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POPULATION AND GENETIC STRUCTURE OF THE WEST AFRICAN RAIN FOREST LIANA *ANCISTROCLADUS KORUPENSIS* (ANCISTROCLADACEAE)[1]

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Ancistrocladus korupensis D. W. Thomas & Gereau (Ancistrocladaceae) is a recently described liana from Cameroon. Its leaves yield the alkaloid michellamine B, which shows in vitro activity against HIV. The only known population is limited to ~ 15 000 ha within Korup National Park and its immediate surroundings. This study: (1) describes ecological patterns (geographic range, population density, stage and size class distributions, host tree characteristics, and seed dispersal patterns) of *A. korupensis*; (2) quantifies patterns of genetic variation on species and subpopulation levels and fine-scale genetic structure; (3) describes variation in michellamine B content; and (4) makes conservation recommendations based on ecological and genetic data. Ecological data from 457 individuals from seven sites indicate that the *A. korupensis* population is dominated by canopy-climbing individuals. Population densities are low with values ranging from 2.5 to 12.9 individuals/ha. Reproduction data suggest limited seed dispersal, episodic fruiting, and no vegetative reproduction. Allozyme data indicate low genetic diversity with only 7.1% of the 14 loci polymorphic. Values for H_{obs} and H_e were 0.022 (± 0.000 SE) and 0.041 (± 0.000 SE), respectively. Wright's *F* statistics analysis suggests that *A. korupensis* is highly inbred (F_{IS} = 0.455) with moderate levels of subpopulation differentiation (F_{ST} = 0.1153). Michellamine B content was best predicted by leaf type but also showed a significant relationship for stage class. The occurrence of rare, private alleles in most of the sites, low overall population size and density, and low availability of individuals for recruitment into the adult stage class are important considerations for the rational management of *A. korupensis.*

Key words: allozyme; Ancistrocladaceae; *Ancistrocladus korupensis*; Cameroon; electrophoresis; genetic conservation; HIV; Korup National Park; liana; michellamine.

Ancistrocladus korupensis (Ancistrocladaceae) is a recently described liana endemic to Southwest Province, Cameroon and neighboring Cross River State, Nigeria (Thomas and Gereau, 1993). In 1991 a novel alkaloid, michellamine B (Bringmann et al., 1993, Bringmann, 1995), displayed in vitro anti-human immunodeficiency virus (HIV) activity (Manfredi et al., 1991). The alkaloid was extracted from material initially thought to be *A. abbreviatus* but which was later determined to belong to a new species, *A. korupensis* (Thomas and Gereau, 1993). Michellamine B acts by inhibiting both the enzymatic activity of reverse transcriptase and cellular fusion for HIV I and II but also inhibits human DNA polymerases (McMahon et al., 1995). Michellamine B is also active against AZT-and pyridinone-resistant HIV strains (Boyd et al., 1994). In addition, monomeric michellamine precursors called korupensamines are active against the malarial parasites, *Plasmodium falciparum* and *P. berghei* (Hallock et al., 1994a, b; Bringmann et al., 1994b). Hobbs et al. (1996) report the stereospecific synthesis of michellamine B using totally synthesized korupensamines. Medicinal uses of Ancistrocladaceae are also reported for the Asian species, *A. extensus* (Mabberley, 1993), *A. heyneanus* (Sharma, Shukla, and Tandon, 1975), and *A. tectorius* (Ruangrungsi et al., 1985). No previous medicinal use of *A. korupensis* is known (D. Thomas, Oregon State University, personal communication).

The Ancistrocladaceae are a family of 21 palaeotropical liana species characterized by the presence of sympodial hooks (Treub, 1883; Massart, 1896; Keng, 1967; Hallé, 1973; Cremers, 1974), alternate or rosette leaves, and winged fruits. While the taxonomic position of the family is uncertain, chemical and pollen data place it next to the Dioncophyllaceae in the Violales (Erdtman, 1958;

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Bringmann, 1985; Bringmann et al., 1990). Recent analysis using *rbcL* sequences further supports this relationship (Cameron, Chase, and Swensen, 1995). Much of the work on the family has examined properties of the naphthylisoquinoline alkaloids and their synthesis (Bringmann, 1985, 1986, 1995; Bringmann et al., 1990; Bringmann, Pokorny, and Zinsmeister, 1991; Rizzacasa and Sargent, 1991a, b; Ruangrungsi et al., 1985; Sharma, Shukla, and Tandon, 1975). Ecological and systematics data have been confined to regional floras (Steenis, 1948; Ramamoorthy, 1976; Léonard, 1982, 1986), new species descriptions (Léonard, 1984; Thomas and Gereau, 1993), family overviews (Gilg, 1925; Hutchinson, 1973; Cronquist, 1981) and observations (Erdtman, 1958, Keng, 1970, Bringmann et al., 1994a).

In order to manage this potentially important liana species it is necessary to understand both the ecology and genetics of its known population. Although the terms ''population'' and ''subpopulation'' are used inconsistently in the literature, we will use Wright's (1951) convention and consider the known species distribution of *A. korupensis* in Cameroon as the population and divisions within its distribution as subpopulations. Ecological data important to the management of a plant species include: size of the population, structure of various age or stage classes, dispersal and seedling recruitment patterns, and growth habits. These data indicate whether the population is stable, the number of individuals that could be harvested without threatening the population, and growth requirements important for the successful cultivation of the species.

Genetic data are necessary for ensuring that the species' genetic integrity is maintained during propagation, cultivation, and harvesting procedures. Species-specific measurements of the level and distribution of genetic variation are important because patterns of genetic variation cannot be predicted for rare plants based on life history traits (Hamrick and Godt, 1994). In addition no genetic data have been reported for a liana making it impossible to generalize from other species. Therefore, this synthesis of ecological and genetic data is critical for the management and conservation of *A. korupensis* (Caughley, 1994).

The most common approach to quantifying the amounts and distribution of genetic variation within and among subpopulations across a species' range is the use of Wright's hierarchical *F* statistics (Wright, 1951). These statistics compare the levels of inbreeding at the individual level compared to the subpopulation (F_{IS}) and subpopulation compared to the total population (F_{ST}) (Hartl, 1988). Allozyme data are particularly amenable to these statistics, and several algorithms have been developed (Weir and Cockerham, 1984; Slatkin, 1985; Slatkin and Barton, 1989; Weir, 1990; Nei, 1972; Cockerham and Weir, 1993). To obtain more fine-scale information on gene flow at a scale below the subpopulation level, it is useful to examine the spatial distribution of individual genotypes. This fine-scale genetic structure can reveal to what degree isolation by distance (IBD) has occurred for the population. Heywood (1991) defines IBD as local deviations from the globally expected gene frequencies due to nondirected processes. Spatial autocorrelation techniques examine the deviance of individuals at given distance categories from the expected frequencies (Sokal and Oden, 1978a, b). Significant IBD patterns indicate the influences of gene flow and genetic drift on the population. Spatial autocorrelation statistics often utilize Moran's *I* directly (Dewey and Heywood, 1988; Campbell and Dooley, 1992; Sork, Huang, and Wiener, 1993) or incorporate Moran's *I* into calculations for an estimator of Wright's coefficient of coancestry, ρ_{ij} (Loiselle et al., 1995).

This study describes several aspects of the population biology of *A. korupensis* in Korup National Park, Cameroon. The major objectives are to: (1) describe the geographic range, stage and size class distributions, densities, host tree characteristics, and seed dispersal patterns of *A. korupensis*; (2) quantify the amount and distribution of genetic variation within and among subpopulations using genetic diversity measures, *F* statistics, and spatial autocorrelation statistics; (3) describe levels of michellamine B and examine ecological characters to explain patterns of variation; and (4) make conservation recommendations based on ecological and genetic data.

MATERIALS AND METHODS

Study site—We undertook this study in the southern portion of Korup National Park, Southwest Province, Cameroon (5°00′N, 8°45′E) and adjacent to its southeastern boundary. The 125 000-ha park ranges in elevation from 40 to 1 079 m. Twelve-year meteorological data from the nearby Ndian Estate indicate a mean annual rainfall of 5 382 mm with the most precipitation falling from June through September and the drier months occurring from December through February (Ministry of Plan and Regional Development, Republic of Cameroon, 1989).

