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– dry season plant selection in Burkina Faso

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Abstract

Human plasmodian species, transmitted by mosquitoes of the genus *Anopheles,* are still one of the leading causes of human mortality in Sub-Saharan Africa. To supplement current vector control strategies, more knowledge about mosquito ecology is required. Central to studies is how malaria vectors relate to their blood hosts. Yet, while we know that sugar is fundamental to male survival and indirectly to female fitness, the relationship between sugar and the highly anthropophilic females of the principal vectors *An. gambiae* s.s. and *An. coluzzii* is debated. Sugar, acquired from floral nectaries and plant fluids, is important to replenish and maintain somatic energy reserves, is known to extend female longevity and is more readily available for flight. However, recent sugar meals inhibit attraction to blood, and sugar in the crop reduces the intake of blood and thus female fecundity. Nonetheless, studies on sugar source preferences on females of the principal malaria vectors have demonstrated the existence of sugar attraction and feeding. Females of *An. gambiae* s.l. appear to display polymorphism for blood host preference, which has led to the suggestion that their response to nectar stimuli is similarly genetically based. If so, they might display an intrinsic ranking of host plant based on the local composition of available plants. This study was aimed at testing the discriminative sugar source selection of males and females of *An. coluzzii* among plant species found during the dry season in South-western Burkina Faso. In the field, data was collected on visits to glue traps containing six common fruit- or flower bearing plant species of the area, by malaria vectors of locally present anopheline species as well as non-malaria vectors of the Culex genus. The results from the field were used to select plants of two combinations for a four-choice bioassay with adult males and females of *An. coluzzii* in the laboratory. The results indicate that wild culicine and anopheline mosquitoes display discriminative sugar source preferences, differing between genera. The same preference ranking of sugar hosts was found among *An. coluzzii* mosquitoes in the laboratory regardless of sex, indicating a preference for *Carica papaya* and *Delonix regia* over *Mangifera indica* and *Thevetia neriifolia.* Fruit received the most feeding mosquitoes, while preferred plants generally attracted more resting and probing mosquitoes. The study was badly affected by climatic factors, which reduced mosquito activity. Nonetheless, it functioned as a pilot study on which further studies could be based, and was able to decisively demonstrate discriminative sugar source selection among female *An. coluzzii* in the laboratory.

Keywords: Malaria, Burkina Faso, dry season, Anopheles, Culex, *Anopheles gambiae*, *Anopheles coluzzii*, sugar source selection, preference, preference ranking, *Delonix regia, Carica papaya, Mangifera indica, Thevetia neriifolia, Jatropha curcas, Azadirachta indica, Banana musa,* probing, feeding, perching.

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Technical terms

* *Anautogenous*	A female insect that requires vertebrate blood for egg maturation
* *Anthropophilic*	Prefers humans
* *Circadian rhythm*	Endogenous, biological and physiological changes that follow a 24-hour cycle
* *Crepuscular*	Active in twilight
* *Crop*	An organ in the foregut that primarily functions as a reservoir for nutrients
* *Endemic*	A disease with high perennial transmission within a certain geographical area
* *Endogenous*	‘built-in’
* *Endophagy*	Prefers to feed indoors
* *Endophily*	Prefers to rest indoors
* *Ephemeral*	Lasts for a short time
* *Exophily*	Prefers to rest outdoors
* *Feeding*	Perching, proboscis in contact with the plant surface
* *Gonotrophic cycle*	The period between a blood meal and the succeeding oviposition
* *Halophilic*	Lives in high salt concentrations
* *Nocturnal*	Active at night
* *Perching*	Resting, the proboscis not in contact with the plant
* *Phytophagous*	Organism that feeds on plants
* *Probing*	Testing the surface with the proboscis, but not perching
* *Proboscis*	Elongate, sucking mouthpart
* *Scotophase*	The dark phase of the light/darkness cycle (antonym: photophase)
* *Sympatric*	Occur in overlapping geographical areas
* *Teneral*	A ‘freshly’ moulted arthropod, cuticula not yet hardened
* *Vectorial capacity*	Measurement of the transmission efficiency of a vector
* *Zoophilic*	Prefers non-human vertebrates

1 Introduction

Malaria is currently one of the leading causes of human mortality in Sub-Saharan Africa (World Health Organization, 2012). Despite many elimination successes of this preventable and treatable vector-borne disease, the World Health Organization estimated that in 2010 alone, there were approximately 219 million cases of malaria worldwide. Out of these cases, an estimated 660,000 were fatal, 90 per cent of which occurred in Sub-Saharan Africa (World Health Organization, 2012). Malaria is caused by parasites of the genus *Plasmodium,* which are transmitted to humans by infected female anopheline mosquitoes (Diptera: Culicidae). Only five species of parasites affect humans, namely *P. falciparum, P. knowlesi, P. malariae, P. ovale,* and *P. vivax,* while approximately 35 anopheline mosquitoes are considered primary vectors of the human malaria parasite and many more function as subsidiary vectors (World Health Organization, 2012; White, 1982). The latter are either local vectors that can maintain limited endemic malaria without a primary vector; or so-called incidental vectors that cannot maintain malarial endemicity on their own (White, 1982). Human plasmodian species are entirely dependent on their mosquito vectors and human hosts to fulfil their natural life cycle, and the epidemiology of malaria is therefore contingent on the ecological success of the anopheline mosquito.

1.1 Malaria control strategies

To tackle the disease, the two main control strategies that the World Health Organization places focus on today are case management, focused to work against the development of severe disease and illness; and prevention of the transmission of the *Plasmodium* parasite, from mosquito to human and vice versa, through vector control (World Health Organization, 2012). The vector control strategies that are most broadly applied today to prevent malaria transmission are indoor residual spraying (IRS) and insecticide-treated nets (ITNs) (World Health Organization, 2012; Ferguson et al, 2010). These strategies are efficient at reducing the contact between humans and vectors and at lowering the mosquito's competence as a vector because its lifespan is reduced, and they are considered the most powerful control strategies applied today. However, as Ferguson et al (2010) point out, these two alone are not enough to eliminate transmission entirely, particularly not in many parts of Sub-Saharan Africa where malaria remains endemic. To achieve elimination, they argue, mosquitoes need to be targeted outside of human dwellings, and strategies that target resources other than blood feeding need to be integrated (Ferguson et al, 2010).

1.2 The malaria vector

Anopheline mosquitoes belong to the subfamily Anophelinae of the family Culicidae. They are holometabolous insects, with larval habitats that require still or slowly moving water bodies that are small or shallow (Clements, 2000). Adult mosquitoes are fluid feeders, and their mouthparts have evolved into elongate composite proboscises used to probe surfaces to obtain fluids. Culicine and anopheline females are anautogenous, meaning that they require protein to produce eggs, which is obtained from vertebrate blood. After emergence, the primary activities of an adult mosquito are mating, feeding, resting and oviposition. At what time these activities are performed is controlled by the circadian rhythm of the mosquito. Anopheline mosquitoes are predominantly crepuscular and nocturnal, with most blood meals taken by females around midnight. The period between a blood meal and the succeeding oviposition is called a gonotrophic cycle, the length duration of which is predominantly controlled by temperature. (Clements, 2000)

For a high vectorial capacity, anopheline mosquitoes require a strong preference for human blood, a high population density and longevity, and the physiological ability to support the development of the malaria parasite from acquisition to transmission (White et al, 2011). The closely related sibling species *Anopheles gambiae* Giles sensu stricto (s.s.) and *An. coluzzii* Coetzee & Wilkerson sp.n. possess all these traits, and due to their highly anthropophilic tendencies where as many as 90 per cent of their blood meals are taken from humans, they are considered the principal malaria vectors on the African continent (Gary and Foster, 2004; Scott and Takken, 2012).

An. gambiae s.s. and *An. coluzzii* were until recently considered to be two molecular forms of *An. gambiae* s.s.: the ancestral S (Savannah) form, now assigned the name *An. gambiae* s.s.; and its derived M form (Mopti), now named *An. coluzzii* after the late professor Mario Coluzzi (Coetzee et al, 2013). These formerly incipient species still exhibit incomplete reproductive isolation and various degrees of gene flow. However, they also exhibit clear ecological divergence, including premating barriers, and this is considered to be the main driver behind the speciation (Diabaté et al, 2009; Constantini et al, 2009; Reidenbach et al, 2012; Coetzee et al, 2013). While largely sympatric in West Africa, these species display distinct larval habitat preferences, which segregate them temporally and spatially – *An. gambiae* s.s. is generally restricted to the rainy season where it prefers ephemeral, rain-dependent water bodies; whereas the drought-tolerant *An. coluzzii* is able to expand its reproductive capacity throughout the dry season, exploiting more temporally stable waters with higher environmental stressors such as pollutants or predators (Diabaté et al, 2002, 2009; Touré et al, 2004; Huestis et al, 2011; Reidenbach et al, 2012).

