

## Isolating mechanisms among five sympatric species of *Aneilema* R. Br. (Commelinaceae) in Kenya

R. B. FADEN\*

### ABSTRACT

The reproductive biology and ecology of five non-hybridizing, sympatric species of *Aneilema* in Kenya is investigated. The high number of species at the study site is believed to be due to the ecological diversity at this locality. Differences in ecology and seasonal flowering periods appear to be inadequate to reproductively isolate these species. However, differences in daily flowering times, pollinators, floral morphology and opening behaviour, and in chromosome number, alone or in combination (perhaps also coacting with other factors not fully evaluated), are effective isolating mechanisms.

### RÉSUMÉ

MÉCANISMES D'ISOLATION PARMIS CINQ ESPÈCES SYMPATRIQUES D'ANEILEMA R. BR. (COMMELINACÉES) AU KENYA

La biologie et l'écologie de la reproduction de cinq espèces sympatriques d'*Aneilema* qui ne peuvent s'hybrider sont étudiées au Kenya. Le nombre élevé d'espèces dans le site de l'étude est dû, croit-on, à la diversité écologique de cet endroit. Les différences dans l'écologie et dans la phénologie de la floraison paraissent être inadéquates pour isoler ces espèces par voie de reproduction. Cependant, des différences dans les durées journalières de la floraison, les agents pollinisateurs, la morphologie florale et le mode d'épanouissement, ainsi que dans le nombre chromosomique, ces facteurs considérés isolément ou combinés (peut-être aussi en association avec d'autres facteurs qui n'ont pas encore été complètement établis) constituent de réelles causes d'isolement.

### INTRODUCTION

The reproductive biology of species of Commelinaceae has been little studied in the field. This may be due to some of the following: there are relatively few species in temperate regions, e.g. none is native to Europe; taxa within the family are often difficult to identify even to genus; individual flowers are short-lived, and therefore plants are frequently encountered (or overlooked) in a nonflowering condition; reports in the literature of unusual reproductive syndromes in the family are scarce and obscure. The few records in the literature, e.g. Barnes (1949), the increasingly recognized diversity in floral morphology in the family as a whole and, in particular, within such recently studied genera as *Tripogandra* (Handlos, 1970, 1975) and *Aneilema* (Faden, 1975), and the accumulating information from observations of cultivated plants (Faden, unpublished; Owens, 1981) indicate that field studies of sexual reproduction in members of this family are likely to yield interesting data. In this paper reproductive characters of five species of *Aneilema* from one locality in Kenya are considered as potential isolating mechanisms among these taxa.

### MATERIALS AND METHODS

In 1974, in the course of doing field work in Africa for a Ph.D. dissertation on *Aneilema* (Faden, 1975), we encountered five species of the genus at one locality in Kenya. Because this was the largest number of taxa found at any site, and four of the species belonged to the same section (section *Lamprodithyros*) of *Aneilema*, we decided to make

detailed observations on the floral biology of these species.

The locality is in Kenya, K7, Tana River District, Garsen-Malindi road, 1.5 km towards Malindi from the turn off to Oda, 2° 32'S, 40° 07'30"E, altitude 5-15 m. Observations and collections were made between the 22nd and 24th of July, 1974. Buds for meiotic chromosome counts were collected in a mixture of chloroform, absolute ethanol and glacial acetic acid (4:3:1, v/v). Vouchers for the plants will be found at the Missouri Botanical Garden (MO) and other institutions. A set of bees and voucher slides for the chromosome counts are in the possession of the author. Some bee collections are in the Snow Entomological Museum, the University of Kansas.

The names used herein follow Faden (1975), with unpublished names in inverted commas: *A. hockii* De Wild. (Faden & Faden 74/1182); *A. petersii* (Hassk.) C. B. Clarke subsp. *petersii* (Faden & Faden 74/1183); *A. 'indehiscens'* Faden subsp. *'indehiscens'* (Faden & Faden 74/1184); *A. 'tanaense'* Faden (Faden & Faden 74/1185); *A. 'succulentum'* Faden (Faden & Faden 74/1186).

