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Spatial distribution of mycelia of the liana (L-) biotype of the agaric *Crinipellis perniciosa* (Stahel) Singer in tropical forest

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SUMMARY

A detailed investigation of the liana biotype (L-biotype) of *Crinipellis perniciosa* (Stahel) Sing. was conducted in Ecuador. Basidiocarps of the L-biotype were consistently found in association with stems or debris of the liana *Arrabidaea verrucosa* (Standl) A. Gentry, although no symptoms associated with witches' broom disease were observed. Debris colonized by the L-biotype were often connected via pseudosclerotial pads to living stems of *A. verrucosa*. Pairings between 87 isolates obtained from basidiocarp stipe tissues or liana bark cores from three sites permitted 37 somatic compatibility groupings (SCGs) to be defined. In all cases these were found to be very restricted in geographical distribution, a situation which contrasts markedly with the distribution of SCGs in the pathogenic and non-outcrossing cocoa biotype. Mating pairings between single basidiospore isolates confirmed that the outcrossing mechanism of the L-biotype is multiallelic and that no significant biological barriers to gene flow exist between coastal and Amazonian populations. Several lines of evidence suggested that mycelia belonging to the same SCG (= genet) were isogenic. Correlations between field observations and the distributions of both SCGs and mating type factors are discussed in relation to patterns of dispersal, establishment and spread of this fungus.

Key words: *Crinipellis perniciosa*, somatic compatibility, witches' broom disease, basidiomycete mating type, fungal population biology.

INTRODUCTION

The agaric fungus *Crinipellis perniciosa* (Stahel) Sing., causal agent of witches' broom disease (WBD) of cocoa, is an important and destructive pathogen of the crop throughout tropical South America (Lass, 1985). Symptoms include the development of brooms from infected meristems, both at shoots apices and flower cushions. In addition to causing WBD on cocoa and its wild relatives in the genera *Theobroma* and *Herrania* (Sterculiaceae) (Bastos, Andebrhan & de Almeida, 1988), *C. perniciosa* has also been associated with WBD symptoms in several solanaceous hosts (Bastos & Evans, 1985) and the shrub *Bixa orellana* (Bixaceae) (Bastos & Andebrhan, 1986) in Brazil. Basidiocarps of *C. perniciosa* have also been found on both live and dead liana vines, as well as associated plant debris, in Amazonian Ecuador (Desrosiers & van Buchwald, 1949), coastal Ecuador (Evans, 1977, 1978; Hedger, Pickering & Aragundi, 1987) and Brazil (Bastos, Evans & Samson, 1981). However, unlike the other hosts, broom development has never been observed on lianas.

Attempts have been made to infect cocoa plants with basidiospores from basidiocarps formed on lianas (Evans, 1977, 1978; Hedger *et al.*, 1987; Bastos *et al.*, 1988; Purdy & Dickstein, 1990). Only mild symptoms (slight swelling and/or abnormal bark formation) were occasionally observed, although Evans (1978) was able to reisolate the fungus from some infected seedlings. Evans (1977) suggested that the liana biotype (L-biotype) might be a symptomless parasite of lianas and other hosts, while Hedger *et al.* (1987) concluded that it was a non-taxon-selective saprotroph.

Although Pegler (1978) made no mention of significant morphological differences between herbarium specimens of basidiocarps found on liana and cocoa material from Ecuador, Hedger *et al.* (1987) were able to distinguish basidiocarps of *C. perniciosa* in Ecuador from different hosts, noting that L-biotype basidiocarps were larger, more darkly pigmented, and bore stouter basidiospores and cheilocystidia than those of the cocoa biotype (C-biotype).

Previous studies of the structure of C-biotype

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populations have indicated that they consist of a comparatively small number of somatic compatibility groupings, which are geographically extensive (Wheeler & Mepsted, 1982, 1984; McGeary & Wheeler, 1988; Griffith, 1989). Such a clonal population structure is consistent with the non-outcrossing (primary homothallic) breeding strategy of the C-biotype (Delgado & Cook, 1976*a*). In contrast, Griffith & Hedger (1994) found that the L-biotype was outcrossing with a bifactorial heterothallic breeding strategy. Attempts to produce hybrids between C-biotype and L-biotype were unsuccessful (Griffith, 1989), indicating, as suggested by Hedger *et al.* (1987), that genetic exchange between the two biotypes is minimal.

The present study involved field observations in Ecuador and the collection of both mycelial and single basidiospore isolates from L-biotype basidiocarps. Mycelial isolates were paired to assess their somatic incompatibility reactions, while matings between non-sib single spore isolates (SSIs) were used to examine the distribution of mating type factors. The correlation between field and cultural studies has permitted a detailed understanding of host specificity, the dynamics of mycelial establishment and the ecological niche of the fungus.

MATERIALS AND METHODS

Field sites

Sampling was conducted at three sites in Ecuador between February and May 1987 (the rainy season in coastal Ecuador). The primary field site was a 1 km^2 area of seasonal moist tropical forest called the *Bosque Viejo* which remains within the grounds of the INIAP (*Instituto Nacional de Investigaciones Agropecuarias*) Tropical Research Station at Pichilingue in Los Rios Province (Fig. 1*a*). The floristic composition of this disturbed forest was similar to that described by Dodson & Gentry (1978) at the nearby *Rio Palenque* forest site. Most large timber trees had been removed from the *Bosque Viejo* and in places cocoa trees had been planted in the understorey some 80 years previously (Carmen Suarez, INIAP, personal communication). Annual rainfall at Pichilingue is 2000 mm (Evans, 1981), mostly falling between February and May.

A second site in Los Rios Province near the village of Jauneche (50 km south of Pichilingue; Fig. 1*b*) was visited on two occasions (3 Mar. 1987 and 26 Mar. 1987). This is a reserve of disturbed semi-deciduous tropical forest covering an area of about 1·5 km^2. Its vegetation has been studied in detail by Dodson, Gentry & Valverde (1978). The annual rainfall of this forest reserve is slightly lower (1855 mm) than that of the *Bosque Viejo* and it has a more pronounced dry season.

A single visit was made to a primary non-seasonal tropical moist forest site in Amazonian Ecuador (9 April 1987). This site was 500 m away from the San Carlos INIAP field station in Napo Province (Fig. 1*b*). The annual rainfall in this area is high (3123 mm) and distributed evenly throughout the year. No description exists of the vegetation at this site; although its floristic composition was typical of *tierra firme* forests in Western Amazonia (Richards, 1952).