To study these two principal malaria vectors is complex, as they are two out of several sibling species of the *An. gambiae* sensu lato (s.l.) complex, species that are morphologically indistinguishable and can be identified only at a cytogenetic basis (Esposito and Habluetzel, 1997). Ecologically, however, these sibling species are highly diverse, differing in larval habitats as well as in host preference and choice of feeding and resting environments. Females of *An. gambiae* s.s. and *An. coluzzii* are generally considered to be endophagic and highly endophilic, i.e. to have a high tendency to blood feed and to rest indoors (see e.g. Scott and Takken, 2012; Esposito and Habluetzel, 1997; Gary and Foster, 2004; Takken and Knols, 1999). However, in the savannah zone of West Africa, there also exists another abundant and sympatric subgroup of *An. gambiae* s.s., which appears to be wholly exophilic (Riehle et al, 2011). This discovery is in stark contrast to how the species is generally characterised and will likely have implications on the main strategies of vector control currently applied in this region. *An. arabiensis* Patton, a more zoophilic yet important vector of the *An. gambiae* s.l. complex, is able to adapt to endophilic or exophilic patterns depending on its hosts (Mahande et al, 2007). Where the geographical distribution of *An. gambiae* and *An. arabiensis* overlaps, the species display competitive exclusion – *An. arabiensis* is generally a more drought-tolerant species and is therefore considered to be the dominant vector species in many arid regions, whereas *An. gambiae* prevails in more humid habitats (Gibson, 1996; Mahande et al, 2007; Ayala and Coluzzi, 2005). However, in West Africa *An. arabiensis* was shown to be more common during the rainy season, while *An. coluzzii* was the predominant species during the dry season (Huestis et al, 2011). For further details about the species of the *An. gambiae* s.l. complex and their characteristics and ecological requirements, see table 1.

Table 1. Characteristics summary of the species of the An. gambiae *s.l. complex.*

Species	Former name	Ecology	Geographical distribution	Breeding sites	Vectorial status
***An. gambiae* s.s.** Giles	*An. gambiae* s.l. species A	Highly anthropophilic Endophilic. One West African subspecies known to be exophilic[7] Prevails in mesic habitats, particularly when geographic distribution overlaps with *An. arabiensis.*[6] Mainly active during rainy season in West Africa	Sub-Saharan Africa	Freshwater Ephemeral waters	Principal vector
An. coluzzii Coetzee & Wilkerson sp.n.[4]	*Anopheles gambiae* molecular M form	Highly anthropophilic Endophilic Drought-tolerant, predominant species during the dry season in West Africa[8]	Sub-Saharan Africa	Freshwater Temporally stable waters; tolerant to pollutions and predators	Principal vector
An. arabiensis Patton	*An. gambiae* s.l. species B	Anthropophilic, zoophilic Exhibits endophilic and exophilic plasticity[5] Drought-tolerant[4] The ancestral species of *An. gambiae* s.l.	Sub-Saharan Africa, the African Horn, the Arabian peninsula	Freshwater	Primary vector
An. quadriannulatus Theobald	*An. gambiae* s.l. species C	Zoophilic. Exophilic[2]	Southern Africa	Freshwater	Non-vector
An. amharicus Hunt, Wilkerson & Coetzee sp.n.[4]	*An. quadriannulatus* species B[4]	Anthropophilic, zoophilic Endophilic	Ethiopia	Freshwater	Non-vector[9]
An. melas Theobald	-	Anthropophilic. Believed to exhibit endophilic and exophilic plasticity[12] Drought-tolerant[11]	Costal swamps, West Africa[3]	Halophilic, salt tolerant	Secondary vector[9]
An. merus Dönitz	-	Anthropophilic and zoophilic Displays both endophilic and exophilic tendencies[10]	Coastal East Africa[3]	Halophilic, salt tolerant	Secondary vector[9, 10]
An. bwambae White[3]	*An. gambiae* s.l. species D	Anthropophilic, facultatively zoophilic Endophilic tendencies [3]	Semliki Valley, Uganda[3]	Geothermal hot springs Halophilic[3]	Secondary vector[9,10]

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[1]Esposito and Habluetzel, 1997; [2]Hunt et al, 1998; [3]White, 1985; [4]Coetzee et al, 2013; [5]Mahande et al, 2007; [6]Ayala and Coluzzi, 2005; [7] Riehle et al, 2011; [8]Huestis et al, 2011; [9]Oyewole et al, 2005; [10]Kipyab et al, 2013; [11]Jawara et al, 2008; [12]Tuno et al, 2010.

1.3 Sugar feeding in mosquitoes

An important first step in recognizing the vector's environmental requirements is to look closer at the mosquitoes' host seeking rhythm. Sugar is overall the basic source of nutrition for mosquitoes, and a necessity for male mosquitoes (Foster, 1995). The energy gained from sugar feeding by males is essential for swarming, and is thus indirectly crucial for female fitness. Conversely, for the anautogenous females, blood is a necessary source of protein in order to produce eggs, and thus sugar-feeding by females mainly serves to fill up and maintain somatic energy reserves, primarily made up of lipids and glycogen (Foster, 1995; Van Handel, 1965). While a blood meal is indispensable for females and normally initiates a gonotrophic cycle, the somatic energy reserves are also important for egg development – so much in fact that insufficient reserve levels may cause the allocation of a blood meal to reserve production to take precedence over egg development (Foster, 1995). Consequently, because sugar is used to synthesise components of the somatic reserves, it also indirectly contributes to egg production.

Mosquitoes acquire sugar from floral nectaries and extra-floral plant fluids, as well as from for instance honeydew, tree sap, and damaged and rotting fruit (Foster, 1995). Although a poor source of sugar, mosquitoes of the genera *Anopheles*, *Aedes* and *Culex* have also been found to regularly feed on plant tissues (Müller and Schlein, 2005). While sugar probably is the more common nutrient source for females of many species (Foster, 1995), the frequency of sugar feeding by females of some species remains unclear. The conflicting views on this issue are largely due to observations carried out in different parts of the world (Foster, 1995), yet there are also contradictions regarding sugar feeding by females of *An. gambiae* s.l.. While some argue that sugar serves as a common and optional supplement to vertebrate blood in this species (see e.g. Foster, 1995), Beier (1996) inferred from observations in the field that females sugar feed rarely if at all. Females can in principle forego sugar altogether in favour of vertebrate blood – although sugar is a more readily available energy source, the female mosquito is able to synthesise glycogen from blood as well, but at a considerably lower rate (Van Handel, 1965). Furthermore, for anautogenous mosquitoes, sugar feeding can be detrimental in terms of fitness – an engorged crop limits the volume of blood that a female mosquito can take in, consequently reducing the size of the egg clutch (Foster et al, 1989; Mostowy and Foster, 2004). In a laboratory study on the effect of meal and energy reserve sizes on the egg-batch size of *Aedes aegypti,* Mostowy and Foster (2004) considered a full crop to have been emptied after approximately 2-3 days of sugar starvation at 27±1°C. Sugar in the crop is generally digested at an exponential rate that is dependent on meal size and sugar concentration, the presence of a blood meal, the flight activity of the mosquito and the size of its somatic energy reserves (Foster, 1995). In their study, Mostowy and Foster (2004) found that substantial energy reserves increased the egg batch size, as did a large blood meal, which was generally imbibed when the energy reserves were low or when there was little sugar present in the crop. Furthermore, when the *Ae. aegypti* female had a large somatic energy reserve, she imbibed less sugar (Mostowy and Foster, 2004), thus making room for a larger blood meal. In *Ae. aegypti* – a species so ecologically similar to *An. gambiae* s.s. and *An. coluzzii* that they have been suggested to be physiologically convergent (Foster and Takken, 2004) - Jones and Madhukar (1976) found that sugar inhibited the female's attraction to vertebrate blood shortly after sugar feeding. For this species it also appears that feeding exclusively on human blood results in a higher fitness than when feeding on both blood and sucrose (Scott et al, 1997; Gary and Foster, 2001). Similarly, Gary and Foster (2001) found that the biting frequency and daily fitness of *An. gambiae* s.s. and *An. coluzzii* increased when sugar was unavailable and the females fed exclusively on blood. However, they also found that sugar appears to extend female longevity, and she would feed on sugar if it were accessible (Gary and Foster, 2001). Several studies have also shown other positive effects of sugar. For instance, with a larger energy reserve, most easily obtained from sugar (Van Handel, 1965), the female is able to produce larger egg clutches (Foster and Hancock, 1994; Mostowy and Foster, 2004). An increased energy reserve due to intake of sugar, while not a prerequisite for blood feeding, also results in a shortened response-time and an enhanced response to human volatiles (Foster and Takken, 2004; Hancock and Foster, 1997). In a study on flight substrates for two species of *Aedes* mosquitoes, crop sugar was found to be preferred and given priority during flight and was metabolised approximately four times as quick as when the mosquito was in rest (Nayar and Van Handel, 1971). Only after the crop sugar had been exhausted was glycogen from the somatic reserves used. Triglycerides were found not to be mobilised at a fast enough rate to be used for short and vigorous flights (Nayar and Van Handel, 1971), and thus mainly glycogen serve as short-flight fuel. Only for extended flight periods are females of *An. gambiae s.s.* and *An. coluzzii* able to utilise a portion of their lipid reserves (Kaufmann and Briegel, 2004).