### SITE AND HABITATS

The site straddles the present Garsen-Malindi road and the old alignment which is parallel to and east of it. Three distinct habitats occur. To the west of the new road, and abutting almost on it, is *Euphorbia robecchii*-*Commiphora boiviniana*-*Strychnos decussata* bushland (habitat 1). The soil is sandy, well-drained and there are scattered termite mounds. Some other characteristic species are *Calyptrorhiza teitensis* (Pax & Vatke) Brenan, *Ochna inermis* (Forssk.) Schweinf. and *Vitex* sp. ?

\* Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, DC. 20560, USA.

TABLE 1. — Ecological and reproductive characters of five sympatric species of *Aneilema* in Kenya

Character	<i>Aneilema hockii</i>	<i>Aneilema petersii</i> subsp. <i>petersii</i>	<i>Aneilema 'indehiscens'</i> subsp. <i>'indehiscens'</i>	<i>Aneilema 'tanaense'</i>	<i>Aneilema 'succulentum'</i>
Habitats <sup>1</sup>	2	1 & 2	1, 2 & 3	2 & 3	1 & 2
Exposure	full sun	shade or partial shade	shade or partial shade	partial shade	shade in 1, full sun in 2
Abundance	common	common in 1, locally common in 2	local & uncommon	common in 3, local & uncommon in 2	locally common
Habit	perennial	perennial	perennial	annual	perennial
Flowering condition	mature flowering, some mature fruits	mostly early flowering, no mature fruits	mostly early flowering, few mature fruits	mostly pre-flowering, few mature fruits	mostly early flowering, few mature fruits
Flower types present	pistillate, perfect, staminate	perfect, staminate (most)	perfect, staminate (few)	perfect, staminate	perfect, staminate (few)
Flower width (mm)	(18–)24–30	15–19	14,5–16	10–14,5	9–14
Paired petal colour <sup>2</sup>	very pale lavender, basal white 'V' (85D)	lilac (87D)	white	pink (84B–C, 84C)	very pale lilac (76C–D)
Flower scent	fragrant	none	none	none	none
Lateral stamen position	divergent	crossed	parallel	divergent	parallel
Lateral stamen filament length <sup>5</sup>	dimorphic	uniform <sup>6</sup>	uniform <sup>6</sup>	uniform <sup>6</sup>	dimorphic
Medial stamen pollen fertility <sup>3,4</sup>	sterile	fertile	fertile	fertile	fertile
Flowering times (h)	0615–1330	0556–1130	0600–1230	0830–1330	1150–1515
Speed of flower opening	slow	slow	slow	rapid	rapid
Lateral stamen retention <sup>1</sup>	not retained	not retained	not retained	retained	retained
Compatibility <sup>4</sup>	self-incompatible	self-incompatible (?)	self-compatible (?)	self-compatible	self-incompatible (?)
Autogamy <sup>4,7</sup>	–	– (?)	–	+	–
Chromosome number	$n = 16$	$n = 13$	$n = 26$	$n = 13$	$n = 13, 2n = 26^8$

<sup>1</sup> See text.

<sup>2</sup> Figures in parentheses are based on the *Royal Horticultural Society Colour Chart* (1966).

<sup>3</sup> Based on staining ability with cotton blue.

<sup>4</sup> Determined from populations of these taxa other than those at the study site.

<sup>5</sup> Dimorphic = lateral stamen filaments longer in staminate than in pistillate flowers (if present); uniform = lateral stamen filaments not noticeably different in length in the different flower types.

<sup>6</sup> Stamen filaments were not measured, so a very slight dimorphism could have been overlooked.

<sup>7</sup> Based on observed fruit set: autogamous (+), xenogamous (–).

