and thus totally reliant on pollinator visits for seed set. Opportunistic nectar-feeding birds appear to be the most important pollinators of this plant. In particular, Drakensberg Siskins (*Crithagra symonsi*) and Yellow Canaries (*Crithagra flaviventris*) were the most frequent visitors and carried the highest pollen loads. The Malachite Sunbird (*Nectarinia famosa*), although often feeding on *Kniphofia caulescens*, carried very little pollen, presumably on account of its long bill which reduces contact between the floral anthers and its head feathers. Honey bees were also frequent flower visitors and made a secondary contribution to fruit set. Flowers of *K. caulescens* contained copious amounts (c. 30 µl) of very dilute (c. 9%) hexose-rich nectar which is consistent with the pollination syndrome found in plants pollinated by opportunistic avian nectarivores.

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**Keywords:** Bird pollination; Breeding system; Drakensberg; Drakensberg Siskin; Honeybee; Nectar; Sugar composition

### 1. Introduction

Ideas about bird pollination systems in Africa have been rapidly evolving. It is now clear that two distinct systems occur — specialist systems, where plants are co-evolved with specialist nectar-feeding birds, and generalist systems, where plants are co-evolved with opportunistic nectar-feeding birds (Johnson et al., 2006; Johnson and Nicolson, 2008; Symes and Nicolson, 2008; Symes et al., 2008; Botes et al., 2008, 2009). The flowers in these two systems tend to not only differ in their overall dimensions, but also to have different nectar properties in terms of volume, concentration, and in most cases, sugar type (Johnson and Nicolson, 2008). For most of these traits, differences are apparent even when controlling for phylogenetic effects, suggesting that they are

adaptive (Johnson and Nicolson, 2008). Although short-billed opportunistic birds have often been recorded worldwide feeding on nectar (Oatley, 1964; Fisk, 1972; Fisk and Steen, 1976; Spofford and Fisk 1977; Ford et al., 1979; Jacot Guillaumod et al., 1979; Franklin, 1999; Franklin and Noske, 1999), relatively few studies have addressed their importance as pollinators (Toledo, 1977; Feinsinger et al., 1979; Morton, 1979; Toledo and Hernandez, 1979; Gryj et al., 1990; Gill et al., 1998; Kunitake et al., 2004; Raju and Rao, 2004; Rangaiah et al., 2004). Recently there have been several papers examining the role of these occasional nectar-feeding birds in the genus *Aloe* (Johnson et al., 2006; Symes et al., 2008; Botes et al., 2008, 2009). However, the paucity of published case studies of pollination of African plants by opportunistic birds may not reflect the true extent of this pollination system. It is thus necessary for more studies to be conducted in as many lineages as possible to establish the general validity of the patterns identified by Johnson and Nicolson (2008).

We initiated a detailed study of the pollination biology of *Kniphofia caulescens*, a high altitude red hot poker exhibiting

<sup>\*</sup> Corresponding author. Tel.: +27 33 260 5661; fax: +27 86 515 2114.

E-mail address: [brownma@ukzn.ac.za](mailto:brownma@ukzn.ac.za) (M. Brown).

ornithophilous flower characteristics, after initial observations suggested that opportunistic nectar-feeding birds are its main flower visitors. The specific aims of this study were to 1) establish the breeding system of *K. caulescens*, 2) quantify key floral traits, such as nectar volume, concentration and composition, which may reflect adaptations to particular pollinators, 3) establish the main flower visitors, and 4) determine the relative contributions of birds and insects to fruit and seed production.

## 2. Materials and methods

### 2.1. Study site and species

*Kniphofia caulescens* Baker, is a range restricted species that occurs between 1800 m and 3000 m in the Drakensberg mountains of South Africa. It grows in marshes on damp mountainsides. Flowering takes place during the summer months from December to March. We studied a natural population of ~500 plants at Sani Top Chalets, at the summit of Sani Pass, KwaZulu-Natal, during Jan–Feb 2008 and Dec–Jan 2009. Some data were also collected from a transplanted population (~100 plants) located 100 m away from the main natural.

### 2.2. Flower morphology and nectar properties

We arbitrarily selected 10 plants in this population for morphological measurements, including style length, flower depth and flower width. Nectar characteristics (volume and concentration) were measured as standing crop from 28 plants (5 flowers from each plant) at 8 am, 10 am, 12 pm and 6 pm. Nectar availability in open flowers from which animal visitors were excluded for 24 h was measured for 5 plants bagged overnight, with 3 flowers from each plant used to quantify nectar volume and concentration at 8 am. Flowers sampled were chosen randomly from each inflorescence, but only from the areas on the inflorescence with flowers at the developmental stages where they are visited by birds (determined by visual observation). We determined volume with 100- $\mu$ L micropipettes and concentration with a handheld refractometer (Bellingham and Stanley, Tunbridge Wells, Kent, UK). Nectar composition was determined using a Shimadzu (LC-20AT) high-performance liquid chromatograph (HPLC). Detection was by refractive index (RID-10A) with a Phenomenex column (Rezex RCM-Monosaccharide, 200  $\times$  780 mm 8 micron). Isocratic separation was accomplished using ultrapure water as the mobile phase. HPLC analysis was conducted on 5 samples taken from 5 different plants.

### 2.3. Breeding system experiments

We determined the compatibility system and dependency of *K. caulescens* on animal vectors for seed production by performing controlled pollination experiments. Inflorescences were bagged from the bud stage to exclude all potential pollinators. We then hand-pollinated 15 flowers from 5 inflorescences with pollen from the same plant (self-compatibility

test), and another 15 flowers from 5 inflorescences with pollen from another plant as a cross-pollinated control. Other flowers were left unmanipulated to test for autogamy. We then examined fruit set and seed set for these flowers.

### 2.4. Pollinator effectiveness

To determine the effectiveness of various pollinator groups, we performed two exclusion experiments. Firstly, we placed mosquito-netting exclusion bags on 20 inflorescences, which excluded all pollinators. Secondly, we placed plastic mesh (aperture diameter of 12.5 mm) bird exclusion cages (Hargreaves et al., 2004; Botes et al., 2009; Wilson et al., 2009; Symes et al., 2009-this issue) over an additional 20 inflorescences. We then determined fruit set and seed set for both these groups, in addition to natural fruit and seed set taken from 100 unmanipulated inflorescences. We analysed fruit set and seed set data using ANOVA with post-hoc Tukey tests. Fruit set data were arcsine square root transformed prior to analysis.

### 2.5. Floral visitor abundance and pollen loads

We carried out incidental observations of birds feeding on the flowers, recording approximate numbers of individuals per hour as an index of visitor abundance. Total numbers of each species mist-netted was also used as an estimate of abundance. We identified insect visitors by collecting samples of all insects seen either collecting pollen, or drinking nectar. To determine the efficiency of birds as pollinators, we collected pollen loads from birds trapped while moving between flowers in the population. Birds were mist-netted, ringed and released after pollen loads were taken. Pollen was collected from the heads and bills of each bird using fuchsin-stained gel, which was then melted onto microscope slides in the field to produce permanent slides (Beattie, 1971). Using reference slides of pollen from *K. caulescens*, we quantified the total count of pollen from each slide.

## 3. Results

### 3.1. Flower morphology and nectar properties

*Kniphofia caulescens* (Fig. 1a) has relatively short, wide flowers, with protruding stamens (Table 1). Standing crop nectar volume and concentration at 8 am, 10 am, 12 pm and 6 pm are presented in Table 2, along with the bagged flower nectar crop at 8 am and 10 am. *K. caulescens* produces relatively large amounts of dilute nectar. Sugar analysis revealed the composition of *K. caulescens* nectar to be made up of  $50.2 \pm 0.07\%$  glucose,  $48.9 \pm 0.18\%$  fructose and  $0.9 \pm 0.25\%$  sucrose sugars (mean  $\pm$  standard error).

### 3.2. Breeding system experiments

Self-pollinated flowers and control flowers set no fruits (0%,  $n=15$  for each group), while 93.3% ( $\pm 0.13\%$ ;  $n=15$ ) of cross-pollinated flowers set fruit. This difference was significant



Fig. 1. (a) Population of *Kniphofia caulescens*; (b) a male Drakensberg Siskin (*Crithagra symonsi*) drinking nectar; (c) a close up of a male Drakensberg Siskin (*Crithagra symonsi*), showing good anther contact around the face; (d) a Cape Weaver (*Ploceus capensis*) drinking nectar; (e) a male Malachite Sunbird (*Nectarinia famosa*) drinking nectar, making little contact with anthers.

(Fisher's exact test;  $p < 0.001$ ) and indicates that *K. caulescens* is self-incompatible.

### 3.3. Pollinator effectiveness

Fruit set was significantly affected by pollinator exclusion ( $F_{2, 38} = 166.43$ ,  $p < 0.0001$ ; Fig. 2a). Inflorescences set significantly more fruits when exposed to pollination by both birds and insects (open flowers) than just insects (Tukey:  $p = 0.0001$ ) or no

pollinators at all (bagged flowers; Tukey:  $p = 0.0001$ ). Caged inflorescences (insect pollinators allowed) also set more fruit than bagged (all pollinators excluded) inflorescences (Tukey:  $p = 0.0001$ ). Seed set was also significantly affected by pollinator access ( $F_{2, 28} = 56.410$ ,  $p < 0.0001$ ; Fig. 2b).

Table 1  
Summary of flower morphology of *Kniphofia caulescens*.

	Mean (mm)	Standard error	N
Flower depth	21.10	0.354	40
Flower width	14.45	2.285	40
Stamen length	33.48	0.895	40

Table 2  
Volume and concentration of nectar in *Kniphofia caulescens* flowers.

Open	Bagged		
	Volume (μl)	Concentration	n
Time of day	Volume	Concentration	n
8 am	17.3 (±1.31)	8.6 (±0.29)	40
10 am	19.4 (±3.57)	9.4 (±0.43)	25
12 pm	25.3 (±2.75)	15.1 (±0.74)	25
6 pm	32.4 (±3.75)	9.4 (±0.47)	50

Values (means ± SE) are given for standing crop in open flowers at different times of the day and for flowers bagged for 24 h.

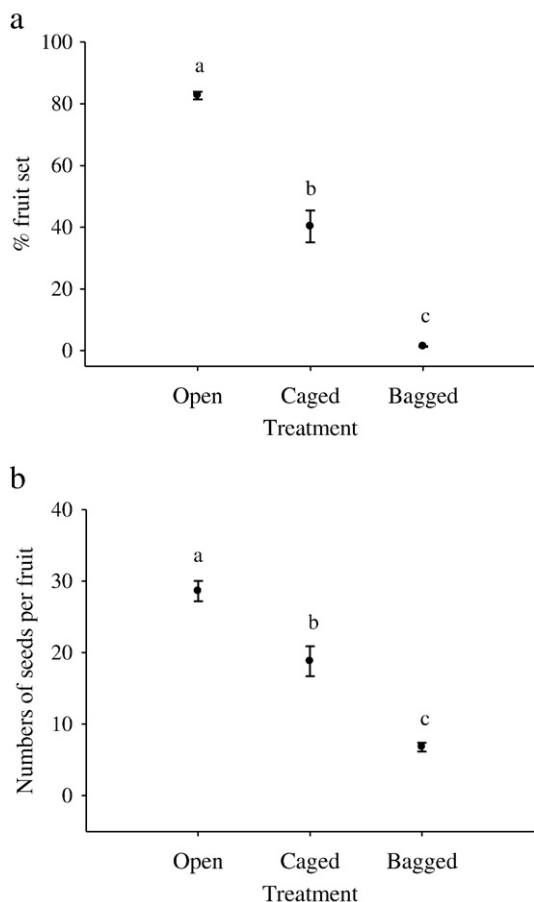


Fig. 2. Effect of pollinator type on (a) fruit set (% per inflorescence) and (b) seed set (total number per fruit) in *Kniphofia caulescens*. Open inflorescences were available to birds and insects, caged inflorescences excluded birds, and bagged inflorescences excluded all pollinators. Means with different letters are significantly different (Tukey test). Symbols represent means  $\pm$  standard error.

### 3.4. Floral visitor abundance and pollen loads

Drakensberg Siskins (*Crithagra symonsi* — Fig. 1b, c) and Yellow Canaries (*Crithagra flaviventris*) were the most

Table 3  
Number of birds netted and observed feeding on *Kniphofia caulescens* and their pollen loads.

Species	Netted	Observed (#/h)	Mean	Standard error	N
Drakensberg Siskin ( <i>Crithagra symonsi</i> )	76	7.9	439	134.2	55
Yellow Canary ( <i>Crithagra flaviventris</i> )	3	4.5	529	521.5	2
Red-winged Starling ( <i>Onychognathus morio</i> )	0	1.5			
Cape Sparrow ( <i>Passer melanurus</i> )	10	1.1	48	27.5	9
Malachite Sunbird ( <i>Nectarinia famosa</i> )	5	1.4	41	19.5	5
Cape Bunting ( <i>Emberiza capensis</i> )	4	0.1	42	36.1	4
Sentinel Rock Thrush ( <i>Monticola explorator</i> )	3	0	0	n/a	1
Cape Weaver ( <i>Ploceus capensis</i> )	1	0.7	3	n/a	1

Feeding observations represent average number of birds seen feeding per hour ( $n=22$  h).

common visitors (Table 3) and carried the highest pollen loads of all birds netted in *K. caulescens* stands (Table 3). The highest count was 6900 pollen grains, collected off a Drakensberg Siskin. Other seed-eaters, like sparrows and weavers (Fig. 1d; Table 3) were recorded visiting flowers. The only common flower visitor not netted was Red-winged Starlings (*Onychognathus morio*). Large pollen loads were visible through binoculars on the starlings, and they are probably also important pollinators on this species (M. Brown pers. obs.). Malachite Sunbirds (Fig. 1e) fed in moderate numbers on inflorescences, but carried low pollen loads (Table 3). Inflorescences were frequently visited by both honey bees (*Apis mellifera scutellata*) and flies (Sarcophagidae, Calliphoridae, Muscidae and Syrphidae). Most bee observations involved pollen collection, although some were occasionally seen drinking nectar. All fly observations involved nectar drinking.

### 4. Discussion

This study showed that opportunistic nectar-feeding birds are the main pollinators of *Kniphofia caulescens*. These birds, especially the Drakensberg Siskin, were by far the most abundant visitors, made effective contact with the sexual parts of the flowers, and carried very large pollen loads — up to 6900 grains, which is comparable to pollen loads reported for sunbirds (Hargreaves et al., 2004). The flower and nectar characteristics of *K. caulescens* conform to the general pattern (syndrome) found in other plants pollinated by these birds (Johnson et al., 2006; Botes et al., 2008, 2009; Johnson and Nicolson, 2008; Symes et al., 2008; Symes and Nicolson, 2008). In particular, the flowers have large volumes of dilute hexose dominant nectar, relatively short, wide corollas, and exerted anthers. Malachite Sunbirds, while encountered feeding on *K. caulescens* relatively frequently, carried low pollen loads and appear not to be important pollinators. Indeed, with their long bills (26.8–39 mm) these birds mostly rob nectar of *K. caulescens*.

Pollinator exclusion experiments, designed to determine the role of bees as opposed to birds, have now been conducted for several southern African ornithophilous plant species. Results so far have been mixed. In *Protea roupelliae*, seed set when birds were excluded was negligible, suggesting that insects played little or no role in this specialist bird pollinated species (Hargreaves et al., 2004). Botes et al. (2009) found that fruit set when birds were excluded was negligible in three generalist bird pollinated aloes (*Aloe africana*, *A. speciosa* and *A. ferox*) which are visited by pollen-collecting bees. However, Botes et al. (2009) found that bees did contribute somewhat to seed set in two specialist bird pollinated aloes (*Aloe pluridens* and *A. lineate* var. *murii*) which have tubular flowers into which bees crawl in search of nectar (Botes et al., 2008). Bees played only a small role in seed set in *Aloe vryheidensis*, a generalist bird pollinated plant, but in this case the plant uses bitter-tasting nectar to selectively exclude nectar-feeding bees and specialist nectar-feeding birds (Johnson et al., 2006). Interestingly, Wilson et al. (2009) found that bird exclusion had no significant effect on

seed set in *Aloe pruinosa*, suggesting that, in this species, birds and bees play an equal role as pollinators.

From data obtained in bird exclusion experiments, Symes et al. (this volume) suggest that bees might be more important than sunbirds as pollinators of *A. greatheadii* var. *davyana*, while they make little contribution to fruit set in *Aloe marlothii*, a species pollinated primarily by opportunistic nectarivores. Our study, on *Kniphofia caulescens*, showed that fruit set in open inflorescences is more than double that in caged inflorescences, indicating that birds played a greater role than bees in pollination in this plant. This effect in *K. caulescens* is even greater in terms of seeds produced per flower ( $\bar{X} = 18.8$  in bird excluded plants and  $\bar{X} = 28.6$  in open-pollinated controls).

Generally, fruit set in bird pollinated species in southern Africa is fairly low: 15–55% in aloes (Botes et al., 2009; Symes et al., 2009-this issue) and 10–40% in proteas (Hargreaves et al., 2004). However, we found relatively high natural fruit set (>80%) in this generalist bird pollinated plant species, which is consistent with the extraordinary high levels of bird visitation that we observed (sometimes more than 30 birds were observed feeding in the population at the same time).

It is apparent that many plant species in southern Africa, across several genera, are adapted to pollination by short-billed occasional nectar-feeding birds (Johnson et al., 2006; Botes et al., 2008; Symes et al., 2008; Botes et al., 2009). These studies have tended to support Johnson and Nicolson's (2008) proposal that there are two quite distinct bird pollination systems in Africa and that they differ markedly in terms of nectar characteristics. Further studies on other *Kniphofia* species, particularly those that are predominantly pollinated by specialist nectarivores, will be conducted to determine if nectar sugar composition is phylogenetically constrained in this genus, as it appears to be in the genus *Aloe* (Van Wyk et al., 1993; Johnson and Nicolson, 2008).

