10 GENERAL DISCUSSION

10.1 Introduction

Hypoxis, like many other lilioid groups provides a challenge for taxonomists. The fairly uniform flower structure in the genus means that greater emphasis is placed on vegetative characters, mainly the leaves, and often these characters overlap between closely related species and thus hamper identification. In a few species, the changing appearance of leaves during the growing season leads to a fair amount of variation not always accounted for in available descriptions. More importantly, the difficulty in providing a practical key to species is due to the high level of polymorphism in the genus. Earlier works on the cytology and reproduction in *Hypoxis* point out that variability in chromosome numbers is caused through polyploidy and apomixis. Since hybridization is known to promote apomixis (Stebbins 1950), it is plausible to consider it as a further mechanism of genetic variability in the genus. Hybridization, polyploidy and apomixis in combination seems to drive speciation in *Hypoxis*. In short, the hybrid and apomictic forms derived through these phenomena end up with characters that no longer align with the original parent species. The derived character sets of these polymorphs start to obscure species limits.

The main purpose of this study was to recognise diagnostic character states for species in the richest *Hypoxis* region of the world, and in so doing provide accurate names that will form the basis for cytological, DNA and other studies within the genus and family. This Chapter presents a synopsis of the outcomes of this study in accordance with the objectives outlined in Chapter 1; the main result being a formal taxonomic treatment for southern African *Hypoxis*. A summary of the diagnostic morphological characters and a schematic representation of the infrageneric groupings described by Singh (2004) for the genus are included. As part of the discussion, relationships inferred from leaf anatomy, rhizome chemistry and seed micromorphology are compared with the infrageneric groupings. Putative hybrids involving three species of *Hypoxis* and an intergeneric hybrid with *Rhodohypoxis* in nature are discussed. This Chapter based mainly on earlier publications describes how hybridization, polyploidy and apomixis bring about chromosome variation in the genus which is in turn responsible for a fair amount phenotypic variation. Evidently, the phylogeny of such an intricately variable group cannot be resolved with certainty on external morphology alone. Cytotaxonomic and molecular studies, for instance are necessary to

establish polyploid taxa and confirm reticulate evolution in the genus. At the end of the Chapter, facets for follow up study in *Hypoxis* are suggested.

10.2 Synopsis of outcomes

10.2.1 Revision of *Hypoxis* in southern Africa

Based on the morphological species concept, 28 species of *Hypoxis* are recognised in the Flora of southern Africa region. The study succeeded in recognising macromorphological characters of diagnostic value in circumscribing species in *Hypoxis*. The most significant characters for separating species were found to include habit, arrangement of leaves from point of emergence (false stem forming or not); leaf shape, length and width, texture, number of veins raised on the upper surface of the leaf, distribution, density and types of leaf hairs; inflorescence type, diameter of open flowers, texture of tepals and stigma type. As character ranges overlap between closely related species, the characters selected for separating species work most effectively when applied in combination. Morphological characters were selected from the discussion on vegetative, and flower and fruit characters provided in Chapters 5 and 7, and presented for use in future phenetic analyses. Table 10.1 provides a list of potential diagnostic characters and their states for the southern African species of *Hypoxis*. The reason the analyses were not run in this study is that only 28 of a total of about 85 *Hypoxis* species world-wide were surveyed. The data are provided so that they can be incorporated in a monographic treatment for the group in future.

Due to the extensive field work undertaken in South Africa for the present study, variation in several common species was studied. Six species namely *H. flanaganii*, *H. floccosa*, *H. kraussiana*, *H. ludwigii* and *H. uniflorata* are known from very few herbarium specimens and although the general morphology of the species is understood, they have not been fully studied in the wild. Phenotypic variation within species in relation to dimensions of vegetative parts was considered to have little value for infraspecific classification. Only in *H. angustifolia*, two varieties are recognised with emphasis on leaf width, but it is also noted that the status of varieties in this species needs to be revisited. Species limits were expanded in some cases to incorporate slight variation in ranges of macromorphological characters like rhizome and leaf dimensions. This resulted in species like *H. cordata* Nel being considered to represent the extreme end of the range in leaf width for *H. rigidula* (Singh 2007) [Appendix 1.2]. *H. cordata* was described by Nel (1914) as a new species based on its broad leaves in comparison to the typical leaves of *H. rigidula*. Unlike leaf dimensions, density of leaf hairs was found to be extremely useful for infraspecific

Table 10.1.—Diagnostic characters and character states of *Hypoxis* in southern Africa