Sampling procedure

Only limited sampling was conducted at the Jauneche and San Carlos field sites, where detailed notes were made of the substrata and relative positions of basidiocarps prior to their collection. In the *Bosque Viejo* at Pichilingue, sites were visited regularly at 1–5 d intervals. The positions of basidiocarps were marked with small, numbered flags and the plant substrata noted. At one site (LA), 2 cm diameter cores (1–2 cm thick) were cut from living lianas at 1 m intervals, in order to isolate mycelium of the fungus and further assess its distribution.

Isolation of C. perniciosa

Media. A novel selective medium was developed to permit isolation of mycelial cultures of *C. perniciosa* from basidiocarp tissues or infected plant material. This consisted of a basal Malt-Yeast Extract Agar (MYEA; see below) medium made with double strength agar (3 %). After autoclaving (15 min, 121 °C), the following filter-sterilized solutions were added to the cooled, molten medium: 10 ml l^{-1} of 1 % benomyl (Benlate, Zeneca, Bracknell, UK), 1 ml l^{-1} of o-phenyl-phenol (2-hydroxybiphenyl, Sigma, Poole, UK; 3 % (w/v) ethanolic stock solution) and 10 ml l^{-1} of an antibiotic cocktail (2 mg ml^{-1} each of streptomycin sulphate [Glaxo Ltd., Middlesex, UK], chlorotetracyclin [Cyanamid Ltd., Gosport, UK], benzylpenicillin [Glaxo] and ampicillin [Sigma]). Routine subculturing was conducted on MYEA (3 % dark malt extract powder, 0·5 % yeast extract, 1·5 % agar [all Lab M Ltd., Bury, UK]).

Isolation from fruit body tissue. Isolation from basidiocarps was conducted within a few hours of picking from the field. Stipes dissected away from the pilei were surface sterilized by dipping in ethanol and briefly flamed. Small pieces of stipe tissue (1–2 mm^3) were cut with a sterile scalpel and plated onto the selective medium (5 pieces per plate). The plates were incubated inverted at 25°C until the distinctive white, fluffy mycelium of *C. perniciosa* had grown onto the medium. The mycelial outgrowths were then subcultured onto MYEA after 4–8 d.

Isolation from liana bark tissue. Having removed the outer flaky bark, bark cores were cut into several

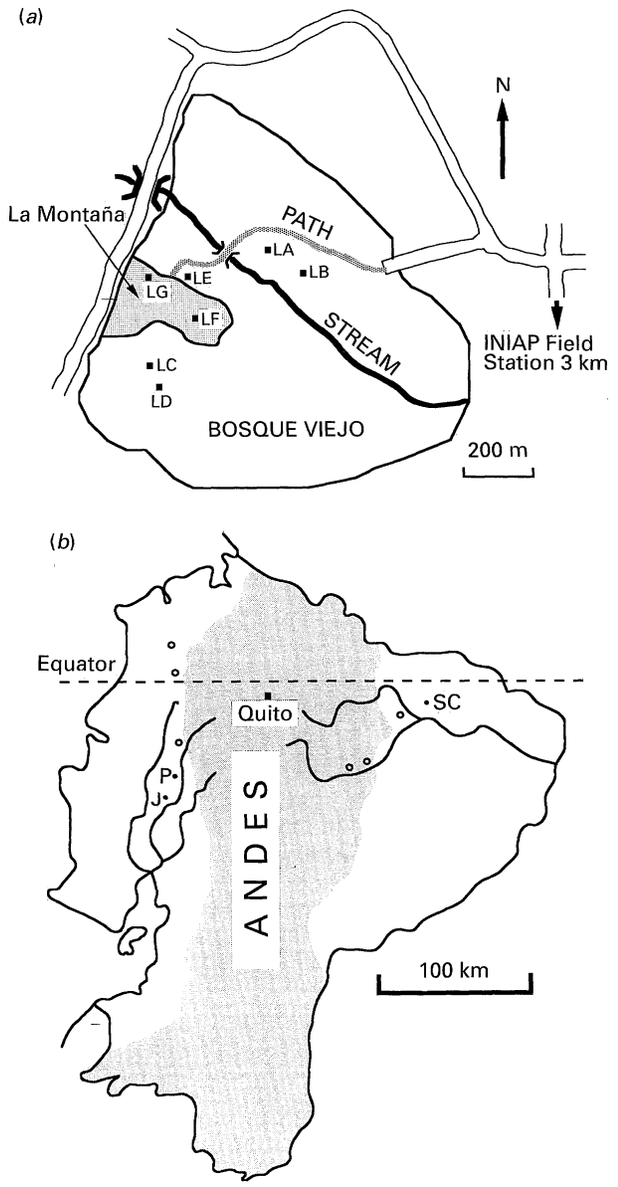

Figure 1. (*a*) Map of the *Bosque Viejo* wood at Pichilingue. The locations of the seven sites (labelled LA to LG) in the *Bosque Viejo* at which basidiocarps of the L-biotype were found are shown. The *La Montaña* area of the wood had previously been cleared and planted with cocoa, as described in the text. (*b*) Map of Ecuador showing sampling sites. The location of the forest sites (●) at which basidiocarps of the L-biotype were found (Pichilingue, P; Jauneche, J; San Carlos, SC) are shown. A further six sites which were also visited, but at which no basidiocarps were found, are labelled (○). The approximate extent of the Andean mountain range is indicated by shading.

pieces (about 0·5 cm^3), surface sterilized as above and plated on the selective medium. Incubation and subculturing was conducted as above.

Isolation from single basidiospores. Basidiocarps were either collected in the field or produced artificially on a bran-vermiculite medium from cultures of the fungus, as described by Griffith & Hedger (1993). Fresh basidiocarp pilei were stuck to the lid of a petri dish with Vaseline and left overnight at 25 °C to deposit a spore print onto a Petri dish of 1·5% tap water agar. Spores from the prints were streaked on MYEA. After 3–5 d incubation at 25 °C, single, well-spaced germlings were picked off and subcultured on MYEA to obtain single basidiospore isolates (SSIs), as described by Griffith & Hedger (1994).

Mating pairings

Matings were conducted as described by Griffith & Hedger (1994). Plugs (5 mm diameter) of each of the two SSIs to be mated in each cross were placed 2 cm apart on a 9 cm petri dish of MYEA and incubated inverted at 25 °C for 4 wk. Plates were sealed with insulating tape to reduce both water loss and the possibility of contamination. After 3 wk incubation and subsequently at weekly intervals, 5 mm plugs were taken from the line of contact between the two colonies, and from 1–2 cm either side of this, and subcultured onto a fresh plate of MYEA. After 3–5 d incubation at 25 °C, 7 mm plugs were taken from the margins of these colonies and examined directly with a 40× objective. The presence/absence of clamp connections and the general morphology of the mycelium was recorded.