The reproductive biology of most *Kniphofia* species remains unstudied. The present study and another on *K. laxiflora* in this volume (Johnson et al., 2009-this issue) appear to be the first to document the breeding systems and identify the primary pollinators for any species in the genus. This is surprising, considering the diversity in the genus (~70 species; 45 species in South Africa; Ramdhani et al., 2006, 2008). As a genus, *Kniphofia* appears to have species pollinated by insects, specialist nectar-feeding birds (sunbirds), and opportunistic nectar-feeding birds (M. Brown unpublished data), and therefore warrants further research. Indeed, it would appear as if the genus *Kniphofia* shows evolutionary shifts between different pollination systems that parallel those found in the genus *Aloe*.

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to the late Prof Steven Piper, whose enthusiasm and passion for both the birds and the plants of Sani Pass was infectious.

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# Pollination of the red-hot poker *Kniphofia laxiflora* (Asphodelaceae) by sunbirds

M. Brown\*, C.T. Downs, S.D. Johnson

School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

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## Abstract

Most of the species in the large African genus *Kniphofia* have floral traits that conform to the bird pollination syndrome, however there has been very little empirical work to confirm that birds are effective pollinators of *Kniphofia* species. From selective exclusion experiments, behavioural observations and pollen load analyses, we identified Amethyst Sunbirds (*Chalcomitra amethystina*) and Malachite Sunbirds (*Nectarinia famosa*) as the primary pollinators in two populations of *Kniphofia laxiflora*, with bees playing a smaller role. Bird visitors obtain moderate volumes (~9 µl) of relatively concentrated (~15%) hexose-rich nectar in the flowers. This species is shown to be genetically self-incompatible, and thus reliant on pollinator visits for seed set. Although mountain pride butterflies (*Aeropetes tulbaghia*) have been identified as important pollinators of this species at two other populations, they did not occur at the two populations we studied.

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**Keywords:** Amethyst Sunbird; Bird pollination; *Kniphofia laxiflora*; Malachite Sunbird; Red-hot poker

## 1. Introduction

*Kniphofia* (Asphodelaceae), a large African genus of ~70 species (45 species in South Africa; Codd, 1968), is closely related to *Aloe* (Treutlin et al., 2003). Like *Aloe*, most species in *Kniphofia* have tubular red or orange flowers that conform to the syndrome of bird pollination, while a minority have very small flowers suggestive of pollination by insects. While pollination systems in *Aloe* have attracted considerable attention recently (Johnson et al., 2006; Botes et al., 2008, 2009; Symes and Nicolson, 2008; Symes et al., 2008, 2009; Brown et al., 2009; Wilson et al., 2009), those in *Kniphofia* have scarcely been investigated.

There is considerable variation in floral tube length even among *Kniphofia* species that conform to the bird pollination syndrome. A recent study of *Kniphofia caulescens* Baker, a species with relatively shallow flowers (c. 20 mm depth), identified short-billed generalist birds as pollinators (Brown et al., 2009). This mirrors the pattern in *Aloe* in which a distinct polli-

nation system involving generalist birds has been identified (Johnson et al., 2006; Botes et al., 2008, 2009; Symes et al., 2008, 2009). *Kniphofia* species with long narrow flowers (c 30–40 mm depth), by contrast, are likely to be pollinated by sunbirds. There are, however, few data available to test this idea. Johnson (1994) observed visits by mountain pride butterflies (*Aeropetes tulbaghia*) to flowers of *K. uvaria* L. (Hook), but suggested that sunbirds were likely to be the primary pollinators of this species. Johnson and Brown (2004) made anecdotal observations of visits by Malachite Sunbirds (*Nectarinia famosa*) to flowers of *Kniphofia laxiflora* Kunth in one population, while Johnson et al. (2009) found that mountain pride butterflies (*A. tulbaghia*) were the main flower visitors and pollen vectors in two other populations of this same species. Bees also visit flowers of this species to gather pollen, but their precise role as pollinators has not been established.

The present study was initiated with the broad aim of establishing the pollinator effectiveness of various visitors to the flowers of *K. laxiflora*. The specific aims were to 1) determine whether this species has a breeding system that makes it dependent on pollinator visits for seed set, 2) quantify key floral traits, such as nectar volume, concentration and composition, which

\* Corresponding author. Tel.: +27 33 2605661.

E-mail address: brownma@ukzn.ac.za (M. Brown).

may reflect adaptations to particular pollinators, 3) establish the main flower visitors and their pollen loads, and 4) determine the relative contributions of birds and insects to fruit and seed production.

## 2. Methods

### 2.1. Study site and species

*K. laxiflora* is a relatively common and variable species with a distribution from Port St Johns inland and as far north as southern Mpumalanga (Codd, 1968). We studied two populations of *K. laxiflora* in the Midlands of KwaZulu-Natal, South Africa. The main study site, near Curry's Post (hereafter called Curry's Post, 2924S 3011E) is where all categories of data presented below were recorded. We collected flower and nectar morphology data from the second population (between Boston and Bulwer, hereafter called Boston, 2944S 2955E), and made visual observations of pollinator visits. Voucher specimens from both populations (M. Brown 4 and M. Brown 5), were lodged at the Bews Herbarium (NU), University of KwaZulu-Natal, Pietermaritzburg. The study was conducted in February 2008 and January to February 2009.

### 2.2. Flower morphology and nectar properties

We selected 11 plants in the Curry's Post population, and 4 plants in the Boston population for morphological measurements, including style length, flower depth and flower width. Nectar characteristics (volume and concentration) were measured as standing crop from 10 plants (5 flowers from each plant) at 9 am, 12 pm, 3 pm and 6 pm at each population. We determined volume with 100- $\mu$ L micropipettes and concentration with a handheld Bellingham and Stanley refractometer (Tunbridge Wells, Kent, UK). Nectar sugar composition was determined using a Shimadzu (LC-20AT) high-performance liquid chromatograph (HPLC). Detection was by refractive index (RID-10A) with a Phenomenex column (Rezex RCM-Monosaccharide, 200  $\times$  780 mm 8 micron). Isocratic separation was accomplished using ultra pure water as the mobile phase. HPLC analysis was conducted on 5 samples taken from 5 different plants of the Curry's Post population.

### 2.3. Breeding system experiments

We determined the compatibility system and dependency of *K. laxiflora* on animal vectors for seed production by performing controlled pollination experiments at the Curry's Post population. Fifteen inflorescences were bagged from the bud stage to exclude all potential pollinators. After anthesis, we hand-pollinated two flowers on each of five inflorescences with pollen from the same plant (self-compatibility test), and another two flowers on each of five different inflorescences with pollen from different plants as a cross-pollinated control. Other marked flowers were left unmanipulated to test for autogamy. Fruit set frequencies for selfed- and cross-pollinated inflorescences were compared statistically using a Chi-square contingency test.

### 2.4. Pollinator effectiveness

To determine the effectiveness of various pollinator groups, we performed two exclusion experiments at the Curry's Post population. We firstly placed mosquito-netting exclusion bags on 10 inflorescences, which excluded all pollinators. Secondly, we placed plastic mesh (aperture diameter 12.5 mm) bird exclusion cages (Hargreaves et al., 2004; Botes et al., 2009; Brown et al., 2009; Wilson et al., 2009) over an additional 10 inflorescences. These mesh cages freely admit bees and other small flying insects (Botes et al., 2009; Wilson et al., 2009). We later determined fruit set and seed set for both these groups, in addition to natural fruit and seed set taken from 30 un-manipulated control inflorescences. We analysed fruit set and seed set data using plant means as replicates using ANOVA with post-hoc Tukey tests, using STATISTICA, Statsoft, Tulsa, USA. Fruit set data was arcsine square root transformed prior to analysis.

### 2.5. Floral visitor abundance and pollen loads

We carried out incidental observations of all species encountered on the flowers at both populations. Birds were observed from a distance of 25 m or less, with binoculars, while insects were observed on focal inflorescences from a distance of 5 m or less. We identified insect visitors that were seen either collecting pollen, or drinking nectar. To determine the efficiency of birds as pollinators, we collected pollen loads from birds mist-netted in the Curry's Post population. Birds were ringed and released after pollen loads were taken. Fuchsin-stained gel was used to collect pollen from the heads and bills of each bird, and then melted onto microscope slides in the field to produce permanent slides (Beattie, 1971). Using reference slides of pollen from *K. laxiflora* for initial identification, we then quantified the total number of *K. laxiflora* pollen grains present on each slide.

## 3. Results

### 3.1. Flower morphology and nectar properties

*K. laxiflora* (Fig. 1a) has relatively long, narrow flowers, with protruding stamens (Table 1). *K. laxiflora* produces relatively small amounts of concentrated nectar. There was no significant difference in the mean volume ( $F_{1,3}=1.086$ ,  $p=0.374$ ) or mean concentration ( $F_{1,3}=5.552$ ,  $p=0.100$ ) of nectar between the two populations. Both populations showed distinct peaks in nectar standing crop (Boston  $F_{3,57}=57.6$ ,  $p<0.0001$ ; Curry's Post  $F_{3,12}=10.1$ ,  $p<0.005$ ), with significantly more nectar available at 9 am and 6 pm than at 12 pm and 3 pm (Table 2). Sugar analysis revealed the composition of *K. laxiflora* nectar to be made up of 50.4% glucose, 48.7% fructose and 0.9% sucrose.

### 3.2. Breeding system experiments

Fruit set frequencies differed markedly between self- and cross-pollinated inflorescences: four of the cross-pollinated inflorescences set two fruits apiece (the maximum possible as two flowers per inflorescence were pollinated), and one set a



Fig. 1. (a) Population of *Kniphofia laxiflora* at Curry's Post; (b) a male Amethyst Sunbird (*Chalcomitra amethystina*) probing flowers of *K. laxiflora*.

single fruit, while no fruits were set by the five self-pollinated inflorescences. This difference in the frequencies of the three possible fruit set categories (zero, one or two fruits) for self- and cross-pollinated inflorescences was significant (Chi-square = 10,  $P=0.006$ ) and indicates the presence of a genetic self-incompatibility system in *K. laxiflora*.

### 3.3. Pollinator effectiveness

Pollinator exclusion significantly affected fruit set ( $F_{2,18}=340.5$ ,  $p<0.0001$ ; Fig. 2a) and seed set ( $F_{2,18}=75.2$ ,  $p<0.0001$ ; Fig. 2b). Inflorescences set significantly more fruits when exposed to pollination by both birds and insects (open flowers) than to only insects, or no pollinators at all (bagged flowers). Caged inflorescences also set more fruit than bagged inflorescences (Fig. 2a).

### 3.4. Floral visitor pollen loads

Amethyst Sunbirds (*Chalcomitra amethystina* — Fig. 1b) and Malachite Sunbirds were the only two bird species recorded feeding on *K. laxiflora* during 14 h of observations at the two study sites. The Curry's Post population was visited exclusively by Amethyst Sunbirds (27 individuals over 10 h), while the Boston population was visited by both species (12 Malachite Sunbirds and 10 Amethyst Sunbirds over 4 h). Pollen loads

collected from four Amethyst Sunbirds netted at Curry's post were 3200, 2900, 1250, and 975 grains of *Kniphofia* pollen, respectively. Large pollen loads were visible through binoculars on both sunbird species at Boston. During 10 h of insect observations, inflorescences were also infrequently visited by honey bees (*Apis mellifera scutellata*). Most bee observations involved pollen collection, and no observations of bees drinking nectar were made.

## 4. Discussion

This study showed that plants in these two populations of *K. laxiflora* were pollinated mainly by sunbirds. *K. laxiflora* has long, narrow, orange corollas and produces small volumes (2.4–16  $\mu\text{l}$ ) of relatively concentrated nectar (12.5–17.5%). These floral and nectar characteristics, with the exception of sugar type, conform to Johnson and Nicolson's (2008) patterns for plants pollinated by specialist avian nectarivores.

Fruit and seed sets (Fig. 2) were significantly lower (~60%) in inflorescences at the Curry's Post population from which birds were excluded, indicating that insects contribute less than birds to seed set in this population of *K. laxiflora*. However, recent studies (Wilson et al., 2009; Hargreaves et al., in press)

Table 1  
Summary of flower morphology of *Kniphofia laxiflora*. Values represent means  $\pm$  standard errors.

(mm)	Curry's post		Boston		<i>t</i>	<i>df</i>	<i>p</i>
	Mean	<i>n</i>	Mean	<i>n</i>			
Flower depth	34.46 $\pm$ 0.321	54	33.9 $\pm$ 0.626	20	0.88	72	0.391
Flower width	4.78 $\pm$ 0.082	54	4.5 $\pm$ 0.063	20	1.98	72	0.052
Stamen length	39.06 $\pm$ 0.564	29					

Table 2  
Volume and concentration of the standing crop of nectar in flowers of *Kniphofia laxiflora* over the day, in two populations. Values represent means  $\pm$  standard errors. Letters indicate significant differences (based on Tukey tests) among mean values for different times in each population.

Summary	Curry's post			Boston		
	Volume ( $\mu\text{l}$ )	Concentration	<i>n</i>	Volume	Concentration	<i>n</i>
9 am	12.7 <sup>a</sup> $\pm$ 0.84	15.1 $\pm$ 0.35	50	12.1 <sup>a</sup> $\pm$ 1.01	15.0 $\pm$ 0.55	25
12 pm	2.4 <sup>b</sup> $\pm$ 0.35	15.6 $\pm$ 0.35	25	3.6 <sup>b</sup> $\pm$ 0.55	12.9 $\pm$ 0.47	20
3 pm	6.7 <sup>b</sup> $\pm$ 1.17	17.2 $\pm$ 0.41	25	5.2 <sup>b</sup> $\pm$ 0.46	13.8 $\pm$ 0.61	25
6 pm	16.0 <sup>a</sup> $\pm$ 2.11	15.5 $\pm$ 0.57	5	14.0 <sup>a</sup> $\pm$ 1.16	14.6 $\pm$ 0.54	25

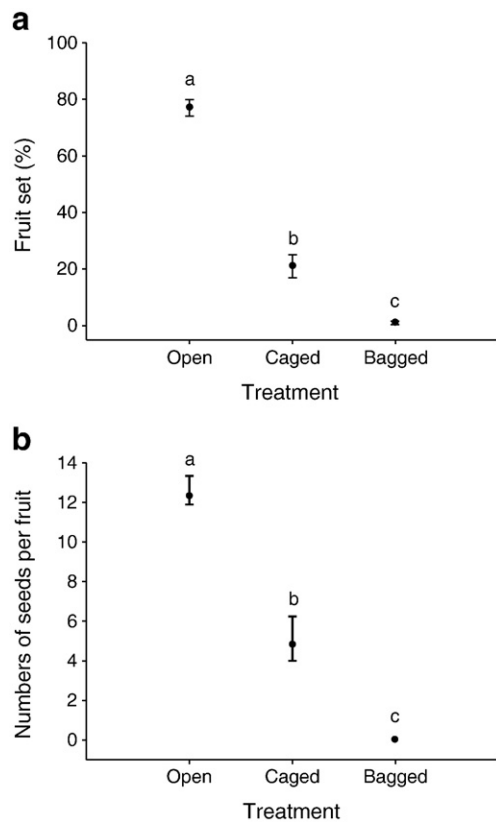


Fig. 2. Effect of pollinator type on (a) the percentage of flowers that set fruit per inflorescence and (b) the number of seeds per fruit in *Kniphofia laxiflora*. Open inflorescences were available to birds and insects, caged inflorescences excluded birds and butterflies, and bagged inflorescences excluded all pollinators. Means with different letters are significantly different (Tukey test). Symbols represent means  $\pm$  standard error.

have shown that the relative contribution that bees make to pollination of primarily bird-pollinated plants can vary from site to site. This means that firm conclusions about the role of bee-pollination in *K. laxiflora* can only be reached if exclusion experiments are conducted in several populations. The relatively small contribution of bees to seed set of *K. laxiflora* in the Curry's Post population contrasts with results of Botes et al. (2009), who found that bees made significant contributions to seed set in two specialist bird-pollinated aloes (*Aloe phuridens* and *A. lineata* var. *muirii*) which have tubular flowers into which bees crawl in search of nectar (Botes et al., 2008), and Wilson et al. (2009) who found that bees were effective pollinators of *Aloe pruinoso*. The differences between the results of these studies may be because the narrow corollas of *K. laxiflora* (Table 1) prevent bees from entering and brushing over inserted reproductive parts of the flowers. *K. laxiflora* is clearly genetically self-incompatible, as has been shown for *K. caulescens* (Brown et al., 2009) and many species in the related genus *Aloe* (Johnson et al., 2006; Botes et al., 2008, 2009; Symes et al., 2008; Wilson et al., 2009).

The results of the present study differ from those of Johnson et al. (2009) who found that mountain pride butterflies (*A. tulbaghia*) were the main visitors to flowers of *K. laxiflora* in the Karkloof mountains, and that sunbirds were only occasional visitors. Mountain pride butterflies were completely

absent from the areas where we studied *K. laxiflora*. However, these butterflies are notoriously habitat specific (preferring rocky mountainous terrain) and may differ strongly in abundance even over a habitat gradient of hundreds of metres (Johnson and Bond, 1992). Since our study sites were more representative of the typical habitat of *K. laxiflora* than were the mountainous sites selected by Johnson et al. (2009) we think it is likely that sunbirds are the primary pollinators of this species. In particular, both Malachite Sunbirds and Amethyst Sunbirds are abundant through most of the range of *K. laxiflora*, and both occur within the grassland shrub mosaic habitat where the species occurs. Both sunbirds are long-billed species with bill lengths (mean  $\pm$  standard error: Amethyst Sunbird  $29.15 \pm 0.098$  ( $n=261$ ); Malachite Sunbird  $34.09 \pm 0.136$  ( $n=349$ ) in Kwa-Zulu-Natal M. Brown unpub. data) that allow access to the nectaries of *K. laxiflora* (tongue extension will allow both species to reach the bottom of the nectary). Geerts and Pauw (2009) recently identified a specialized guild of long-tubed plants in the Cape Floristic Region which is pollinated by Malachite Sunbirds. This guild with a corolla tube length range of 30–50 mm extends to the eastern grassland region, and would include species such as *K. laxiflora*. However, this study and others (e.g. Botes et al., 2008; Ford and Johnson, 2008) suggests there can be considerable overlap in pollination by Malachite and Amethyst sunbirds among long-tubed bird-pollinated plants in the eastern region, although Malachite Sunbirds tend to predominate in higher altitude grassland habitats, while Amethyst sunbirds are more common in lower altitude savanna and thicket habitats.