1	Rhizome large, yellow or orange within (0); small, white within (1)
2	Leaves clasped together forming a false stem (0); spreading upwards and outwards from base (false stem absent (1)
3	Leaves broadly lanceolate (0); linear-lanceolate (1); narrowly linear (2)
4	Leaves rigid (0); firm (1); soft (2); membranous (3)
5	Leaf indumentum absent (0); present (1)
6	Indumentum evenly spread over lamina, midrib and margins (0); dense along midrib and margins only (1)
7	Hair bifurcate (0); stellate (1); mixture of bifurcate and stellate (2)
8	Veins on upper surface almost evenly thickened (0); unevenly thickened, 1–3 veins close to margins raised on upper surface (1)
9	Leaf hairs white (0); golden yellow (1); red-brown (2)
10	Inflorescence racemose (0); corymbose (1); single-flowered (2)
11	Tepal colour yellow (0); white (1)
12	Diameter of open flowers 20 mm or more (0); 12 mm or less (1)
13	Tepal length 12–20 mm long (0); 4–15 mm long (1)
14	Stigma pyramidal (0); spherical (1)
15	Filaments and style subulate (0); filiform (1)
16	Capsule dehiscing by circumscissile split only (0); by circumscissile split and longitudinal split (1)
16	Seed smooth (0); papillate (1)
17	Papillate seeds lack secondary papillae (0); with secondary papillae (1)

classification. In *H. argentea*, *H. rigidula* and *H. sobolifera*, two varieties are distinguished on intensity of hairs. The approach of creating complexes for validly published species that are hardly distinguishable from closely related species was avoided; one such example being *H. ludwigii* which is very similar to *H. obtusa*. Such species were retained as valid and it was noted that decisions on their status should be taken following thorough population sampling combined with cytological studies.

10.2.2 Infrageneric groupings for southern African *Hypoxis*

Singh (2004) [Appendix 2.3] classified 35 species of *Hypoxis* into eight informal groups and two subgroups based on habit, leaf, inflorescence and flower characters. Representatives of each group are illustrated in Figure 10.1. For descriptions of the groups refer to Singh (2004). The allocation of species to groups remains similar to that proposed in this publication, except for a few changes based on taxonomic decisions and data from seed micromorphology. The taxonomic changes include a new species *H. nivea* added to Group 7, and the reduction of a few species (*H. cordata* = *H. rigidula*, *H. dinteri* = *H. argentea*, *H. oblonga* = *H. rigidula*, *H. obconica* = *H. hemerocallidea*, *H. limicola* = *H. parvula*, *H. patula* = *H. hemerocallidea*) to synonymy in Singh (2007) [Appendix 1.2] and in the treatment presented in Chapter 12. Seed surface characters suggest the placement of *H. kraussiana* and *H. parvifolia* in Group 2 and *H. argentea* into Group 5a. The reasons for repositioning the species are discussed in 10.2.3.

10.2.3 Data from leaf anatomy, rhizome chemistry and seed micromorphology

Leaf anatomy, rhizome chemistry and seed micromorphology in *Hypoxis* provided key characters that broadly support the groups and confirm relationships of species. From the study on internal leaf anatomy, two characters were found to be useful for grouping species, namely the thickness of mesophyll cell layers and the sclerenchyma in the inner bundle sheath. Species with membranous leaves have few (2–4) cell layers in the mesophyll, while coriaceous leaves have many more mesophyll layers, confirming differences in leaf texture. The arrangement of sclerenchyma varies depending on the species and it is useful in separating species with rigid or firm leaves from those with soft leaves. However, it is impossible to apply this character to the morphological groupings in Singh (2004).

From the chemical analysis of rhizome extracts of 14 species, three fingerprint types were recognised for *Hypoxis*: the *hemerocallidea*-type found in *H. colchicifolia*, *H. galpinii*, *H. rigidula*, *H. acuminata*, *H. hemerocallidea*, *H. obtusa*, *H. costata* and *H. multiceps* has hypoxoside as the main compound; the *filiformis*-type is present in *H. argentea*, *H. filiformis*, *H. membranacea* and *H. parvula* and has a characteristic unknown compound; and the *angustifolia*-type which has a unique combination of compounds. Species showing *hemerocallidea*-type chemical profile have robust rhizomes that are distinctly yellow or orange internally. Further, these species are related in tepal size and shape. The tepals in these species are 12–20 mm long and the inner tepals are broadly ovate and about twice as wide as the outer tepals which are linear or linear-acuminate. In contrast, species showing *filiformis*- and *angustifolia*-type profiles have delicate rhizomes that are white internally. They are further related by their tepals being 4–15 mm long, linear shaped with inner and outer tepals of almost equal width. *H. sobolifera* var. *pannosa* has *filiformis*-type profile but the rhizomes of the species are white or yellow internally. This species is however more closely related to those of the *filiformis*-type by the size and shape of its tepals. Chemical profiles confirmed the close relationship between species with robust rhizomes and large leaves of a coriaceous, firm texture (*hemerocallidea*-type) and correlates with Groups 1 to 4 in Singh (2004); those with delicate rhizomes and small coriaceous or soft leaves (*filiformis*- and *angustifolia*-type) associate well with Groups 5 to 8 (Singh 2004). In the grouping of species on morphology, *H. angustifolia* is placed with *H. membranacea* and