Somatic incompatibility pairings

The expression of somatic incompatibility between mycelial isolates of *C. perniciosa* was assessed by plating isolates derived from stipe or liana bark tissue on 20% clarified V-8 juice medium (2CVJA; Aragundi, 1982). Fifteen g $CaCO_3$ was added to 1 l V-8 juice (Campbell's Soups Ltd., Norwich, UK), stirred vigorously for 5 min and centrifuged at 10000 ***g*** for 10 min. Two hundred mls of clarified V-8 juice and 20 g agar was made up to 1 l and autoclaved (15 min, 121 °C). Inoculum plugs (5 mm diameter) from four or five different isolates were paired on 9 cm vented Petri dishes containing 25 ml medium; dishes were sealed with insulating tape and incubated inverted for up to 8 wk.

RESULTS

Field distribution of basidiocarps

Over 130 basidiocarps were observed at seven separate sites in the *Bosque Viejo* (denoted LA to LG in Fig. 1 *a*) during the three month sampling period. Basidiocarps were generally produced in flushes, usually 1–3 d after the end of a period of dry weather. At five of the sites (LA, LB, LC, LD, LE) basidiocarps were found on, or closely associated with, living and dead stems of the liana species *Arrabidaea verrucosa* (Standl) A. Gentry (Bignoniaceae). Identification of this liana on the basis of leaf and stem characters (in the absence of flowers) was confirmed by C. Dodson and A. Gentry (personal

Figure 2. (*a*) Part of site LA at Pichilingue. Portions of lianas L1 and L2 from the central portion of site LA are shown. Note the dead but attached sidebranch on liana L1. Five basidiocarps were collected from this sidebranch (as indicated by the flags), although pure cultures from mycelia from stipe tissues were only obtained in three cases (all scgA). (*b*) A view from the north of site LC at Pichilingue. Lianas of several species, including *Arrabidaea verrucosa*, were growing around the trunk of a canopy tree at this site.

Figure 3. (*a*) Basidiocarps of the L-biotype on liana L2 at site LC at Pichilingue. (*b*) A pseudosclerotial pad (PSP) connecting two pieces of liana stem.

communication). Routine identification was based on the flaky bark and distinctive cruciform shape of the vascular tissues in cross-section.

A. verrucosa lianas were widespread within the *Bosque Viejo* but, despite frequent examination, basidiocarps of *C. perniciosa* were never found on the majority of the lianas. This liana species generally occurred in discrete thickets (i.e. a tangle of liana stems), often intertwined with lianas of other species and usually supported by the branches of understorey or canopy trees. Basidiocarps of *C. perniciosa* were generally restricted to the densest (longest established) thickets.

The diameter of the *A. verrucosa* stems ranged between 2 and 10 cm and in one case a single stem was traced for a distance of over 50 m. However, most stems could only be traced for 10 to 20 m due to their growth either underground or into the forest canopy. Green shoots were very occasionally seen emerging from the liana stems within a few metres of ground level, although dead but attached sidebranches (< 1 cm diameter) were more common. Examination of both green and woody parts of lianas on which L-biotype basidiocarps were present showed no symptoms associated with WBD on cocoa and attempts to isolate the fungus from green shoots were unsuccessful.

Sites LA and LC (Fig. 2) yielded the majority of basidiocarps. Both sites were well-established liana thickets, apparently consisting of several separate stems of *A. verrucosa*. *C perniciosa* was previously identified on lianas at these two sites by Hedger *et al.* (1987) and probably also by Evans (1977, 1978). The majority of basidiocarps at these sites were found on the bark of living lianas of *A. verrucosa* (Fig. 3*a*), although many were also found on debris of the same species. A number of basidiocarps were found on dead but attached sidebranches of the lianas but it was not clear whether death of the branches was due to the presence of the fungus.

In those cases where the substratum consisted of debris of *A. verrucosa* or some other species, it was usually found that the debris was attached by yellow or purple pseudosclerotial pads (PSPs; Fig. 3*b*) to either living or dead material of this species. Similar PSPs were noted to be associated with both the C- and L-biotypes of *C. perniciosa* by Evans (1977, 1978) and Hedger *et al.* (1987), and have also been observed in *Hymenochaete corrugata* colonizing aerial *Corylus* debris (Ainsworth & Rayner, 1990). Considerable amounts of plant debris could be seen hanging from stems of *A. verrucosa* in the forest understorey, often held in place by these PSPs. Basidiocarps were observed on living stems 5 m or more above the ground, although collection was not possible.

At three sites (LB, LD and LE), each consisting of a small thicket of young lianas (2–4 cm diameter), basidiocarps were found on only a single occasion. At two sites (LF and LG) the basidiocarps were found on the dead branches of two different but unidentified hardwood trees. Debris were attached to these branches by PSPs but there was no evidence of *A. verrucosa* in the vicinity. This part of the forest had been cleared in 1982 for the planting of cocoa and the undergrowth kept back by periodic herbicide applications. Although the cocoa trees in this area were heavily infected with WBD, all the basidiocarps at this site were morphologically typical of the L-biotype (Hedger *et al.*, 1987).

Lianas of *A. verrucosa* were widely distributed in the Jauneche forest reserve, as was previously recorded by Dodson *et al.* (1978). Basidiocarps of *C. perniciosa* were consistently associated with lianas of this species and generally found on the more mature liana stems. At San Carlos the associated liana was very similar in appearance to *A. verrucosa* but identification was not confirmed. Six other forest sites in Ecuador were visited (Fig. 1*b*) but no basidiocarps of *C. perniciosa* associated with liana were found, despite the presence of *A. verrucosa* lianas at five of the sites. The fact that Evans (1977) reported basidiocarps of *C. perniciosa* on lianas at one of these sites (*Rio Palenque*) suggests that the fungus may have been present but not sporulating when these sites were visited.