Floral traits such as corolla length, corolla width, and nectar composition are known to be correlated with pollinator type (Fenster et al., 2004; Ford and Johnson, 2008). Johnson et al. (2009) reported a mean tube length of 38.3 mm for butterfly-visited flowers of *K. laxiflora*, which is c. 10% longer than that recorded in the two bird-visited populations in the current study. However, we do not know yet with any certainty whether differences in the pollinator fauna between populations of *K. laxiflora* has resulted in divergence in floral traits, or conversely whether pre-existing differences in floral traits are responsible for the differences in the pollinator fauna.

While few *Kniphofia* species have been studied so far (Brown et al., 2009; Johnson et al., 2009; current study), it appears as if sugar composition may be phylogenetically constrained in the genus. Within bird-pollinated systems, species pollinated by specialist nectarivores tend to have sucrose rich nectar, whilst those pollinated by generalist nectarivores tend to have hexose rich nectar (Johnson and Nicolson, 2008). Both *K. caulescens* (generalist nectarivore pollinated; Brown et al., 2009) and *K. laxiflora* (specialist nectarivore pollinated; current study) have hexose dominant nectar, with negligible amounts of sucrose (<1%). Phylogenetically constrained hexose nectars are also found in the closely related genus *Aloe* (Johnson and Nicolson, 2008). Corolla tube length and bird bill morphology appear to be correlated within *Kniphofia* (Brown unpub. data), which suggests that both flower morphology and nectar characteristics (with the exception of sugar type) within bird-pollinated members of the genus *Kniphofia* have responded to selective pressure by their pollinators.

## Acknowledgement

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**Covariation of flower traits and bird pollinator  
assemblages among populations of *Kniphofia  
linearifolia* (Asphodelaceae)**

Submitted to Plant Systematics and Evolution

Mark Brown<sup>\*</sup>, Colleen T. Downs & Steven D. Johnson

School of Biological & Conservation Sciences; University of KwaZulu-Natal; Private Bag X01;  
Scottsville; 3209; RSA.

<sup>\*</sup>Corresponding author: brownma@ukzn.ac.za; +27 33 2605661; fax +27 865152114

**Abstract**

Globally, bird pollinated plants can be separated into those pollinated by specialist nectarivores and those pollinated mainly by occasional nectarivores. There are marked differences in nectar properties among the two groups, implying that there has been pollinator mediated selection on these traits. This raises the possibility that variation in bird assemblages among populations of a plant species could lead to the evolution of intra-specific variation in floral traits. We examined this hypothesis in *Kniphofia linearifolia*, a common and widespread bird pollinated plant in southern Africa. We showed that bird pollinator assemblages differ markedly between 5 different

populations of *K. linearifolia*, and that variation in flower morphology and nectar properties between these populations were associated with the dominant guild of bird visitors at each population. We identified two distinct ecotypes, based on corolla length, nectar volume and nectar concentration, which reflect the bird assemblages found in each type. Further work is needed to establish if a natural geographic mosaic of bird assemblages are the ultimate cause of differentiation in floral traits in this species.

**Key words:** bird pollination; pollination ecotypes; red-hot poker; honeybee; nectar

## **Introduction**

It is generally accepted that adaptation to different pollinators has played a role in diversification within different plant groups (Johnston 1991; Johnson 1996; 2004; Kephart and Theiss 2003; Hargreaves et al. 2004; Anderson and Johnson 2009). In addition to this, geographic variation in flower traits, reflecting differences in pollinator types, have been recorded within a number of species (Robertson and Wyatt 1990; Galen 1989; Johnson 1997; Johnson and Steiner 1997; Alexandersson and Johnson 2002; Boyd 2004; Herrera 2005), including those pollinated by birds (Boyd 2002; 2004; Nattero and Cocucci 2007; Brown et al. in press). Such intra-specific differences in flower traits, correlated with different pollinators, can lead to pollination ecotypes (Johnson 1997; Nattero and Cocucci 2007). This suggests pollinator mediated selection on intra-specific flower morphology, which is of particular interest to evolutionary biologists (Harder and Johnson 2009; Johnson 1997; Nattero and Cocucci 2007). It has also been shown that such pollinator-mediated evolution can occur rapidly (Galen 1996).

Our current understanding of bird pollination systems suggest that plants can be separated into two categories, namely those pollinated by specialist nectar feeding birds and those pollinated by generalist or occasional nectar feeding birds (Johnson and Nicolson 2008). Plants pollinated by specialist avian nectarivores typically have small volumes (10-30 $\mu$ l) of relatively high concentration (15-25% w/w) sucrose rich (40-60% sucrose content) nectar, whilst those pollinated by generalist avian nectarivores have high volumes (40-100 $\mu$ l) of dilute (8-12%) hexose rich (sucrose content 0-5%) nectar (Cruden 1997; Johnson and Nicolson 2008). These differences have been found across the Americas and Africa, and even occur when phylogeny is accounted for. Indeed, apart from sugar type composition, which may be phylogenetically constrained in some genera (e.g. *Aloe*), good evidence now exists to suggest that these nectar characteristics can accurately be used to predict bird pollinators (Botes et al. 2008; Symes et al. 2008; Brown et al. 2009), and that the differences in nectar composition are found within many different bird pollinated genera, including *Aloe* (Johnson et al. 2006; Botes et al. 2008; Symes et al. 2008; Wilson et al. 2009), *Erythrina* (Baker and Baker 1982; Bruneau 1997; Cruden and Toledo 1977) and *Kniphofia* (Brown et al. 2009; Brown et al. in press). In addition, flower morphology is also found to be different between these two functional bird pollination groups, with occasional nectarivore pollinated plants exhibiting shorter, wider flowers than specialist nectarivore pollinated plants (Botes et al. 2008; Brown et al. 2009; Symes et al. 2008; 2009).

The family Asphodelaceae is divided into two subfamilies, Alooideae and Asphodeloideae. The genus *Kniphofia* consists of approximately 70 species, distributed around Africa, Madagascar (2 species) and Yemen (one species) (Ramdhani et al. 2006; 2008). The phylogenetics of this group, despite recent molecular and morphological studies, remains problematic (Ramdhani et al. 2006). *Kniphofia* appears to show similar pollinator diversification

with the closely related genus *Aloe*, in terms of containing species adapted to specialist avian nectarivore (Brown et al. submitted), occasional nectarivore (Brown et al. 2009) and insect (Brown unpublished data) pollinators. However, very few species have been studied to date, especially in terms of pollination ecology.

The present study examined ecotypic variation in flower traits and avian pollinator assemblages in *Kniphofia linearifolia*. Preliminary observations suggested that different populations may exhibit different bird feeding guilds. We set out to determine if floral traits, in terms of flower morphology and nectar properties, differed between populations, and to test whether such differences were mirrored by differences in avian pollinators. The specific aims were to 1) determine whether this species has a breeding system that makes it dependent on pollinator visits for seed set, 2) quantify key floral traits, such as corolla length, corolla width, nectar volume, concentration and composition for five different populations, 3) establish the main flower visitors and their pollen loads, and 4) determine the relative contributions of birds and insects to fruit and seed production.

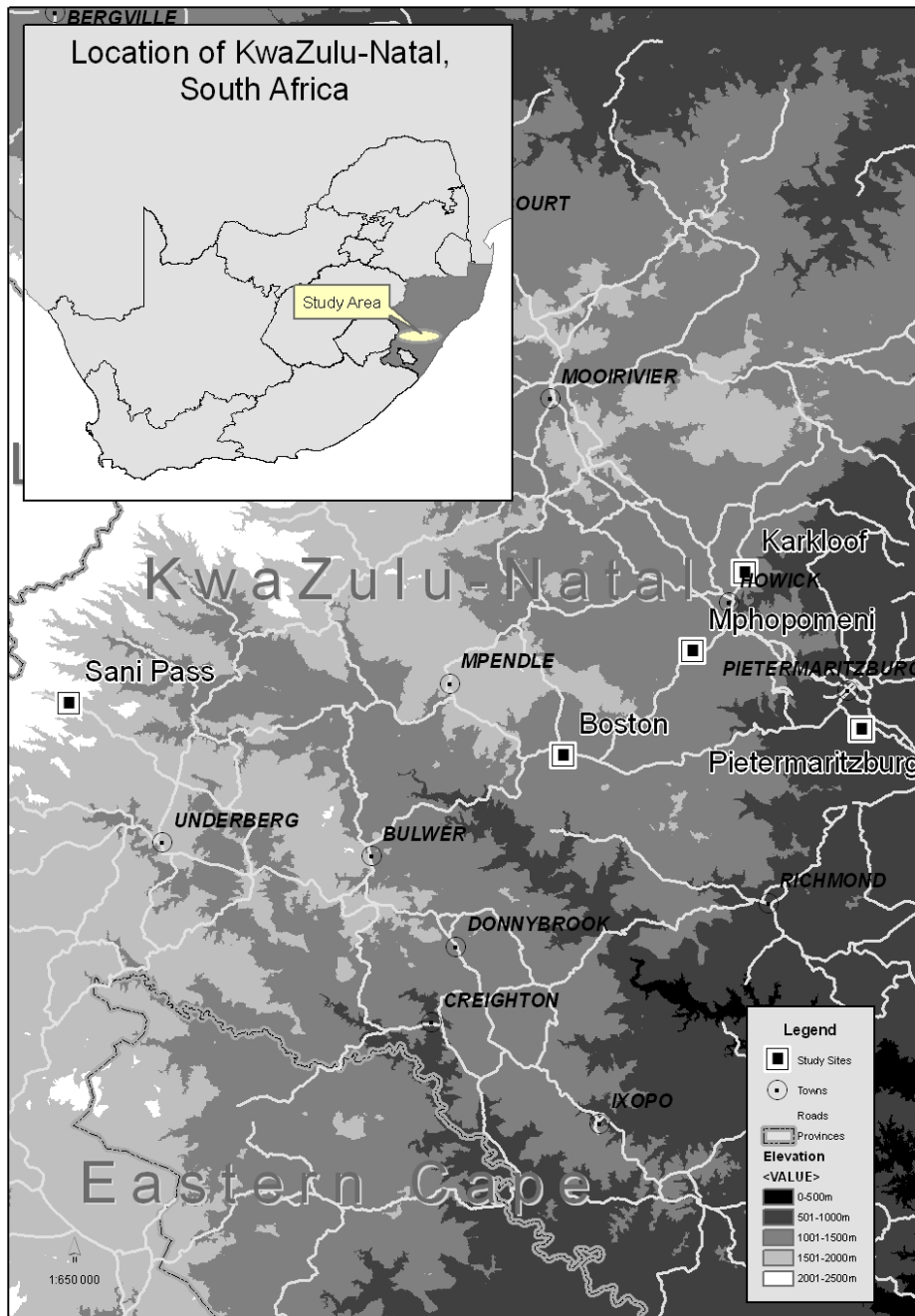
## **Materials & Methods**

### *Study site and species*

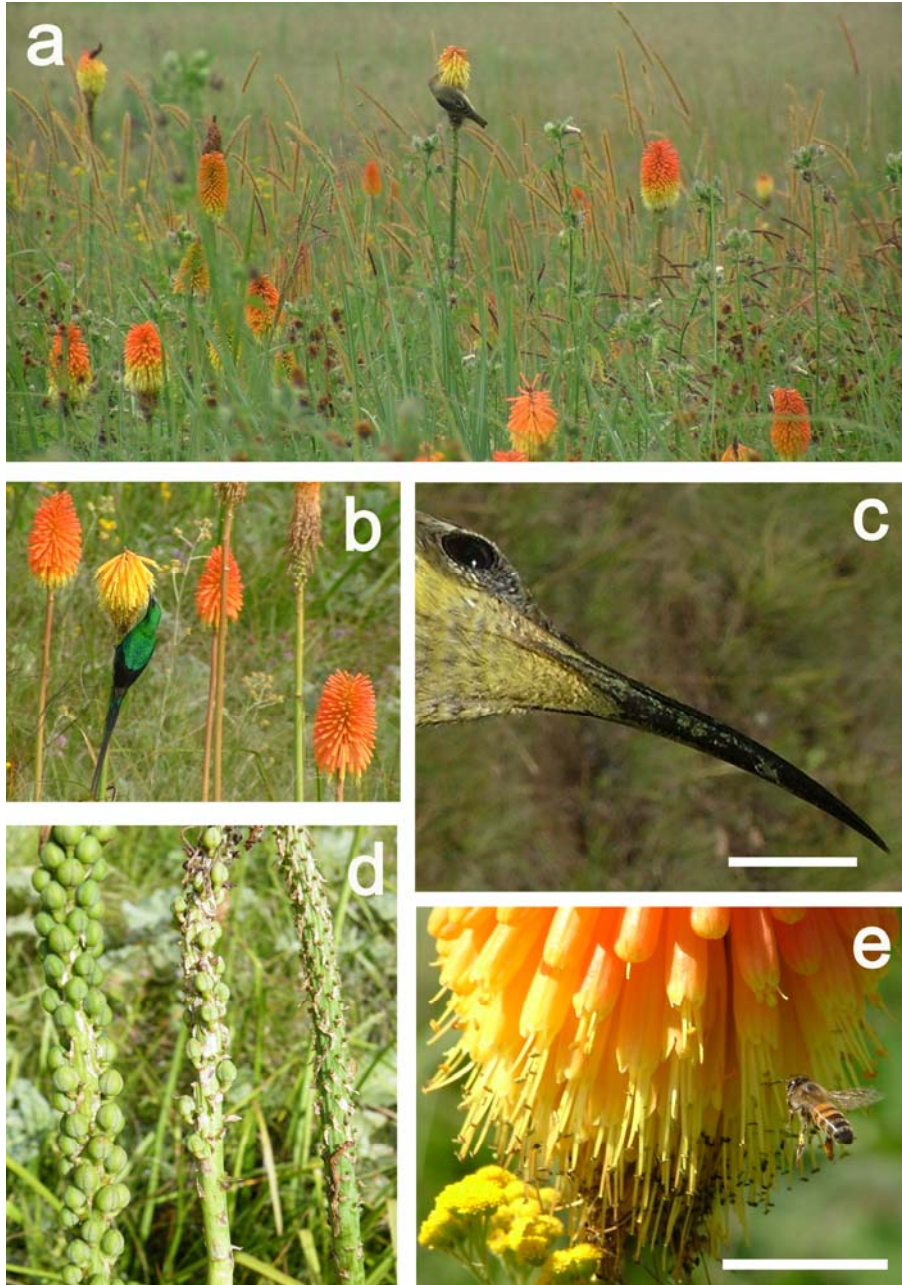
*Kniphofia linearifolia* is the most common and widespread red-hot poker species in South Africa and exhibits variation in stature of plant, size and color of inflorescences, and width and colour of leaves (Codd 1968). *K. linearifolia* has relatively long, narrow flowers, with protruding stamens. We studied five populations of *K. linearifolia* in KwaZulu-Natal, South Africa. The populations were located near the Sani Pass South African border post (hereafter called Sani

Chap. 9 Bird Pollination of *K. linearifolia*

Pass: 2937S 2923E), at Boston (2941S 3001E), near Mphophomeni (2933S 3011E), along the Karkloof Road (hereafter called Karkloof: 2927S 3015E) and along Murray Road, Pietermaritzburg (hereafter called Pietermaritzburg: 2939S 3024E) (Figure 1).



**Fig. 1:** Distribution of the 5 populations of *Kniphofia linearifolia* studied in KwaZulu-Natal



**Fig. 2:** a – Village Weaver (*Ploceus cuculatus*) feeding at the Boston population of *Kniphofia linearifolia*; b – Male Malachite Sunbird (*Nectarinia famosa*) drinking nectar at Sani Pass population; c – high visible pollen load on female Malachite Sunbird at Sani Pass; d – Fruit set on open (left), caged (middle) and bagged (right) inflorescence of *K. linearifolia* at Sani Pass; e – Honey Bee (*Apis mellifera*) collecting pollen on *K. linearifolia* at Sani Pass. Scale bar = 10mm.

*Chap. 9 Bird Pollination of K. linearifolia**Flower morphology and nectar properties*

We selected between 5 and 14 plants from Sani Pass, Boston, Mpophomeni, Karkloof and Pietermaritzburg populations for morphological measurements (5 flowers from each plant), including flower depth and width. Nectar volume and concentration were measured from between 5 and 10 plants (5 flowers per plant) as standing crop (08h00 and 12h00) and bagged overnight (08h00) from each population. Volume was determined using a 100- $\mu$ L micropipettes and concentration with a handheld Bellingham and Stanley refractometer (Tunbridge Wells, Kent, UK). Grand means based on mean values per plant were used for analysis. Nectar sugar composition of 3 flowers from each of 3 plants was determined for Sani Pass using a Shimadzu (LC-20AT) high-performance liquid chromatograph (HPLC). Detection was by refractive index (RID-10A) with a Phenomenex column (Rezex RCM-Monosaccharide, 200 x 780mm 8micron). Isocratic separation was accomplished using ultrapure water as the mobile phase.

*Breeding system experiments*

We performed controlled pollination experiments at the Sani Pass and Pietermaritzburg populations, in order to determine the compatibility system and dependency of *K. linearifolia* on animal vectors for seed production. Sixteen inflorescences at each of these populations were bagged from the bud stage to exclude all potential pollinators. We hand-pollinated 15 flowers on five inflorescences with pollen from the same plant to test self-compatibility, and another 15 flowers from five inflorescences with pollen from different plants as a cross-pollinated control. Other flowers were left unmanipulated to test for autogamy. We then examined fruit set and seed set for these flowers.