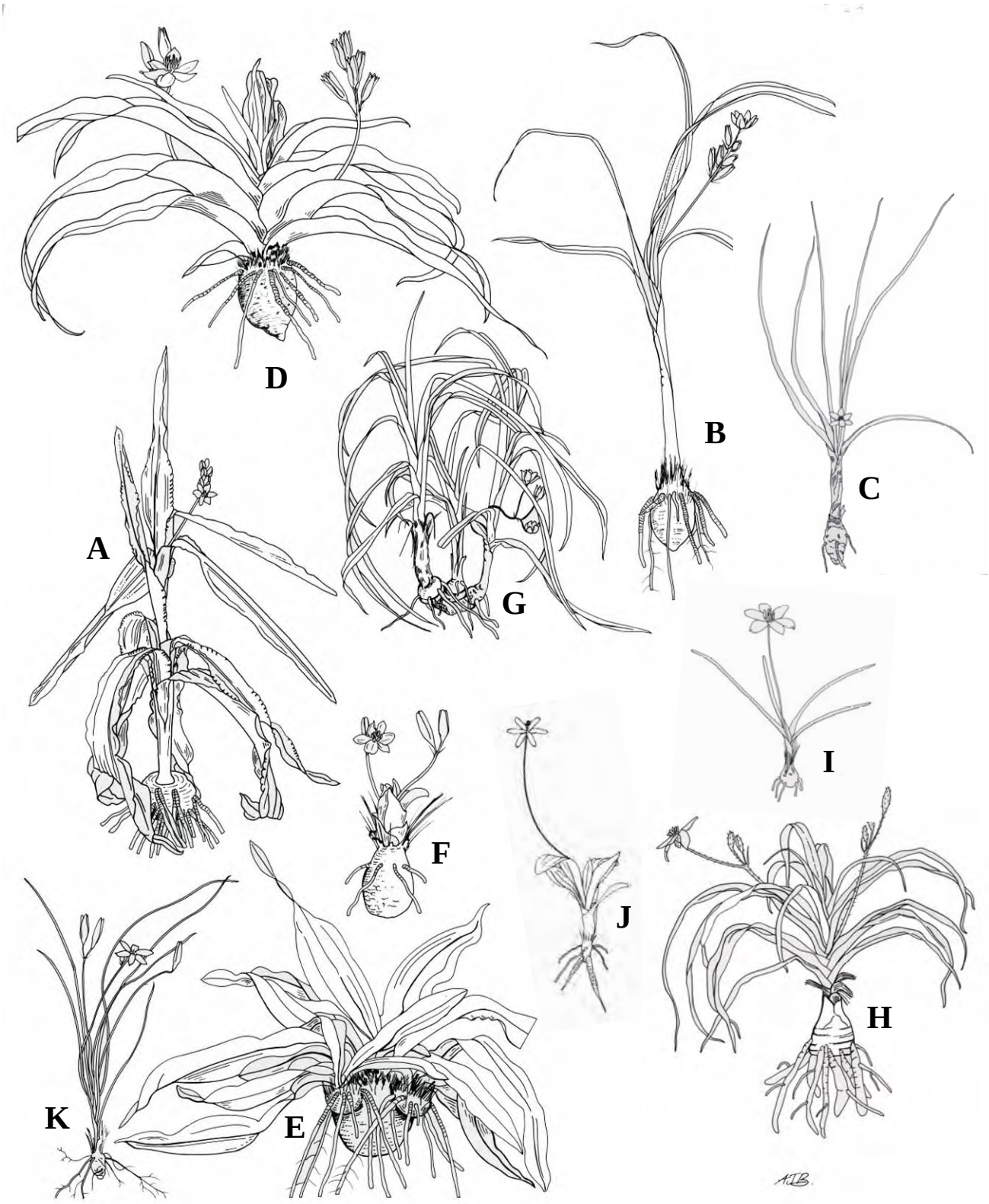

Figure 10.1—Species representing eight groups recognised for southern African *Hypoxis*. A, Group 1a, *H. colchicifolia*; B, Group 1b, *H. rigidula*; C, Group 2 *H. acuminata*; D, Group 3, *H. hemerocallidea*; E, F, Group 4, *H. multiceps*, mature and young; G, Group 5a, *H. sobolifera*; H, Group 5b, *H. stellipilis*; I, Group 6, *H. flanaganii*; J, Group 7, *H. parvula*; K, Group 8, *H. filiformis*.