Isolation into pure culture

Younger basidiocarps, whose pilei had not fully expanded and which were dissected within a few hours of picking, gave the highest success rate of isolation into pure culture. The same basidiocarps were also found to deposit a spore print more reliably for isolation of single (basidio-) spore germlings (SSIs). SSIs were more successfully obtained from artificially fruited basidiocarps. The gills of naturally occurring basidiocarps were usually infested with flies and larvae and it is likely that these were responsible for the microbial contaminants which frequently overgrew the germlings. The selective medium was found to be effective in suppressing the growth of most microbial contaminants, except for fast-growing Zygomycetes (e.g. *Mucor* spp.). Attempts to isolate *C. perniciosa* from liana bark cores were largely unsuccessful, possibly because the mycelium was present at a low density, compared with other micro-organisms.

A total of 82 mycelial pure cultures were obtained from basidiocarp stipe tissues, as well as five isolates from bark cores of liana L1 at site LA (Table 1). Both mycelial and SSI cultures were subcultured into minivials of MYEA prior to import to Wales. The gross appearance and hyphal characteristics of all isolates were examined to confirm that they were *C. perniciosa*. Clamp connections were present on the hyphae of all stipe and liana bark isolates, while no clamps were present on the hyphae of most SSIs.

Table 1. *Origin of mycelial isolates of the Liana biotype of* Crinipellis perniciosa *belonging to each somatic compatibility grouping*

SCG	Origin	Basidiocarps on live liana	Basidiocarps on dead liana	Basidiocarps on other debris	Bark core isolates	Total number of isolates
scgA	P, LA	9	4+	1−	1	15
scgB	P, LA	1	0	0	0	1
scgC	P, LA	2	0	0	0	2
scgD	P, LA	0	1+/1−	0	0	2
scgE	P, LA	0	2+	0	0	2
scgF	P, LA	2	1+/1−	0	1	5
scgG	P, LA	1	1+	0	0	2
scgH	P, LA	1	0	5−	0	6
scgI	P, LA	1	0	0	0	1
scgJ	P, LA	0	1−	0	0	1
scgK	P, LA	3	1+	0	1	5
scgL	P, LA	0	2+	0	0	2
scgM	P, LA	1	1+	0	2	4
scgN	P, LA	0	1−	0	0	1
scgO	P, LA	0	1+	0	0	1
scgP	P, LC	3	1+	0	0	4
scgQ	P, LC	4	2+	0	0	6
scgR	P, LE	0	1+	0	0	1
scgS	P, LF	0	0	1−	0	1
sctT	P, LF	0	0	5−	0	5
scgU	P*	1	0	0	0	1
scgAA	J	0	1+	0	0	1
scgBB	J	1	1+	0	0	2
scgCC	J	0	1+	0	0	1
scgDD	J	2	0	0	0	2
scgEE	J	0	0	1−	0	1
scgFF	J	0	0	1−	0	1
scgGG	J	0	1+/1−	0	0	2
scgHH	J	0	1−	0	0	1
scgII	J	1	0	0	0	1
scgJJ	J	0	0	1+	0	1
scgKK	J	0	1−	0	0	1
scgLL	J	0	1+	0	0	1
scgMM	SC	0	1+	0	0	1
scgNN	SC	1	0	0	0	1
scgOO	SC	1	0	0	0	1
scgPP	SC	1	0	0	0	1
	Total	36	24+/7−	1+/14−	5	87

Abbreviations: P = Pichilingue; J = Jauneche; SC = San Carlos; LA, LC, LE, LF = sites within the *Bosque Viejo* at Pichilingue; P* = Pichilingue, site unknown; + = basidiocarps on dead liana or debris attached via pseudosclerotial pads (PSP) to a living liana stem; − = basidiocarps on dead liana or debris attached with no visible connection to any living liana stem.

The occasional occurrence of fused and unfused clamp connections on the hyphae of SSIs of the L-biotype is discussed elsewhere (Griffith & Hedger, 1994).

In addition to the isolates obtained during 1987, a number of SSIs were obtained from four L-biotype basidiocarps produced on three liana stem sections (denoted LX, LY and LZ). These stems were collected from the *Bosque Viejo* in 1984 and 1985. Basidiocarp production was induced by use of a 'broom cabinet' in which tropical rainfall conditions were simulated, as described by Suarez (1977) and Rocha & Wheeler (1985). A single mycelial isolate was obtained from the stipe tissues of one of these basidiocarps (LZ1).

Somatic compatibility groupings

Mycelial isolates from the field were paired in a configuration which permitted assessment of all pairing combinations between four or five isolates on a single Petri dish (Fig. 4). Although incompatible reactions were visible on MYEA, the use of a V-8 juice-based medium (2CVJA) was found to enhance the visibility of the mycelial barrages and zones of pigmentation. This was attributed to the smaller amount of aerial mycelium that was produced on this nitrogen-poor medium. Media containing a high carbon : nitrogen ratio were found to give the clearest distinction between compatible and incompatible reactions (Griffith, 1989; data not shown).