With the exception of the study by Beier (1996), it appears that most of our knowledge on sugar feeding by females of *An. gambiae* s.l. is pointing towards the view that sugar is a facultative yet relatively common and beneficial source of nutrition. However, the frequency of sugar feeding is not merely a question of magnitude; it is also a temporal question – as the requirements and physiological state of the mosquito changes over its lifetime, so does its host seeking rhythm.

Even when mosquito larvae are able to develop under optimal conditions, the energy reserves of *An. gambiae* s.l. only allow them to survive without food for 2-3 days after emergence at 27° Celsius (Takken et al, 1998; Foster and Takken, 2004). The first two days after emergence are therefore particularly critical when it comes to obtaining nutrition. Although responsive to human volatiles, teneral females do not take blood within 24h of emergence from the pupae (Foster and Takken, 2004). During this period and up to about two days after emergence, females appeared to respond more strongly to honey than human volatiles in a study by Foster and Takken (2004). If both blood and sugar are readily available, a larger proportion of females will choose to feed on sugar first, then blood after mating (Stone et al, 2011). However, females of *An. gambiae s.s.* and *An. coluzzii* appear to be generally opportunistic regarding their first choice of meal, and will choose whichever energy source is most easily obtainable (Stone et al, 2011). Over time, the female's response to volatiles associated with sugar meals decreases radically, and at an age of 5 days the response to sugar is low to non-existent in previously sugar-fed females, due to a shift in physiological state (Foster and Takken, 2004). However, despite the direct conflicts between sugar and blood, sugar still generally appears to function as an optional nutritional supplement to vertebrate blood between gonotrophic cycles, and may become a vital food-source when environmental constraints cause the interval between the cycles to extend (Gary and Foster, 2006).

2 Aims and objectives

It is clear that the malaria control strategies applied through time have made massive progress but are limited in their success of achieving total control, and that integrated strategies that target various resources important for different stages of the mosquito life cycle are necessary to eliminate malaria (Ferguson et al, 2010). One such important resource is sugar, as it is not only a necessary resource for male survival, but also appears to have an effect on female longevity and thus vector competence (Gary and Foster, 2001). Knowledge on which sugar hosts the malaria vector utilises may therefore be important for the development of new vector control strategies, as it contributes to our basic understanding of vector ecology and could potentially be applied to the development of new trapping methods.

Which sugar host the mosquito selects depends on a range of factors – from the internal state of the mosquito, including gonotrophic state of females and energetic reserves; to for instance stimulus strength, the combination and composition of host volatiles, host proximity and which plant species that are available (Foster and Takken, 2004). Manda et al (2007a) were the first to experimentally show discriminative behaviour of *An. gambiae* among plant species, and studies on wild anopheline mosquitoes have similarly found discriminative behaviour among plant species in the field (Müller and Schlein, 2006; Müller et al, 2010; Gouagna et al, 2010). Females of *An. gambiae* have previously been found to display a degree of polymorphism for blood host preference (Gillies, 1964), and it has therefore been suggested that their response to nectar stimuli might be similarly genetically based (Stone et al, 2011). If so, it is possible that the mosquito has an intrinsic ranking of host plants that should be dependent on the local composition of available plants. The aim of this study was therefore to investigate the sugar feeding behaviour of males and females of anopheline mosquitoes, with an emphasis on the behaviour of the principal vector *An. coluzzii,* on local plants commonly occurring during the dry season in South-western Burkina Faso. It was hypothesised that the mosquitoes will discriminate between different local sugar sources, and that the discriminative feeding behaviour will differ between the sexes due to different physiological and ecological requirements. Ultimately, the aim of this study was to contribute to the knowledge on the local population of anopheline mosquitoes, and to serve as a pilot study for further investigation on the discriminative host-seeking behaviour of the local malaria vectors.

3 Methods

The data collection of this study was divided into two parts: the *picnic test* and the *cafeteria test.* The picnic test was conducted in the field in villages in Vallée du Kou, in order to determine the preference for the most commonly occurring plants by mosquitoes that are active in the region during the dry season. Based on the initial results of the picnic test, two combinations of four plant species were chosen for the cafeteria test, which was performed under semi-controlled conditions in the laboratory. A four-choice preference test was conducted on females and males of *An. coluzzii*, respectively.

3.1 Study area

The field study was carried out in Bama (11°24' N, 04°24'59 W), Vallée du Kou, 30 km west of Bobo-Dioulasso in the humid Sudan savannah region of South-western Burkina Faso. Bama is a rice cultivation area that was developed during the early 1970's, and comprises seven discrete villages (VK1-VK7) that are surrounded by wooded savannah (Dabiré et al, 2007, Robert et al, 1991). The area has a mean annual rainfall of about 1,200 mm and is characterised by two seasons, the rainy season that extends from May to October, and the dry season that extends from November to April. There are two rice crops per year (July-November and January-May), and because the Kou River supplies permanent irrigation water for the area, the rice fields provide breeding sites for mosquitoes even during the dry season. Of the mosquito species populating this area, *An. coluzzii* is the predominant vector during most of the year. It almost exclusively populates the area during the dry season, while it occurs in sympatry with *An. gambiae* s.s. during the rainy season, along with lower densities of *An. funestus* and *Culex quinquefasciatus* (Dabiré et al, 2007; IRSS/Centre Muraz/Bobo-Dioulasso, unpublished). The sampling sites for this study were located in villages VK7, VK3, and VK5. Throughout Vallée du Kou, the major crops are interspersed with small areas of root crops, and vegetables such as cabbages and onions. VK3 and VK5 are the two smallest villages of Bama and are both surrounded by rice fields interspersed with fields of maize, with a moderately high density of mango trees (*Mangifera indica*, Anacardiaceae) within the village perimeters. VK7, on the other hand, is located on the North-western border of Bama with rice and maize fields on one side and cotton fields on the other. (figure 1)

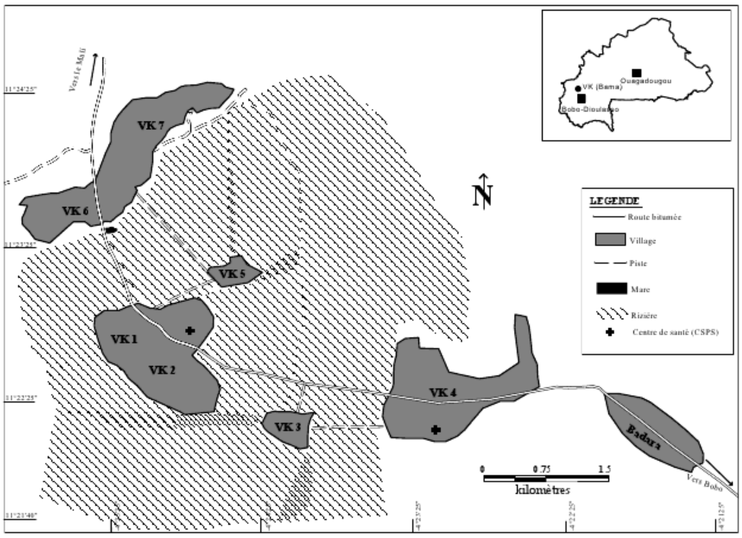

Figure 1. Schematic map of the Kou Valley (IRSS/Centre Muraz/ Bobo-Dioulasso, unpublished).

3.2 Plant species

To determine which plant species were the most common in the villages of Vallée du Kou during the dry season, two transects were drawn in each of the villages VK1, VK3, VK5 and VK7 – north to south and west to east, using the outermost huts as village perimeters. The plant species found within a ten meter radius of the transects were identified and counted, after which the six most commonly occurring plant species growing in the vicinity of human dwellings that bore flowers or fruit during the sampling period, were selected for the picnic test (table 2, figure 2).

Table 2. List of commonly occurring plants species used in the picnic test and the cafeteria test, respectively.