H. parvula (Singh 2004), but the chemical profile suggests that it is unique in the group. However, wider chemical sampling of members of Hypoxidaceae is needed to determine the phylogenetic relationships of the different varieties of *H. angustifolia* within Group 7 and with related genera *Spiloxene* and *Empodium*, with which it shares the character of soft-textured leaves.

Seed micromorphology contributed characters useful in separating species [see Singh & Van Wyk [Appendix 1.5]. It broadly supported the phylogenetic relationships of species surmised from macromorphology (Singh 2004) but suggested the repositioning of *H. argentea*, *H. kraussiana* and *H. parvifolia*. These species were considered to be morphologically similar to members of Group 6 on the basis of their narrow leaves (usually less than 6 mm). Seed characters indicate that *H. argentea* is closer to species in Group 5a in having cuticular wings as secondary sculpturing and that *H. parvifolia* and *H. kraussiana* are related to members in Group 2 in their lack of secondary sculpturing. On revisiting the morphological characters, leaf hair and inflorescence type were found to support a closer relationship of these species with taxa in Group 5 and Group 2 respectively. *H. argentea* shares with *H. villosa* (Group 5a) the characters of long, silky hairs on leaves and corymbose inflorescences. Leaf hairs in *H. kraussiana* are similar to those of *H. longifolia* (Group 2); hairs in both species are stellate with 4–6 arms. *H. parvifolia* shares with *H. acuminata* (Group 2) the two-flowered inflorescences (sometimes 3 or 4) with flowers held on short pedicels. *H. argentea* is better placed in Group 5a and *H. kraussiana* and *H. parvifolia* in Group 2.

10.2.4 *Hypoxis* species of uncertain taxonomic status

The taxonomic status of nine species, namely *H. beyrichii* Nel, *H. exaltata* Nel, *H. jacquinii* Baker, *H. longipes* Baker, *H. mollis* Baker, *H. nigricans* Baker, *H. sagittata* Nel, *H. setosa* Baker and *H. uniflorata* Markötter remains unresolved. The problems associated with resolving these taxa are mentioned at the end of the taxonomic treatment in Chapter 12.

10.3 Evidence of hybridization, polyploidy and apomixis

During field work in South Africa, stands of vigorous plants were found among populations of *H. angustifolia* var. *buchananii*, *H. hemerocallidea*, *H. rigidula* var. *rigidula* and *H. sobolifera*. These plants were two to three times larger than the regular form in the area. The larger plants were considered to represent variation in morphology, and the ranges for these species were therefore expanded to incorporate the deviations. Although the larger forms are suspected of being polyploids, in the absence of cytological data for these populations it is not possible to link this variation to polyploidization.

10.3.1 Putative ybrids in the wild in southern Africa

In southern Africa, *Hypoxis* shows mainly sympatric patterns of distribution. In a number of single sites on farms or Nature Reserves throughout South Africa, three to five species were recorded to grow sympatrically. The sympatric species common in grasslands throughout the region are those with a wide distribution and include *H. angustifolia*, *H. argentea*, *H. filiformis*, *H. hemerocallidea*, *H. obtusa*, *H. longifolia H. multiceps* and *H. rigidula*. Though not widely distributed, *H. colchicifolia*, *H. galpinii* and *H. obliqua* may also occur sympatrically with the widespread species. During this study, possible hybrids were observed at a few sites in South Africa. Putative hybridisation was noted to be most common between *H. obtusa* and *H. rigidula* (*Singh & Govender 440*, *Singh 569*, *Singh & Wiland 662*, *773*, *Singh 605*, all in NH). Hybrids of *H. colchicifolia* and *H. rigidula* (*Singh & Govender 560* in NH) were recorded only at one site, the QwaQwa National Park in the Free State. At this site, large populations of both species overlap with each other and a number of plants were observed to display characters of both species. In Vryheid, populations of *H. colchicifolia*, *H. rigidula* var. *rigidula* and *H. argentea* were observed to grow sympatrically with no obvious hybrids between taxa. However, at the edge of the *H. rigidula* var. *rigidula* population, a group of plants typical of the species (*Singh 435* in NH) was found to have broader leaves, close to 20 mm wide. This entity was described by Nel (1914) as a distinct species, *H. cordata*, which was subsequently sunk (Singh 2007). Such examples of morphological variation may be due to hybridization between distinct species (in this case *H. colchicifolia* and *H. rigidula*) or polyploidy in the genus (see 10.3.2).