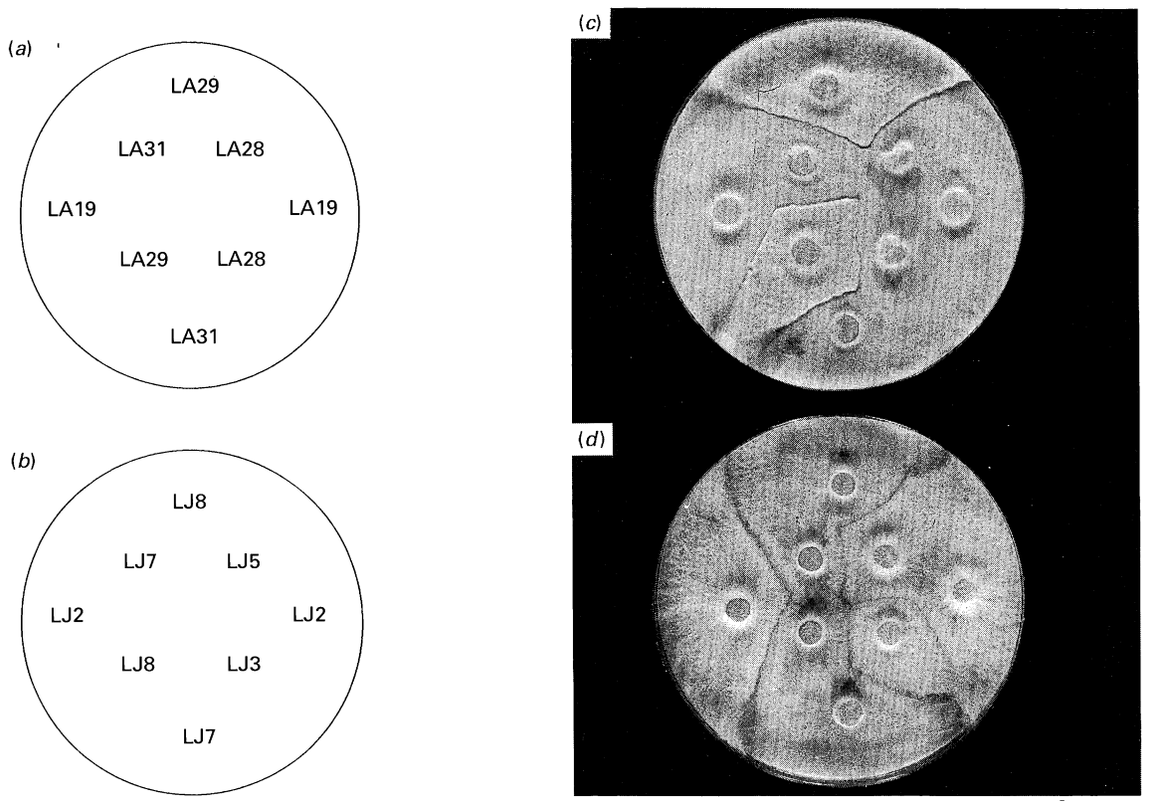

Figure 4. Expression of somatic incompatibility between mycelial isolates of the L-biotype. Diagrams (a) and (b) indicate how plugs (5 mm diameter) of mycelial isolates of the L-biotype from site LA at Pichilingue (*a*, *c*) and Jauneche (*b*, *d*) were inoculated on 9 cm Petri dishes containing 2% clarified V-8 juice agar. This pattern of inoculation permitted somatic incompatibility reactions to be observed in all possible combinations between four and five isolates respectively. The corresponding plates (*c* and *d*) show the expression of somatic incompatibility after incubation at 25 °C for 4 wk. The upper plate (*c*) indicates that isolates LA19, LA28 and LA31 were assigned to scgH, while the fourth isolate on this plate (LA29) was assigned to scgI. The lower plate (*d*) indicates that isolates LJ2 and LJ5 were assigned to scgBB, LJ3 to scgCC and LJ7 and LJ8 to scgDD (scg = somatic compatibility grouping).

The antagonistic reactions associated with somatic incompatibility in the L-biotype were much clearer than those obtained from comparable pairings between isolates of the C-biotype (Wheeler & Mepsted, 1984; Griffith, 1989). All L-biotype pairing results were clearly transitive, e.g. if isolate A was compatible with isolates B and C, then isolates B and C were also compatible with each other (cf. Grosberg, 1988). Each isolate was paired against progressively more geographically distant isolates, until all could be placed in groupings of compatible isolates (Somatic Compatibility Groupings = SCGs). These groupings were denoted scgA, scgB etc., as summarized in Table 1.

A total of 37 SCGs were defined among mycelial isolates of the L-biotype from Ecuador but compatible reactions were only observed between isolates obtained from basidiocarps collected less than 7 m apart in the field. The majority of SCGs from the *Bosque Viejo* sites were represented by several isolates, although due to the more limited sampling from Jauneche and San Carlos, SCGs from these sites were generally represented by only a single isolate. All isolates belonging to the same SCG were morphologically identical in culture, further suggesting that they were isogenic or near isogenic. The term SCG is therefore equivalent to the term genet, first used by Kays & Harper (1974) to describe vegetatively reproducing plants, while isolates belonging to the same SCG correspondingly represent different ramets of a single genet. The validity of using these terms in a mycological context is discussed by Brasier & Rayner (1987).

Having established the SCGs, it was possible to make correlations with field observations and hence assess the field distribution of the L-biotype genets. Members of the same SCG were often present on the same substratum (i.e. the same living liana stem or piece of debris; Table 1 and Fig. 5). Where this was not the case, physical connection between the substrata was visible and, at the point of contact, yellow-purple PSPs were often present. In the case of the four isolates belonging to scgM (Fig. 5*a*), three were from a living liana (L1), while the fourth was found on a piece of liana debris connected by a PSP to L1. Attempts to isolate *C. perniciosa* from dissected PSPs were unsuccessful.

In the case of scgD (Fig. 5*a*), one basidiocarp was found on liana debris on the ground almost directly below the other basidiocarp produced on a separate