*Chap. 9 Bird Pollination of K. linearifolia**Pollinator effectiveness*

We performed exclusion experiments at the Sani Pass and Pietermaritzburg populations to determine the effectiveness of various pollinator groups. Firstly, we placed mosquito-net exclusion bags on 16 inflorescences, in order to exclude all pollinators. We also placed plastic mesh (12.5 mm aperture diameter) bird exclusion cages (Hargreaves et al. 2004; Botes et al. 2009; Brown et al. 2009; Wilson et al. 2009) over an additional 10 inflorescences. After flowering, we determined fruit and seed set for both groups, and natural fruit and seed set from an additional 25 (Sani Pass) and 20 (Pietermaritzburg) inflorescences. Fruit set and seed set data was analysed using ANOVA, followed by post-hoc Tukey tests. We performed analyses on arcsine square root transformed fruit set data.

*Floral visitor abundance, pollen loads and bird culmen length*

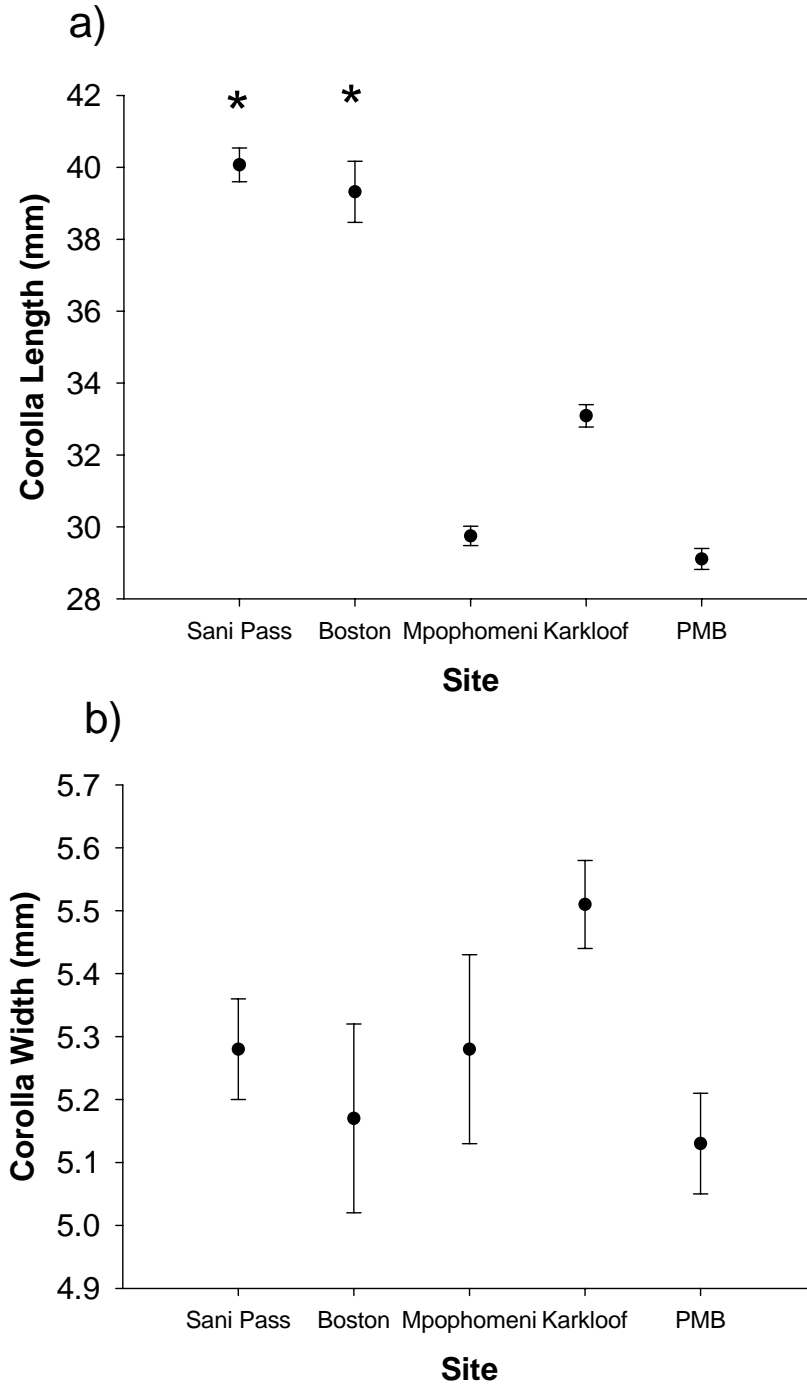
All species encountered on the flowers at any of the five populations were recorded. Insect visitors seen either collecting pollen or drinking nectar were collected for identification. Approximate numbers of birds (identified to species level) were assessed on each trip. Mean numbers of each species recorded are reported. Pollen loads were collected from birds mist-netted at Sani Pass and Pietermaritzburg, in order to determine the efficiency of different birds as pollinators. We used Fuchsin-stained gel to collect pollen from heads and bills of all birds captured. This was then melted onto microscope slides in the field (Brown et al. 2009), and compared to reference slides of *K. linearifolia* pollen for identification and quantification. In addition, we present culmen length data (collected in KwaZulu-Natal between 1999 and 2009) for each of the bird species recorded feeding on *K. linearifolia*.

## Results

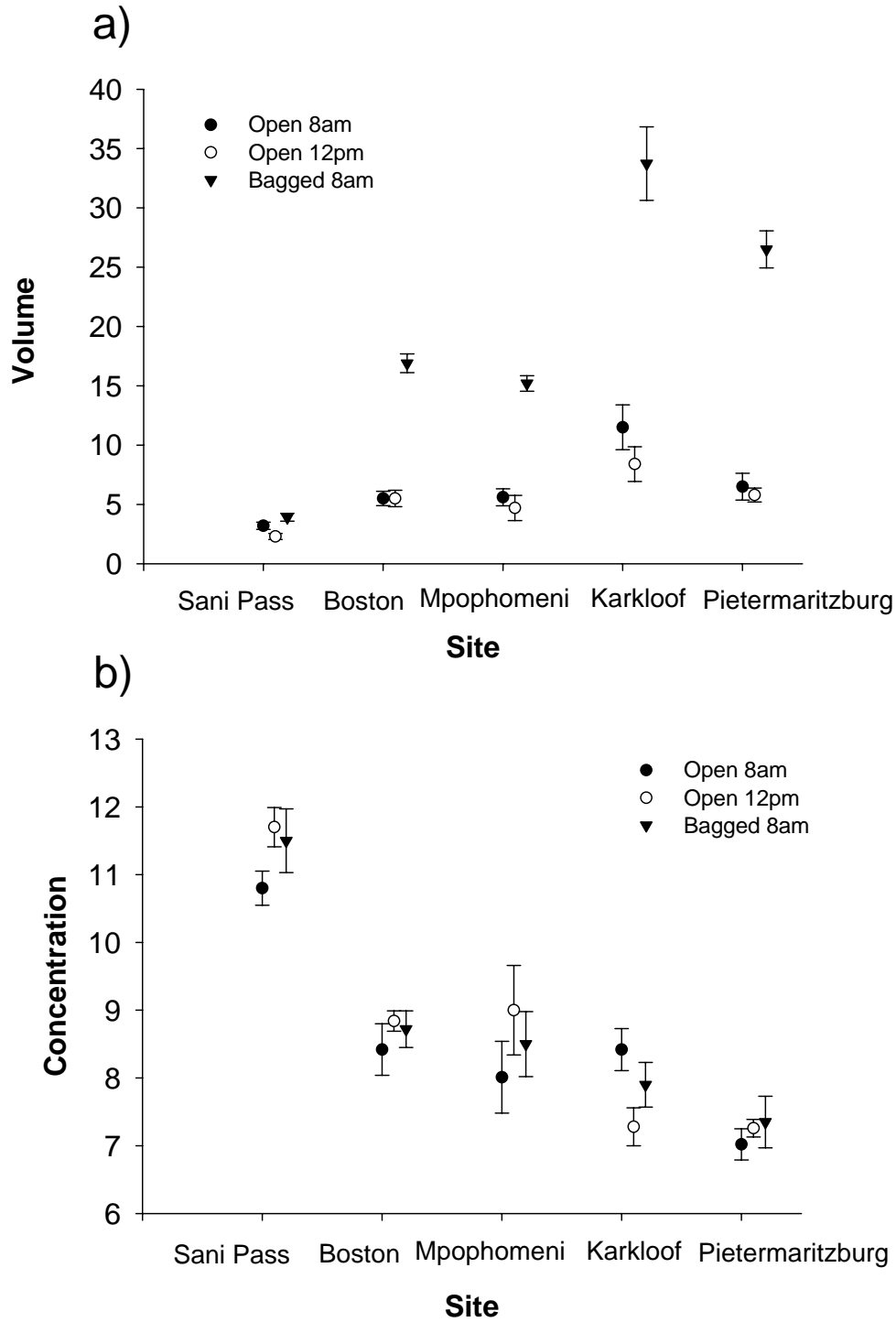
### *Flower morphology and nectar properties*

Corolla length in *K. linearifolia* was highly variable ( $F_{4, 96} = 72.804$ ,  $p < 0.001$ ; Figure 3a), and varied on average by 27.5% between the largest and smallest means from different populations (Sani Pass and Pietermaritzburg). Corolla length was significantly longer in Sani Pass and Boston than in Mpophomeni, Karkloof and Pietermaritzburg (Figure 3a). Corolla width was less variable (Figure 3b) and did not show variation between populations ( $F_{4, 96} = 1.94$ ,  $p = 0.11$ ; Figure 3b).

Nectar produced in *K. linearifolia* flowers bagged overnight varied among populations in volume ( $F_{4, 56} = 27.76$ ,  $p < 0.001$ ; Figure 4a) and concentration ( $F_{4, 56} = 16.38$ ,  $p < 0.001$ ; Figure 4b) nectar. Flowers bagged overnight in Boston and Mpophomeni produced similar volumes (Tukey:  $p = 0.996$ ), those in Sani Pass produced significantly less nectar (Tukey:  $p < 0.01$ ), and those in Karkloof and Pietermaritzburg produced significantly more (Tukey:  $p < 0.01$ ). Open flowers in all five populations were found to have similar standing crop volumes, irrespective of time of day ( $F_{4, 56} = 1.97$ ,  $p = 0.11$ ; Figure 4a). Flowers bagged overnight at Sani Pass produced significantly more concentrated nectar than all other populations (Tukey:  $p < 0.001$ ; Figure 4b). Standing crop concentration of open flowers within populations was not affected by time of day ( $F_{1, 14} = 0.04$ ,  $p = 0.88$ ; Figure 4b). Sampled nectar of *K. linearifolia* was composed of  $51.1 \pm 0.10\%$  glucose,  $47.8 \pm 0.13\%$  fructose and  $1.1 \pm 0.13\%$  sucrose sugars (mean  $\pm$  standard error,  $n = 9$ )



**Fig. 3:** Variation in corolla length (a) and width (b) among five populations of *Kniphofia linearifolia*. The Sani Pass population is visited by specialist avian nectarivores only, while all other populations are visited by specialist and generalist avian nectarivores. \* indicates populations with significantly longer corollas.



**Fig. 4:** Nectar volume and concentration from five different populations of *Kniphofia linearifolia* taken from open flower at 08h00 and 12h00, and from bagged flowers at 08h00.

*Chap. 9 Bird Pollination of K. linearifolia**Breeding system experiments*

Self-pollinated plants set negligible levels of fruit set (Sani Pass 13.3%; Pietermaritzburg 6.7%), while cross-pollinated flowers had high fruit set (Sani Pass 80%; Pietermaritzburg 73.3%). This difference was significant (Fishers Exact Test;  $P < 0.01$  for both populations) and indicated the presence of a genetic self-incompatibility system in *K.linearifolia*.

*Pollinator effectiveness*

Pollinator exclusion significantly affected both fruit set ( $F_{2, 18} = 418.83$ ,  $p < 0.001$ ; Figure 5a) and seed set ( $F_{1, 14} = 54.67$ ,  $p < 0.001$ ; Figure 5b) of *K. linearifolia*. Inflorescences set significantly more fruits when exposed to pollination by both birds and insects (open flowers) than to only insects, or no pollinators at all (bagged flowers). Caged inflorescences also set more fruit than bagged inflorescences. This relationship did not differ between populations (fruit set:  $F_{1, 9} = 0.13$ ,  $p = 0.73$ ; Figure 5a; Seed set:  $F_{1, 14} = 0.02$ ,  $p = 0.89$ ; Figure 5b). Fruits contained significantly more seeds when exposed to all pollinators than when birds were excluded (Figure 5b).

*Floral visitor abundance, pollen loads and bird culmen length*

Composition of avian species feeding on *K. linearifolia* flowers differed between different populations (Table 1). Plants at Sani Pass were visited only by specialist avian nectarivores (3 species), and those at Boston by specialists (2 species) and one species of generalist avian nectarivore. The remaining populations were all visited almost equally by both specialist and

Chap. 9 Bird Pollination of *K. linearifolia*

generalist nectar feeding birds (Table 1). Honey bees (*Apis mellifera scutellata*) were observed collecting pollen at all five populations. The only other nectar drinker observed was the mountain pride butterfly (*Aeropetes tulbaghia*) which was observed visiting flowers infrequently at Sani Pass (highest total recorded was three individuals in one day, only recorded during two of ten days field work). Pollen loads taken from birds mist-netted at Sani Pass and Pietermaritzburg were very high (Table 2). Culmen length for avian species recorded feeding on *K. linearifolia* are given in Table 3.

Table 1: Daily mean ( $\pm$  standard error) number of each bird species recorded feeding at five different populations of *Kniphofia linearifolia* in KwaZulu-Natal.

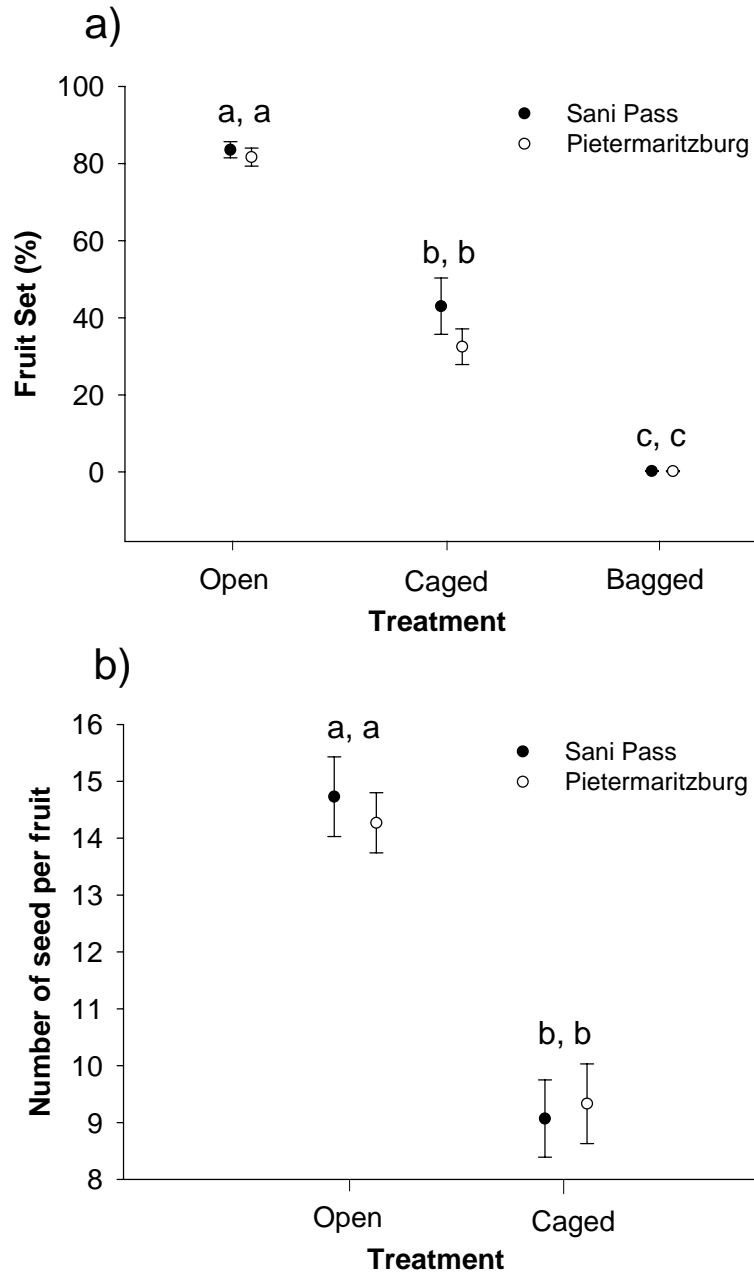
Species	Sani Pass (n=4)	Boston (n=3)	Mpophomeni (n=3)	Karkloof (n=2)	Pietermaritzburg (n=4)
Gurney's Sugarbirds	2.5 $\pm$ 0.5	-	-	-	-
Malachite Sunbird	67.5 $\pm$ 13.2	7 $\pm$ 0.7	1 $\pm$ 0.6	-	-
Greater Double-collared Sunbird	3.67 $\pm$ 0.6	-	-	-	-
Amethyst Sunbird	-	5 $\pm$ 1.8	4 $\pm$ 0.3	8.5 $\pm$ 4.5	6 $\pm$ 2
White-bellied Sunbird	-	-	-	-	0.5 $\pm$ 0.5
Cape White-eye	-	-	3 $\pm$ 1.2	2 $\pm$ 0	3 $\pm$ 0.5
Dark-capped Bulbul	-	-	2 $\pm$ 0.9	2 $\pm$ 1	2 $\pm$ 2
Village Weaver	-	11 $\pm$ 4.4	8 $\pm$ 1.3	2 $\pm$ 1	5 $\pm$ 5
Cape Weaver	-	-	-	4.5 $\pm$ 0.5	-

Table 2: Pollen loads collected from birds trapped while feeding in *Kniphofia linearifolia* populations in KwaZulu-Natal

Species	Pollen count	n	Range	Location
Malachite Sunbird	969 ± 274	30	27-7752	Sani Pass
Amethyst Sunbird	779	1	-	Pietermaritzburg
Dark-capped bulbul	995	1	-	Pietermaritzburg
Cape White-eye	650	1	-	Pietermaritzburg
Village Weaver	570 ± 180	3	256-880	Pietermaritzburg

Table 3: Mean culmen lengths (± standard error) for specialist (\*) and occasional nectar feeding birds seen feeding on *Kniphofia linearifolia* in KwaZulu-Natal (Brown, M. unpublished data collected in KwaZulu-Natal).