Soils from *Ancistrocladus korupensis* habitat are characteristically sandy (71–89% sand), strongly acidic (pH 4.3–4.6), low in clay content (4–20%), and generally low in available phosphorus (0.002–0.005 g/kg) (Gartlan et al., 1986). Newbery et al. (1988), however, found higher levels of extractable phosphorus (0.0074–0.0136 g/kg) during the dry season for the same area. Gartlan et al. (1986) suggest that low phosphorus availability would favor tree species with ectomycorrhizal associations. Indeed, Newbery et al. (1988) found that ectomycorrhizae are associated with ten caesalpinioid genera from the low-phosphorus site. Although these genera represent only 8% of the species, they account for 28% of the basal area at the site. More recently, Newbery and Gartlan (1996) report that three ectomycorrhizal, caesalpinioid species (*Microberlinia bisulcata, Tetraberlinia bifoliolata,* and *T. moreliana*) dominate and are restricted to the nutrient-poor habitat of *A. korupensis.* Thomas and Carpenter-Boggs (1995) report a vesicular–arbuscular mycorrhizal fungi association in *A. korupensis.*

The most common tree species found in association with *A. korupensis* include *Oubangia alata* (Scytopetalaceae), *Microberlinia bisulcata* (Leguminosae), *Strephonema pseudocola* (Combretaceae), and *Cola rostrata* (Sterculiaceae) (Gartlan et al., 1986). Families represented by the highest number of species of common trees at the Ekundu Kundu site include Leguminosae, Euphorbiaceae, and Olacaceae (D. Thomas, Oregon State University, personal communication). Data from 0.1-ha transects from 11 lowland African forest sites show Korup to be the most diverse with 100 tree and 39 liana species representing 43 families ≥ 2.5 cm diameter at breast height (dbh) (Gentry, 1993).

Species description–*Ancistrocladus korupensis* D. W. Thomas & Gereau is a rain forest liana restricted to southwestern Cameroon with a single, isolated collection from neighboring Nigeria (Thomas and Gereau, 1993). Individuals occur at elevations between 40 and 160 m (Thomas and Gereau, 1993; R. Gereau, Missouri Botanical Garden, personal communication). *A. korupensis* grows free-standing until reaching

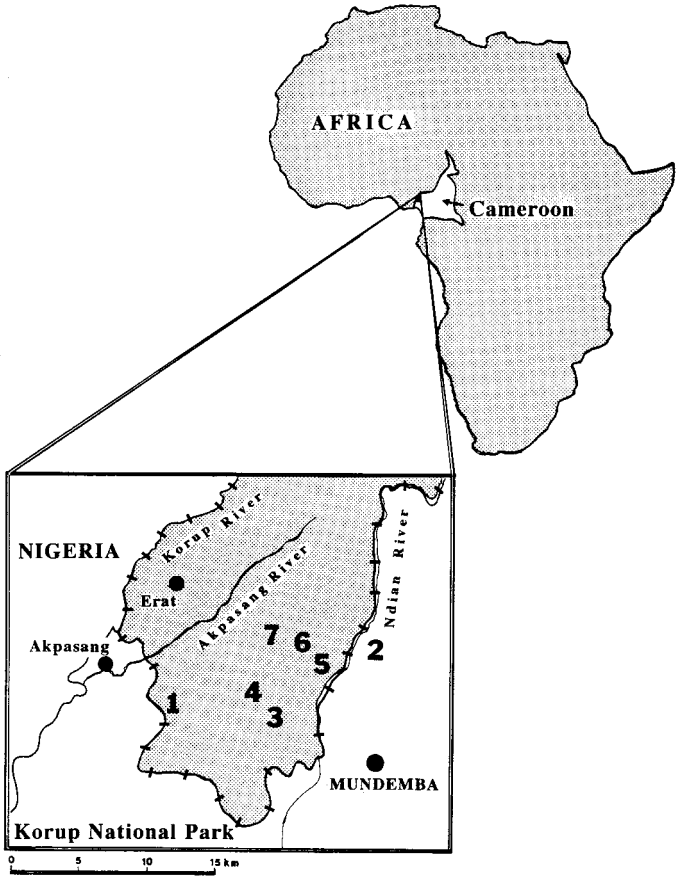

Fig. 1. Site map for Korup National Park. See Table 1 for site codes.

heights of 3–4 m when a lateral bud somewhat below the apical meristem sprouts and sends an aerial, hooked stem upwards. Once climbing, the original sapling arches over and the former apex dies back leaving a continuous, unbranched liana stem (Thomas and Gereau, 1993; P. Foster, personal observation). *A. korupensis* reroots only if the original rooting point is damaged (P. Foster, personal observation).

Large, climbing individuals have a black, rough bark, violet red cambium layer with no exudate, and are usually confined to one host tree. There are two types of leaves. Most leaf biomass consists of rosettes, or clusters of eight to ten leaves at the end of the stem. Rosette formation may be due to the accumulation of auxin in the pendant branches forming shortened internodes. Erect, climbing shoots bear simple, alternate, and much longer leaves. Free-standing saplings have similar, long leaves and are pachycaulous. Flowers are perfect with dimorphic pale yellow-green sepals; shorter, pale yellow to pink petals; ten stamens in two whorls; and three styles (Thomas and Gereau, 1993; R. Gereau, Missouri Botanical Garden, personal communication). Flowering phenology is poorly known and difficult to assess because flowering occurs within the canopy and flower parts are not deciduous. Reports from local residents indicate that adults may not flower annually (R. Gereau, Missouri Botanical Garden and D. Thomas, Oregon State University, personal communication). The characteristic five-winged fruits appear in late February on the forest floor. The fruit's wings appear to be more effective in orienting the fruit so that the radicle emerges adjacent to the ground (i.e., right side down) than dispersing the fruit over large distances (R. Gereau, Missouri Botanical Garden, personal communication).

Field collection—In April 1992 four host trees were felled in the Ekundu Kundu site and leaves of *A. korupensis* harvested for michellamine B extraction. The discovery of significant michellamine B levels in leaf litter by the National Cancer Institute in March 1993 led to the collection of fallen leaves for michellamine B extraction beginning in October 1993 by employees of the Korup Project (hereafter ''leaf collectors'') (Thomas et al., 1994). Their work led to the development of excellent search images of both the fallen leaves and liana stem as well as the discovery of several dozen individuals.

TABLE 1. Sites of *Ancistrocladus korupensis* with site numbers, abbreviations, and types of analyses.

	Site	Abbreviation	Types of analyses
1	Akpasang	AK	ecological, genetic
2	Ekundu Kundu	EK	ecological, genetic
3	Ikassa	IK	ecological, genetic
4	Last Bush	LB	ecological
5	Mana River	MA	ecological, genetic
6	Rengo Camp	RC	genetic
7	Rengo Rock	RR	ecological

Mapping of individuals and collection of material for this study began in February 1994. Seven sites were selected to represent the geographic range of the species in both known and unexplored areas (Fig. 1). Ecological data were recorded at six of the sites, while genetic material was collected at five sites (Table 1). The seven sites were: (1) Akpasang (AK), located just east of the Akpasang River (only seven individuals were collected and measured here due to the remoteness of the site); (2) Ekundu Kundu (EK), located east of Korup National Park along the Ekundu Kundu Road (this area is currently unprotected); (3) Ikassa (IK), located on the southern border of the park along the trail to Akpasang Village; (4) Last Bush (LB), located north of IK along a trail connecting the village of Ikassa Last Bush to the main east–west corridor through the Park (Transect P); (5) Mana River (MA), located parallel to the Ndian/Mana River and west of the EK site; (6) Rengo Camp (RC), located along the northern and western limit of the species' range including Chimpanzee camp; and (7) Rengo Rock (RR), located along a trail from Transect P north towards Rengo Rock. In addition, 30 individuals were randomly selected from the 2000 seedlings growing in the Korup Project nursery in Mundemba. These seedlings were grown from seeds collected in March 1993 from the EK, MA, RC, and IK sites. A sample size of 30 seedlings was selected based on limitations in freeze-drying capacity and the goal of maximizing sampling from the natural population.

Seven sites were selected throughout the entire geographic range of the plant's distribution. Within each site we wanted to maximize the geographic area covered. Therefore, we sampled by walking parallel to and within $\sim$ 100 m on either side of a trail. In order to calculate density using nearest-neighbor methods, the closest unsampled individual was always the next to be sampled. This method was the most efficient way to maximize the number of individuals sampled over the geographical range of the species. The disadvantage of this approach was that it created subpopulations with elongated shapes and distances greater than genes flowed through pollination and seed dispersal. We, therefore, used maps of the individuals at each site to create subpopulations formed from naturally occurring clusters of individuals and separated by areas where no individuals occurred.