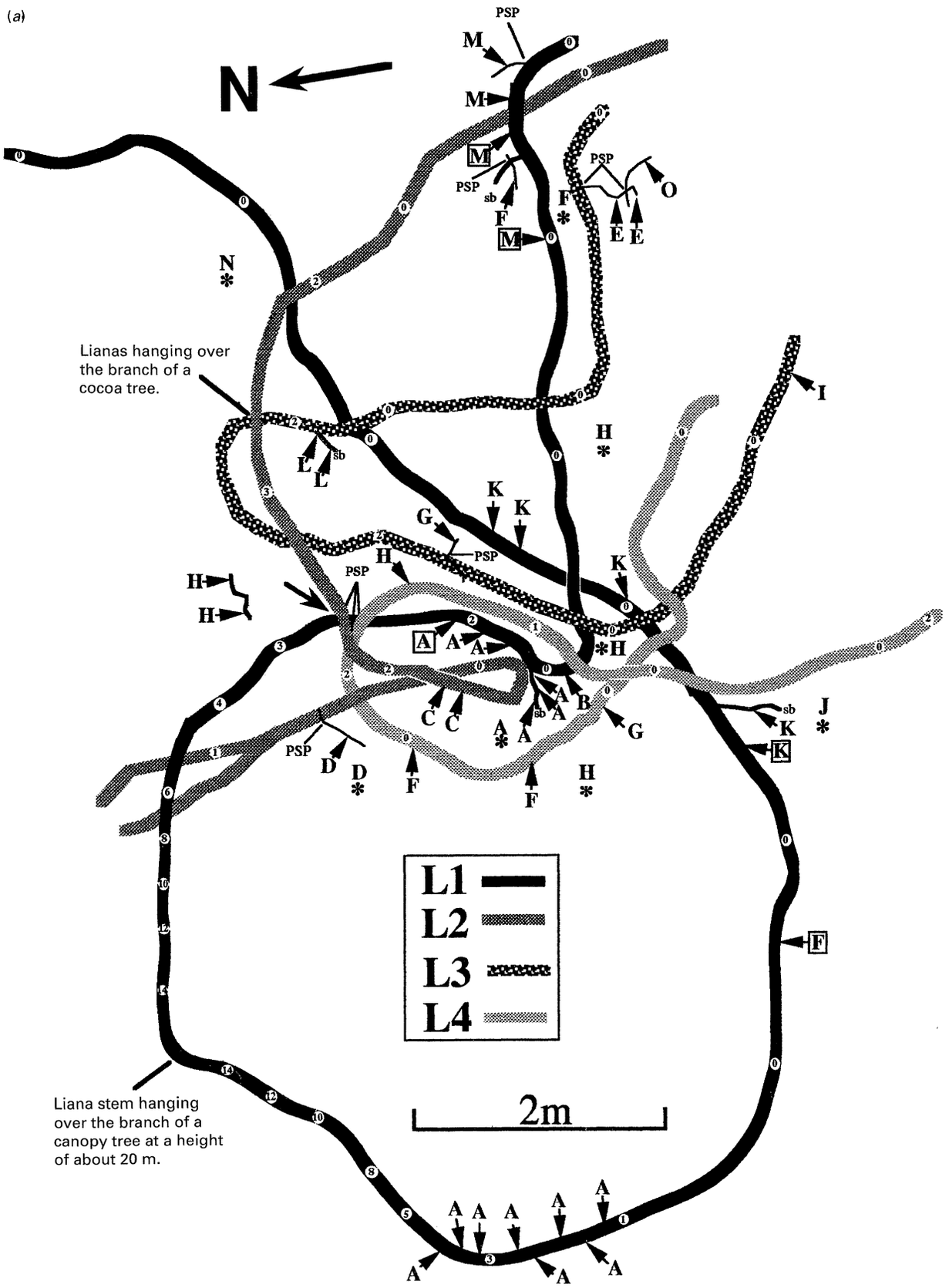

Figure 5. (*a*) For legend see opposite.

piece of liana debris connected by a PSP to liana L2. A similar vertical connection can be made for members of scgH; one was found on liana L4, while the other five basidiocarps of scgH were located on debris on the ground within a horizontal radius of 2 m. It is possible that the substrata on which these

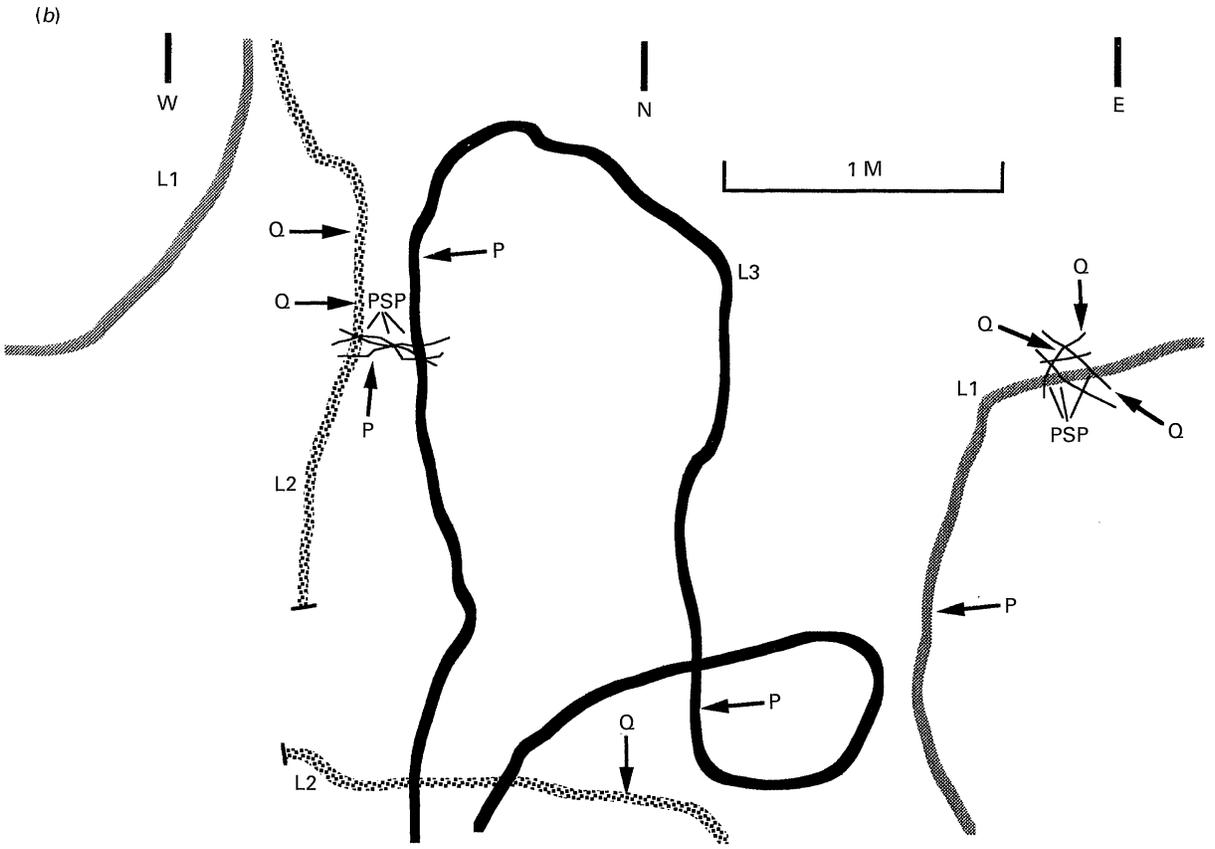

Figure 5. (*a*) A schematic plan view of site LA in the *Bosque Viejo* at Pichilingue. The paths of four lianas, labelled L1 to L4 are shown. The numbers within the white circles indicate the distance above ground (in metres, to the nearest metre) of the lianas at 2 metre intervals along the stems. The locations of basidiocarps on lianas or connected debris, which were found by somatic compatibility pairings to belong to the same genet (scgA to scgO), are shown by arrows. The position of individual basidiocarps which were found on debris unconnected to living lianas is indicated by asterisks (*). Letters within squares indicate mycelial isolates obtained from liana bark cores. The location of sidebranches (sb) from the main liana stems, and also of pseudosclerotial pads (PSP) connecting lianas and debris, are also indicated (scg = somatic compatibility grouping). (*b*) A schematic representation of site LC in the *Bosque Viejo* at Pichilingue. Two-dimensional plan of the paths of three liana stems (L1 to L3) around a large canopy tree is shown. Cardinal points of the compass are indicated (W, N, E). One of the stems (L1) almost encircled the tree. Arrows indicate the positions of basidiocarps from which mycelial isolates were obtained. The positions of pseudosclerotial pads (PSP) connecting stems and pieces of plant debris are shown. A cut in liana stem L2 indicates the likely origin of a piece of liana stem (LX) obtained in 1985, as described in the text.

basidiocarps were found were previously contiguous but had been disturbed and detached, for instance by debris falling from the canopy.