Species	Culmen length (mm)	n
Gurney's Sugarbird (*)	30.87 ± 0.19	175
Malachite Sunbird (*)	34.09 ± 0.134	349
Amethyst Sunbird (*)	29.15 ± 0.10	261
Greater Double-collared Sunbird (*)	28.08 ± 0.28	42
Village Weaver	19.78 ± 0.04	1273
Cape Weaver	21.96 ± 0.10	185
Dark-capped Bulbul	18.27 ± 0.09	326
Cape White-eye	11.53 ± 0.04	656



**Fig. 5:** The influence of pollinator type on a) fruit set (percentage of flowers to set fruit per inflorescence) and b) numbers of seeds per fruit in two populations of *Kniphofia linearifolia*. Open inflorescences were available to birds and insects, caged inflorescences excluded birds and butterflies, and bagged inflorescences excluded all pollinators. Means with different letters are significantly different (Tukey test). Symbols represent means  $\pm$  standard error.

## Discussion

This study showed that bird pollinator assemblages differed between 5 different populations of *K. linearifolia*, and that variation in flower morphology and nectar properties between these populations were associated with the dominant guild of bird visitors at each population. The Sani Pass population was visited exclusively by long-billed specialist nectar feeding birds, while other populations had mixed assemblages of specialist and short-billed occasional avian nectarivores.

Two distinct ecotypes appeared to occur: one where flowers have long (39-40mm) corolla lengths, small volumes (~5 $\mu$ l) of more concentrated (~11%) nectar, and where specialist nectarivores were the main pollinators; and one where flowers have shorter (29-33mm) corollas, larger volumes (15-35 $\mu$ l) of more dilute (~8%) nectar, and where a combination of specialist and occasional nectar feeding birds were the main pollinators. Nectar sugar composition was similar to that of other *Kniphofia* species (Brown et al 2009; in press), and was hexose dominant with negligible amounts of sucrose. The floral and nectar characteristics of these two ecotypes, with the exception of nectar sugar composition (which appears to be phylogenetically constrained to produce hexose rich nectar irrespective of pollinator type - Brown et al 2009; in press; Johnson and Nicolson 2008), conform to Johnson & Nicolson's (2008) predictions for specialist and generalist avian nectar feeding pollination systems.

Both flower morphology (e.g. corolla length and width) and nectar properties (volume, concentration and sugar type) are known to be correlated with pollinator type (Boyd 2002; 2004; Fenster et al. 2004; Ford and Johnson 2008; Johnson and Nicolson 2008). The range of bill lengths for the main bird species recorded visiting *K. linearifolia* flowers in our study area were 28-34mm for specialist species and 11-22mm for generalist species (Table 3). There appears to

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be a distinct relationship between bird assemblages and floral traits across the various populations, but we can only speculate as to whether the differences in bill lengths of the pollinator fauna between populations of *K. linearifolia* has resulted in divergence in floral traits (pollinator mediated selection), or conversely whether pre-existing differences in floral traits, e.g. as a result of phenotypic plasticity, are responsible for the differences in the pollinator fauna. Interestingly, although corolla width is generally wider in *Kniphofia* species pollinated by generalists (Brown et al. 2009) than in those pollinated by specialists (Brown et al. in press), it appears relatively fixed in *K. linearifolia*, even though flower width is sometimes mediated by selection by birds (Campbell et al. 1996). However, Nattero and Cocucci (2007) similarly found no effect of pollinator type on corolla width, and suggest that it is not easily mediated by pollinator pressure.

Fruit set (c. 80%) and seed set were high and similar in populations from both ecotypes, indicating that both pollinator suites were equally effective (cf Boyd 2004). Fruit set in both ecotypes was similar to that recorded in other *Kniphofia* species pollinated by generalist (Brown et al. 2009) and specialist (Brown et al. in press) avian nectarivores. Insects played a minor role in fruit set (Figure 4a), particularly in comparison with birds (~50% reduction in fruit set when birds excluded). Apart from the mountain pride butterfly, which probably is an effective pollinator at Sani Pass (although visitation rates were low), the most abundant insect recorded on flowers were honey bees, which were only recorded collecting pollen. Narrow corolla tubes (~5.25mm) may have prevented bees from entering and brushing over inserted reproductive parts of the flowers, as is the case in *K. laxiflora* (Brown et al. in press).

Our results concur with those found in other bird pollinated species (Boyd 2004; Nattero and Cocucci 2007) which show a correlation between pollinator bill length and corolla length

across populations. In addition, we found evidence of changes in nectar concentration and volume corresponding to changes in bird assemblages. Further experiments, including reciprocal transplantation between populations are required to confirm that pollinator mediated selection led to the formation of different ecotypes within this red hot poker species.

### **Acknowledgments**

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## **Can bird sugar preferences explain plant nectar properties?**

The idea that sugar preferences of birds provide an evolutionary explanation for the nectar properties of the plants they pollinate is not novel. Indeed, several attempts to correlate avian pollinator characteristics and nectar traits have been made in the past, based either on bird phylogeny (Baker & Baker 1982; 1983; Baker et al. 1998) – which can be termed the ‘phylogenetic hypothesis’ – or, more recently, based on bird feeding guild (specialist vs. occasional nectarivores; Johnson & Nicolson 2008) – which can be termed the ‘feeding niche’ hypothesis. I set out to test the latter hypothesis in this work.

### **Do specialist and occasional nectarivores differ in terms of sugar preferences?**

This thesis was comprised of two parts. In the first section, I examined sugar preferences, concentration preferences and apparent assimilation efficiencies of a specialist avian nectarivore (Chapter 3) and several occasional avian nectarivores (Chapters 4-6; appendix 1). Species were selected to reflect a range of feeding guilds, and to be representative of both passerine and non-passerine lineages. My results, when considered together with those of other authors (Schondube & Martínez del Rio 2003; Fleming et al. 2004; Fleming et al. 2008), revealed some differences between passerine specialist nectarivores and passerine occasional nectarivores. In general, specialist nectarivores show a concentration dependant switch from hexose preference at low concentrations to sucrose preference or no preference at high concentrations (Chapter 3; Schondube & Martínez del Rio 2003; Fleming et al. 2004; Fleming et al. 2008), while occasional passerine nectarivores generally prefer hexose at low concentrations, and show either hexose preference, or no preference at high concentrations (Chapters 4, 6; Appendix 1). However, the one species of non-passerine occasional nectarivore studied showed similar preferences to those of specialist nectarivores (Chapter 5), suggesting that phylogeny may play a role too.

Many specialist avian nectarivores appear to be physiologically constrained when feeding on solutions below about 8% sugar by mass, and are unable to meet daily energy demands, consequently losing body mass (Chapter 3; Schondube & Martínez del Rio 2003; Fleming et al.

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2004; Fleming et al. 2008). Furthermore, plants pollinated by these birds do not generally have nectar with concentrations below around 8-10% (Johnson & Nicolson 2008). Indeed, specialists show a strong preference for solution of the highest sugar concentrations when given a choice (Chapter 3). These observations suggest that specialist avian nectarivores in the field are unlikely to interact with plants with nectars of very low concentrations in a consistent enough manner to apply selection for hexose sugars. Occasional avian nectarivores, however, show either a preference for low concentration solutions, or lack a concentration preference, and are able to maintain energy balance on low concentration solutions. This, coupled with their preference for hexose solutions, suggests they may have been responsible for the evolution of high proportions of hexose sugars in the low concentration nectar in plants they pollinate (Johnson & Nicolson 2008).

Given that sugar preferences of birds are known, in many cases, to be dependent on sugar concentrations of solution, it is necessary to statistically disentangle the effects of the bird feeding niche and sugar concentration when attempting to explain sugar preference. To do this I extracted data from Chapters 3-6, Appendix 1, and from published literature where authors have examined sugar preference using equicaloric solutions at several concentrations (Schondube & Martínez del Rio 2003; Fleming et al. 2004; Fleming et al. 2008). I then used Analysis of Covariance (ANCOVA), using type III sum of squares, with 12 species (8 specialists and 4 occasional nectarivores nested within these two feeding niches) and sugar solution concentration as the covariate. The response variable was arcsine square-root transformed sucrose preference data for solutions ranging from 3-25% sugar concentration (Figure 1). I found a significant overall effect of concentration on sugar preference ( $F_{1, 38.9} = 7.86, p < 0.01$ ), with no effect of species ( $F_{1, 38.9} = 1.85, p = 0.079$ ). Surprisingly, I found no significant difference in sucrose preference between specialist and occasional nectarivores ( $F_{1, 38.9} = 0.721, p = 0.415$ ). This result thus raised questions about the validity of the feeding niche hypothesis developed by Johnson and Nicolson (2008). I then developed a new hypothesis ('body size hypothesis') by considering the relationship between body size and sucrose preference for these 12 species, using regression analysis for four different concentrations (Figure 2). Here I excluded data from concentrations below 8%, as the inability of several specialist nectarivores to maintain energy balance at these

low concentrations may skew the results, and also excluded published data above 25% as most bird pollinated plants have a concentration range of 8-25% (Johnson & Nicolson 2008).

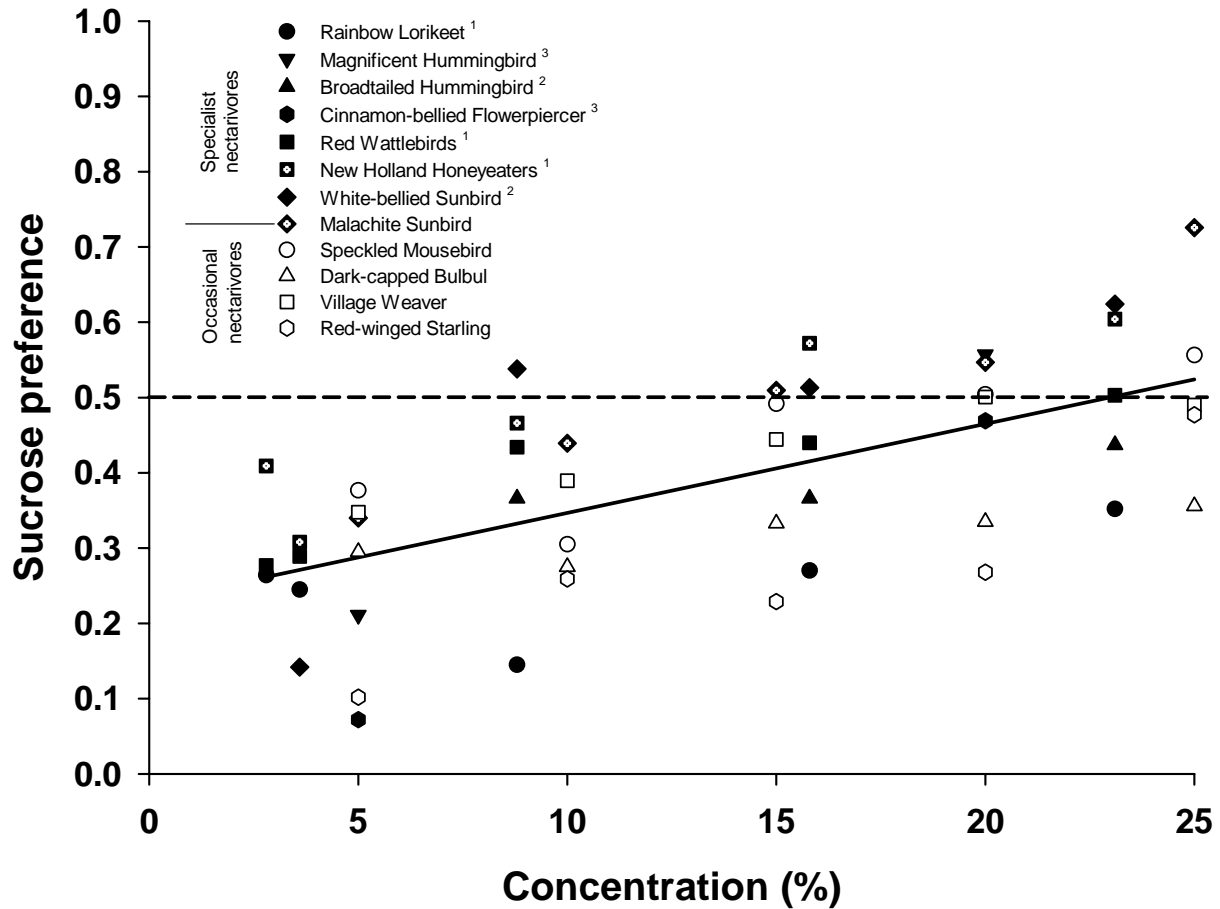


Figure 1: The relationship between diet concentration and sucrose preference for 12 species of nectar feeding birds. Data extracted from Chapters 3-6; Appendix 1; <sup>1</sup>Fleming et al. 2008; <sup>2</sup>Fleming et al. 2004; <sup>3</sup>Schondube & Martínez del Rio 2003).

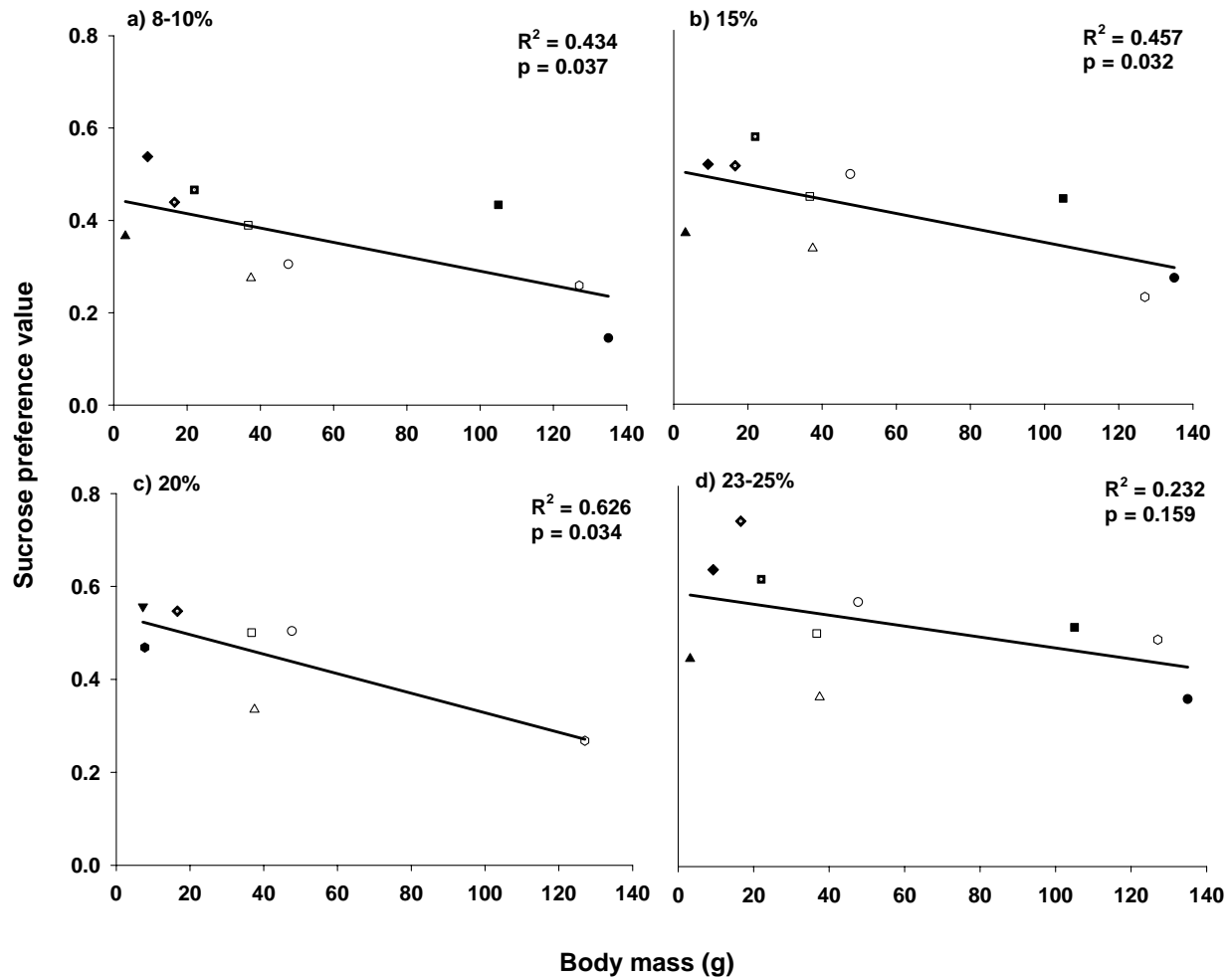


Figure 2: The relationship between bird body size (g) and sucrose preference across four different concentrations of sugar solutions. Data extracted from Chapters 3-6; Appendix 1; Schondube & Martínez del Rio 2003; Fleming et al. 2004; Fleming et al. 2008). Legend details same as Figure 1).

Sucrose preference was significantly correlated with bird body mass at 8 - 10% (Figure 2a), at 15% (Figure 2b) and at 20% (Figure 2c), but not at 23 - 25% (Figure 2d). Correlation analyses based on raw values can lead to errors because of non-independence of data points due to phylogenetic relationships between species (Felsenstein 1985; Garland et al. 2005). To test the effect of body mass on sugar preference after correcting for the effects of phylogeny, I ran

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phylogenetically independent contrast (PIC) analyses on the data using the PDAP module in Mesquite (Midford et al. 2005; Maddison & Maddison 2006). These analyses were based on a phylogeny constructed from Hackett et al. (2008) for non-passerines, and Sibley and Ahlquist (1990) for passerines (Figure 3). For statistical analysis branch lengths were adjusted by the arbitrary method of Pagel (1992) as this normalised the residuals. Subsequent regression analysis confirmed that there are significant relationships between the contrasts of body size and sucrose preference values at 8 - 10% (Figure 4a), 15% (Figure 4b), and 20% (Figure 4c) using two-tailed tests. There was a marginally non-significant effect at 23 - 25% (Figure 4d), however, this effect was significant using a one-tailed test ( $p = 0.032$ ) based on the specific hypothesis of a negative relationship between body mass and sucrose preference. The similarity of the phylogenetically corrected analysis to the original analysis was striking, and indicated that the relationship is likely to reflect adaptive evolution. These new findings suggest that small birds (< 20g) should exhibit sucrose preference at high (20 - 25%) concentrations, while larger birds (> 40g) should exhibit a hexose preference at low concentrations (8 - 15%) and either hexose preference or no preference at higher concentrations.