<sup>8</sup> Mitotic count from plants in cultivation.

nov. (Faden & Faden 74/1191.) Two Commelinaceae, a coastal form of *Commelina africana* L. and *Cyanotis* sp. 'A' of Faden & Suda (1980), are restricted to this habitat.

East of the old alignment and somewhat removed from it is *Commiphora campestris* — *Acacia bussei* bushland (habitat 3). This habitat differs from habitat 1 both in species composition and soil type. Some distinctive species are *Thespesia danis* Oliv., *Combretum hereroense* Schinz, *Grewia tenax* (Forssk.) Fiori, *Maerua* sp. 'C' of FTEA, *Lannea greenwayi* Kokwaro and *Sansevieria powellii* N. E. Brown. The soil is a seasonally waterlogged, black cotton clay mixed with some sand. Termite mounds are absent.

Between habitats 1 and 3, and including all of the area between the two road alignments, is a greatly disturbed, mostly open habitat that was originally cleared as a right-of-way for telephone lines and is here designated as habitat 2. Some scattered shrubs and trees, e.g. *Thespesia danis*, and occasional patches of thicket occur, but the area is characterized by a great diversity of herbs which are mostly absent from the other two habitats. Examples include *Ipomoea mombassana* Vatke, *Portulaca* spp., *Endostemon tereticaulis* (Poir.) Ashby, *Chloris roxburghiana* Schult., *Abutilon guineense* (Schumacher) Bak. f. & Exell and the Commelinaceae *Aneilema hockii*, *Commelina erecta* L., *C. benghalensis* L., *C. forskalaei* Vahl, *C. imberbis* Hassk. and *C. sp. aff. erecta* L. (Faden & Faden 74/1187). The soil is similar to that of habitat 3, but with some admixture of sandy soil near the new road. A drainage line is present on the western side of habitat 2, parallel to the roads and closer to the new alignment. Termite mounds are also absent in this habitat.

#### ECOLOGICAL ISOLATION

Differences in ecology may effectively isolate sympatric species. From Table 1 it may be seen that only three of the five species occur in habitat 1 and only two of the five in habitat 3. Although all five species are present in habitat 2, *A. hockii* and *A. 'succulentum'* occur in open, sunny spots in this habitat, whereas the other three species are found in shade or partial shade. *Aneilema petersii* subsp. *petersii* grows mainly in sandy soils. It is at least as common in habitat 1 as it is in habitat 2. *Aneilema 'tanaense'*, in contrast, grows chiefly in clayey soils. It is the only *Aneilema* which is most common in habitat 3.

From these data it appears that each of the species, with the possible exception of *A. 'indehiscens'*, has a preferred habitat. The large number of *Aneilema* species at this locality is clearly due to the variety of habitats present. The occurrence of all five taxa in habitat 2 suggests that differences in habitat preference may not be sufficient in themselves to reproductively isolate species.

#### TEMPORAL ISOLATION

Species can be temporally isolated by flowering either during different seasons of the year or at

different times of the day. In this part of Kenya highly seasonal rainfall separated by long dry periods causes related species to flower at the same time of year. Some phenological differences in flowering condition were noted among the five *Aneilema* species (Table 1). *Aneilema hockii* was clearly most advanced in flowering, and all four perennials were more precocious than the annual *A. 'tanaense'*. Yet, overall, these phenological differences appeared to be slight — four of the five species had at least a few mature fruits — and seasonal differences in flowering would seem to be ineffectual as isolating mechanisms at this site.

Non-overlapping daily flowering times can very effectively isolate species. Even partial temporal separation of flowering can greatly reduce the probability of interspecific pollen transfer. Among the *Aneilema* species at this locality, there are examples of both total and partial reproductive isolation by means of differences in flowering times (Table 1; Fig. 1). The daily flowering times of *Aneilema petersii* subsp. *petersii* and *A. 'succulentum'* do not overlap at all. The flowering times of *Aneilema 'indehiscens'* and *A. 'succulentum'* overlap briefly thereby probably effectively isolating these species. The situation for *A. 'tanaense'* is more complex. Its flowering time overlaps with all of the other species, but its flowers open some two and a half hours later than the three early-opening species. Similarly, although *A. 'tanaense'* and *A. 'succulentum'* have a combined flowering period of six and a half hours, they flower simultaneously for only about one and a half hours.