Some SCGs appeared to have a more complex distribution. Members of scgA were with one exception found on liana L1, albeit in two discrete groups. The intervening portion of liana stem, estimated to be 20 m in length, could not be sampled since it rose high into the understorey. It is possible that mycelium of scgA was present along the whole of this length of stem (i.e. forming a single contiguous mycelial system over 30 m in length), although it is equally possible that mycelial spread via PSPs could have occurred if the two portions of liana stem had been in contact at some time in the past. Members of scgF had the widest distribution of any genet at site LA, with basidiocarps being found on two different living lianas, as well as on unconnected debris several metres away. Factors such as animal or human disturbance may explain this distribution.

Most of the basidiocarps found on living liana stems at site LA were present on liana L1 and it was isolates from these basidiocarps which were subsequently found to belong to the most extensive genets. The fact that L1 was the thickest liana at site LA, and therefore probably the longest established, suggests that fruiting of the fungus may occur only after the development of a significant amount of bark tissue. The infrequent occurrence of basidiocarps on younger liana stems (e.g at sites LB, LD and LE) further indicates that this may be the case.

Non-sib matings

SSIs were obtained not only from field-collected basidiocarps but also by fruiting of mycelial isolates using a bran-vermiculite medium (Griffith &

(*a*)

		A				E	L		H				M	U				P				Q			
		1	3	2	6	1	1	2	2	4	3	5	3	7	10	14	5	1	5	6	2	1	4	10	3
scgA	1	-	+	-	f	+	▫	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	+	-	f	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	-	f	-	+	+	▫	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	◦	◦
	6	f	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	◦	◦
scgE	1					-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	◦	◦
scgL	1						-	f	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2						f	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2								-	+	-	f	+	+	▫	+	▫	+	+	+	+	+	+	+	+
scgH	4								+	-	f	-	+	+	+	+	+	+	+	+	+	+	+	+	+
	3								-	f	-	+	+	+	▫	+	▫	+	+	+	+	+	+	◦	◦
	5								f	-	+	-	+	+	+	+	+	+	+	+	+	+	+	◦	◦
scgM	3												-	+	+	+	+	+	+	+	+	+	+	+	+
	7													-	+	-	f	+	+	+	+	+	+	+	+
scgU	10													+	-	f	-	+	+	+	+	+	+	+	+
	14													-	f	-	+	+	+	+	+	+	+	◦	◦
	5													f	-	+	-	+	+	+	+	+	+	◦	◦
	1																	-	+	-	f	▫	f	▫	+
scgP	5																	+	-	f	-	+	+	+	+
	6																	-	f	-	+	▫	+	▫	+
	2																	-	-	+	-	+	f	f	+
	1																					-	+	-	f
scgQ	4																					+	-	f	-
	10																					-	f	-	+
	3																					f	-	+	-

(*b*)

		FF				HH			II			KK	
		1	5	7	4	1	2	9	3	7	1	2	3
scgFF	1	-	+	-	f	+	+	+	+	+	+	+	▫
	5	+	-	-	-	+	+	+	+	+	+	+	+
	7	-	-	-	+	+	+	+	+	+	+	+	+
	4	f	-	+	-	+	+	+	+	+	+	+	▫
scgHH	1					-	+	-	+	+	+	+	*
	2					+	-	-	+	+	+	+	*
	9					-	-	-	+	+	+	+	+
scgII	3								-	+	-	+	*
	7								+	-	-	+	+
	1								-	-	-	+	+
scgKK	2											-	-
	3											-	-

(*c*)

		MM				OO				PP				NN
		1	21	5	10	5	6	15	1	1	6	14	2	1
scgMM	1	-	+	-	f	+	+	+	+	+	+	+	+	+
	21	+	-	f	-	+	+	+	+	+	+	+	+	+
	5	-	f	-	+	+	+	+	+	+	+	+	+	+
	10	f	-	+	-	+	+	+	+	+	+	+	+	+
scgOO	5					-	+	-	f	▫	+	▫	f	+
	6					+	-	-	-	+	+	+	+	+
	15					-	-	-	+	▫	+	▫	*	+
	1					f	-	+	-	f	+	+	▫	+
scgPP	1									-	+	-	f	+
	6									+	-	f	-	+
	14									-	f	-	+	+
	2									f	-	+	-	+
scgNN	1													-

Figure 6. (*a–c*) For legend see opposite.

Hedger, 1993). Sib-matings had previously established the distribution of the parental mating type factors among these SSIs (Griffith & Hedger, 1994). In non-sib matings between SSIs derived from basidiocarps which were found to belong to the same SCG, only about 25% gave rise to secondary mycelia bearing clamp connections (data not shown). This is consistent with the segregation of

(*d*)

		FF		HH		II		KK	
		1	5	1	2	3	7	2	3
scgA	1	+	+	+	+	+	+	+	+
	3	+	+	+	+	▫	+	+	+
scgE	1	+	+	+	+	+	+	+	+
scgL	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
scgH	2	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+
scgM	3	+	+	+	+	+	+	+	+
scgU	7	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+
scgP	1	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+
scgQ	1	+	+	+	+	+	+	*	*
	4	+	+	+	+	+	+	*	*

(*e*)

		MM		OO		PP		NN
		6	8	1	1	6		1
scgA	1	+	+	+	+	.		+
	3	+	.	*	+	.		*
scgE	1	+	*	+	+	.		.
scgL	1	*	.	*	+	.		*
	2	*	.	.	+	.		*
scgH	2	+	+	+	+	+		.
	4	*	*	.	*	+		+
scgM	3	+	*	+	*	.		+
scgU	7	+	*	*	+	+		*
	10	+	*	*	+	.		*
scgP	1	+	+	*	+	+		*
	5	+	+	*	+	.		+
scgQ	1	*	*	*	+	+		*
	4	+	*	+	+	.		*
scgFF	1	+	*	*	*	.		*
	5	+	+	+	*	.		.
scgHH	1	+	+	.	+	.		+
	2	+	*	*	*	*		+
scgII	3	+	+	*	+	.		*
	7	+	.	+	+	.		*
scgKK	2	+	*	*	+	.		*
	3	.	+	*	+	.		*