Test	Plant part	Plant family	Species name	Common name
Picnic, cafeteria	Leaves, flowers	Fabaceae	*Delonix regia* (Hook.)	Flamboyant
Picnic	Leaves, flowers, fruit	Euphorbiaceae	*Jatropha curcas* (L.)	Jatropha
Picnic, cafeteria	Fruit	Anacardiaceae	*Mangifera indica* (L.)	Mango
Picnic, cafeteria	Leaves, flowers, fruit	Meliaceae	*Azadirachta indica* (Juss.)	Neem
Picnic, cafeteria	Fruit	Caricaceae	*Carica papaya* (L.)	Papaya
Picnic, cafeteria	Leaves, flowers	Apocynaceae	*Thevetia neriifolia* (Juss.)	Tevetia
Cafeteria	Fruit	Musaceae	*Banana musa*	Banana

The plants selected were the following: Flamboyant, *Delonix regia* Hook. (Fabaceae; figure 2), a species native to Madagascar, is a large ornamental tree with large, scarlet to orange-red flowers (JSTOR Global Plants). It flowers towards the end of the dry season and is found in most villages of Vallée du Kou, where it mainly functions as a shade tree. Jatropha, *Jatropha curcas* L. (Euphorbiaceae; figure 2), a shrub native to tropical America that is now dispersed throughout the tropics, flowers during the rainy season, after which it produces small, green fruit (Achten et al 2008). It has recently been recognised as a suitable plant for biodiesel production (Achten et al 2008), and as is often the case in West Africa, it is primarily found along the perimeter of the villages of Vallée du Kou where is functions as a natural hedge between human dwellings and fields. Mango, *M. indica* L. (Anacardiaceae; figure 2), a species originating in the Indo-Burmese region and is believed to have been introduced to West Africa some time during the 19th century, is much appreciated for its fruit and shade, and the fruit serves as an important export product in Burkina Faso (Rey et al, 2006). Its fruiting period begins at the end of the dry season and onset of the rainy season, during which time there is an excess of ripe fruit in the villages. It is a large, abundant tree with a large, dense canopy, and grows in close proximity to human dwellings in the villages of Vallée du Kou. Neem, *Azadirachta indica* Juss. (Meliaceae; figure 2), an evergreen tree native to Southern to Southeast Asia, has long been considered an important medicinal plant, known for containing many secondary compounds with insecticidal, anti-bacterial and anti-malarial properties in its roots, seeds, leaves and bark. Many of these properties are attributed to a class of compounds called isoprenoids (Schmutterer, 1990; Biswas et al, 2002). These include azadirachtin, known for its highly deterrent, growth regulating, antifeedant, anti-ovipositional, and fitness-reducing effects on phytophagous insects; and gedunin, a compound with inhibitory activity on the asexual stages of *P. falciparum* (Schmutterer, 1990;

Khalid et al, 1989; McKinnon et al 1997). This large tree is found in all villages of Vallée du Kou, most often growing on the perimeter of the villages. Thevetia, *Thevetia neriifolia* Juss. (Apocynaceae; figure 2), native to tropical America, is a small tree that is found in close proximity to human dwellings in Vallée du Kou. It bears large, singular, yellow flowers at the end of the dry season (JSTOR Global Plants). Papaya, *Carica papaya* L. (Caricaceae; figure 2), the last of the plants selected for the picnic test, is native to tropical America and is believed to have reached Africa some time during the sixteenth century (JSTOR Global Plants). It is a tall and for the most part branch-less tree, which produces large, sweet and juicy fruit and grows near human dwellings in Vallée du Kou. Banana, *B. musa* (Musaceae), native to Southeast Asia, is a giant perennial monocotyledonous herb that produces one of the most common fruits found in the villages of Vallée du Kou during the dry season. While not found to be among the most common bush or tree in the villages, it was chosen for combination B of the cafeteria test to contrast the fruit of *C. papaya*.

Figure 2. Plant species selected for the picnic test in the field. Top left to right: *J. curcas, D. regia, M. indica.* Bottom left to right: *A. indica, C. papaya, T. neriifolia.* (Photo: Louise Malmgren)

3.3 Picnic test

The attraction of wild mosquitoes to six local plant species (table 2) was determined using sticky traps, placed on the outer village perimeters between the agricultural fields and the human dwellings in different locations in villages VK3, VK5 and VK7 in Vallée du Kou. The design of the traps used in the picnic test was for the most part based on the traps used in the study by Müller et al (2010), but some modifications were made due to local conditions. The picnic test was constructed as follows: Two tables (approximately 1.5 m in height) and two wooden boards (placed on the ground) were placed on the ground in one straight line, one board next to one table. On the flat surfaces, identical netted containers covered in sticky glue (Tangle Foot, Israel) were placed upside-down, 600 mm apart. 1.5 L plastic bottles were cut in half, filled with local well-water and placed inside the containers, the bottom-half of which were covered in mesh (figure 3). Freshly cut branches of the plants that included flowers or fruit were collected in the local area and placed in the cut plastic bottles. For *M. indica* and *C. papaya*, approximately 0.2 kg of ripe fruit from local trees was cut to pieces and placed on 250 mm long wooden stakes in the plastic bottles. The control consisted of a cut plastic bottle containing well water. For each trial there were two sticky traps for each plant species, one standing on the tables and one on the boards on the ground. The placement of the different plants and fruit was randomised for each new trial. Each new trial was prepared before midday by adding fresh branches, fruit, and water; changing the placement of the sticky traps, removing any previously caught insects or material and adding fresh glue to the surfaces of the sticky traps to ensure proper stickiness and to remove any residual dirt. The number of mosquitoes caught by the glue was counted, mosquitoes were identified and their sex was determined the following morning. *Culex* mosquitoes were identified down to genus level, while anopheline mosquitoes were identified down to species level with the exception of the members of the *An. gambiae* s.l. complex, as they are morphologically indistinguishable from one another. Species identification by PCR of the *An. gam-*

Figure 3. Sticky trap used in the picnic test in Vallée du Kou (Photo: Louise Malmgren).

biae s.l. complex was not performed due to limiting circumstances. After approximately three trials in the same location in the village, the tables and boards were moved to a new location. The picnic test was conducted during four consecutive weeks in April-May 2012.

3.4 Mosquito colony

The mosquitoes used in the cafeteria test were of the species *An. coluzzii*. Adults were obtained from a colony that had recently been established from eggs of wild gravid females collected in human dwellings in VK5 in Bama, in South-western Burkina Faso. The colony was reared in standard 30*30*30 cm mesh-covered cages under controlled conditions (LD 12:12h cycle, 27 ± 1°C, 80 ± 10% relative humidity) in the insectaries of Institut de Recherche en Sciences de la Sante (IRSS) in Bobo-Dioulasso. Adults were offered 6% glucose solutions *ad libitum*, and wild female specimens were allowed to feed on the arms of human volunteers while subsequent generations of female adults were continuously fed rabbit blood.

Table 3. Plant species of combinations A and B of the cafeteria test.

Combination A	Combination B
Delonix regia (Hook)	*Delonix regia* (Hook)
Carica papaya (L.)	*Carica papaya* (L.)
Mangifera indica (L.)	*Azadirachta indica* (Juss.)
Thevetia neriifolia (Juss.)	*Banana musa*

3.5 Cafeteria test

The cafeteria test was in part based on the methodology of Manda et al (2007a). In this four-choice assay, adult mosquitoes of the same sex and cohort were enclosed with four plant species in a one cubic meter mesh-covered cage, and the number of visits to each plant was observed. Each trial was performed between sundown and midnight, during two consecutive weeks in April-May 2012. The initial results from the picnic test were used as a basis for which plant species should be selected for further investigation in the laboratory. Two combinations were chosen, each combination consisting of four species, and it was decided to select two flowering plants and two fruits for each combination for the sake of contrast. Combination A included the species *D. regia, C. papaya, T. neriifolia* and *M. indica* (table 3).

For combination B (table 3), the species *C. papaya, A. indica, D. regia* and *B. musa* were chosen. Despite not having been included in the preceding picnic test, *B. musa* was included as an alternative fruit to *M. indica* in combination B, to see whether this would have an effect on the mosquitoes' attraction to *C. papaya*. In each trial, approximately 50-70 grams of freshly cut branches of the plants were used. For the fruit, one ripe fruit of each species was cut in half and the seeds were per standard removed to facilitate a clearer view for the observers, after which the fruit was placed on 250 mm long wooden stakes.

Figure 4. Mesh-covered cage used into which mosquitoes were released for the laboratory bioassays for the cafeteria test (Photo: Louise Malmgren).