#### REPRODUCTIVE ISOLATION BY POLLINATORS

Whether partially overlapping flowering times function to isolate species depends mainly upon the behaviour of the insects which visit the flowers. From Table 2 it can be seen that each of the four *Aneilema* species on which bees were observed — no other potential pollinators were noted — was visited principally by one genus (and one species?) of bees. *Aneilema hockii* and *A. petersii* subsp. *petersii* shared the same principal pollinator, whereas *A. 'tanaense'* and *A. 'succulentum'* were visited mainly or exclusively by other bee genera. *A. 'tanaense'*, however, was occasionally visited by *Amegilla*, the main pollinator of *A. hockii* and *A. petersii*, so pollen transfer among these three species would appear possible. *Aneilema 'succulentum'* shared no bee visitors, and this species was thereby reproductively isolated from the other three taxa. During the observation period, the flowers of *A. 'indehiscens'* had pollen removed from the anthers by 0850 h, but the pollinator was not observed.

#### REPRODUCTIVE ISOLATION BY FLORAL MORPHOLOGY

Differential visits by pollinators to related sympatric species may be due to differences in floral morphology. The flowers of all five species present at this locality differ among themselves in numerous attributes, such as size, petal colour, scent, position of lateral stamens at anthesis, fertility of the medial

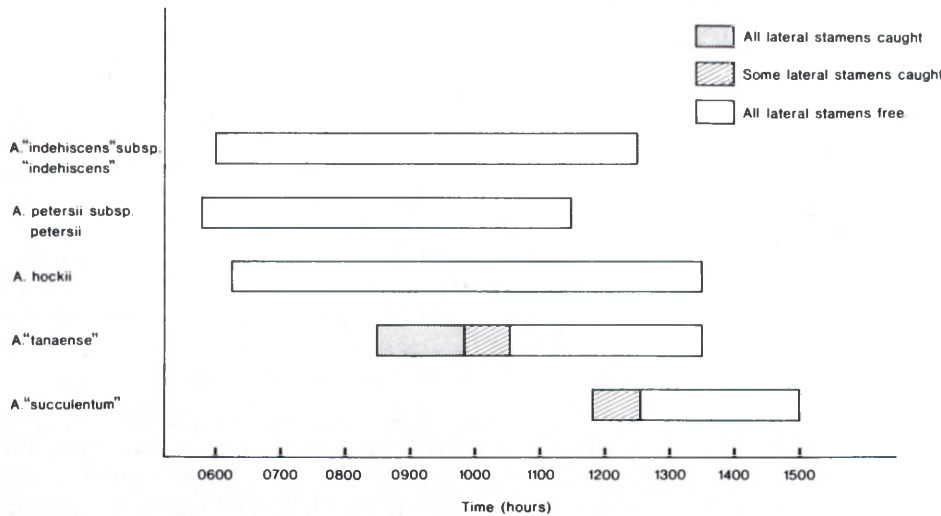


FIG. 1.—Flowering times of five sympatric species of *Aneilema* in Kenya.

stamen pollen, and the presence or absence of lateral stamen filament dimorphism in the different types of flowers (Table 1). How these various differences affect pollination are largely unknown, but a few generalizations may be made. Based on numerous observations made in 1974 of bees visiting several *Aneilema* species, including *A. hockii* and the annual subspecies of *A. petersii*, in the vicinity of Tsavo National Park East, Kenya, it appears that medium to large bees, e.g. *Amegilla*, preferentially visit flowers in which the stamens extend forwards to a considerable degree. At the present study site such species would be *A. hockii*, *A. petersii* and *A. 'indehiscens'*, which also happen to have the largest flowers. Smaller bees (< 10 mm long), e.g. *Lasioglossum*, *Nomia* and *Nomioides*, are most often observed visiting smaller-flowered species, e.g. *A. 'tanaense'* and *A. 'succulentum'*. They may also visit larger-flowered species, but in those flowers they frequently fail to make contact with the stigmas.