Intergeneric hybridization occurs between *Hypoxis* and *Rhodohypoxis*, although it is rare in the wild. Cultivated hybrids between the genera are being referred to as *Rhodoxis hybrida* which does not appear to be validly published and has the common name ‘Hebron Farm Pink’. Hilliard & Burtt (1978) recorded hybrids between *Hypoxis parvula* and *Rhodohypoxis baurii* (Baker) Nel and *R. milloides* (Baker) Hilliard & Burtt, and reported that crossing with the latter species is rare. Hybridization between the two genera was observed during this study at a site on the Ukhlahamba-Drakensberg Mountains at Sentinel Peak, Free State, where populations overlap (Figure 10.2A). Only one plant (*Singh 555* in NH) [Figure 10.2B] being a likely cross between *Hypoxis parvula* (*Singh 556* in NH) (Figure 10.2C) and *Rhodohypoxis baurii* (*Singh 554* in NH) [Figure 10.2D] was found. The hybrid plant (Figure 10.2B) resembles the larger plants of *Rhodohypoxis* more closely in its leaf texture and, pink and white colouration of tepals, but the tepals are free to the base as in *Hypoxis*. In the field, it was noticed that the older inflorescences on the plant did not produce fruit but were drying out without setting seed. In contrast, dehisced capsules were common among plants of the putative parents, *Hypoxis parvula* and *Rhodohypoxis baurii*.

10.3.2 High polymorphism in *Hypoxis* due to polyploidy

During the sexual cycle, organisms double their number of homologous chromosomes (ploidy) after fertilization and reduce their ploidy by half at meiosis. Polyploidy refers to the process of chromosome duplication that gives rise to multiple sets of chromosomes in an organism. The process can occur naturally in a number of ways but the two main modes for polyploidy are somatic doubling in mitosis and non-reduction in meiosis. Modes of polyploid formation have been discussed extensively by many different general works for example Grant (1971), Ramsey & Schemske (1998) and Wendel & Doyle (2005).

Polyploidy is common in mosses, ferns and angiosperms and less prevalent in liverworts and gymnosperms (Stebbins 1950; Grant 1971). In providing frequency values of polyploidy in higher plants, Grant (1971) classified species with 14 or more chromosome pairs as polyploids and those with fewer than 14 pairs as mainly diploids. For the monocot families, Goldblatt (1980) suggested a lower number; species with 11 or more chromosome pairs are polyploids and those with 9 or 10 chromosome pairs are aneuploid (having one or more extra or less chromosomes and the set is not an exact multiple of the haploid number) derivatives of ancestors with higher diploid numbers. He concluded that although needing confirmation, a relatively high percent (62%) of the 13 species surveyed for the family Hypoxidaceae, mainly belonging to *Hypoxis* had a count of 13 chromosome pairs or more, implying high polyploidy in the family. It is accepted that there is a high frequency of polyploidy in *Hypoxis* following the cytological reports by Wilsenach & Papenfuss (1967), Nordal *et al*. (1985) and Zimudzi (1994). From the chromosome records available for *Hypoxis* (Table 10.2), all species except *H. filiformis* and *H. stellipilis* are polyploids.

The development of knowledge in *Hypoxis* cytology over the years indicates high ploidy levels for species and diverse levels for some taxa, and makes a correlation between polyploidy and robustness of species. Significant reporting on chromosome numbers in the genus took place between 1955 and 1975 and counts for 11 species were published (Table 10.2), mainly through the work of Wilsenach and co-workers (1967). Counts for a further six species emerged in later years (Table 10.1). In a series of cytological papers, Wilsenach and co-workers (1967) presented data to

Figure 10.2.—Putative hybrid between *Hypoxis* and *Rhodohypoxis*. A, Plants of *Hypoxis parvula* var. *parvula* and *Rhodohypoxis baurii* growing sympatrically; B, x *Rhodoxis hybrida*; C, *Hypoxis parvula* var. *parvula*; D, *Rhodohypoxis baurii.*

support that polyploidy drives speciation in *Hypoxis* and strongly suggested the occurrence of apomixis in the genus. The first article by Wilsenach (1967) proposed a basic chromosome number of 7 or 8 for the genus and deduced that counts of 2n = 16, 32, 36, 72, and 96 represented polyploids. He concluded that it was difficult to establish a basic number for the genus and although x = 8 suited four of the species studied, it could not explain 2n = 36 for *H. multiceps*. In the follow up article, Wilsenach & Papenfus (1967) reported low chromosome numbers and normal meiosis in species of *Hypoxis* with smaller growth forms and higher chromosome numbers and abnormal meiosis in those with larger growth forms, and mention that this is consistent with the hypothesis of sexual reproduction in the smaller forms and apomixis in the larger forms. They also confirmed that there are numerous abnormalities during meiosis in the polyploid species.