Plant locations were mapped using a compass coordinate and distance to a known reference point (usually a kilometre reading along a path) or to a previously mapped individual. Due to security concerns raised by the Park Conservator, individuals were not permanently tagged but were numbered with a small red plastic tag attached to a tree sapling branch $\sim$ 1.5 m from the ground and within 2 m of the liana stem. We collected the following data for each individual: stem diameter, stage class, distance and compass bearing to host tree, host tree diameter at breast height (dbh), and number of *A. korupensis* seedlings within 5 m of the host tree. Because *A. korupensis* usually loops several times on

TABLE 2. Enzymes and their buffer systems used in genetic diversity survey of *Ancistrocladus korupensis*. Full names of enzymes are given in text.

	System		
	8-	11	34
Enzymes	AAT ME TPI	IDH PGM 6-PGD	ACP CE FE LAP PGI
Gel buffer ingredients	0.04 mol/L LiOH 0.029 mol/L Boric acid 0.033 mol/L Tris 0.006 mol/L Citric acid	0.009 mol/L L-Histidine HCl monohydrate	0.04 mol/L Citric acid monohydrate
Tray buffer ingredients	0.388 mol/L LiOH 0.263 mol/L Boric acid	0.4 mol/L Citric acid trisodium salt	0.3 mol/L Boric acid 0.1 mol/L NaOH
pH	7.6	7.0	8.6
Power	40 A	45–55 A	150–200 V
Run time (h)	3.0	4.5	5.5
Reference	Soltis and Soltis, 1989 (modified by J. Hamrick, University of Georgia, personal communication)	Soltis and Soltis, 1989 (modified by J. Hamrick, University of Georgia, personal communication)	Soltis and Soltis, 1989 (modified by J. Hamrick, University of Georgia, personal communication)

the ground before climbing, stem diameter measurements were taken from the point on the stem where it emerges from the ground rather than at breast height or its maximum thickness. Life stage classes were defined as: (1) free-standing $\geq$ 2 m; (2) climbing with leaf biomass in the subcanopy; or (3) climbers with leaf biomass in the canopy.

Entire rosettes of leaves (usually two to three rosettes per plant) were collected from each individual and transported back to the park headquarters in Mundemba for freeze drying. Two to three leaves per rosette were stored in plastic bags in a cooler with ice packs at the headquarters until freeze dried (not more than 3 d). Leaves were processed for freeze drying by cutting the lateral half of the leaf nearly to the midvein and labeling with a piece of paper attached with a paper clip. Leaf samples were typically 4 $\times$ 10 cm. Two leaf samples per individual were stacked neatly in the bottom chamber of a 3-L secondary vacuum trap (Labconco Corporation, Kansas City, MO). The upper chamber was filled with dry ice (transported from the Brasseries du Cameroun in Douala) and $\sim$ 20 mL of 95% ethyl alcohol. The chamber was then attached to a Maxima D4C vacuum pump (Fisher Scientific, Pittsburgh, PA), creating a bench top freeze dryer. Thermocouple temperature readings ranged from $-50°$ to $-60°$C during freeze drying. After 24 h the vacuum was released slowly, the leaves inspected, and the frozen condensation removed from the bottom of the upper chamber. Brittle leaves were removed while those that were still flexible were freeze dried for an additional 12–24 h. Freeze-dried leaves were stored in manila coin envelopes placed in resealable bags with desiccant and transported to St. Louis. In addition one leaf from each individual was air dried and sent to the National Cancer Institute, Natural Products Division, Frederick, MD for michellamine B analysis.

Locations of *A. korupensis* seedlings beneath two adults were mapped in the permanent plot of D. Newbery (University of Stirling) just west of the RR site. Each set of seedlings was mapped with a 10 m wide transect. The transect began at the parent's root and passed directly through the host tree continuing until no other seedlings were encountered. We recorded each seedling's distance along the transect, height, and number of leaves.

Electrophoresis—Freeze-dried leaves were stored in desiccant at room temperature until enzyme extraction. Extracts were prepared by grinding leaves with liquid nitrogen using a mortar and pestle. The extraction buffer consisted of 0.2 mol/L sucrose, 0.001 mol/L EDTA, 0.003 mol/L DTT, 0.005 mol/L ascorbic acid (sodium salt), 0.003 mol/L sodium bisulfite, 5% PVP-40, 0.1% β-mercaptoethanol, and 0.016 mol/L sodium phosphate buffer (Wendel and Parks, 1982, modified). This buffer was added to the pulverized leaf, the slurry allowed to absorb onto 4 $\times$ 6 mm filter paper wicks, and the wicks then stored at $-70°$C. Using standard horizontal starch-gel electrophoresis procedures (see Hamrick and Loveless, 1986; Weeden and Wendel, 1989; Wendel and Weeden, 1989; Kephart, 1990; Acquaah, 1992; Soltis and Soltis, 1989; Liengsiri, Piewluang, and Boyle, 1990), we surveyed 21 enzymes across a total of six buffer system combinations. The 11 enzymes and 14 putative loci we selected were based on clarity of resolution (name followed by abbreviation and E.C. number): aspartate aminotransferase (AAT 2.6.1.1), malic enzyme (ME 1.1.1.40), triose phosphate isomerase (TPI 5.3.1.1), isocitrate dehydrogenase (IDH 1.1.1.42), phosphoglucomutase (PGM 2.7.5.1), 6-phosphogluconic dehydrogenase (6-PGD 1.1.1.44), acid phosphotase (ACP 3.1.3.2), colorimetric esterase (CE 3.1.1.-), fluorescent esterase (FE 3.1.1.-), leucine aminopeptidase (LAP 3.4.11.1), and phosphoglucoisomerase (PGI 5.3.1.9) (Table 2).

Data analysis—We used SAS (SAS Institute, Cary, NC) to calculate descriptive statistics including: numbers of individuals in each stage class, stem diameter, and distance to and dbh of host tree across sites. An analysis of variance (ANOVA) tested the effect of stem diameter on stage class with stem diameter $\log_e$ transformed. Additional analyses of variance tested the differences between Stages 2 and 3 on distance between liana and host and dbh of the host tree. Both distance and tree dbh were $\log_{10}$-transformed to equalize variances among the stage classes (Sokal and Rohlf, 1981). We used circular statistics to test for nonrandomness in compass bearing to host tree using a Rayleigh test (Batschelet, 1981):

$$r = \frac{1}{N}\left[\left(\sum \cos \phi_i\right)^2 + \left(\sum \sin \phi_i\right)^2\right]$$

where r = the length of the mean vector, N is the sample size, and ϕ is the compass bearing to the tree. Goodness of fit for proportions among stage classes was tested using a G test (Sokal and Rohlf, 1981):

$$G = 2 \sum^{a} f_i \ln\left(\frac{f_i}{\hat{f}_i}\right)$$

where f_i = the observed frequency at the ith observation from 1 to a and $\hat{f}_i$ is the expected frequency calculated by multiplying the stage

TABLE 3. Frequences of stage classes for four sites of *Ancistrocladus korupensis.* Data include number of individuals within a stage class (*N*), their percentage within site (%), mean diameter (D, in cm), and its SE. Stage classes are: 1 = free-standing ≥2 m, 2 = subcanopy climber, 3 = canopy climber.

	Stage class											
	1				2				3			
Site	*N*	%	D	SE	*N*	%	D	SE	*N*	%	D	SE
EK	15	11.0	2.8	0.19	22	16.9	4.3	0.39	97	72.1	7.4	0.24
IK	23	23.5	3.0	0.19	12	11.8	4.0	0.26	66	64.7	8.2	0.32
LB	1	2.6	2.0	—	4	10.5	2.2	0.31	33	86.8	7.1	0.47
MA	6	7.1	1.9	0.27	10	11.8	4.0	0.54	69	81.1	7.0	0.26
Combined	45	12.7	2.8	0.23	48	13.5	4.0	0.23	265	73.7	7.4	0.14

class proportion by the total number of individuals for the site. We calculated densities from average nearest-neighbor distances using the following formula (Southwood, 1978):

$$m = \frac{1}{4r^2} \times 10\,000,$$

where m = density (number of stems per hectare) and r is the mean nearest-neighbor distance. Isolated individuals >150 m from a nearest neighbor were excluded when calculating the mean distance. These excluded individuals never accounted for >5% of the number of individuals but had a strong effect on underestimating density.