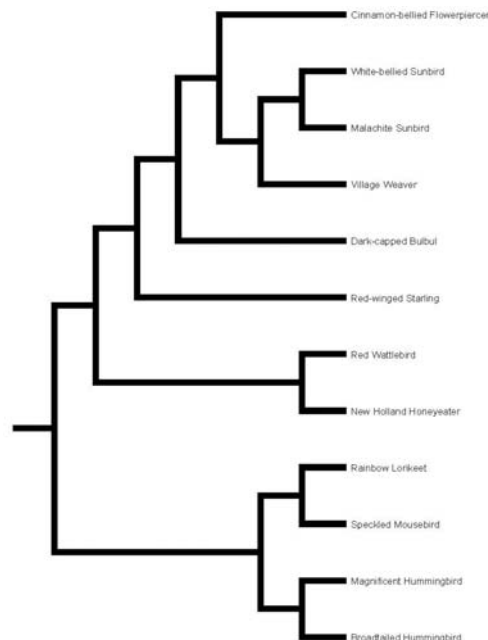


Figure 3: Phylogenetic tree for 12 species of nectar feeding birds used in phylogenetically independent contrast analysis of sucrose preference and body mass. Reconstructed using Sibley and Ahlquist (1990) for passerines and Hackett et al. (2008) for non-passerines.

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Feeding niche and body mass tend to be correlated in birds. In general, specialist avian nectarivores tend to be relatively small, the exception being the psittacines and several of the honeyeaters (Meliphagidae). The hummingbirds (Trochilidae), flowerpiercers (Thraupidae), sunbirds (Nectariniidae) and sugarbirds (Promeropidae) are all relatively small (with a few notable exceptions). In contrast, many occasional avian nectarivores are larger (> 30 - 40g). Examples from southern Africa include the bulbuls (Pycnonotidae), mousebirds (Coliidae), weavers (Ploceidae), starlings (Sturnidae), drongos (Dicruridae) and orioles (Oriolidae), although there are exceptions here too, for example the white-eyes (Zosteropidae) that are around 10-12g. These overall differences in mean body size between specialist and occasional nectarivores probably explain why there is support for the feeding niche hypothesis (Johnson & Nicolson 2008), i.e. the idea that nectar in bird-pollinated plants diverges according to whether the pollinators are specialist or occasional nectarivores. However, Johnson and Nicolson's (2008) data set only included plants from Africa and the Americas, and excluded the large specialist nectarivores from Australia.

My preliminary analyses suggest that body size is the best available predictor of bird sugar preference, and may thus be used to predict the nectar characteristics of plants birds feed on (body size hypothesis). The notion that body size affects behavioral choice for different sugars in birds should not be a surprise, as body size affects more aspects of animal biology than any other characteristic (Brown et al. 1978; Karasov & Martínez del Rio 2007). Indeed, Karasov and Martínez del Rio (2007) argue that almost any comparative analysis of physiological and life history variables should start with a consideration of size. That size of birds could influence selection on plant nectar is perhaps also not surprising, considering the well known relationship between pollinator body mass and nectar secretion rate per flower (Brown et al. 1978) and well documented examples of bird pollinator communities being segregated by size, with corolla length in particular correlating well to bird body size and in particular bill size (Boyd 2002; 2004; Nattero & Cocucci 2007). Indeed, it is perhaps surprising that the relationship between bird body size and sugar preference, followed by inferences of the role of bird body size as a selective force on floral and nectar characteristics, has not been fully examined before.

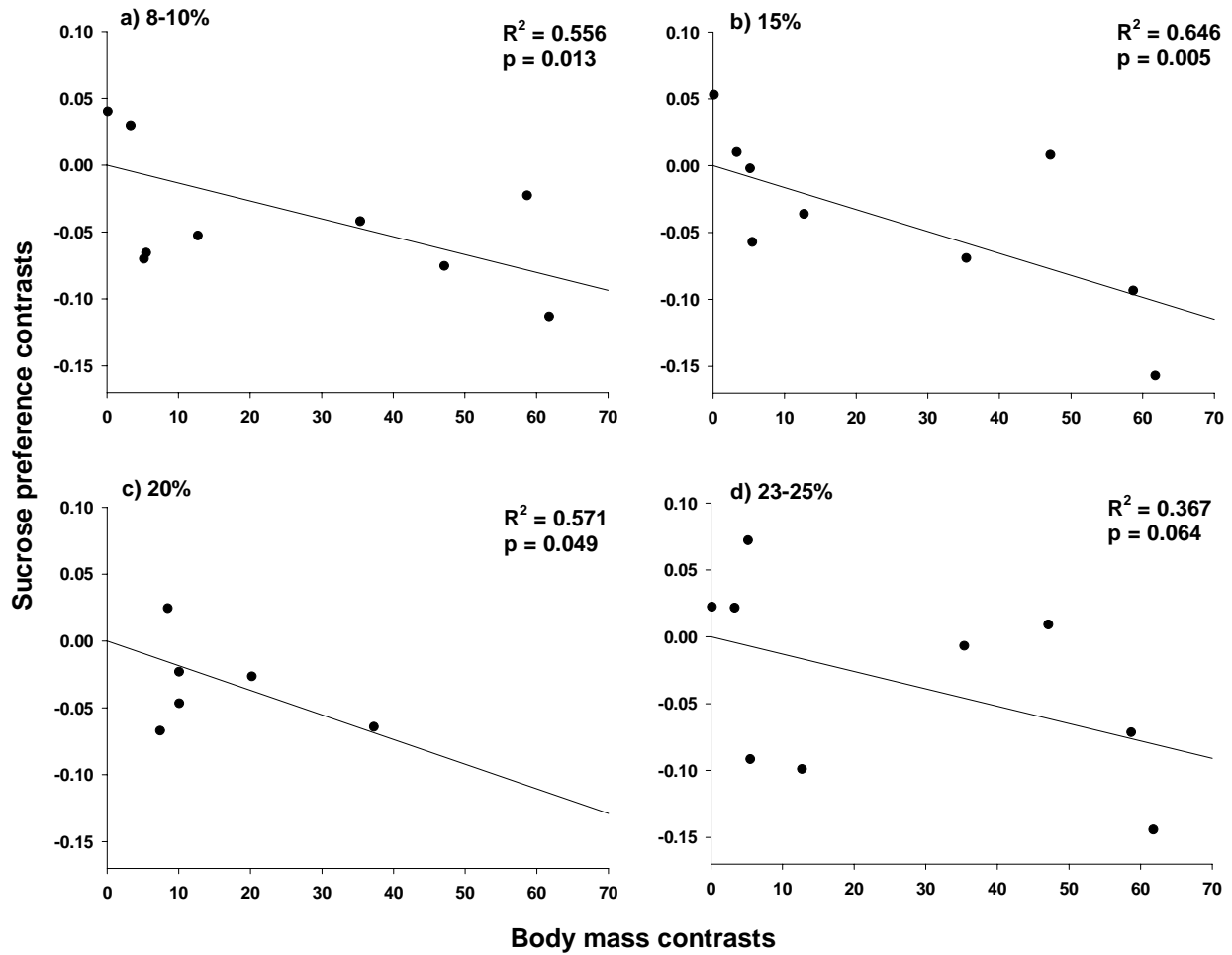


Figure 4: The relationship between positivised standardised phylogenetically independent contrasts of bird body size (g) and sucrose preference across four different concentrations (a – 8-10%; b – 15%; c – 20% and d – 23-35%). Significance values are given for two-tailed tests.

### Does digestive physiology explain differences in sugar preferences?

Bird diet choice is often based on digestive capabilities (Karasov & Martínez del Río 2007). In order to understand bird sugar preferences, it is therefore necessary to examine their digestive physiology. I recorded apparent assimilation efficiencies of occasional avian nectarivores when fed sugar solutions of different concentrations (Chapter 4-6; Appendix 1). Apart from the Red-winged Starling *Onychognathus morio* (Chapter 6), which did not assimilate

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sucrose as expected by its membership of the Sturnidae-Muscicapoidea lineage, these species showed reasonable ability to assimilate sucrose. Of particular interest is the effect of concentration on assimilation efficiency that was found in Dark-capped Bulbuls (Chapter 4). Generally, specialist avian nectarivores show no effect of concentration on apparent assimilation efficiency, all consistently exhibiting very high efficiencies (Downs 1997a; Markman et al. 2006; McWhorter and Lopez-Calleja 2000). Other occasional nectarivores tested (Speckled Mousebird *Colius striatus* – Chapter 5; Red-winged Starling – Chapter 6; Village Weaver *Ploceus cucullatus* – Appendix 1) show no significant effect of concentration on apparent assimilation efficiency.

To further explore the relationship between bird feeding guilds and assimilation efficiency, I extracted data on apparent assimilation efficiency for several specialist and occasional nectarivores from the literature and combined these data with my own to determine if significant differences occurred between the two groups for sucrose or hexose sugars (Table 1). I only used data from studies where birds were fed sugar solutions, rather than including studies where fruit or agar cubes were used. I also excluded species from the Sturnidae-Muscicapoidea lineage from analyses of sucrose assimilation efficiency. As these data are proportions, they were arcsine square root transformed prior to analysis. There was no significant difference in apparent assimilation efficiency for sucrose and hexose sugars in specialist avian nectarivores ( $t = 1.19$ ;  $p = 0.247$ ). Likewise, there was no significant difference in apparent assimilation efficiency for sucrose and hexose sugars in occasional avian nectarivores ( $t = 0.85$ ;  $p = 0.427$ ). Specialist avian nectarivores, however, assimilate sucrose sugars more efficiently than do occasional avian nectarivores (mean  $\pm$  standard deviation:  $98.08\% \pm 2.17$  versus  $87.92\% \pm 7.83$ ,  $t = 4.41$ ;  $p < 0.001$ ), while there was no difference between the groups for hexose sugars ( $95.63\% \pm 7.72$  versus  $87.60\% \pm 11.44$ ,  $t = 0.75$ ;  $p = 0.474$ ).

Given that body mass was found to be an important predictor of sugar preference (Figure 2 and Figure 4), its relationship with assimilation efficiency was also explored. Regression analyses indicated that hexose assimilation efficiency, but not sucrose assimilation efficiency, can be significantly predicted by bird body mass (Figure 5). To further test the joint influence of feeding guild (specialist vs. occasional nectarivores) and body mass on apparent assimilation

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efficiency, I used Analysis of Co-variance (ANCOVA), using type III sum of squares, with 22 species (16 specialists and 6 occasional nectarivores nested within these two feeding niches). The response variable was arcsine square-root transformed apparent assimilation efficiency data for sucrose and hexose sugars (Table 1). This indicated no significant interaction of feeding guild and body mass for either sucrose or hexose assimilation efficiency. The interaction was therefore removed from the models. In the models with main effects only, sucrose assimilation efficiency was significantly affected by feeding guild ( $F_{1, 18} = 29.34$ ,  $p < 0.001$ ) but not by body mass ( $F_{1, 18} = 1.48$ ,  $p = 0.24$ ). In contrast, hexose assimilation efficiency was significantly affected by body mass ( $F_{1, 8} = 28.55$ ,  $p < 0.005$ ) but not by feeding guild ( $F_{1, 8} = 4.96$ ,  $p = 0.06$ ). Log transforming the body mass data led to similar results which are not reported here. These results indicate that specialist avian nectarivores are better adapted to process sucrose rich nectars, but that there is no difference in hexose processing ability between specialist and occasional nectarivores.

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Table 1: Apparent assimilation efficiencies (percentage of diet digested) of birds fed sugar solutions at various concentrations. S = sucrose, G = glucose, F = fructose, GF = glucose:fructose mix.

Specialist Nectarivores	Mass (g)	S	G	F	GF	Method	Reference
Red Lory ( <i>Eos bornea</i> )	180	99				1	Downs (1997b)
Rainbow Lorikeet ( <i>Trichoglossus haematodus</i> )	135	90.5	80			2	Karasov & Cork (1994, 1996)
Cinnamon Hummingbird ( <i>Amazilia rutila</i> )	7.74	98				5	Martínez del Rio (1990a)
Magnificent Hummingbird ( <i>Eugenes fulgens</i> )	7.23	99	99			1	Schondube & Martínez del Rio (2003)
Broad-billed Hummingbird ( <i>Cynanthus latirostris</i> )	3.17	98				5	Martínez del Rio (1990a)
Golden-crowned Emerald ( <i>Chlorostilbon canivetii</i> )	2.2	98				5	Martínez del Rio (1990a)
Cape Sugarbird ( <i>Promerops cafer</i> )	36	99.8	99.8	99.8		1, 3	Jackson et al. (1998)
Gurney's Sugarbird ( <i>Promerops gurneyi</i> )	25.7	99				1	Downs (1997a)
Singing Honeyeater ( <i>Lichenostomus virescens</i> )	25	99				1	Collins & Morellini (1979)
Malachite Sunbird ( <i>Nectarinia famosa</i> )	16.6	99				1	Downs (1997a)
Amethyst Sunbird ( <i>Nectarinia amethystina</i> )	14.4	99				1	Downs (1997a)
Brown Honeyeater ( <i>Lichmera indistincta</i> )	11	99				1	Collins et al. (1980)
Bananaquit ( <i>Coereba flaveola</i> )	9.5	97	97	97		1	Mata & Bosque (2004)
Lesser Double-collared Sunbird ( <i>Nectarinia chalybea</i> )	8.7	97				1	Lotz & Nicolson (1996)
Cinnamon-bellied Flowerpiercer ( <i>Diglossa baritula</i> )	7.74	99			99	1	Schondube & Martínez del Rio (2003)
Palestine Sunbird ( <i>Nectarinia osea</i> )	7.2	99			99	1	Roxburgh & Pinshow 2002
<b>Occasional Nectarivores</b>							
Red-winged Starlings ( <i>Onychognathus morio</i> )	127	0			69	4	Chapter 6 Martínez del Rio & Stevens (1989)
European Starling ( <i>Sturnus vulgaris</i> )	75	0				6	
Rufous-backed Robin ( <i>Turdus rufopalliatus</i> )	74	0				4	Martínez del Rio (1990b)
Speckled Mousebirds ( <i>Colius striatus</i> )	47.64	86			91	4	Chapter 5
Orange-billed Nightingale-thrush ( <i>Catharus auratirostris</i> )	27	0				4	Martínez del Rio (1990b)
Dark-capped Bulbuls ( <i>Pycnonotus tricolor</i> )	37.47	76			85	4	Chapter 4
Village Weaver ( <i>Ploceus cucullatus</i> )	36.7	92			97	4	Appendix 1
House Finch ( <i>Carpodacus mexicanus</i> )	20	97			96	1	Avery et al. (1999)
Cape White-eye ( <i>Zosterops virens</i> )	12.1	88.6				7	Brown & Downs (2003)

Method: 1 – refractometry; 2 – double isotope method; 3 – HPLC; 4 – sucrose analysis; 5 – apparent assimilated mass coefficients; 6 – blood glucose concentration; 7 – bomb calorimetry.

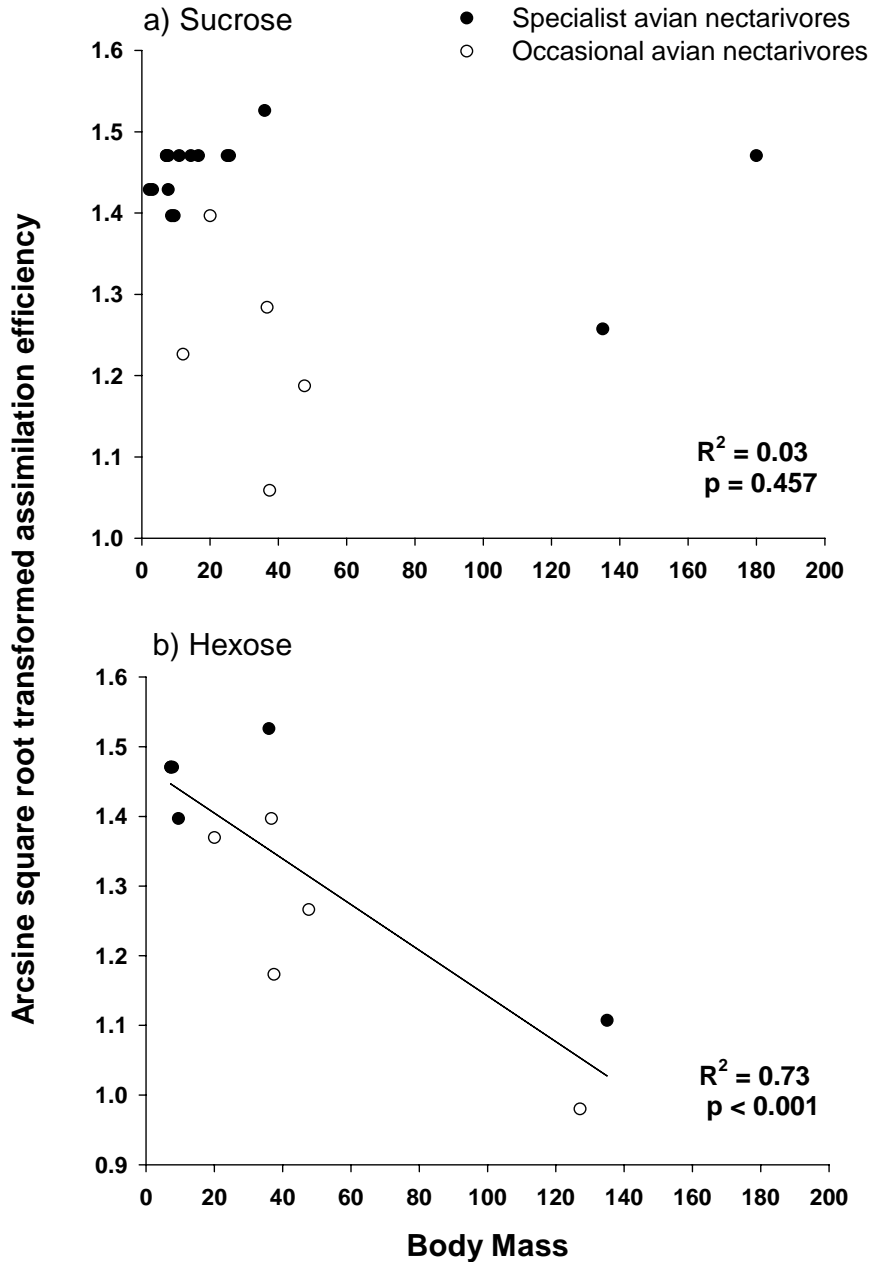


Figure 5: The relationship between bird body size (g) and apparent assimilation efficiency across four different concentrations. Data sources are given in Table 1.