The position of the lateral stamens at anthesis determines the manner in which a flower can be

'worked' by visiting bees and also which part of the bee's anatomy is dusted with pollen. Larger bees visiting *A. hockii* flowers (Fig. 2.1) frequently grasp the long, divergent stamen filaments near the base and draw them together while backing towards the anthers (and usually contacting the stigma), so that the pollen of both lateral stamens can be collected simultaneously. The crossed lateral stamen filaments of *A. petersii* (Fig. 2.2) cause the pollen of these anthers to be presented in a direction different from that of all other species of the genus.

The avoidance of the flowers of certain species by particular bees may be wrongly attributed to an 'unworkable' floral morphology. It may be due instead to the opening of the flowers of completely unrelated taxa. *Amegilla* was seen visiting flowers of *Abutilon guineense* at 1216 h, just after they had opened, and also the flowers of *Plectranthus longipes* Bak. at 1640 h. This bee may not have been 'avoiding' *A. 'succulentum'* because its flowers were unattractive or difficult to manipulate, but merely because they were less attractive or rewarding than those of other available species.

TABLE 2.—Visits by bees to *Aneilema* species in a mixed colony in Kenya

Species of <i>Aneilema</i>	Bee <sup>1</sup>	Frequency of visits
<i>A. hockii</i>	<i>Amegilla</i> sp. <sup>2</sup> (Anthophoridae, Anthophorinae)	numerous
	Undetermined, uncollected bee	twice
<i>A. petersii</i> subsp. <i>petersii</i>	<i>Amegilla</i> sp.	very numerous
	<i>Xylocopa</i> or <i>Bombus</i> sp.? (not collected)	once
<i>A. 'indehiscens'</i> subsp. <i>'indehiscens'</i>	Not observed	
<i>A. 'tanaense'</i>	<i>Lasioglossum</i> sp. (Halictidae)	frequent
	<i>Amegilla</i> sp.	occasional
<i>A. 'succulentum'</i>	<i>Nomia</i> sp. (Halictidae)	numerous
	<i>Nomioides</i> sp. (Halictidae)	once

<sup>1</sup> Bees were determined only to genus.

<sup>2</sup> All specimens of *Amegilla* from this site are very similar and may well belong to a single species.