For the genetic analysis we used GeneStrut (Weir, 1990) to calculate allele frequencies, percentage polymorphic loci, average number of alleles per locus, and expected and observed levels of heterozygosity. Genetic diversity estimates at the site level were calculated from all individuals from that site including those which had not been assigned to subpopulations. The data set for overall species diversity included all wild-growing individuals and excluded the nursery sample. The nursery diversity estimates were calculated in a separate analysis.

For calculating Wright's *F* statistics, the three largest sites were subdivided into subpopulations as previously described (three within EK, three within IK, and one within MA). *F* statistics and standard errors for the *F* statistics using jackknife procedures were generated using a program suggested by Weir (1990) and modified by T. Holtsford (University of Missouri-Columbia, personal communication) using formulas from Weir and Cockerham (1984). Chi-square values testing the level of significance for F_{IS} values for each locus were calculated using the following formula:

$$\chi^2 = (F_{IS})^2 \times N \times (k - 1)$$

where N = sample size and k = number of alleles and the degrees of freedom = $k(k\text{-}1)/2$ (Li and Horvitz, 1953). The mean F_{IS} and F_{ST} values across loci were tested compared to zero using a two-tailed Student's *t* test (Sokal and Rohlf, 1981) based on the jackknife standard error values.

Fine-scale genetic structure of *A. korupensis* was examined using programs that estimate the degree of coancestry between individuals (ρ_{ij}) developed by J. Nason (University of Georgia) (Loiselle et al., 1995). These programs use a randomization procedure based on allelic frequencies to generate populations under the null hypothesis of no spatial genetic structure (Loiselle et al., 1995). We selected a 99% confidence interval for each pairwise distance class to minimize the number of spurious correlations due to the large number of classes. Values outside the confidence interval may be due to nonrandom gene dispersal through localized pollen or seed dispersal (isolation by distance) or microhabitat adaptation.

Michellamine B analysis was undertaken in the laboratory of T. McCloud (Program Resources Institute, Frederick, Maryland) where, in early 1994, a quantitative assay was developed using high-pressure liquid chromatography (HPLC) to determine percentage dry mass of michellamine B. Several sampling experiments have since been undertaken by D. Thomas (Oregon State University) as part of a cultivation feasibility project. These experiments include examining michellamine B levels across different leaf types, parts of the leaf, and within a plant. The factors examined here include leaf type, stem diameter, life stage class, and site. Leaf types were defined as follows (D. Thomas, Oregon State University, personal communication): (1) old, fallen, brown; (2) old, senescent, mottled green/yellow/brown; (3) old leaf on vine, dark green with many epiphyllae; (4) mature dark green leaf; (5) young, fully expanded leaf, pale green; (6) very young leaf, not fully expanded, pale green to +/- red. The response variable used for comparing differences among leaf type was the mean percentage dry mass of michellamine per leaf type per plant. Results of 736 separate assays were used. To test the differences in the mean michellamine B values for stage class effects the data set was limited to the most common leaf type collected (leaf type 4). To test differences among sites we limited the data set to Stage 3 individuals and leaf type 4 in order to avoid confounded effects.

RESULTS

Ecology of Ancistrocladus korupensis*—Geographic range*—Individuals were sampled from seven sites (Table 1, Fig. 1). Individuals from the RR and RC sites were well known to the leaf collectors before this study, while sites EK, MA, LB, and IK were partially known (i.e., some individuals had been located but without thorough searches for all individuals ≥ 2 m). The AK site was previously unknown. In addition, areas were searched west of the Akpasang River near the village of Erat and west to the Nigerian border without finding any *A. korupensis.* Moreover, an area 50 km south of Korup National Park along the Wangay River (4°17′55″N 8°57′58″E) was searched, and although many associated species were present (e.g., *Oubangia alata*), *A. korupensis* was not.

Stage class, stem diameter frequencies, and densities—The distribution of individuals among stage classes shows that the majority (75%) of *A. korupensis* individuals ≥ 2 m are canopy climbers and that the two lesser stage classes are nearly equal in representation (13% each) (Table 3, Fig. 2a). The *G* test results for goodness of fit for frequencies among stage classes across sites was highly significant ($G = 70.22$, df = 11, $P < 0.001$). Because the *G* test is additive (Sokal and Rohlf, 1981), subtotals for sites and life stage classes can be analyzed individually. Likewise, all seven of these separate *G* tests were highly significant ($P < 0.001$). The most common diameter class among all individuals is 4–6 cm (Fig. 2b). There are approximately half as many smaller individuals, but larger individuals decrease in frequency more gradually. Mean values for stem diameters increase

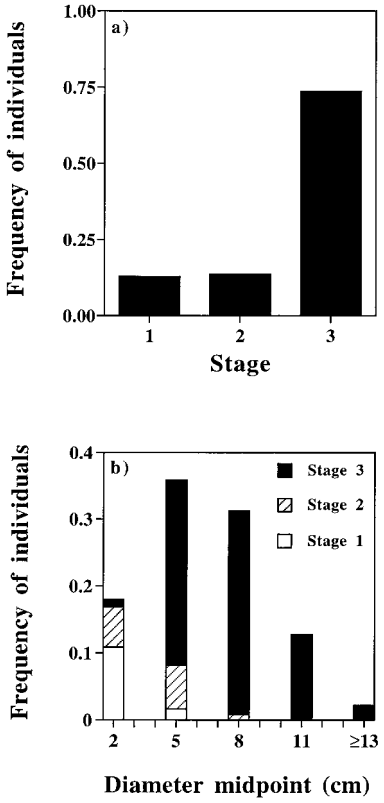

Fig. 2. (a) Frequencies of stage classes across all sites, and (b) frequencies of stem diameters for all individuals (excluding Nursery sample) grouped by stage class. Stage 1 = free-standing ≥ 2 m, 2 = subcanopy climber, 3 = canopy climber.

steadily with stage (Table 3) with significant differences among stage classes for $\log_e$-transformed diameter values (ANOVA, $F_{2, 365} = 216.9$, $P < 0.0001$).

Density estimates varied across sites (Table 4). The LB and MA sites were least dense with 2.6 and 2.5 *A. korupensis* stems of any stage classes per hectare, respectively. The EK and RR populations contained 11.29 and 12.87 individuals/ha respectively across any stage classes. The IK site was intermediate with 5.7 individuals/ha. For individual stage classes with sufficient sample sizes results were inconsistent. In the IK site the most dense stage were free-standing (Stage 1), while the canopy climbers (Stage 3) were most dense in the EK site. Thomas and Gereau (unpublished data) estimate that the total area of suitable habitat for *A. korupensis* is 10 000 ha. This estimate would give a total population size of between 25 000 and 128 700 individuals ≥ 2 m.

Liana and host tree interactions—The relationship between *A. korupensis* and its host tree differs between the two climbing stages. Stage 3 individuals are found in significantly larger trees (back-transformed means with 95% confidence interval: 26.8 cm ≤ mean dbh ≤ 30.9 cm) than Stage 2 individuals (9.4 cm ≤ mean dbh ≤ 16.8 cm) (ANOVA, $F_{1, 323} = 51.69$, $P < 0.0001$). Similarly, Stage 2 individuals were growing in trees located significantly closer to their rooted base (back-transformed means with 95% confidence interval: 1.55 m ≤ mean distance ≤ 2.56 m) than Stage 3 individuals (3.01 m ≤ mean distance ≤ 3.55 m) (ANOVA, $F_{1, 323} = 13.11$, $P < 0.001$). Results of the Rayleigh test show no deviation from randomness for orientation of the climbing tree in relation to the rooted *A. korupensis* stem for all climbing individuals (df = 333, $r = 0.082$, $P > 0.05$) nor for Stage 2 (df = 39, $r = 0.132$, $P > 0.05$) nor Stage 3 (df = 293, $r = 0.076$, $P > 0.05$) individuals analyzed separately.

TABLE 4. Estimates of densities (no. individuals/ha) of *Ancistrocladus korupensis* by stage class based on mean distances from nearest neighbor within same stage class and from nearest neighbor of any stage class. Empty cells indicate insufficient sample size to estimate density.