Among the first 11 species listed in Table 10.2, Naranjo (1975) noted that *H. filiformis* and *H. stellipilis* were diploid in having n = 7 and 2n = 16 respectively, and the remaining species were polyploids. Based on W.D. Jackson's unpublished count for the Australian *H. pusilla*, Darlington & Wylie (1955) proposed x = 11 for *Hypoxis*. Naranjo (1975) argued that the chromosome numbers recorded for the genus do not support 11 as the basic chromosome number. He postulated that for a wide group like *Hypoxis*, four basic numbers are likely to exist namely 7, 8, 9 and 19, the last mentioned being suggested earlier by Fernándes & Neves (1962) following their counts for *H. hemerocallidea* (*H. rooperi*).

The association of higher ploidy levels and robust habit was confirmed by Nordal *et al*. (1985). These authors described in detail the chromosome kinetics for various stages of meiosis and reported on irregular chromosome separation in populations of their *H. obtusa*-complex in which they incorporated 21 species. In this article, the authors reported a correlation between extensive morphological variation and cytological variability in the *H. obtusa*-complex, which was resolved by Wiland-Symańska & Nordal (2006) to incorporate six valid species, listed in Table 10.2. Now that there is agreement that the species in the *H. obtusa*-complex are morphologically distinct (Wiland-Symańska & Nordal 2006), the chromosome counts produced by Nordal *et al*. (1985) can now be compared to determine relationships. However, the voucher specimens cited in the publication on chromosome counts (Nordal *et al*. 1985) are not recorded in the treatment for the Flora of Tropical East Africa (Wiland-Symańska & Nordal 2006). This poses a challenge to match the species with a chromosome number without studying the vouchers.

Table 10.2.—Summary of chromosome counts for species of *Hypoxis* from the literature

Species	n	2n	Authors
H. hemerocallidea (*H. rooperi*)		38, 76	Fernándes & Neves (1962)
H. pusilla		22 28	Darlington & Wylie (1955) Beuzenberg & Hair (1963)
H. stellipilis		16	Wilsenach (1967)
H. cf. *zeyheri*		32	Wilsenach (1967)
H. multiceps		36	Wilsenach (1967)
H. longifolia		72	Wilsenach (1967)
H. hemerocallidea (*H. rooperi*)	± 43-58	96	Wilsenach (1967)
H. obtusa (*H. nitida*)		48, 80	Wilsenach (1967)
H. filiformis	7		Wilsenach & Papenfus (1967)
H. acuminata	18, 20		Wilsenach & Papenfus (1967)
H. aurea		54	Mehra & Sachdeva (1971)
H. decumbens		22, 44 42	Stenar (1925) Naranjo (1975)
H. angustifolia		14, 28	Nordal *et al.* (1985)
H. goetzei		62	Nordal *et al.* (1985)
H. filiformis (*H. malosana*)		14	Nordal *et al.* (1985)
H. obtusa pro parte, *H. fischeri* pro parte, *H. gregoriana* pro parte, *H. nyasica* pro parte, *H. urceolata* pro parte, *H. rigidula* pro parte. [*H. obtusa*-complex sensu Nordal *et al.* (1985)]		40-50, 75, 92, 98, 108, 130–135, 160–200	Nordal *et al.* (1985)
H. angustifolia		28	Zimudzi (1994)
H. filiformis		14	Zimudzi (1994)
H. cuanzensis (= *H. schimperi*)		53	Zimudzi (1994)
H. galpinii		54, 55	Zimudzi (1994)
H. multiceps (= *H. bampsiana*)		70	Zimudzi (1994)
H. rigidula		56, 67, 80, 81, 87, 76	Zimudzi (1994)
H. *villosa*-complex sensu Zimudzi (1997) (= *H. obtusa* pro parte, *H. nyasica* pro parte)		41, 42, 55, 56, 70, 76, 78, 80, 85, 89, 92, 94, 96, 98, 102, 105	Zimudzi (1994)