I then ran phylogenetically independent contrasts analyses using the PDAP module in Mesquite (Midford et al. 2005; Maddison & Maddison 2006) on the data using a phylogeny constructed from Hackett et al. (2008) for non-passerines, and Sibley and Ahlquist (1990) for passerines (Figure 6). For statistical analysis branch lengths were adjusted by the arbitrary method of Pagel

(1992) as this normalised the residuals. Subsequent regression analysis confirmed that there are significant relationships between the contrasts of body size and hexose apparent assimilation efficiency (Figure 7) using two-tailed tests. From past studies on disaccharide digestion, we know that maltase activity, but not sucrase activity, is positively correlated with body mass (Martinez del Rio 1990b). While these studies have examined the effect body size has on the breakdown of disaccharides, they do provide a framework to suggest that even monosaccharide digestion may be affected by body mass in birds. The strength of the relationship between hexose assimilation efficiency and bird body mass is surprising, and suggests that the body size hypothesis introduced above may extend to physiological parameters of monosaccharide digestion, not just behavioural patterns of sugar preference. The underlying biochemical and/or physiological mechanisms that may explain this relationship need further exploring.

While the difference in sucrose assimilation efficiency between specialist and occasional avian nectarivores may play a role in shaping bird sugar preference, it is important to note that assimilation efficiency for this sugar is as high in some occasional nectarivores as it is in specialist nectarivores (see Lotz & Schondube 2006 and Fleming et al. 2008 for global summaries). The lack of the enzyme sucrase in the Sturnidae-Muscicapoidea lineage suggests that these birds will place selective pressure for hexose sugars in the plants they feed on provided they are effective pollinators and therefore contribute to plant fitness. While differences in assimilation efficiencies between specialist and occasional avian nectarivores may therefore be significant at a local scale (in terms of species placing different selective pressure on plants for different sugar types), they may not play a major role in explaining patterns at a global scale. Instead, bird body size, and the effect it has on hexose assimilation efficiency, may be more important.

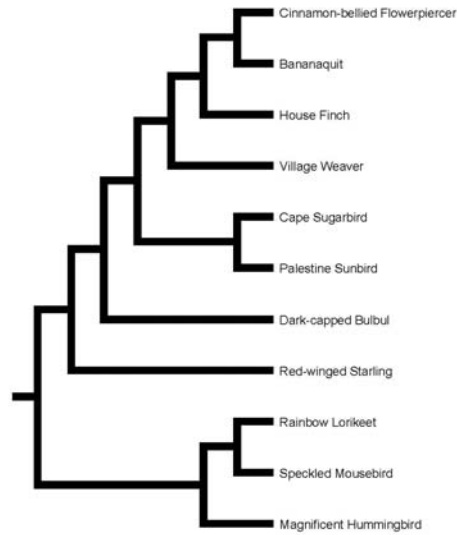


Figure 6: Phylogenetic tree for 11 species of nectar feeding birds used in phylogenetic contrasts analysis of hexose assimilation efficiency and body mass. Reconstructed using Sibley and Ahlquist (1990) for passerines and Hackett et al. (2008) for non-passerines.

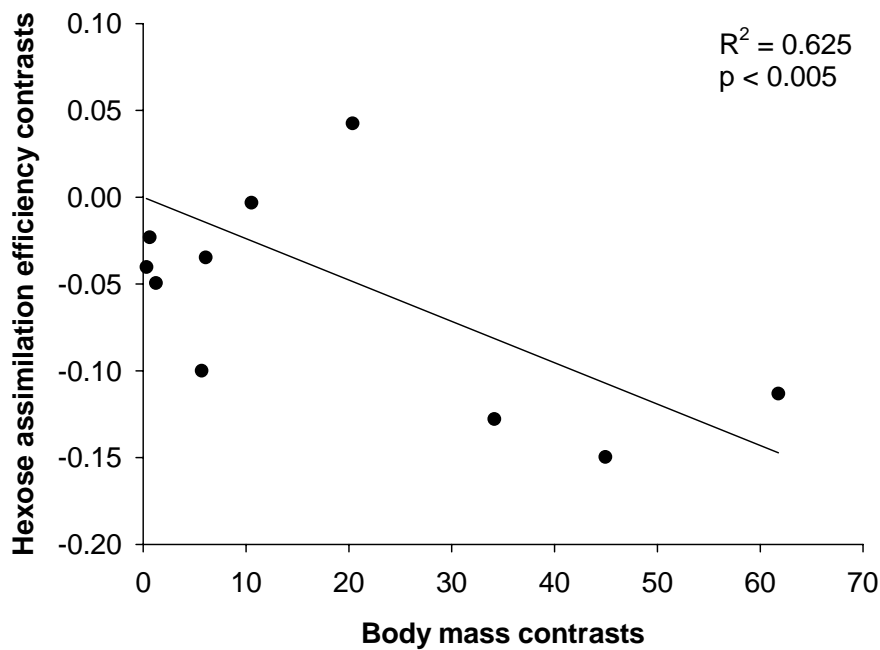


Figure 7: The relationship between positivised standardised phylogenetically independent contrasts of bird body size (g) and hexose assimilation efficiency.

**Do birds place selective pressure on plant nectar characteristics?**

In the second part of the thesis, I documented the pollination systems of several *Kniphofia* species and corroborated recent findings that plant nectar characteristics correlate well with bird pollinator group (specialist or occasional nectarivores), even within a genus. By studying several species of *Kniphofia*, I was able to show that nectar volume and concentration, but not sugar type (which appears to be phylogenetically constrained in the genus) correlated well with sugar preferences of the birds that pollinate them. Importantly, I was able to show that these correlations occur between different species within the genus (Chapters 7 and 8), and also within a single species (Chapter 9), suggesting that nectar concentration and volume are relatively labile traits that can be selected for by avian pollinators. These results are similar to findings of ecotypic divergence in traits of other assemblages of bird-pollinated plants (Boyd 2002; 2004; Nattero & Cocucci 2007), as well as in plants with other pollinator types (Robertson & Wyatt 1990; Galen 1989; Johnson 1997; Johnson & Steiner 1997; Alexandersson & Johnson 2002; Herrera 2005).

**Summary**

Taken together, the two parts of this thesis suggest that bird sugar preferences have been an important selective force behind differences in nectar characteristics in plants pollinated by birds, and that bird body size has played a role in shaping this. However, I view this thesis as only a starting point, as it generated new questions that will need to be addressed by further research. More sugar preference work needs to be done on occasional nectar feeding birds from both passerine and non-passerine lineages and from several continents in order to test the generality of the results of the current work. In particular, it will be important to further examine the role bird body size has on sugar preference within both specialist and occasional nectarivore groups. Studying sugar preferences of large specialist nectarivores, and small occasional nectarivores, will be particularly important to further test this theory. Given that feeding niche and body size are strongly correlated in birds (Johnson & Nicolson 2008), statistical approaches will be required to determine if they have effects independent of one another, and this will

require much larger species sample sizes than what I had at my disposal. The intriguing lack of a concentration preference, or preference for low concentration solutions, in occasional nectarivores needs further research. In addition, more in depth ecological field-based studies of both specialist and occasional nectarivores, and the plants they feed on, at both interspecific (within a family) and intraspecific (ecotype) levels are needed to examine selection mediated by birds on the plants they pollinate.

In conclusion, my work suggests that, while accounting for differences in nectar characteristics in bird pollinated plants, the feeding niche hypothesis on its own does not fully explain pollinator mediated selection by birds on plant nectars. The body size hypothesis, which I propose, may, in conjunction with the feeding niche hypothesis, help to solve this outstanding problem in evolutionary ecology.

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## Errata

The following errors were noticed by examiners and are highlighted here as these papers are published:

Chapter 2: Brown, M., Downs, C. T. & Johnson, S. D. 2008. Sugar preferences of nectar feeding birds - a comparison of experimental techniques. *Journal of Avian Biology* 39: 479-483.

- The methods section states that birds were weighed before and after each trial. This should have been omitted as we do not present body mass data in the results.

Chapter 4: Brown, M., Downs, C. T. & Johnson, S. D. 2010. Sugar preferences and digestive efficiency in an opportunistic avian nectarivore, the Dark-capped Bulbul. *Journal of Ornithology* doi: 10.1007/s10336-010-0498-8.

- Methods: banana appears twice under Animal husbandry.
- Methods: “volumetric sucrose preference values” should read “sucrose preference index”.
- Methods: under the section on assimilation efficiency “crystallisation” should read “crystallization”.
- Results: volumetric intake results of the concentration preference trial should refer to Fig. 2, not Fig. 3.

Chapter 7: Brown, M., Downs, C. T. & Johnson, S. D. 2009. Pollination of the red-hot poker *Kniphofia caulescens* by short-billed opportunistic avian nectarivore. *South African Journal of Botany* 75: 707-712.

- Materials and methods: under section 2.1, last line, should read “located 100m away from the main natural population.
- Results: section 3.1 incorrectly states there were measurements of bagged flower nectar crop at 8am and 10am. This should read 8am only.
- Table 3: “Mean” refers to mean number of pollen grains collected off each species.

## Sugar preferences and digestive efficiency of the village weaver: a generalist avian pollinator of African plants

T. C. Odendaal, M. Brown\*, C. T. Downs and S. D. Johnson

School of Biological and Conservation Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, 3209, South Africa

\*Author for correspondence (brownma@ukzn.ac.za)

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### SUMMARY

Recent research has shown that nectar properties of flowers pollinated by generalist avian nectarivores differ markedly from those of flowers pollinated by specialist avian nectarivores. In particular, flowers pollinated by generalist avian nectarivores tend to have very dilute nectar dominated by hexose sugars. To establish whether pollinator-mediated selection can explain these traits, we tested nectar sugar preferences and digestive capabilities of the village weaver (*Ploceus cucullatus*), a common generalist passerine nectarivore in South Africa. When offered pairwise choices of equicaloric hexose and sucrose solutions, village weavers preferred hexose solutions at 5% and 10% sucrose equivalents (SE) but did not show significant preference for either type of sugar when higher concentrations were offered (15%, 20% and 25% SE). Birds were less efficient at absorbing sucrose than hexose sugars, as revealed by high-performance liquid chromatography (HPLC) analysis of excreta sugar content. This was true at both concentrations tested (8.22% and 25%), although apparent sucrose assimilation rates were still relatively high (89.6±2.9% at low concentrations and 93.6±1.7% at high concentrations). Transit times indicated that sucrose also passes through the digestive tract faster than hexose sugars, particularly when consumed at high concentrations. This may limit the rate at which sucrose can be hydrolyzed before absorption. These results indicate that hexose preferences in generalist avian nectarivores may help explain the low sucrose content in flowers pollinated by these birds. Moreover, the preference for hexose sugars in weavers was most evident at the low concentrations (ca. 9% sugar by mass) that are typical of nectar in flowers pollinated by generalist avian nectarivores.

Key words: apparent absorption efficiency, sucrose, hexose, transit time, *Ploceus cucullatus*.

### INTRODUCTION

Earlier literature on bird pollination systems emphasized a dichotomy in nectar properties between hummingbird (Apodiformes: Trochilidae) and passerine (Passeriformes) systems (Cruden and Toledo, 1977; Baker and Baker, 1983). More specifically, hummingbird-pollinated plants were shown to have sucrose-rich nectar, while passerine-pollinated plants were found to have hexose-rich nectars (Baker and Baker, 1983). Initial research into sugar preferences of these two groups of birds found that hummingbirds preferred sucrose solutions (Stiles, 1976; Hainsworth and Wolf, 1976; Martínez del Rio, 1990; Martínez del Rio et al., 1992) and passerines preferred hexose solutions, and indeed in several cases passerines were shown to be sucrose intolerant (Martínez del Rio et al., 1988; Martínez del Rio et al., 1992; Martínez del Rio and Stevens, 1989; Martínez del Rio, 1990; Brugger and Nelms, 1991; Brugger et al., 1993).

However, recent research has shown that not only are nectar properties in flowers pollinated by specialized passerines strongly convergent with those of hummingbird-pollinated flowers (Johnson and Nicolson, 2008) but that they too show preferences for sucrose-rich solutions (Downs and Perrin, 1996; Lotz and Nicolson, 1996; Jackson et al., 1998a; Jackson et al., 1998b). By contrast, plants pollinated by generalist avian nectarivores [also referred to as 'occasional nectarivores' because they often utilize nectar only as a secondary food source (cf. Johnson et al., 2006)] tend to produce a higher volume of nectar, with a lower sugar concentration and a lower proportion of sucrose, than do plants pollinated by specialist avian nectarivores (Johnson and Nicolson, 2008; Brown et al., 2009;

Symes et al., 2009). Specifically, Johnson and Nicolson found that plants pollinated by specialist nectarivores are characterized by low (10–30 µl) volumes of relatively concentrated (15–25% w/w) sucrose-rich (40–60% of total sugar) nectars while plants pollinated by occasional bird pollinators are characterized by large volumes (40–100 µl) of very dilute (8–12%) nectar, with low (0–5%) sucrose content (Johnson and Nicolson, 2008). In the Americas, this distinction fits the classic hummingbird–passerine dichotomy (Cruden and Toledo, 1977). However, in Africa, where hummingbirds do not occur, this dichotomy applies to passerine-pollinated plants, raising the interesting possibility that generalized and specialized passerine nectarivores differ in their nectar preferences.

It has long been suggested that pollinator preference drives selection on nectar rewards (Wykes, 1952; Martínez del Rio et al., 1992), and hence preference experiments have been conducted quite extensively on specialist nectarivorous birds (Downs and Perrin, 1996; Lotz and Nicolson, 1996; Roberts, 1996; Downs, 1997a; Jackson et al., 1998a; Jackson et al., 1998b; Schondube and Martínez del Rio, 2003; Fleming et al., 2004; Lotz and Schondube, 2006). These studies indicate that specialized passerines such as sunbirds have sugar preferences and digestive capacities that are similar to those of hummingbirds (Fleming et al., 2004; Lotz and Schondube, 2006). Most specialized avian nectarivores exhibit either a preference for sucrose, or no preference at high concentrations, no preference at intermediate concentrations, and switch to a hexose preference at low concentrations (Fleming et al., 2004; Fleming et al., 2008; Brown et al., 2010a). However, these switches to hexose

preference generally occur at very low concentrations (<10%) that are not found naturally in flowers pollinated by these birds, and may therefore not be biologically relevant to selection on nectar traits (Brown et al., 2010a).

Primary causes of the concentration-dependent preferences in specialists are suggested by authors to be elements of digestive physiology, specifically: (1) accommodation of high osmotic concentrations when birds consume concentrated hexose; and (2) sucrose hydrolysis rate limitations when large volumes of dilute solutions are consumed (Martínez del Rio et al., 2001; Fleming et al., 2004; Lotz and Schondube, 2006).

Sugar preference experiments have been conducted on a number of generalist avian nectarivores species. These can be divided into those concerned only with members of the Muscicapoidea superfamily, lacking sucrose activity (Schuler, 1983; Martínez del Rio and Stevens, 1989; Brugger, 1992; Malcarney et al., 1994; Gatica et al., 2006), and those concerned with other species (Martínez del Rio et al., 1989; Franke et al., 1997; Lane, 1997; Mata and Bosque, 2004; Brown et al., 2010b). From the latter group, it is evident that generalist avian nectarivores, although they possess some ability to digest sucrose, prefer hexose sugars. However, apart from recent work on bulbuls by Brown et al. (Brown et al., 2010b), only comparisons at single concentrations have been reported, and these concentrations have not been representative of the very dilute nectar found in flowers pollinated by these birds. Also, test solutions in earlier studies were not equicaloric; thus, conflating tests of sugar preference with an energy difference between test solutions (Brown et al., 2008).

The avoidance of sucrose, particularly by members of the Muscicapoidea superfamily, has been shown to be the consequence of a limited activity (Martínez del Rio et al., 1988; Martínez del Rio et al., 1989) or a complete lack (Martínez del Rio and Stevens, 1989) of the digestive enzyme sucrose, necessary for the hydrolysis and subsequent assimilation of this sugar. If such a physiological constraint was a general trend among occasional nectarivores, then hexose preference would be expected. Because specialist nectarivore preferences do not adequately explain the low proportion of sucrose and dilute nature of some nectars (Fleming et al., 2004; Brown et al., 2010a), it has been suggested that preferences of occasional nectarivores may be a contributing factor to selection for nectar sugars (Dupont et al., 2004; Fleming et al., 2004; Brown et al., 2010b). Johnson and Nicolson propose that a better understanding of foraging preferences and digestive abilities of occasional nectarivores is important to explain why nectars of generalist bird-pollinated plants are hexose rich (Johnson and Nicolson, 2008).