Each plant species was placed in 1.5 l plastic bottles that had been cut in half. The cafeteria test was conducted in darkness in a climate-controlled room at the Institut de Recherche en Sciences de la Sante (IRSS) in Bobo-Dioulasso (27 ± 1°C, 80 ± 10% relative humidity). A one cubic meter aluminium-framed cube covered by removable mesh was constructed, with one closable opening on one side to enable easy access to the mosquitoes and plant species in the cage. To avoid that the mosquitoes were influenced by human volatiles, the front of the cage had been fitted with Plexiglas, which was kept between the observers and the mosquitoes during each trial (figure 4). Approximately 12 hours before each repetition of the cafeteria test, 50 ± 5 adult mosquitoes of the same sex and cohort were collected in small paper cups, 100 mm in diameter and covered in mesh, and set aside in a climate-controlled room without food but with a cotton wick soaked in water to avoid dehydration. Before each trial, fresh plants and fruit were placed in each corner of the cube, the placement of which were randomised for every trial. Before the beginning of the trial, starved mosquitoes were released into the centre of the cage for a 30-minutes acclimatisation period. Observations were subsequently carried out for 15 minutes per trial by two observers, each using a hand-held torch with red light. During the trial, the number of mosquitoes perching (resting, the proboscis not in contact with the plant), probing (testing the surface with the proboscis, but not perching), feeding (perching, proboscis in contact with the plant surface), and prolonged feeding, was recorded. When 15 minutes had passed, the mosquitoes were collected using a mouth aspirator and the number of mosquitoes with an observed abdominal distension was recorded. The plant- and fruit material were then removed and replaced by new cuttings.

3.6 Statistical analyses

3.6.1 Picnic test

The data collected for the picnic test was initially going to be analysed using ANOVA, or a non-parametric equivalent depending on how the numbers were distributed. However, a χ^2 –test in a 2*7 contingency table analysis was deemed most appropriate due to the size of the dataset, and was used in order to test the randomness of the number of mosquito visits to the plant species. The following analyses were performed:

- The number of mosquito visits, with sex and anopheline species distinguished; disregarding plant species placement and height – is there a difference in the number of visits to the plant species available, according to sex and/or mosquito species?
- The number of mosquito visits, with sex and species pooled for each genus, *Anopheles* and *Culex*, respectively; disregarding the placement and level of which the plant species were placed – is there a difference in the number of visits to the plant species according to genus?
- The number of mosquito visits, with plant species placement and height distinguished – is there a difference in visits to the plants or fruit present, according to their placement or if they were on the ground or on the tables?

Additionally, Fischer's exact test was used to test the number of visits to each plant species against the control, per genus, species, and sex.

3.6.2 Cafeteria test

The data on mosquito visits were discrete counts following a Poisson distribution. For each of the two combinations of plant species, the effect of plant species and other explanatory variables on the number of mosquito visits was assessed using a Poisson Generalized Linear Model (GLM), with a quasi-likelihood to allow for over-dispersion. Which model that best described the response variable was determined using stepwise deletion, followed by model comparisons for each variable. The overall model fit was tested using a likelihood ratio test (LRT), performed using ANOVA with a χ^2 –distribution to calculate the p-value. When significant differences were observed, the fitted models were used in General Linear Hypotheses tests (glht) to run multiple pair-wise comparisons of means with Tukey contrasts, to distinguish between groups. This was applied particularly on the effect of plant species on the number of visits, in order to make an attraction ranking.

Those variables that could be standardised were tested as explanatory variables against the response variable (the number of visits). The following explanatory variables were tested against the number of visits:

- Effect of plant species – does the number of visits differ depending on the plant species?
- Effect of sex – does the number of visits differ depending on the sex of the mosquito?
- Effect of the placement of the plant species present – is there a placement effect on the number of visits?
- Effect of time – does the number of visits differ depending on if the trial was performed during the crepuscular period (i.e. twilight) and onset of scotophase (i.e. darkness) (approximately 6 p.m.-7:30 p.m.), or during scotophase (approximately 7:30 p.m.-9 p.m.)?

Furthermore, to test the mosquitoes' responses to the plant species, the number of visits to each plant or fruit and the number of mosquitoes performing any of the three responses feeding, probing and perching, were tested against one another. Feeding, probing and the two responses combined were also tested as explanatory variables against the total number of mosquitoes with abdominal distension.

Due to an observed decrease in activity from combination A to combination B, the total number of visits by all mosquitoes was tested as a response variable against the combination, to determine whether this had been a statistically sound observation. Climatic hourly surface data from April 19th until May 20th 2012, collected at the DFOO (655100 99999; 11°17' N, 4°30' W; elevation 460.6 m) weather station in Bobo-Dioulasso, was subsequently acquired from the national Climatic Data Center/National Oceanic and Atmospheric Administration (NCDC/NOAA). To see if the possible decrease in activity was an effect of climatic variables, the total number of visits per day for each combination and both combinations combined was tested against the following explanatory variables:

- The daily average temperature (presented in Fahrenheit, converted to degrees Celsius by one decimal's accuracy)
- The daily average calculated relative humidity (per cent)
- The daily average sea level pressure (hPa)

All data was entered and processed in Microsoft Excel for Mac 2011, which was also employed to perform χ^2-tests and create figures. The remainder of the statistical analyses were performed with R version 3.1.0 in RStudio, Inc., version 0.98.983, using a significance level of 5%.

4 Results

4.1 Picnic test

The total number of mosquitoes collected from the glue traps in the field was sparse, and comprised of 63 *Culex* sp. (49 females, 14 males) and 36 *Anopheles* sp.. *An. gambiae* s.l. comprised 63.9% (n=23) of all mosquitoes of the *Anopheles* genus collected in the field. The remainder of the collected anopheline mosquitoes were *An. pharoensis* (9/36), *An. funestus* (2/36), and *An. costeii* (2/36). The number of mosquitoes found on each glue trap was generally low for all villages and locations, but a majority of the mosquitoes were collected in VK3. On average, eleven mosquitoes of any genus were collected in VK3 per trial, three per trial in VK5 and seven in VK7. Due to the scarcity of data, the number of variables that could be taken into account during the statistical analyses was limited. Pooling data across both sexes, all plant species placements, levels that the plant species

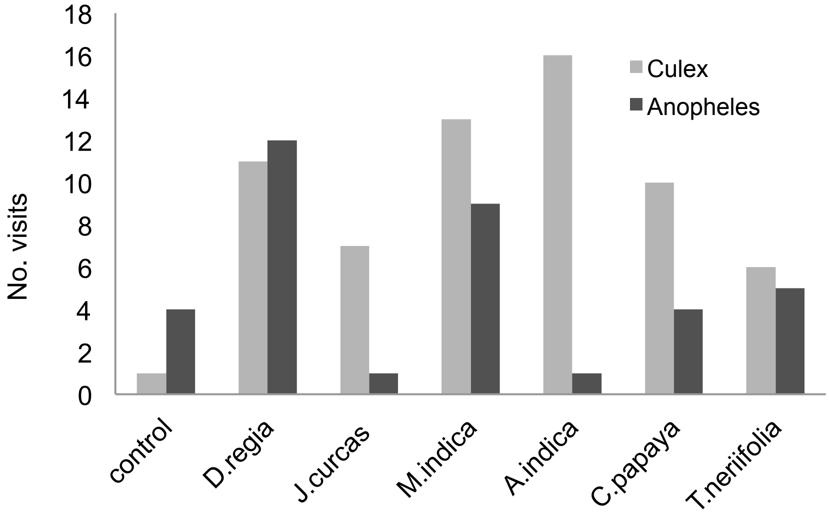

Figure 5. The total number of mosquitoes collected in the field in Vallée du Kou of the *Anopheles* and *Culex* genera, sex and species pooled, according to plant species.

were placed (ground or tables) and all anopheline species, the number of collected anopheline mosquitoes was found to differ depending on plant species (χ^2=19.22; d.f.=6; p<0.01) - from *D.regia* as the most common plant species, to *J. curcas* as the least common (figure 5). The *Culex* mosquitoes displayed a similarly uneven distribution on the plant species tested, with *A. indica* as the most common and the control as the least commonly visited plant species (χ^2=17.11; d.f=6; p<0.01) (figure 5). Furthermore, there was a significant difference between the plant species that the anopheline mosquitoes were collected from, compared with *Culex* mosquitoes (χ^2=16.78; d.f.=6; p<0.01) (figure 5). Fischer's exact test, that would have been used to test the number of mosquitoes on each plant species against the control, in order to rank the plant species from most attractive to least attractive, could not be performed because the dataset was too small.

4.2 Cafeteria test

4.2.1 Combination A

The results of the cafeteria test trials with plant species combination A (i.e. *D. regia, C. papaya, M. indica* and *T. neriifolia*) showed a significant difference in attraction of the mosquitoes to the different plant species (LRT χ^2=-305.97, d.f.=110, p<0.001) (table 4). While males and females displayed similar plant species preferences with no significant differences, there was a sex effect on the total number of visits (LRT χ2=-43.183, d.f.=108, p<0.01), with the males being significantly more active than the females. Note, however, that because the availability of mosquitoes in the insectarium was limited, more trials were performed with males than females (males: 15 trials; females: 13 trials). Nonetheless, the males were on average significantly more active than the females. Whether the trials were performed during the crepuscular period and onset of scotophase, or during scotophase, the time did not have an effect on the mosquitoes' visits, nor did the placement of the plant species inside the cage.