	Stage class			
Site	1	2	3	Any
EK	2.97	2.64	4.20	11.29
IK	3.75	0.41	2.63	5.70
LB	—	—	—	2.62
MA	—	—	2.12	2.50
RR	—	—	—	12.87

Seed dispersal—Nearly 75% of the Stage 2 and 3 individuals had five or fewer *A. korupensis* seedlings within 5 m of the host tree. However, ~ 6% of the 324 Stage 2 and 3 individuals had ≥50 seedlings beneath their host trees, indicating potentially high reproductive output (Fig. 3). Seedling distribution patterns indicate very limited dispersal. Transects show most seedlings within 10 m of the host tree (Fig. 4). Seedlings beneath Tree I averaged 14.3 (± 1.27 SE) cm tall and had 4.4 (± 0.25 SE) leaves per individual. Mean height and mean leaf number of seedlings beneath Tree II were 14.8 (± 1.12 SE) and 4.3 (± 0.14 SE), respectively.

Genetic diversity and patterns of variation—An initial screening of 20 enzymes yielded 14 putative isozyme loci and 27 alleles over 11 enzyme systems. Of the 14 putative isozyme loci identified through starch gel electrophoresis eight expressed more than one allele (Table 5). However, rare alleles accounted for most of this polymorphism, with only one locus, ACP-1, approaching a 1:1 allelic frequency ratio. Moreover, diversity was distrib-

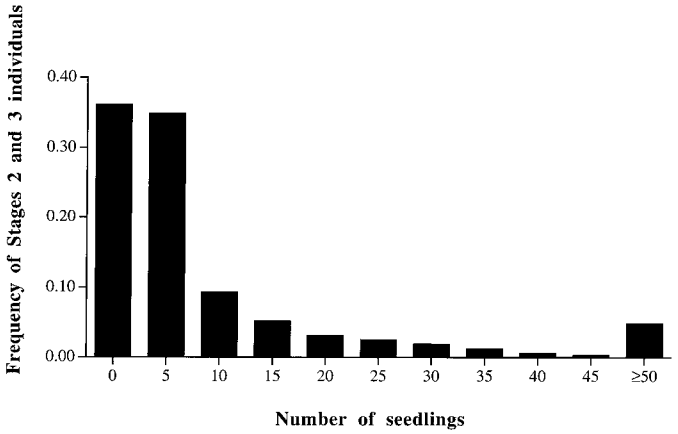

Fig. 3. Frequency of number of seedlings found within 5 m of Stage 2 and 3 individuals ($N = 324$).

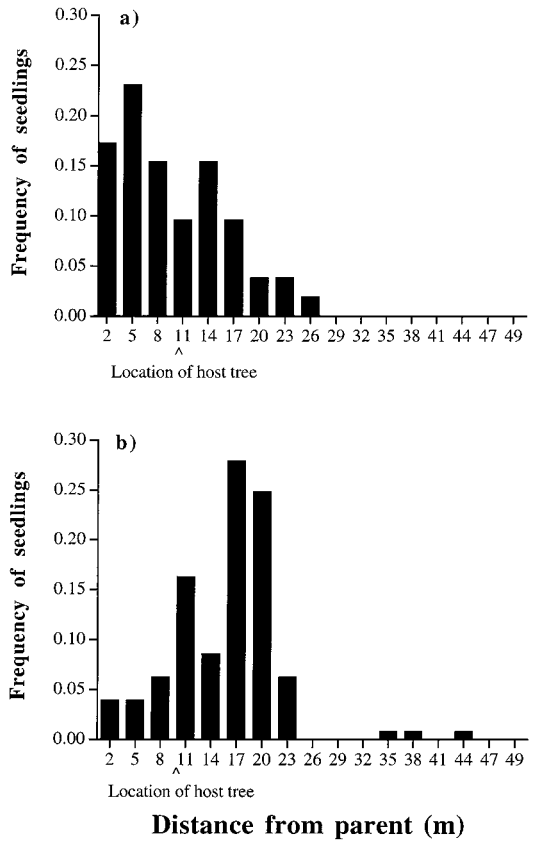

Fig. 4. Frequency of number of seedlings found along transects (a) I $N = 52$ and (b) II $N = 129$.

uted among sites with EK, IK, and MA all harboring rare, private alleles.

The low frequency of these alleles is reflected in the low percentage polymorphic values across sites (Table 6). Values of P_P ranged from 7.1 to 14.3 (one to two polymorphic loci). Variation in the number of alleles among sites is reflected in the number of alleles per locus (A_P). This variation disappears, however, when only polymorphic loci are considered (A_{PP}) except in the Nursery sample which contained all three alleles for the 6-PGD locus. Mean observed heterozygosity (H_{obs}) ranged from 0.008 in the MA site to 0.044 in the AK site. Mean expected heterozygosity (H_e) values were higher in all but the AK site and ranged from 0.033 to 0.062.

At the species level measures of genetic variation were low (Table 7). Only one of the 14 loci was polymorphic (P_P) at the 95% criterion. H_e and H_{obs} values were 0.041 and 0.022, respectively. The mean number of alleles per locus (A_P) was 1.93, while the average number of alleles per polymorphic locus (A_{PP}) was 2.0.

Wright's F statistics suggest high levels of inbreeding within subpopulations (Table 8). The unbiased, weighted mean of F_{IS}, which is a measure of the amount of inbreeding relative to the subpopulation (Hartl, 1988), is 0.4550 (Student's t, $t_7 = 5.01$, $P < 0.01$). Chi-square analysis reveals that four loci, ACP-1, FES-2, LAP-1, and ME-1, were significantly different from zero. The weighted mean of F_{ST} across all loci, a measure of differentiation among subpopulations, is moderate (0.1153) (Table 8) and significantly different from zero (Student's t, $t_7 = 6.55$, $P < 0.001$).

Patterns of fine-scale genetic variation can be revealed using spatial autocorrelation of the distribution of genotypes. One technique is to calculate ρ_{ij}, which is an unbiased estimator of Wright's coefficient of coancestry (Loiselle et al., 1995). An analysis of multilocus genotypes reveals two interplant distances (20–30 m and 260 m) at which individuals are more related than would be predicted by a random mating model (Fig. 5). Only the first peak fits well with the limited seed dispersal patterns suggested by the ecological data. The second peak does not appear to be due to low sample size. Both the number of pairs and pairs per locus values were within one standard deviation of the means for all 50 comparisons.

Michellamine B levels—Michellamine B concentration differs significantly across leaf types (ANOVA, $F_{5,353} = 43.56$, $P < 0.0001$). Concentrations are low for young, newly emerged and senescent leaves but much higher for mature leaves (Fig. 6a). Michellamine B content differed significantly among stage classes (ANOVA, $F_{2, 271} = 4.46$, $P < 0.05$, Fig. 6b). No differences were detected among the five sites (ANOVA, $F_{2, 201} = 1.53$, $P > 0.05$, Fig. 6c).

DISCUSSION

Population ecology—Except for a single collection record from Nigeria (Thomas and Gereau, 1993), *Ancistrocladus korupensis* appears to be limited to a continuous area in the southeastern corner of Korup National Park and adjacent Ekundu Kundu Road (EK site). More recent surveys by R. Gereau have failed to locate any individuals farther south than the IK site (R. Gereau, Missouri Botanical Garden, personal communication). The species ranges in elevation from 40 m in the IK site to 160 m in the northern portion of the RC site. The sites used in this study are thus likely to represent the species' complete distribution within Cameroon.

Most surprising about this study is the relative abundance of larger individuals over small. This patterns is reflected by the deficit in the first diameter size class (0–3 cm) in Fig. 2b. While part of this deficit is because individuals < 2 m were not sampled, the distribution of stage classes reflects the same situation, i.e., larger stemmed individuals are more prevalent than thinner, smaller individuals. This pattern contrasts with reports for other lianas, (e.g., Putz, 1984b in Panama; Caballé, 1984 in Gabon; and Putz and Chai, 1987 and Campbell and Newbery, 1993 in Malaysia) in which the lowest diameter size classes (e.g., < 1 cm) were always the most frequent and frequencies decreased with increased diameters. *A. korupensis* does not have this ''reverse-J-shaped curve'' for frequency of size classes, rather, this study reveals that canopy climbers (Stage 3) were much more common than Stage 1 or Stage 2 individuals.

The proportion of *A. korupensis* individuals in each stage class might represent the length of time spent in that class with Stages 1 and 2 progressing fairly quickly to the long-term Stage 3. This explanation is supported by the paucity of observations of saplings in the initial stages of climbing except in the northern portion of the

TABLE 5. Allele frequences of *Ancistrocladus korupensis* for sites, Nursery sample, and all individuals sampled from the wild. Subpopulations are indicated by subscripts. The subscript ''All'' contains all individuals sampled within the site including those not assigned a subpopulation. The ''All'' column includes all individuals growing in the wild and excludes the Nursery sample. Sample sizes appear below site/subpopulation names in parentheses.