Zimudzi (1994) provided counts for seven species of *Hypoxis* and found *H. filiformis* to be the only diploid species. He points out that the diploid form of *H. angustifolia* common to East Africa

does not occur in Zimbabwe, but that the tetraploid form noted from south-western Tanzania by Nordal *et al*. (1985) is also present in Zimbabwe. He speculated that *H. angustifolia* originated in the tropical parts of Africa and developed tetraploid forms on its migration south, and that its vigour and viability as a polyploid allows it to colonize new habitats compared to the diploid form. From his work, *H. rigidula* and *H. obtusa* were shown to have diverse chromosome numbers (Table 10.2) and their counts represent three ploidy levels namely octoploid, decaploid and dodecaploid. Zimudzi (1997) reported 16 varying counts for his *H. villosa* complex which Wiland-Symańska & Nordal (2006) recognised as *H. obtusa* and *H. nyasica*. This implies that *H. obtusa* shows high ploidy levels, similar to *H. hemerocallidea*, an allied species, and this correlates well with the extensive morphological variation recorded for the species.

Since species of *Hypoxis* with robust habit were shown to have high chromosome numbers, mostly over hexaploid levels, it suggests that diploids like *H. filiformis* and *H. stellipilis*, and those with lower ploidy levels for example *H. angustifolia* may represent species with chromosomes closest to the ancestral basic number. However, in the absence of chromosome counts for the majority of the validly recognised species, it is futile to speculate on the chromosome complements as indicators of primitive or advanced states.

10.3.3. Role of apomixis in polymorphism

Wilsenach & Papenfus (1967) concluded that high ploidy levels, irregular meiosis and morphological variation within a species points to the occurrence of apoximis (formation of seeds without fertilization in the genus). Using data from Wilsenach (1967) and Wilsenach & Papenfus (1967), Wilsenach & Warren (1967) surmised that *H. hemerocallidea* (*H. rooperi*) had the most convincing apomictic attributes, namely a high somatic chromosome number (96), abnormalities during meiosis, pollen grains are formed which contain a variable number of chromosomes (43 to 58) and populations show morphological variation. In contrast, they considered *H. filiformis* with a low chromosome number n = 7 in the group, and constant counts as representing a species with normal sexual reproduction. Following on the probability of *Hypoxis* populations being apomictic, these authors studied embryo sac development in *H. hemerocallidea* (*H. rooperi*) and *H. filiformis*. They found that in both species there is degeneration of the megaspore mother cells and the embryo sac is produced from nucellular cells which strongly suggest apomixis, even in the diploid, *H. filiformis*.

Nordal *et al.* (1985) reported on reproduction trials in two specimens each of *H. angustifolia* and their *H. obtusa*-complex. They found that all plants were autogamous, being able to produce seeds after self-pollination. These authors subjected the same plants to apomixis by emasculation and

isolation and found that all, except one belonging to the *H. obtusa*-complex, did not produce seeds without pollen. They deduced that apoximis was proved in one case at least, but the process cannot be excluded from the other tests as pollen may be required for endosperm production (pseudogamy).

Zimudzi (1994) undertook trials to test apomixis, autogamy and cross fertilization for about seven species of *Hypoxis* in Zimbabwe. His results indicated that the majority of the plants tested for apomixis produced seeds in the absence of pollen. However, the percentage seed set was lower than in plants with pollen and this led him to suggest that the plants may be pseudogamous or facultative apomicts. Plants tested by Zimudzi (1994) for autogamy and cross fertilization also produced seeds, with higher seed sets recorded for the autogamous plants. Zimudzi's trials confirmed that members of *Hypoxis* produce seed by crossing, selfing or apomixis.

Studies on embryo sac development (Wilsenach & Papenfus 1967) and reproductive traits (Nordal *et al*. 1985, Zimudzi 1994) confirm the occurrence of apomixis in a few species of *Hypoxis*.

The reason for the occurrence of apomixis in *Hypoxis* is unknown. It cannot be ascribed to pollen viability as it is recorded as being high in the genus (Nordal *et al*. 1985). However, its association with hybridization and polyploidy in bringing about speciation in *Hypoxis* is complex. Stebbins (1950) provided an in depth review on apomixis in relation to variation and evolution. He explains that polymorphism is caused in apomicts by first, hybridization between the original sexual ancestors of the apomicts; second, hybridization between facultative apomicts, or between apomicts and sexual species, and third, chromosomal and gene changes within the apomictic clones themselves. Other points from the review that help to interpret how hybridization and polyploidy may effect apomixis in *Hypoxis* include:

- Hybridization promotes apomixis by firstly the combination of genes that initiate an apomictic cycle is probably put together most easily by hybridization, either between two species or between different forms of the same species, and secondly, the hybrid genotypes have greater vigour and tolerance for a wide range of ecological conditions in comparison to the parents.
- Although apomixis can be induced in diploids by gene mutation, the action of the apomixis-inducing gene is stronger at the polyploid level.
- The tendency towards pseudogamy may be induced by increasing chromosome number.
- An obvious feature of many apomictic plants is the abnormalities in the meiotic division of both ovules and anthers.