For females, there were significantly more visits to *C. papaya* than to *M. indica*, and although not significant, a trend towards *D. regia* being preferred over *M. indica* and *T. neriifolia* (figure 6a). Because there was only one visit to *T. neriifolia,* this visit was treated as an outlier in figure 6a, hence the insignificant difference between this flower and *C. papaya.* The males were overall more active, and there were therefore clearer differences in preference between the plant species of combination A. While there was no significant difference in the number of visits to *C. papaya* and *D. regia*, both these plant species had significantly more visits than *M. indica* and *T. neriifolia* (figure 6b). Figure 6c shows the number of visits of males and females combined. With males and females pooled, the difference in preferences is more apparent, with significantly more visits to *C. papaya* and *D. regia* than to *M. indica* and *T. neriifolia*.

The responses to the plant species did not differ between the sexes and the following analyses were therefore performed with both sexes pooled. Probing was observed significantly less on both *M. indica* (t=2.863, d.f.=108, p<0.01) and *T. neriifolia* (t=-2.263, d.f.=108, p<0.05) than on *D. regia* and *C. papaya*, while perching was observed significantly less on *M. indica* (t=-3.157, d.f.=108, p<0.01) than on *D. regia* and *C. papaya*. Only on *C. papaya* (t=5.369, d.f.=108, p<0.001) was feeding observed on a significant level. Figure 7 illustrates the number of visits by males and females to the different plant species, according to responses. On *D. regia*, the mosquitoes were observed to probe more than feed (z=-3.157, p<0.01), while probing and feeding were significantly more common responses on *C. papaya* than was resting (z=-2.44, P<0.05; z=-3.51, p<0.001). There was no difference in responses on *M. indica* and *T. neriifolia* (figure 7).

There was a significant correlation between probing and feeding as explanatory variables, respectively, and the number of mosquitoes with abdominal distension as a response variable (t=2.638, d.f.=22, p<0.05; t=3.473, d.f.=22, p<0.01); yet the model with the best fit was with feeding and probing combined as a single explanatory variable (t=3.691, d.f.=22, p<0.01).

Table 4. Mean number of visits, S.E., and the total number of visits for each plant species of combination A for males, females and all mosquitoes pooled.

Plant species	Sex	Mean	SE	n
D. regia	Males + Females	4.32	0.993	121
D. regia	Males	6.00	1.65	90
D. regia	Females	2.38	0.747	31
C. papaya	Males + Females	8.11	1.56	227
C. papaya	Males	10.4	2.24	156
C. papaya	Females	5.46	1.97	71
M. indica	Males + Females	1.21	0.297	34
M. indica	Males	1.20	0.470	18
M. indica	Females	1.23	0.361	16
T. neriifolia	Males + Females	0.43	0.227	12
T. neriifolia	Males	0.147	0.151	11
T. neriifolia	Females	0.0154	0.0344	1

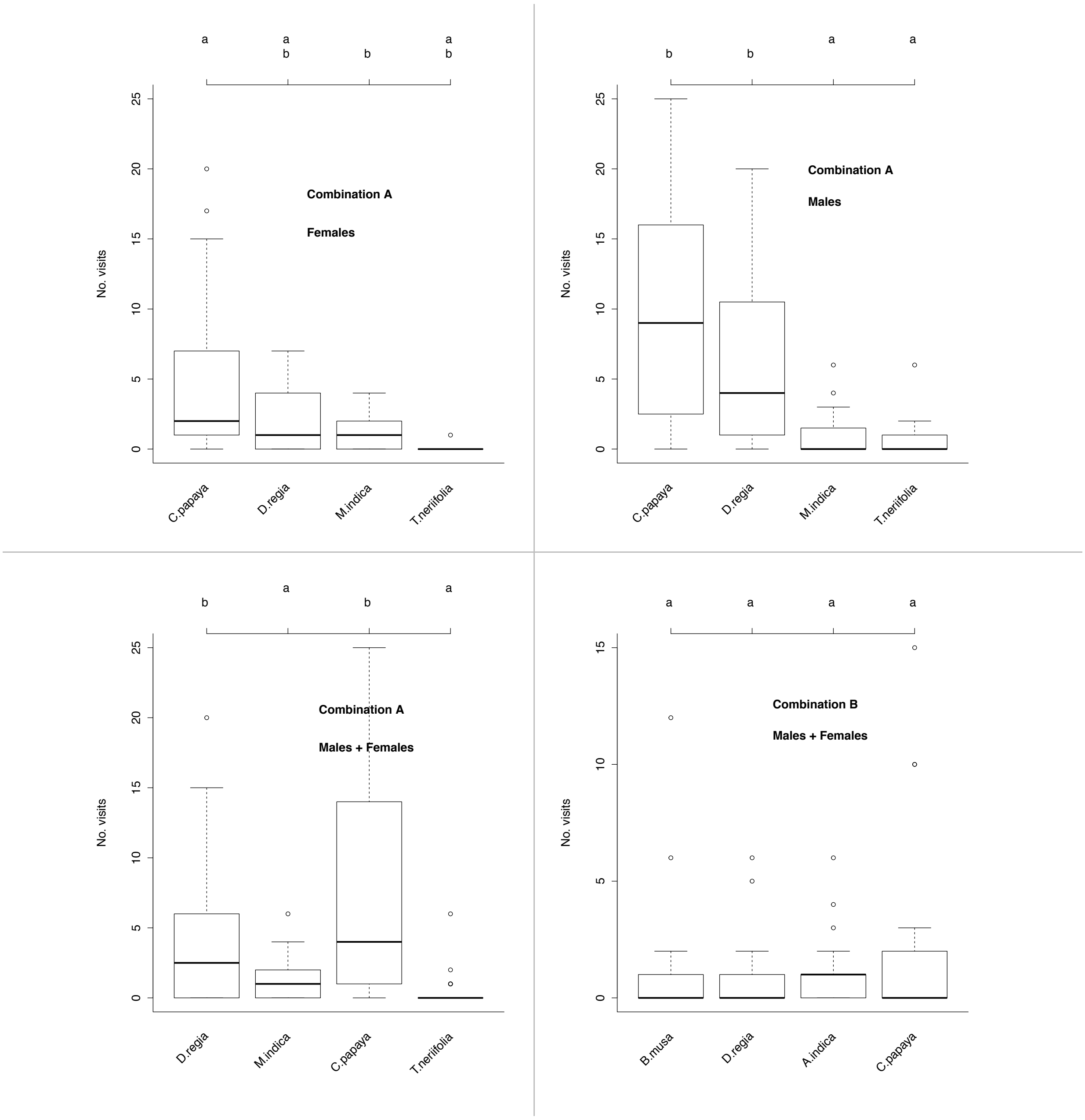

Figure 6. The number of visits to the different plant species according to sex and combination. The boxes extend across the interquartile range between the 25th and 75th percentiles and the whiskers extend to the most extreme value. Each plot illustrates the ranking of the plant species in relation to one another, where plant species that share the same letter are not significantly different at a 5% significance level. A) The number of visits by females to plant species of combination A; *C. papaya* and *M. indica* are significantly different. B) The number of visits by males to plant species of combination A; there were significantly more visits to *C. papaya* and *D. regia* than to *M. indica* and *T. neriifolia*. C) The number of visits by males and females pooled, to plant species of combination A; there were significantly more visits to *C. papaya* and *D. regia* than to *M. indica* and *T. neriifolia*. D) The number of visits by males and females pooled, to plant species of combination B; there was no significant difference between visits to the different plant species.

4.2.2 Combination B

Thirteen trials with females were performed on plant species of combination B, and twenty trials with males. No effect of sex or plant species could be found on the number of mosquito visits in the trials with combination B (i.e. *D. regia, C. papaya, B. musa* and *A. indica*) (figure 6d). The number of visits was also not influenced by the time of the evening that the trials were conducted. There was a placement by sex effect on the number of visits (χ^2=-70.181, d.f.=131, p<0.01), with a significantly higher number of visits by males to the left-hand corner of the cube nearest the observers (t=2.001; p<0.05). However, the placement of the plant species did not affect the number of visits to the different plants or fruit.