		Site													
Locus	Allele	AK_{All} (7)	EK_1 (14)	EK_2 (38)	EK_3 (30)	EK_{All} (135)	IK_1 (33)	IK_2 (43)	IK_3 (9)	IK_{All} (100)	MA_1 (67)	MA_{All} (84)	RC_{All} (19)	Nursery (30)	All (345)
AAT-1	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AAT-2	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AAT-3	a	0.857	0.857	0.987	1.000	0.980	1.000	0.953	1.000	0.955	1.000	1.000	0.974	1.000	0.974
	b	0.143	0.143	0.013	0.000	0.020	0.000	0.047	0.000	0.045	0.000	0.000	0.026	0.000	0.026
ACP-1	a	0.167	0.214	0.057	0.119	0.161	0.045	0.430	0.250	0.293	0.200	0.259	0.368	0.283	0.234
	b	0.833	0.786	0.943	0.881	0.839	0.955	0.570	0.750	0.707	0.800	0.741	0.632	0.717	0.766
CES-1	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FES-1	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FES-2	a	0.000	0.077	0.000	0.095	0.063	0.000	0.000	0.000	0.005	0.037	0.036	0.000	0.000	0.028
	b	1.000	0.923	1.000	0.905	0.938	1.000	0.977	1.000	0.985	0.963	0.964	1.000	1.000	0.968
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.010	0.000	0.000	0.000	0.000	0.004
IDH-1	a	1.000	0.964	1.000	1.000	0.996	1.000	1.000	1.000	1.000	1.000	1.000	0.974	1.000	0.997
	b	0.000	0.036	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.003
LAP-1	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.020	0.000	0.000	0.000	0.000	0.006
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.750	0.980	1.000	1.000	1.000	1.000	0.994
ME-1	a	0.000	0.000	0.000	0.048	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003
	b	1.000	1.000	1.000	0.952	0.992	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.997
6PGD-1	a	0.000	0.000	0.000	0.000	0.000	0.016	0.070	0.188	0.051	0.000	0.000	0.132	0.017	0.027
	b	1.000	0.962	1.000	1.000	0.993	0.984	0.930	0.813	0.949	1.000	1.000	0.868	0.867	0.971
	c	0.000	0.038	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.117	0.002
PGI-1	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.001
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.990	0.993	0.970	0.974	1.000	0.988
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.024	0.000	0.000	0.006
	d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.001
	e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.006	0.000	0.000	0.003
PGM-1	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TPI-1	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

TABLE 6. Genetic diversity statistics and standard errors calculated using GeneStrut for four sites of *Ancistrocladus korupensis*. A_p = mean number of alleles per locus, A_{pp} = mean number of alleles per polymorphic locus, H_e = mean unbiased heterozygosity expected under Hardy-Weinburg equilibrium. H_{obs} = observed mean heterozygosity per locus, P_p = the percentage polymorphic loci, SE in parentheses. Loci are considered polymorphic if frequency of most common allele ≤ 0.95. Subpopulations are indicated by subscripts. The subscript "All" contains all individuals within the site including those not assigned a subpopulation.

	Site											
Statistic	AK_{All}	EK_1	EK_2	EK_3	EK_{All}	IK_1	IK_2	IK_3	IK_{All}	MA_1	MA_{All}	RC_{All}	Nursery
A_p	1.14	1.36	1.14	1.21	1.43	1.14	1.29	1.21	1.57	1.21	1.29	1.36	1.21
(SE)	(0.097)	(0.133)	(0.097)	(0.114)	(0.137)	(0.097)	(0.125)	(0.114)	(0.202)	(0.114)	(0.163)	(0.133)	(0.155)
A_{pp}	2.00	2.00	2.00	2.00	2.00	—	2.00	2.00	2.00	2.00	2.00	2.00	2.50
(SE)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	—	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.500)
H_e	0.40	0.064	0.010	0.035	0.033	0.009	0.055	0.080	0.049	0.030	0.037	0.062	0.047
(SE)	(0.045)	(0.046)	(0.024)	(0.038)	(0.038)	(0.022)	(0.051)	(0.055)	(0.046)	(0.041)	(0.045)	(0.051)	(0.049)
H_{obs}	0.044	0.041	0.006	0.024	0.018	0.009	0.025	0.080	0.032	0.002	0.008	0.038	0.029
(SE)	(0.012)	(0.012)	(0.000)	(0.008)	(0.000)	(0.000)	(0.008)	(0.022)	(0.008)	(0.000)	(0.000)	(0.012)	(0.008)
P_p	14.3	21.4	7.1	14.3	14.3	0.000	14.3	21.4	14.3	7.1	7.1	14.3	14.3
Mean N per locus	6.93	13.7	34.3	20.6	114.9	32.3	43.0	8.8	98.8	63.3	80.1	18.2	26.1
(SE)	(0.07)	(0.13)	(2.12)	(1.54)	(5.23)	(0.07)	(0.00)	(0.11)	(0.92)	(3.7)	(3.9)	(0.79)	(2.3)
Total number of alleles	16	19	16	17	20	16	18	17	22	17	18	19	17

TABLE 7. Species-level genetic diversity measures for 345 individuals of *Ancistrocladus korupensis* calculated using GeneStrut. A_s = mean number of alleles per locus, A_{sp} = mean number of alleles per polymorphic locus, H_e = mean unbiased heterozygosity expected under Hardy-Weinburg equilibrium, H_{obs} = observed mean heterozygosity per locus, P_s = the percentage polymorphic loci (SE in parentheses).

Statistic	A_s	A_{sp}	H_e	H_{obs}	P_s	Mean N per locus	Total number of alleles
	1.93	2.00	0.041	0.022	7.1	318.9	
	(0.305)	(0.000)	(0.000)	(0.000)		(6.76)	27

EK site (P. Foster, personal observation) and the episodic nature of seedling regeneration. The high proportion of Stage 3 individuals may be due to longevity in this stage. Thick-stemmed lianas (i.e., ≥ 10 cm diameter) are possibly the oldest woody plants in the forest, potentially surviving several generations of host trees, and their presence is often considered to be a strong indicator of primary vs. secondary tropical forest (A. Gentry, deceased, personal communication). Balée and Campbell (1990) found only three of 52 liana genera with individuals ≥ 10 cm dbh in a successional liana forest in Brazil.

Low frequencies for Stages 1 and 2 might, however, represent high mortality in those stages. This explanation is supported by the uniformity of the seedling height and number of leaves within the two seedling transects and the uniformity in size of seedlings counted beneath other host trees (P. Foster, personal observation). Escaping mortality, i.e., entering the next stage class could depend on transition rates controlled by uncommon frequency-dependent events (e.g., canopy openings) or density-dependent events (e.g., suitable climbing substrates).

Data from a transect in the northern portion of the EK site (D. Thomas, Oregon State University and R. Gereau, Missouri Botanical Garden, unpublished data) show similar density estimates to those reported in this study. They calculated densities of 4.8 adults (equivalent to Stages 2 and 3 from this study) per hectare in the 5.4 ha area. Densities of *A. korupensis* are lower than for other canopy lianas. Putz (1984b) found densities of canopy lianas

TABLE 8. *F* statistics based on Weir and Cockerham (1984) for eight polymorphic loci from seven subpopulations (EK_1, EK_2, EK_3, IK_1, IK_2, IK_3, and MA_1. SE estimated using a jackknife procedure (Weir, 1990).