Complexity in *Hypoxis* taxonomy relates to polymorphism most likely caused by genetic abnormalities arising from hybridization, polyploidization and apomixis. Polymorphism in the genus makes the treatment of species taxonomically unstable and decisions on whether and when to treat clones as separate species remain a vexing problem. Nordal & Kativu (1999) highlighted this as the most difficult taxonomic problem within the Hypoxidaceae. Suggestions on how some of the taxonomic complexities may be resolved are outlined in the next section.

10.4 Further research in *Hypoxis*

Wilsenach & Warren (1967) cautioned that a revision of *Hypoxis* should not be attempted until apomixis is confirmed through cytological and breeding studies, and they refer to general works on handling agamic complexes. It is tempting to agree with such a statement for a group like *Hypoxis* that displays reticulate variation in morphology, but it should be noted that such studies require critical selection of populations for testing. Critical selection of material is only possible once there is clarity on species even if the boundaries that define them are arbitrary. The greatest investment of species-level taxonomies like the present one is the collection of morphological data from extensive field and herbarium work, and the recording of variation among and between populations and clones. It is this underlying knowledge that guides sampling for research in breeding, population genetics and molecular studies of the genus. The lack of a good taxonomy for *Hypoxis* impedes research in other disciplines. The argument for a revision first is clearly one-sided for a genus where hybridization, polyploidy and apoximis are responsible for variation and drives speciation.

More research is certainly necessary to resolve the taxonomy of *Hypoxis*. For the African members, the starting point will be a cytotaxonomic study of the heterogenous and widespread species like *H. angustifolia*, *H. argentea*, *H. hemerocallidea*, *H. obtusa* and *H. rigidula*, to fully understand the mechanisms of variation. A study using heritable polymorphism and genetic markers is necessary to assess genetic variation in populations (clones) and this should be undertaken for a single species, for example *H. obtusa* initially. These studies when combined with reproductive trials will clarify whether a gene for a particular character is dominant or recessive in offspring arising from hybridization and apomixis and their subsequent generations, and its role on variation. A considerable number of studies linked with flora regions in Africa have been completed for the genus and a monograph of *Hypoxis* through a collaborative effort is inevitable. A major motivation for a monograph is to attain agreement on handling polyploid and agamic clones in the genus. The monograph will also provide a phylogeny for the largest genus of the Hypoxidaceae and should identify cryptic agamic complexes for cytological and DNA analyses.

11 CONCLUSIONS

The results of this study lead to the following principal conclusions for southern African *Hypoxis*:

- Twenty eight species of *Hypoxis* (close to 70% of the genus) occur in the Flora of southern Africa [FSA] region, undoubtedly making it the most pronounced centre of diversity and endemism for the genus world-wide.

- The most significant characters for demarcating species include habit, arrangement of leaves from point of emergence (false stem forming or not); leaf shape, length and width, number of prominent veins, distribution, density and types of leaf hairs and it is necessary to use these in combination with reproductive features like inflorescence type, diameter of open flowers, texture of tepals and stigma type for accurate identifications.

- Other characters that aid species delimitation are the unusual white colour of flowers in *H. membranacea*, *H. parvula* var. *albiflora* and *H. nivea*, rhizome size and anther tips being either entire or split.

- Geographical distribution is valuable in identifying species with restricted ranges, e.g. *H. stellipilis* is restricted to Albany Thicket along the Eastern-Western Cape boundary and *H. kraussiana* is found only in KwaZulu-Natal.

- Species can be separated broadly into two groups: robust characterised by a large habit, tough leaves and a hardy racemose inflorescence (seldom a strong corymb), and delicate species defined by small stature, flaccid leaves and fragile corymbose inflorescence (seldom an abbreviated raceme). Leaves in *Hypoxis* are coriaceous, except in *H. membranacea*, *H. parvula*, *H. angustifolia* and *H. nivea*, in which leaves are soft tending towards membranous.

- Using morphology, species can be placed in eight informal groups. Data from leaf anatomy, rhizome chemistry and seed morphology support the broad grouping of species.

- The most widely distributed species, *H. angustifolia* occurs in Africa, the west Indian

Ocean Islands and Yemen. It is extremely variable and four varieties are recognised on morphology. The species is unique in its rhizome chemistry.

- Spatial distribution and biogeographic knowledge provided in this study contributes to decisions on conservation and sustainable use of species.