The trials with combination A were conducted during the time period April 19th until May 4th, and two trials on May 17th. The trials with combination B were conducted during the time period May 7th until May 20th. During the course of the cafeteria test trials, there was an observed decrease in activity among all mosquitoes, particularly during the transition from the trials with combination A to combination B. The number of visits was tested against the combinations, showing that there indeed was a significantly lower activity during the trials with combination B (LRT χ^2=-184.76, d.f.=59, p<0.001). The age of the mosquitoes was a variable that could not be standardised due to logistical reasons, however, its effect on the decrease in activity was still tested but no effect of age could be found. To see if the combination of plant species had an effect on the activity of the mosquitoes, combination A was re-introduced on May 17th, one trial for each sex, to see if a difference in activity could be observed compared with combination B. While only one trial was conducted per sex, there did not appear to be any increase in activity

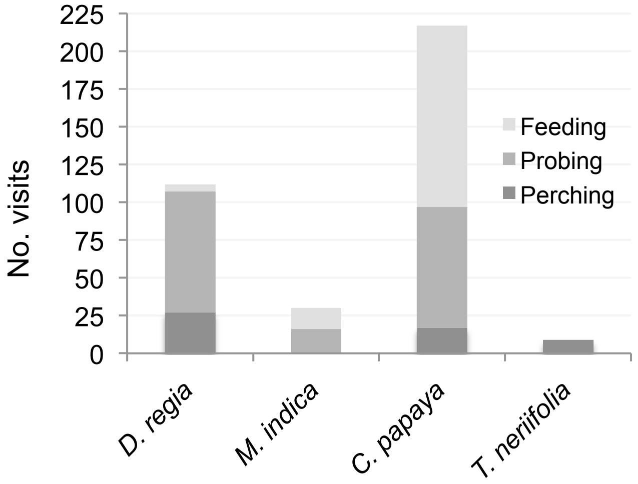

Figure 7. The number of mosquito visits to the four plant species of combination A in the cafeteria test, according to the responses feeding, probing and perching; sexes pooled.

because combination A had been re-introduced. The average daily humidity, average daily sea level pressure and the average daily temperature in degrees Celsius were derived from the data that was acquired from NCDC/NOAA. This was subsequently tested against the number of visits for each of the different combinations and against the entire trial period of the cafeteria test (combination A and combination B together). There was no effect of the average daily temperature on the number of visits for either of the combinations. However, there was clear negative correlation between the average daily humidity level and the number of visits during the entire trial period (LRT $\chi 2$=-165.7, d.f.=59, p<0.001), and during the trials with combination B (LRT $\chi 2$=-39.577, d.f.=32, p<0.01). There was not an effect of humidity on the trials with combination A (figure 8a). There was also an effect of the average daily sea level pressure on the entire trial period (LRT $\chi 2$=-81.843, d.f.=59, p<0.01) and on combination B (LRT $\chi 2$=-60.455, d.f.=32, p<0.001) – the higher the average sea level pressure, the lower the activity of mosquitoes. The average daily sea level pressure did not have an effect on the activity of the mosquitoes tested on combination A (figure 8b).

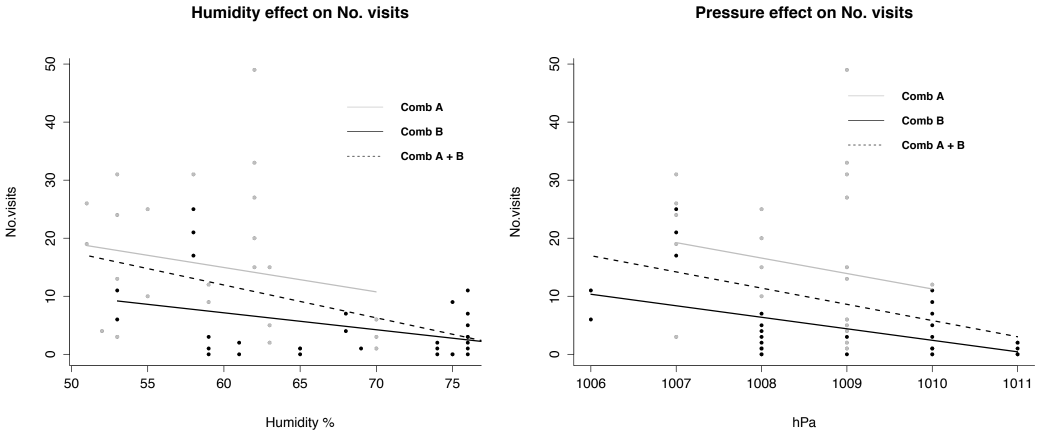

Figure 8. The effect of the average daily humidity (a) and the effect of the average daily sea level pressure (b) on the number of visits of mosquitoes of both sexes of *An. coluzzii* in the cafeteria test. Regression lines were fitted for both plots merely to illustrate the decrease in activity for combination A (not significant for either of the two variables humidity or sea level pressure), combination B (a significant activity reduction for both variables) and the entire trial period (a significant activity reduction for both variables).

5 Discussion

The data collected in the field on wild culicine and anopheline mosquitoes present in Vallée du Kou in South-western Burkina Faso during the dry season; indicate that anopheline mosquitoes display discriminative sugar source preferences, preferences that are dissimilar to those displayed by culicine mosquitoes. Based on results from the laboratory study on laboratory-reared *An. coluzzii*, the mosquitoes appear to display a preference ranking for certain plant species. Of the responses displayed on the plant species in the laboratory, only fruits appear to elicit a feeding response, while any plant species that was visited often prompted probing or perching responses. The preference ranking and plant species responses follow the same pattern among both females and males of *An. coluzzii.*

This study was aimed at investigating the discriminative sugar source selection of males and females of *An. coluzzii* among plant species found during the dry season in South-western Burkina Faso. Studies have previously shown that both sexes of *An. coluzzii* and *An. gambiae* s.s. discriminate between plant species, experimentally (Manda et al, 2007a) as well as for males in the field (Gouagna et al, 2010). The indicative field results of the present study appear to support these findings, suggesting that anopheline mosquitoes may display discriminative selection of sugar sources when presented with several choices of plants. Similarly, the results for the culicine mosquitoes caught in the traps in the field indicate that they display discriminative behaviour, however, they appear to prefer other plant species than the anophelines. For instance, while *A. indica* was the plant with the lowest frequency of anopheline visits, it attracted the highest frequency of culicine visits. Due to the data collected in the field being sparse, all conclusions based on the picnic test should be drawn with caution. Similarly, few trials conducted for each combination of plant species in the cafeteria tests means that no conclusions should be drawn without carefully noting that these are merely indicative results from a pilot study, on which further studies can be based. Nonetheless, the indicative results from the picnic test is further supported by the laboratory findings, which indicate that females and males of *An. coluzzii* display preferences for certain plant species, with similar discriminative behaviour for both sexes. Cafeteria trials using plant species of combination A showed that the mosquitoes displayed a two-parted preference ranking – the fruit of *C. papaya* and flowers of *D. regia* attracted significantly more visits than the fruit of *M. indica* and flowers of *T. neriifolia*. The results of the picnic test appear to contradict this attraction ranking of plant species by showing more visits to *M. indica* than to *C. papaya* by anopheline mosquitoes, however, because there is too little data present, it is not possible to draw any such conclusions without further investigation.

Not surprisingly, the responses to the different plant species in the cafeteria test with combination A of plant species told a similar story as the attraction ranking, with the highest frequency of resting and probing behaviour displayed on *C. papaya* and *D. regia*. Interestingly to note, however, is that while over half of the visits by mosquitoes to *C. papaya* resulted in feeding, the mosquitoes rarely fed on *D. regia*, where most of the visits resulted in a probing response. Without further trials as well as studies where the chemical composition and nutritional content of the different plant species are taken into account, one can merely observe that the most apparent difference between the two plant species was that *C. papaya* was a fruit whereas *D. regia* mainly included flowers. However, there is reason to believe that the two responses may actually be one and the same in the sense that both result in sugar acquisition. There are many previous studies in which probing has been equated to feeding, in many cases because observations have confirmed that one leads to the other (Gary and Foster, 2006; Impoinvil et al, 2004; Manda et al, 2007a). This is further supported by the present study, where it was found that the number of mosquitoes with an abdominal distension at the end of each trial was consistent with the number of mosquitoes that had fed or probed.

Unlike the first combination of plant species, the second combination revealed no support for the presence of any discriminative feeding behaviour at the significance level tested. This deviating result appears to have been attributed to the significant reduction in activity seen among the mosquitoes over time, rather than the absence of discriminative behaviour or attractive stimuli. This reduction was explained by a negative correlation between mosquito activity and the average daily humidity and average daily sea level pressure for the area, respectively. Both combination trials experienced a reduction in activity as the onset of the rainy season drew closer and the humidity and sea level pressure increased, but this only had a significant effect on combination B – hence the inconclusive results.

Although highly indicative, the results show that the number of visits were more equally distributed among the plant species used in combination B, possibly indicating that the four plant species were more equal to one another in terms of attractive stimuli. What is interesting is that *A. indica* appears to have elicited interest equal to that of *D. regia* when tested in this four-choice assay. Because of its repellent and anti-malarial traits, *A. indica* would be expected to repel mosquitoes rather than attract them, but although the results of the picnic and the cafeteria combination B tests were indicative at best, this does still not appear to be the case. However, it is important to note that many of the studies that have demonstrated the effects of secondary compounds of *A. indica* often use extracts and derivatives of the plant rather than direct cuttings of leaves, bark or fruit (Schmutterer, 1990; Biswas et al, 2002; McKinnon et al 1997). It is therefore possible that the insecticidal effects and responses observed were greater than they would have been, had the phytophagous insects been exposed to the naturally occurring concentrations in plant cuttings. For this reason it would be interesting to investigate further the responses of mosquitoes to cuttings of this plant species. Are uninfected *An. coluzzii* attracted to *A. indica* as was indicated in the laboratory trials in this study, how does this differ from infected *An. coluzzii* females, and what are its potential effects on mosquito fitness and fecundity?