Locus	F_{IS}	F_{IT}	F_{ST}
AAT-3	−0.0780	−0.0055	0.0673
ACP-1	0.6320***[a]	0.6860	0.1467
FES-2	0.6027***[a]	0.6066	0.0097
IDH-1	−0.0209	0.0047	0.0250
LAP-1	−0.3197***[a]	0.0437	0.2754
ME-1	1.000***[a]	1.000	0.0208
PGI-1	0.0077	−0.0017	−0.0094
6PGD-1	−0.0855	−0.0089	0.0705
Weighted mean	0.4550**[b]	−0.5178	0.1153***[c]
(SE)	(0.0908)	(0.3616)	(0.0176)

***[a] $P < 0.001$ Chi-square test for individual loci F_{IS} values (Li and Horvitz, 1953).

**[b] $P < 0.01$ Student's two-tailed *t* test. H_0: $F_{IS} = 0$.

***[c] $P < 0.001$ Student's two-tailed *t* test. H_0: $F_{ST} = 0$.

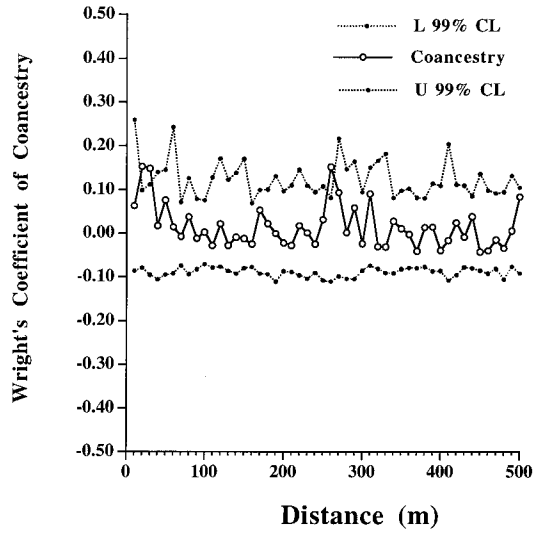

Fig. 5. Correlogram of Wright's coefficient of coancestry (ρ_{ij}) vs. 10-m interplant distances. Dotted line indicates 99% confidence intervals around null hypothesis of no spatial autocorrelation ($\rho_{ij} = 0$).

that were climbing (equivalent to Stages 2 and 3 in this study) on Barro Colorado Island, Panama ranging from 101 individuals per hectare for *Maripa panamensis* (Convolvulaceae) to three for *Anthodon panamense* (Hippocrateaceae) and *Combretum decandrum* (Combretaceae). The median value for the 18 species listed was 22 individuals/ha. However, these higher densities may be due to multiple branching and rerooting. Caballé (1994) found 16.3 individuals/ha ≥ 5 cm dbh of the clonal liana *Dalhousiea africana* (Leguminosae). Density values within stage classes could indicate the degree to which those individuals are randomly distributed. Stage class values below the density of all individuals would indicate that individuals of that size class are not clumped, while values higher than for all individuals would indicate a clumped distribution. Very low values (e.g., IK Stage 2) may indicate hyperdispersal of individuals.

Proximity to the host tree plays a key role in the growth of *A. korupensis*. Smaller *A. korupensis* individuals were found in smaller host trees and were closer to those host trees than larger *A. korupensis* individuals. It appears that individuals grow continuously into a series of progressively larger and more distant trees in a staircase fashion. As a result Stage 2 and Stage 3 individuals differ in host characteristics, a pattern that has not been observed in other liana species. Other studies have identified preferences based on host tree species. Talley, Lawton, and Setzer (1996) found host tree preferences based on tree species for the common, North American liana *Rhus radicans* (Anacardiaceae). *R. radicans* was more abundant than expected on *Carya ovata* (Juglandaceae) and *Quercus rubra* (Fagaceae) and less abundant than expected on *Juglans nigra* (Juglandaceae), *Acer saccharum* (Aceraceae), and *Sassafras albidum* (Lauraceae). Morphological characteristics of the host may be responsible for liana colonization. Putz (1984a) showed that predominantly liana-free trees have greater elasticity and larger leaves, allowing greater flexibility to sway and thus pull away from lianas colonizing from neighboring trees.

Large *A. korupensis* stems are usually coiled on the ground, indicating that they may have been shed by their host trees. On the other hand, Putz (1990) found ''excess'' stem beneath individuals of *Calamus* spp. (Palmae) and attributed it to a ratcheting mechanism whereby the climbing palm used hooked tendrils to ascend and downward slipping of the stem to avoid climbing out of the tops of host trees. *A. korupensis* may be displaying this latter strategy.

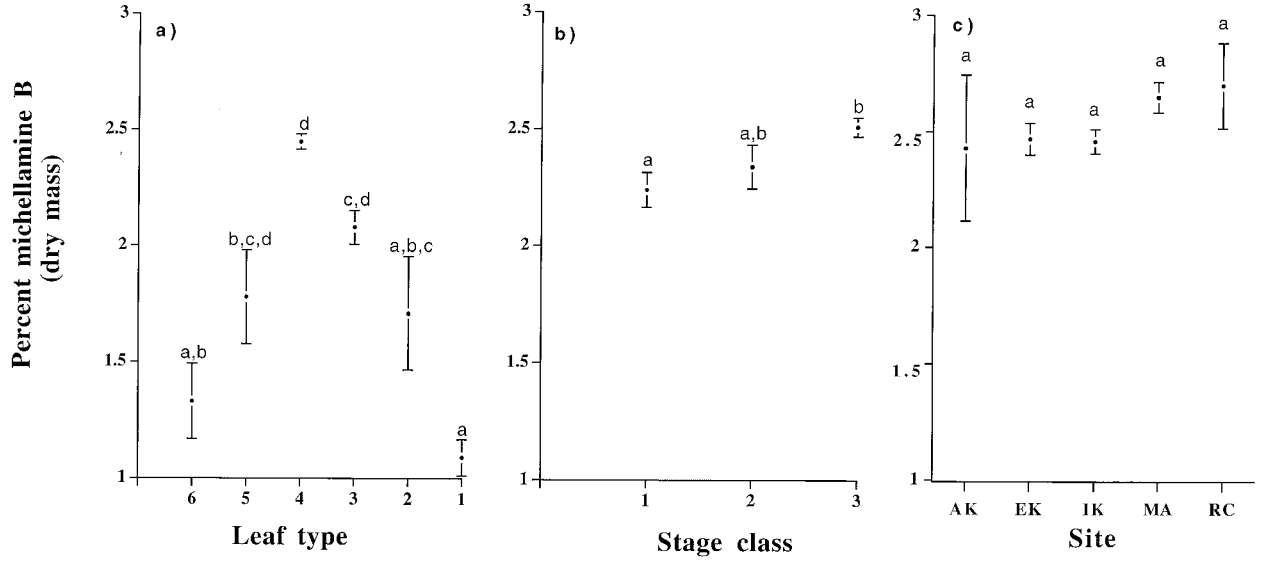

Fig. 6. Mean michellamine B dry mass concentrations by (a) leaf type, (b) stage class, and (c) site (±1 SE). Mean values with the same letter are not significantly different (α = 0.05, Tukey post hoc test). Leaf types defined as follows: 1 = old, fallen, brown; 2 = old, senescent, mottled green/yellow/brown; 3 = old leaf on vine, dark green with many epiphyllae; 4 = mature dark green leaf; 5 = young, fully expanded leaf, pale green; 6 = very young leaf, not fully expanded, pale green–(+/-) red. Stage classes: 1 = free-standing ≥ 2 m; 2 = subcanopy climber; 3 = canopy climber.

Genetic variation within and among sites—Genetic variation in *A. korupensis* is much lower than commonly found in angiosperms or other tropical species: a summary of 322 long-lived woody taxa show much higher levels of genetic variation (Hamrick, Godt, and Sherman-Broyles, 1992) with 59.5 (± 2.6 SE) percentage polymorphic loci (P_s), 2.10 (± 0.07 SE) alleles per locus (A_s), and 0.183 (± 0.011 SE) average heterozygosity (H_{es}). The 38 tropical taxa included show a similar pattern, although the effective number of alleles (A_{es}) is lower. More recently Hamrick (1994) concurred that tropical woody plants have lower levels of within-species (P_s and H_{es}) and within-population (P_p and H_{ep}) variation than do temperate woody taxa. He attributes this difference not to life history characteristics but rather to low densities of reproductive individuals for tropical species. Levels of genetic diversity in locally endemic plants are lower than in widespread taxa (Hamrick, Godt, and Sherman-Broyles 1992), a pattern further supported by this study. Similarly, Godt and Hamrick (1991) attribute high levels of variation for the perennial vine *Lathyrus latifolius* (Leguminosae) to its widespread distribution.

In addition to low population density and small geographic range as explanations of low diversity, homogeneity of soil type, rainfall, and elevation within *A. korupensis* habitat could reduce selective pressure on population differentiation since the time when speciation occurred from a common ancestor. The Korup region appears to be the center of diversity for West African Ancistrocladaceae with three allopatric species occurring in the park region. Moreover, Korup was one of three forest refugia in the Guineo-Congo Region during the Pleistocene (Newbery and Gartlan, 1996). This temporal homogeneity in forest type may have mitigated past selective pressure toward diversification in *A. korupensis.*

Another possible explanation for low levels of genetic variation is apomixis. Novak and Mack (1995) found levels of allozyme diversity similar to *A. korupensis* for an apomictic vine, *Bryonia alba* (Cucurbitaceae) ($A_p = 1.19$, $P_p = 14.9$). While apomictic species are characterized by low seed set (Murawski, 1996), a pattern reflected in the frequency of *A. korupensis* seedlings found beneath most host trees, they also have higher values of observed heterozygosity than those expected under Hardy-Weinberg equilibrium, a relationship not observed in this study.