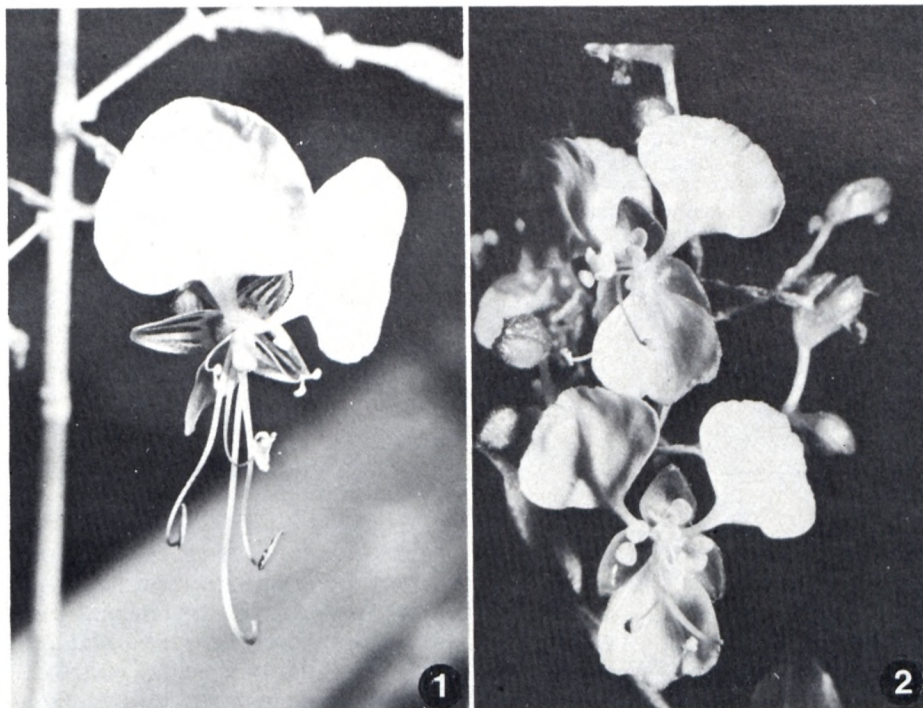


FIG. 2.—Flowers of: 1, *Aneilema hockii* De Wild.,  $\times 1.9$ , from Faden *et al.* 72/234; 2, *A. petersii* (Hassk.) C. B. Clarke subsp. *petersii*  $\times 2.3$  with staminate flower above and perfect flower below (from Andrews s.n.).

#### REPRODUCTIVE ISOLATION BY FLORAL OPENING BEHAVIOUR

If all of the pollen produced by a flower in which pollen is the sole floral reward is made available to insects at the same time, then most of that pollen may be collected soon after the flower opens, and the flower may not again be visited by potential pollinators. When the flowers open in certain species of *Aneilema* section *Lamprodithyros*, the lateral stamens are retained within the cup-shaped medial petal by its involute margins (Fig. 3.1). The margins of this petal gradually unroll and, when the margins are sufficiently far apart, the lateral stamens pop out, either singly or together (Fig. 3.2). The period of stamen retention varies from species to species. In the most extreme case, *A. calceolus* Brenan, both lateral stamens are held in the medial petals of all flowers for at least two hours, or more than half of the entire flowering period. In all cases the medial stamen is always free and available to insects from the time the flowers open.

The stamen retention mechanism may function in several ways: to increase outcrossing, to prolong the period during which the flowers are attractive to pollinators, and as an isolating mechanism. Outcrossing is promoted by the presentation of only a fraction of the flower's total pollen at a given time. This forces the insect to visit more flowers in order to obtain its needs. Attractiveness to pollinators is prolonged by the flower's pollen being presented over a period of time, so that it cannot all be collected when the flower opens. Stamen retention may serve as an isolating mechanism if any of the following are true: prolongation of pollen presentment leads to visitation by different species of bees (e.g. species which are active later) or effective temporal separation of flowering from related species; pollen presentment in smaller quantities per unit time selects for smaller bees.

Four of the five species at this locality — all but *A. hockii* — belong to section *Lamprodithyros*. Stamen retention is absent (apparently primitively)