It was hypothesised that the physiological differences found between female and male mosquitoes would result in different preferences displayed for the two sexes. However, the laboratory results indicated no such difference. It is important to note, however, that the age of the mosquitoes could not be standardised during the cafeteria trials, and that the difference between the sexes was therefore assessed while entirely disregarding age. Foster and Takken (2004) previously found that females of *An. coluzzii* experience a shift in physiological state with age with a vast change in response to sugar as a result, and it is therefore possible that age may have an effect on the response of the female mosquito to the plant species present. The hypothesised difference between the sexes may therefore be present if the age of the mosquitoes is taken into account.

A study on host plant preferences of male *An. gambiae* s.l. was previously conducted in South-western Burkina Faso, performed first in the field, followed by laboratory bioassays (Gouagna et al, 2010). In their study, Gouagna et al found that males of *An. gambiae* s.l. (predominantly *An. coluzzii*) were consistently more attracted to *M. indica* than any other plant species tested, including when trials with pairs of competing plant stimuli were conducted (Gouagna et al, 2010). This is in stark contrast with the cafeteria results of this study, where males and females of *An. coluzzii* displayed little attraction to *M. indica*. Because the large quantities of ripe fruit of *M. indica* constitute an almost inexhaustible potential source of energy for newly enclosed mosquitoes at the onset of the rainy season, the results of the present study are somewhat surprising. Further investigation with more trials as well as one-choice and dual choice assays would therefore be recommended to narrow down the number of competing stimuli to determine if and why the results of the two studies differ.

Unlike the present study, the mosquitoes collected near the investigated plant species by Gouagna et al were collected from suspended clay pots rather than glue traps with plant cuttings, and although the studies were conducted during approximately the same time of the year, the authors were considerably more successful with their trap catches (Gouagna et al, 2010). The main problem that was faced when conducting the picnic test was the apparent lack of mosquitoes in the field - outdoor mosquito collections that concurred with the field trials of the present study appeared to experience a similar mosquito shortage. Because the trials were conducted at the end of the dry season during April and May 2012, it was therefore hypothesised that the apparent lack of mosquitoes was due to low population densities as a result of dry environmental conditions. However, because this study has not previously been conducted in this area or with this setup, it cannot be ruled out that the lack of mosquitoes caught in the traps was due to a faulty methodology rather than environmental factors. The three main sections of the methodology that have been identified as potential weaknesses are the trap design, the trap placement, and the assumption on which the trap location was determined. The design of the glue traps was mainly based on the traps used by Müller et al (2010) and any deviation from the original design was due to local obstacles and limitations, such as lack of material. As a result, the traps constructed were smaller and could not hold as much plant material. This may have had a two-folded effect – firstly, there was less plant material to attract mosquitoes; and secondly, there was a smaller surface area on which to catch mosquitoes. The traps also had to be placed at a considerably shorter distance from one another than those used by Müller et al (2010). While this could potentially have increased the directional stimulus at longer distances, there is the possibility that the proximity may have caused a cocktail effect of stimuli at a short distance. Furthermore, the close proximity may have increased the risk of accidental catches by neighbouring traps. The decision to place the traps between the agricultural fields – which serve as permanent breeding sites – and human dwellings, was based on the assumption that newly enclosed or oviposited mosquitoes flying from the fields may require sugar to fill up their energy reserves, and thus that these were the most appropriate locations in the villages for mosquito catches. If this was a false assumption, however, this may help to explain the low number of caught mosquitoes. It is suggested that the picnic test should be repeated at the peak of the rainy season when the environmental conditions are more favourable and the mosquitoes more abundant, to determine whether the dry conditions or methodology caused the low numbers of mosquito catches.

Time constraint was the limiting factor during the cafeteria test, making it impossible to standardise variables such as mosquito age. Furthermore, because the fieldwork of this study coincided with the shift from dry season to rainy season, the humidity and sea level pressure ended up having major effects on the performance of the mosquitoes, ultimately reducing the quality of the results for the cafeteria trials, particularly with plant species of combination B. The only factor that appeared to have an effect on the number of visits of the mosquitoes to the plants and fruit of combination B, apart from climatic factors, was the placement of the plant species in the cage. This did not affect the number of visits to the different plant species, and was likely caused by an observer bias – either because the volatiles of the observers, although separated from the mosquitoes by Plexiglas, were inadvertently attracting the females; or the plant species nearest to the observers had a visual advantage. However, the placement effect was mainly present among males, and because humans only serve as hosts for females it seems unlikely that human volatiles caused the discrepancy. However, an observer proximity bias seems similarly unlikely, since it should have been equally present among females. What is the cause for this result is therefore yet to be determined.

This study focused on how the mosquitoes responded to different plant choices, and did not go into detail about the chemical composition, nutritional benefits or the plant species' effects on mosquito fitness. The study by Manda et al (2007a), on which the cafeteria test set-up was based, was followed up by a subsequent study where the composition and concentration of sugars of the previously investigated plants was determined, along with their effects on mosquito fitness and fecundity (Manda et al, 2007b). They found that the preference ranking was for the most part similar to the survival and fitness ranking of the plants tested. However, there were cases when the mosquitoes chose to feed on plant parts that were less nutritious and caused a poor performance, although presented with more favourable and nutritious options. This led the authors to conclude that ingestion of plant material does not necessarily imply ‘feeding’, as it does not always have anything to do with obtaining nutrients (Manda et al, 2007b). This is an interesting point, as it implies that preferred plant species that attract mosquitoes do not necessarily contribute to an increased fitness – they may in fact have the opposite effect. A better knowledge about the nutritional composition, which part of the plant the mosquitoes prefer and how this affects the mosquitoes and their performance may therefore be important for the basic understanding of the local populations of the vector, and may be used in vector control efforts. Knowledge about plant-host preference also has great potential to be used more directly for trap baiting. In an Israeli study by Müller and Schlein (2006), it was found that the application of the most preferred plant species as baits in traps with insecticides resulted in an extensive mortality of the mosquito population in their study area.

It can be concluded that the environmental constraints of the dry season not only affected the adult mosquitoes in general; it also appeared to complicate the execution of the field and laboratory tests of this study considerably. Although the picnic test gave an initial indication of which plants were the most common sugar sources found during the dry season in the field, the test needs a complement of trials conducted during the rainy season, to test the robustness of the methodology. The results of the first of the cafeteria test trials, on the other hand, did indicate an attraction ranking of preferred host plants. While this result merely give us an indication of the plant preferences displayed by the mosquito population found in South-western Burkina Faso, it has the potential to be applied in vector control efforts in the region. However, the most important contribution of this study is that it functioned as a pilot study, on which further bioassays on plant host preference could be based.

The role of sugar feeding among female mosquitoes is a debated topic. While it has the potential to conflict with female fecundity, most studies agree that sugar serves many important purposes (Foster, 1995; Gary and Foster, 2001; Mostowy and Foster, 2004). But while many also agree that sugar feeding is present among females of even highly anthropophilic species such as *An. gambiae* s.s. and *An. coluzzii*, many studies (e.g. Gary and Foster, 2001; Impoinvil et al, 2004; Foster and Takken, 2004; Gary and Foster, 2004; Gary and Foster, 2006; Stone et al, 2009) still cite Beier (1996), who inferred from field observations that females of *An. gambiae* s.l. and *An. funestus* sugar feed rarely if at all. Over the last decade, several studies have demonstrated the presence of sugar host preferences among females and males of *An. gambiae* s.s. and *An. coluzzii* in different areas of Sub-Saharan Africa and the Middle East. While some studies were performed under laboratory conditions (Gary and Foster, 2004; Impoinvil et al, 2004; Manda et al, 2007a; Gouagna et al, 2010), many were performed under field conditions (Müller and Schlein, 2006; Müller et al, 2010; Gu et al, 2011;), thus decisively demonstrating an attraction of female mosquitoes to sugar sources. The present study indicates a similar pattern among anopheline and culicine mosquitoes in the field of South-western Burkina Faso, showing a preference for some plant species over others that differ among genera. This is supported by the laboratory assay, which indicates that females and males of the *An. coluzzii* population present in Vallée du Kou display a preference ranking among plant species. It can therefore be concluded, that while this study did not decisively demonstrate that sugar sources attracted female and male mosquitoes in the field, it did so in the laboratory, demonstrating that *An. coluzzii* indeed discriminates among plant species.

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