The inbreeding coefficient among sites was high (mean $F_{IS} = 0.455$). This F_{IS} value is equivalent to full sibling mating or a mixed mating system of half sibling and self-pollination. Low adult densities could promote a high degree of self fertilization. Parker (1988) found that high levels of inbreeding in the annual vine *Amphicarpaea bracteata* (Leguminosae) were due to its cleistogamous flowers and high proportion of seeds produced from selfing. Low population densities and high inbreeding rates for *A. korupensis* support early predictions on mating systems for tropical plants (e.g., Dobzhansky 1950; Fedorov, 1966). More recent reviews (e.g., Murawski, 1996) show that outcrossing rates are high but that high inbreeding rates may be explained by low population densities. The latter is certainly true of *A. korupensis.* If we preclude selfing, the short seed dispersal distances observed in this study could be creating highly related individuals in close proximity to one another. The results of nearest-neighbor matings can create high inbreeding values like those in this study without relying on selection or other deterministic forces (Turner, Stephens, and Anderson, 1982).

The level of differentiation among subpopulations in *A. korupensis* was moderate (mean $F_{ST} = 0.1153$) and conforms to values for other tropical woody species. The average G_{ST} (the weighted mean of F_{ST} values across loci) for tropical woody species is 0.119 or 12% of genetic variation resident in each subpopulation (Hamrick, Godt, and Sherman-Broyles, 1992). Thus, the level of genetic structure of *A. korupensis* is typical of other tropical plant species. This pattern is also supported by *A. korupensis* density values. Williams (1994) found higher subpopulation differentiation and inbreeding values in low-density, patchy populations than in high-density populations of three temperate herb species.

To gain more insight about fine-scale genetic structure, it is useful to examine a genetic autocorrelation analysis. The correlogram for *A. korupensis* displayed a significant degree of relatedness within sites at distance classes of 20–30 m and 260 m . The high r_{ij} values at small distance classes are consistent with the higher F_{IS} values we observed. Loiselle et al. (1995) found significant relatedness at the small distance class in *Psychotria officinalis* (Rubiaceae), and Sork, Huang, and Wiener (1993) found significant relatedness for adult *Quercus rubra* (Fagaceae) between 5 and 20 m. In both studies the authors suggest restricted seed dispersal. Williams (1994) concurs, having found significant levels of relatedness (using Moran's *I*) for both the gravity-dispersed species, *Cryptotaenia canadensis* (Umbelliferae) (1–8 m) and animal-dispersed species, *Sanicula odorata* (Umbelliferae) (1–4 m). Campbell and Dooley (1992) also found significant levels of autocorrelation at short distances (1–4 m) in *Ipomopsis aggregata* (Polemoniaceae), less than they predicted from IBD models. While autocorrelation techniques may therefore be overestimating levels of gene flow, their F_{ST} values were inconsistent with values from this study. Knowles, Perry and Foster (1992) found conflicting autocorrelation patterns in *Larix laricina* (Pinaceae) from two anthropogenically disturbed sites. Significant levels of spatial genetic structure (5–20 m) were present at the recovered clearcut and were attributed to limited seed dispersal. Surprisingly, a regenerated field of the same age showed no spatial genetic structure. Evidence from these studies, therefore, suggests that spatial structure in *A. korupensis* is due to limited seed dispersal.

Michellamine B content—Analysis of michellamine B content reveals two predictors for estimating the alkaloid's content in *A. korupensis.* The most important factor is leaf type. Levels of michellamine B are low in young leaves, increase to peak in fully expanded leaves, and decline as leaves become covered with epiphylls and senesce. Our knowledge of liana leaves is limited, yet some results are important for the understanding and management of *A. korupensis.* Putz (1983) found a significant correlation of 0.819 between total aboveground dry biomass and stem dbh for 12 Venezuelan liana species. Lianas account for a small portion of the total forest biomass but contribute a much larger portion of the total litter biomass. Burnham (1994) found that vines and lianas represented 13–22% of the species in each litter

sample from lowland Peruvian rain forest. Hladik (1974) found that liana leaves represent 36% of the leaffall biomass for a Gabonese rain forest, suggesting that lianas have a much higher turnover rate than trees. Gentry (1983) proposes that tropical lianas have a greater ratio of litter production to wood production than tropical trees and that this explains the ''excess'' of litter production compared to temperate forests. *A. korupensis* appears to have an expectedly high leaf turnover rate as well. Individuals that were stripped of all leaves for michellamine B extraction showed all six leaf types after only 12 mo (Thomas et al., 1994). *A. korupensis* individuals found in treefall gaps covered large areas of the gap with terminal rosette-leafed branches. Erect, hooked branches with single, alternate leaves were found along the edge of the gaps. Similarly, Peñalosa (1983) found the formation of dimorphic shoot types based on light conditions.

Michellamine B content in leaves is also a function of stage class. The analysis showed that Stage 3 plants produced more michellamine B than did free-standing individuals, but Stage 2 individuals did not differ significantly from either of the other two stages. Perhaps additional light resources available to canopy individuals are responsible for the higher michellamine levels. Hladik (1978), however, found that the distribution of alkaloids did not differ between emergent and medium-sized species, between liana and tree species, nor between common and rare species in the Ipassa forest of Gabon.

Conservation of Ancistrocladus korupensis—*A. korupensis* is limited to ~ 15 000 ha with ~ 10 000 ha of suitable habitat in southwestern Cameroon. Most of this area is currently protected within Korup National Park, but the large EK site is unprotected. This site has the second highest density but is much larger in area than the RR site and therefore contains the highest number of individuals. It also contains high levels of genetic variation (P_P = 14.3) and is the only site with the ME-1-a and 6-PGD-1-c alleles. Furthermore, there is significant population differentiation among subpopulations. Habitat fragmentation within subpopulations jeopardizes rare alleles and promotes the loss of diversity through genetic drift. Despite high levels of michellamine B in Stage 3 individuals, destructively harvesting them is imprudent because there are too few Stage 1 and Stage 2 individuals to replace them. These stages each contain only approximately one-third the number of individuals of the Stage 3 class.

The genetic data suggest that variation as measured by allozymes in *A. korupensis* is very low. Such genetic homogeneity may be precarious for an economically important plant. Low levels of genetic variation may constrain adaptation to changing environments, although plants may be phenotypically plastic and respond adequately without changes in gene frequencies. Because the genetic variation occurs at such low frequencies (i.e., rare alleles), efforts should be made to protect all sites and prevent the loss of these alleles through genetic drift. Rare alleles become more threatened by loss through drift as habitats are fragmented (Hamrick, 1994). Efforts should be made to maintain the integrity of the existing population and avoid habitat destruction where *A. korupensis* grows.

In conclusion, *Ancistrocladus korupensis* is a species characterized ecologically by a small geographical range with ~ 25 000–125 000 individuals within an area of 10 000 ha. While this number of individuals is probably stable, threats due to habitat destruction are more serious. Every effort should be made to protect the Ekundu Kundu Road site to avoid the loss of this particularly important site. Stage class and diameter size distributions reflect a species that is dominated by larger individuals. This distribution indicates that harvesting wild individuals jeopardizes the stability of the species since there are too few smaller individuals to replace them. Additional ecological data show that *A. korupensis* is reproductively challenged. For example, sexual reproduction based on seedling distributions and frequencies is episodic and infrequent. Unlike other lianas (Caballé, 1977; Peñalosa, 1984), *A. korupensis* does not appear to reproduce vegetatively in the wild.

Genetic data indicate that *A. korupensis* is somewhat depauperate in genetic variation, with most sites harboring at least one rare allele. We found high levels of inbreeding and expected levels of population differentiation. Fine-scale genetic structure reveals that individuals separated by 20–30 m were more related than would be predicted using a random mating model. Chemical data indicate that the highest michellamine levels are found in mature leaves within leaf type and canopy climbers within stage class. Finally, the rational management of *A. korupensis* will require preservation of its current habitat and cultivation for leaf harvesting.

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