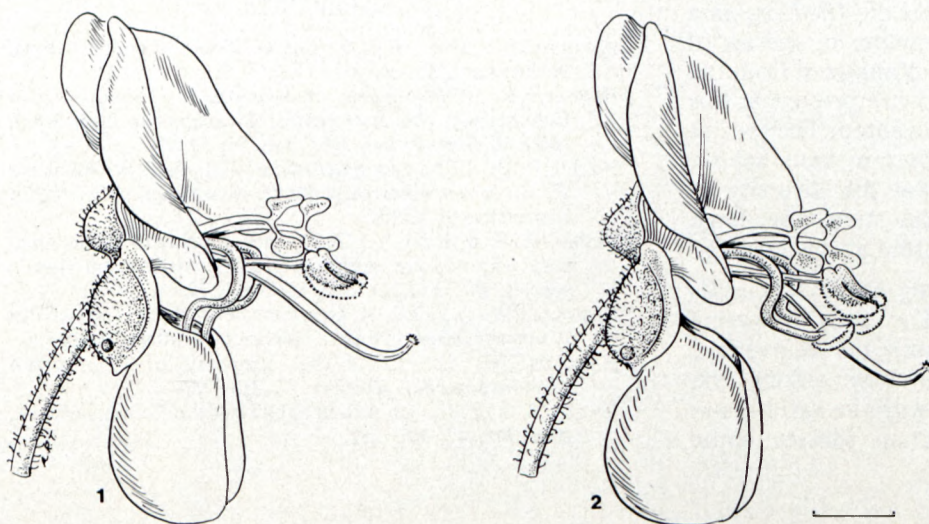


FIG. 3.—Flower of *Aneilema* 'tannaense' Faden. 1, lateral stamens retained in medial petal; 2, lateral stamens after release from medial petal. (From Faden & Faden 77/582.) The bar is 2 mm long.

TABLE 3.—Reproductive isolating mechanisms among five sympatric species of *Aneilema* in Kenya

	<i>A. hockii</i>	<i>A. petersii</i> subsp. <i>petersii</i>	<i>A. 'indehiscens'</i> subsp. <i>'indehiscens'</i>	<i>A. 'tanaense'</i>
<i>A. 'succulentum'</i>	flower size pollinator basic number	flowering time flower size pollinator	flowering time flower size ploidy	flowering time? pollinator
<i>A. 'tanaense'</i>	flower size basic number	flower size	flower size ploidy	
<i>A. 'indehiscens'</i> subsp. <i>'indehiscens'</i>	basic number ploidy	ploidy		
<i>A. petersii</i> subsp. <i>petersii</i>	basic number			

in *A. petersii* subsp. *petersii* and *A. 'indehiscens'* subsp. *'indehiscens'*, well developed in *A. 'tanaense'*, and poorly developed and seemingly disappearing — perhaps it is no longer needed? — in *A. 'succulentum'* (Fig. 1). The effect of these differences remains to be tested.

#### REPRODUCTIVE ISOLATION BY BREEDING SYSTEM AND CHROMOSOME NUMBER

Interspecific, incompatible pollen/stigma reactions can completely isolate species reproductively. Chromosomal incompatibility can also prevent hybridization. For all five species some data are available on self-compatibility, and on the presence or absence of autogamy (Table 1). Unfortunately, no crossing experiments among these species have been conducted, so it is not known whether cross-compatibility barriers exist. Chromosome numbers have been determined for all five species at this site (Table 1). Because *A. hockii* was found to differ in basic number from the other four species, and *A. 'indehiscens'* subsp. *'indehiscens'* was discovered to differ in ploidy from the other three taxa which have the basic number  $x = 13$ , it is probable that reproductive isolation occurs between these two species and the other three taxa.

#### SUMMARY AND CONCLUSIONS

The occurrence of five species of *Aneilema* at a locality in Kenya, the largest number of species of the genus found at a single site, is analysed from the viewpoints of why so many taxa are present at this spot and how hybridization is prevented. The species are found to be somewhat distinct in their habitat preferences. It is concluded that the diversity of habitats is responsible for the unusually large number of *Aneilema* species present at this locality.

Reproductive isolation among these species is achieved by various means. The daily flowering times of some taxa do not or only barely overlap. Dissimilarities in floral morphology among the species, perhaps in conjunction with the variation in flowering times, result in certain species being

visited by different genera of bees, the only pollinators observed. The variable morphological characters include the shape of the medial petal which in certain species of section *Lamprodidithyros* retains the lateral stamens for specific periods of time after the flowers open, a possible isolating mechanism. Differences in basic chromosome number or ploidy probably reproductively isolate some of the taxa. All of these characters alone or in combination, and probably others which cannot yet be evaluated, effectively isolate these five species at this locality. No hybrids were found (Table 3).

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