The aim of this study was to establish whether pollinator-mediated selection can explain the dominance of hexose sugars in the dilute nectar of plants pollinated by generalist avian nectarivores. The village weaver *Ploceus cucullatus* Müller was studied, as this species and several other closely related weavers are important pollinators of plants with flowers adapted to short-billed generalist avian nectarivores (Oatley and Skead, 1972; Daniels, 1987; Botes et al., 2008; Symes et al., 2008; Brown et al., 2009). Village weavers are predominantly granivores but feed on nectar opportunistically throughout of the year. It was predicted that this generalist nectarivore would have a preference for hexose sugars, and that this preference would be more pronounced when it was offered dilute solutions that approximate the concentration of nectar in flowers pollinated by this bird. Because sugar preference may be determined by digestive efficiency, apparent absorption efficiencies and transit times of nectar sugars were also determined. It was predicted that hexose sugars would pass through the digestive tract more slowly

and, hence, together with possible limitations of sucrose activity, be absorbed more efficiently.

## MATERIALS AND METHODS

### Capture and maintenance of village weavers

Ten non-breeding adult village weavers were captured using mistnets (May 2007) at Hilton College, outside Pietermaritzburg, South Africa (29°36'S 30°26'E), under license from the local conservation authority Ezemvelo KwaZulu-Natal Wildlife. The birds were held in outdoor aviaries (4.2 m × 2 m × 1 m) at the University of KwaZulu-Natal Animal House, Pietermaritzburg Campus for ca. three weeks before being moved indoors for the trials. They were fed a maintenance diet of mixed bird seed (*Panicum miliaceum* and *Panicum maximum*), mixed nectar ± 20% [1:1 sucrose and hexose (equal fructose and glucose) w/w], bonemeal (protein source) with water *ad libitum*. Bird mass (36.7 ± 0.9 g mean ± s.e.) was representative of the species in the capture area (38.0 ± 0.1 g; *N* = 1338) (M.B., unpublished data).

All birds were transferred to individual indoor (25°C with a 12 h:12 h L:D) experimental cages (90 cm × 30 cm × 45 cm), and restricted to one half of the cage during experiments. No food or water was available to them overnight before a trial, so as to ensure a post-absorptive state. Each bird had at least two days on the maintenance diet and water between trials. Trials were conducted during June to July.

### Sugar preference

Equicaloric sucrose and hexose (1:1 fructose and glucose) nectars were offered simultaneously to individual birds at five concentrations, 5%, 10%, 15%, 20% and 25% sucrose equivalents (SE). The low concentrations (5% and 10%) are similar to the mean concentration of nectar found in flowers pollinated by generalist avian nectarivores (reviewed by Johnson and Nicolson, 2008). The higher concentrations (15–25%) are similar to the mean concentration of nectar in flowers pollinated by specialist avian nectarivores (reviewed by Johnson and Nicolson, 2008).

Solutions were offered for 12 h (06:00–18:00 h) in a pairwise fashion from two 50 ml glass burettes placed 6 cm on either side of a central perch. The glass burettes were cut at the base and stopped with thick rubber rings that fitted tightly onto white plastic bird feeder bases. The fluids were thus presented to birds from identical cup-like structures with approximately 2 cm<sup>3</sup> surface area each. Initial left and right positions were randomized for each bird and switched after 6 h. On dilute diets some burettes required refilling, done from the same original nectar solution. Control burettes of each solution were placed in the experimental room to account for evaporation.

Birds were weighed to the nearest 0.5 g before and after every trial. In order to account for inter-individual variation of body mass, volumes consumed were divided by the mass of each bird before analysis (ml g<sup>-1</sup>). Preference for sucrose was calculated as a proportion: (ml g<sup>-1</sup> sucrose total ml<sup>-1</sup> g<sup>-1</sup> consumed) and then arcsine square-root transformed.

### Apparent absorption efficiency

Apparent absorption efficiency of the two sugars was tested indirectly following a method similar to that used by Jackson et al. (Jackson et al., 1998b). Each sugar solution was tested at the 8.22% SE and 25% SE concentrations (*N* = 10). The weavers were housed individually in wire-mesh-floored cages (40 cm × 40 cm × 40 cm with mesh 2 cm × 1 cm × 1 cm), placed over a tray containing a layer of liquid paraffin ± 1.5 cm deep. Each sugar solution was available

to birds between 06:00–18:00 h using the same glass burettes above. Excreta were collected at 06:00 h the following morning.

Cloacal fluid was obtained from the liquid paraffin using a needled (1 ml) syringe and weighed to the nearest 0.01 g. Because cloacal fluid was extremely dilute (see below) with a density close to 1.0 and because direct measurements of cloacal fluid volume (to the nearest 0.01 ml) were statistically indistinguishable from estimates based on mass (paired-samples *t*-test: d.f.=9;  $t=-1.14$ ;  $P=0.28$ ), we used the latter. After thorough shaking, a 1.5 ml sub-sample from each bird was extracted and centrifuged at 6088 g for 3 min. Excreta was filtered with a 0.45 µm syringe filter and centrifuged again. 200 µl of the supernatant was analyzed for sugar concentrations using a high-performance liquid chromatograph (HPLC, Shimadzu, Duisburg, Germany). Detection was by refractive index (RID – 10A) with a Phenomenex column (Rezex RCM-Monosaccharide, Aschaffenburg, Germany, 200 mm×780 mm×8 µm). Isocratic separation was accomplished using ultrapure water as the mobile phase.

Apparent absorption efficiency (AE) was calculated as (Jackson et al., 1998a):

$$AE = 100 \times [(sugar_{in}) - (sugar_{out}) / (sugar_{in})],$$

where (sugar<sub>in</sub>) was a function of molar concentration and volume of nectar consumed; and (sugar<sub>out</sub>) was a function of excreta volume and concentration of sugar in the excreta (mg ml<sup>-1</sup>). Square-root transformed data was used to test the overall effects of sugar type and concentration on AE.

#### Transit times

Transit times were measured separately as the time taken from first ingestion of dyed nectar to the first appearance of dye in the excreta (Brown and Downs, 2003). Three ml of red food coloring (Robertsons, Cape Town, South Africa) was added to 500 ml of each of the same four nectar solutions. Birds were observed continuously until the appearance of dye in the excreta, at which time the trial was terminated. Eight birds were tested on each solution, on four different mornings, with maintenance diet being available for the remainder of the day.

Approval for this project was received from the Animal Ethics sub-committee of the University of KwaZulu-Natal. All birds were released at the capture site after the project was concluded.

## RESULTS

### Sugar preference

Village weavers showed significant preference for hexose sugars when offered solutions of 5% (one-sample *t*-test:  $t=2.408$ ,  $P=0.039$ ) and 10% (one-sample *t*-test:  $t=3.066$ ,  $P=0.013$ ) concentrations. They showed no significant preferences when offered solutions at concentrations of 15% (one-sample *t*-test:  $t=0.945$ ,  $P=0.369$ ), 20% (one-sample *t*-test:  $t=0.048$ ,  $P=0.963$ ) or 25% (one-sample *t*-test:  $t=0.165$ ,  $P=0.873$ ) (Fig. 1).

Total daily energy consumption (kJ g<sup>-1</sup> day<sup>-1</sup>) was not significantly different between the five concentrations [repeated-measures analysis of variance (RMANOVA):  $F_{4,28}=1.378$ ,  $P=0.267$ ], as volumetric intake increased with decreasing concentration (Fig. 2). Birds did not lose body mass during any of the trials (RMANOVA:  $F_{4,36}=15.95$ ,  $P=0.06$ ).

### Apparent absorption efficiencies

Apparent absorption efficiencies were affected by sugar type (RMANOVA:  $F_{1,9}=5.73$ ,  $P=0.040$ ), so that efficiency was greater for hexose nectars than for sucrose nectars (Table 1). Significant

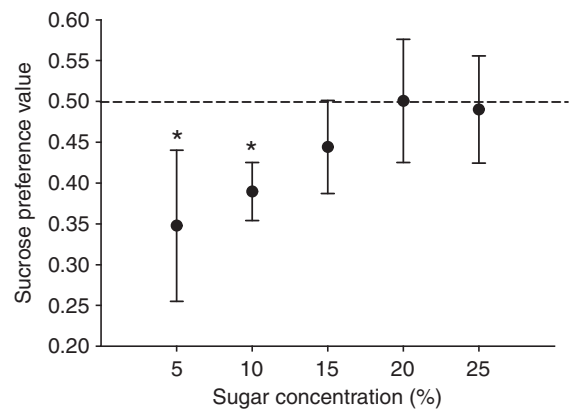


Fig. 1. Sugar preference of the village weaver *Ploceus cucullatus*, expressed as the proportion of ml g<sup>-1</sup> of sucrose nectar consumed in pairwise sucrose–hexose (1:1 fructose and glucose) choices ( $N=10$ ). These were offered at five concentrations, measured in sucrose equivalents (SE). Bars indicate 95% c.i. \* indicate significant preferences.

differences between hexose and sucrose sugars occurred at both low (paired-sample *t*-test:  $t=1.99$ ,  $P=0.039$ ) and high concentrations (paired-sample *t*-test:  $t=2.00$ ,  $P=0.038$ ). Despite lower absorption rates of sucrose, the birds maintained body mass during both 12 h sucrose diet trials [RMANOVA:  $F_{1,9}(\text{time})=0.002$ ,  $P=0.97$ ].

Nectar concentration did not influence absorption efficiency (RMANOVA:  $F_{1,9}=3.87$ ,  $P=0.081$ ), so that both hexose absorption (paired-sample *t*-test:  $t=1.09$ ,  $P=0.15$ ) and sucrose absorption (paired-sample *t*-test:  $t=1.53$ ,  $P=0.08$ ) were independent of concentration.

### Transit times

Nectar sugar composition had a significant effect on transit time (RMANOVA:  $F_{1,7}=12.39$ ,  $P=0.010$ ), with sucrose solutions passing through the digestive tract more quickly (Table 1). Paired-sample *t*-tests showed differences at low [ $P$  (1-tailed)=0.038] and high concentrations [ $P$  (1-tailed)=0.014].

Nectar concentration also affected transit times (RMANOVA:  $F_{1,7}=5.68$ ,  $P=0.048$ ), with lower concentrations passing through the digestive tract more quickly. Paired-sample *t*-tests revealed real differences between concentrations of sucrose [ $P$  (1-tailed)=0.034]

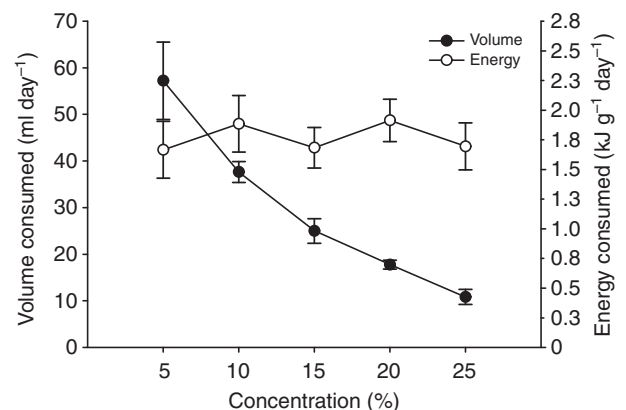


Fig. 2. Adjustment of volumetric intake (left axis) by village weaver *Ploceus cucullatus* resulting in similar energy intake (right axis) across five different concentrations ( $N=10$ ). Values presented are means ± s.e.

Table 1. Effect of sugar type and concentration on both apparent absorption efficiency and retention time in village weavers (mean  $\pm$  s.e.)

	Sugar type	Sugar concentration	
		8.22%	25%
Apparent absorption efficiency (%)	Sucrose	89.6 $\pm$ 2.9	93.6 $\pm$ 1.7
	Hexose	96.0 $\pm$ 1.4	97.5 $\pm$ 0.6
Transit time (s)	Sucrose	41.7 $\pm$ 6.9	56.0 $\pm$ 7.0
	Hexose	67.9 $\pm$ 13.4	132.7 $\pm$ 30.9

and hexose nectars [ $P$  (1-tailed)=0.040]. Transit times were independent of an intake response, as there was no overall relationship between volume consumed and transit time (least-squares regression:  $R^2=0.04$ ,  $P=0.28$ ). For each diet offered separately, this relationship was marginally significant only for the 25% SE hexose solution, with a low percentage of variance explained (regression:  $R^2=0.55$ ,  $P=0.035$ ).

### DISCUSSION

The results of this study are consistent with our prediction that village weavers have a preference for hexose over sucrose sugar when offered solutions as dilute as those found in plants adapted for pollination by these birds (Fig. 1). A preference for hexose sugars is consistent with most of the previous studies on occasional nectarivores, except that tests on most of these species were conducted with high or intermediate concentration solutions, and most did not use equicaloric solutions (Martínez del Río et al., 1989; Malcarney et al., 1994; Franke et al., 1997; Lane, 1997). At high concentrations (20% and 25% SE) village weavers showed no sugar preference (Fig. 1). At 15% [which is at the lower end of the range for nectar concentration in specialist-pollinated flowers (Johnson and Nicolson, 2008), and at low concentrations of 5% and 10% [typical of plants pollinated by generalist avian nectarivores (Johnson and Nicolson, 2008)], village weavers preferred hexose nectar solutions (Fig. 1). This dependence of preference on concentration has been shown for specialist nectarivorous birds (Schondube and Martínez del Río, 2003; Fleming et al., 2004; Fleming et al., 2008; Brown et al., 2010a) but has not been demonstrated previously for a generalist avian nectarivore.

Importantly, birds were able to maintain body mass on low concentrations, and met daily energy requirements, similar to dark-capped bulbuls (Brown et al., 2010b). This contrasts with most specialist avian nectarivore species, which are unable to meet energy demands at very low concentrations (Nicolson and Fleming, 2003; Fleming et al., 2004; Fleming et al., 2008; Brown et al., 2010a). This could be a result of relatively slow transit times, allowing for better absorption at low concentrations.

Slow transit times do not, however, account for why village weavers did not maintain a hexose preference when offered high concentration nectars. In this case birds were probably able to extract sufficient resources from either solution, because of the readily available energy contained therein. This would reduce the limitation caused by possible non-optimal sucrase activity. In addition, sucrose preference at high concentrations in specialist nectarivores has been explained by osmotic pressure differences between the two sugars (Schondube and Martínez del Río, 2003). A high concentration of ingested hexose, consisting of many molecules, causes dehydration by osmotic water movement into the gut (Schondube and Martínez del Río, 2003). When no additional water is offered, as in this study, dehydration can be minimized by drinking sucrose solutions. When offered concentrated solutions, the lack of any particular preference by village weavers may indicate a balancing of the limitations of a sucrase deficiency and dehydration.

Physiological explanations for a hexose preference that have been suggested in the literature include sucrase deficiency (Brugger, 1992), sucrose hydrolysis rate limitations because of short retention times (Schondube and Martínez del Río, 2003) and/or superficial taste (Lotz and Schondube, 2006). At low concentrations any of these factors may be at work in village weavers.

Our results for sucrose absorption efficiency suggest that sucrose sugars were not hydrolyzed and assimilated as optimally as hexose sugars are assimilated (Table 1). This difference was significant at both concentrations, although more pronounced for dilute nectars. Although the method of analyzing sucrose absorption is not a direct assessment of sucrase activity, sugarbirds, sunbirds and white-eyes have been shown to assimilate both hexose and sucrose with nearly 100% efficiency using the same methodology, or with alternative refractometer-based sugar analysis methods that tend to underestimate efficiency (Downs, 1997b; Lotz and Nicolson, 1996; Jackson et al., 1998b; Franke et al., 1997). Compared with these species, village weavers do not effectively assimilate sucrose. However, unlike bird species that cannot assimilate sucrose at all (Muscicapoidea superfamily), and hence avoid sucrose solutions altogether (Gatica et al., 2006; Malcarney et al., 1994), village weavers consumed relatively large volumes of sucrose solutions. Furthermore, even though these birds appear to be less effective at extracting energy from sucrose nectars, sufficient absorption was achieved on sucrose-only diets to enable birds to maintain body mass over 12 h. This is consistent with the suggestion by Martínez del Río et al. (Martínez del Río et al., 1988) that birds that have primarily granivorous diets, containing complex carbohydrates, may have increased enzymatic activity, making them more tolerant of sucrose diets than insectivorous birds.

The second physiological explanation for hexose preference, sucrose hydrolysis rate limitation, is partly supported by transit times for village weavers. Faster transit times for sucrose, which were independent of volume intake (Table 1), may limit the time available for the breakdown of this sugar.

The reason why nectars produced by plants pollinated by generalist birds tend to have a low proportion of sucrose has not previously been satisfactorily explained (Johnson and Nicolson, 2008). It seems likely that differences in nectar properties between plants pollinated by specialist avian nectarivores and those pollinated by generalist ones are due to differences in selection imposed by these two groups of birds but this is still poorly understood (Fleming et al., 2004). The results reported here, along with those of Brown et al. (Brown et al., 2010b), reveal that sugar preferences and digestive abilities of occasional nectarivores like village weavers and dark-capped bulbuls, when offered solutions of biologically realistic concentrations, could explain the evolution of the hexose-dominated nectar in plants pollinated by these birds. Additional studies on generalized avian nectarivores would indicate whether this type of selective pressure is stable and widespread across a diversity of avian taxa.

An outstanding dilemma yet to be satisfactorily explained is the evolution of very dilute nectar in flowers pollinated by generalist

birds (Johnson and Nicolson, 2008). Non-specialist nectarivorous birds have a range of resources from which to access energy. Thus, the use of nectar in their diet may be dependent on the availability of other resources (Franklin and Noske, 1999). It has been hypothesized that preferences for concentrated nectar by these birds may become weak when such resources are not restricting or, alternatively, that nectars are used mainly as a water resource in the dry months (Oatley and Skead, 1972; Johnson and Nicolson, 2008; Symes et al., 2008). Although the sugar preferences and digestive capacities presented here suggest pollinator-mediated selection for hexose sugars when nectar is dilute, experiments that untangle the relative importance of nectar for energy and water balance would contribute further to understanding the nectar properties of flowers pollinated by generalist birds.

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