Figure 6. Non-sib matings between single spore isolates of the L-biotype. The results of non-sib matings between SSIs of the L-biotype from Pichilingue (*a*), Jauneche (*b*) and San Carlos (*c*) are shown. Matings between SSIs from Pichilingue and Jauneche (*d*) and between coastal and Amazonian SSIs (*e*) are also shown. Matings were conducted as described in the text. The outcomes were assessed after incubation at 25 °C for 4–9 wk by microscopic examination. Results indicating that mating type factors were shared by SSIs belonging to different SCGs are shown in 'outline' font. +, compatible mating indicated by the presence of fused clamp connections; −/▫, incompatible mating indicated by the absence of any clamp connections; f/f, hemi-compatible (common B factor) mating indicated by the presence of false (unfused) clamp connections; *, matings in which fused clamp connections were observed only after prolonged or repeated pairings or those 'unilateral' matings in which fused clamp connections were observed only on one side of the plate; ·, mating not conducted.

two unlinked parental mating type factors among sibling SSIs (as observed in sib-matings; Griffith & Hedger, 1994). Confirmatory pairings between other SSIs derived from the same parental isolates gave similar results, indicating that isolates belonging to the same SCG contain identical mating type factors.

The results of matings involving SSIs from the basidiocarps LY1 and LY2 (both produced on liana stem LY in the 'broom cabinet'), and also from isolates belonging to scgP from site LC, indicated that all these sets of SSIs had identical mating type factors. Matings between SSIs from basidiocarp LX1 (produced on liana stem LX) and SSIs derived from scgQ gave a similar result. In the absence of mycelial isolates from the stipe tissues of basidiocarps LX1, LY1 and LY2, it was not possible to conduct somatic compatibility pairings between the parental mycelia. However, the distribution of mating type factors suggests that the mycelia from which the SSIs LX1 and LY(1+2) were derived belonged to the genets scgQ and scgP respectively. As can be seen in Figure 5*b*, a portion of stem from liana L2 at site LC in Pichilingue had previously been removed. Since basidiocarps collected from the same liana were found to belong to scgQ, it is likely that the stem section LX originated from this point.

Most matings between SSIs derived from basidiocarps belonging to different SCGs were compatible, thus indicating that different mating factor complements were generally present in each genet and that the outcrossing mechanism of the L-biotype of multiallelic. However, a small number of matings did not give rise to mycelia bearing fused clamp connections, suggesting that some mating type factors were common to different genets.

Matings between tester SSIs representing each of the four possible combinations of mating type specificities from parental mycelial isolates belonging to different SCGs (eight from Pichilingue and four each from Jauneche and San Carlos) were therefore used to examine the distribution of mating type factors among genets of the L-biotype from each

Table 2. *Distribution of mating type factors among somatic compatibility groupings of the L-biotype of Crinipellis perniciosa*

SCG	A_XB_X	A_YB_Y	A_XB_Y	A_YB_X
scgA	LA1.1 A1 B1	LA1.3 A2 B2	LA1.2 A1 B2	LA1.6 A2 B1
scgE	LA10.1 A3 B3	—	—	—
scgL	LA41.1 A1 B4	—	—	LA41.2 A4 B4
scgH	A43.2 A5 B5	LA43.4 A6 B6	LA43.3 A5 B6	LA43.5 A6 B5
scgM	LA46.3 A7 B7	—	—	—
scgU	LZ1.7 A8 B8	LZ1.10 A5 B9	LZ1.14 A8 B9	LZ1.5 A5 B8
scgP	LC1.1 A9 B10	LC1.5 A10 B11	LC1.6 A9 B11	LY1.2 A10 B10
scgQ	LC3.1 A9 B12	LC3.4 A11 B10	LC3.10 A9 B10	LC3.3 A11 B12
scgFF	LJ11.1 A12 B13	LJ11.5 A13 B14	LJ11.7 A12 B14	LJ11.4 A13 B13
scgHH	LJ14.1 A14 B15	LJ14.2 A15 B16	—	—
scgII	LJ15.3 A2 B17	LJ15.7 A16 B18	LJ15.1 A2 B18	—
scgKK	LJ17.2 A17 B13	—	LJ17.3 A17 B19	—
scgMM	SCFT1 A18 B20	SCFT21 A19 B21	SCFT5 A18 B21	SCFT10 A19 B20
scgNN	SCL1A.1 A20 B22	—	—	—
scgOO	SCL2.5 A21 B23	SCL2.6 A22 B24	SCL2.15 A21 B24	SCL2.1 A22 B23
scgPP	SCL4.1 A21 B25	SCL4.6 A23 B24	SCL4.14 A21 B24	SCL4.2 A23 B25

The following mating type factors were common to two somatic compatibility groupings:

A1 A L A2 A II A6 H U A9 P Q A21 OO PP
B10 P Q B13 FF KK B24 OO PP

Single spore isolates (SSIs) from the somatic compatibility groupings (SCGs) listed above were placed into one of four mating type factor phenotype groups (A_XB_X etc.) on the basis of sib-matings (Griffith & Hedger, 1994). Subsequent non-sib matings using tester SSIs (the accession numbers of these are shown in small print above the mating phenotype) established the distribution of particular mating type factors among genets of the L-biotype obtained in Ecuador. These are numbered sequentially A1 and B2 etc. Dashes (—) indicate where certain mating type factor combinations were not obtained due to the small number of SSIs. It should be noted that scgP and scgQ share two mating type factors (A9 and B10), as do scgOO and scgPP (A21 and B24).

site. In the case of seven out of the 16 SCGs, only one to three mating type factor combinations were identified, due to the small number of sibling SSIs that were obtained. The results of these 'within-site' matings are shown in Figure 6*a–c*. Additional matings, including the use of different SSIs with the same mating genotype (as predicted from sib-matings) were conducted for any pairings which were not fully compatible. The putative mating genotypes of each SCG were labelled sequentially from scgA (= A1B1 A2B2) onwards, as summarized in Table 2.

Of the factors which were found to be common to mycelia belonging to different SCGs, most were shared by mycelia found in close proximity in the field. Mating type factors A9 and B10 were both common to scgP and scgQ from site LC at Pichilingue, while factors A21 and B24 were also common to scgOO and scgPP from San Carlos. In both cases the parental mycelia were found in close proximity and may therefore have been sib-related.

There were three instances where mycelia which did not have any visible mycelial connection shared a single mating type factor. At Jauneche, mating factor B15 was shared by scgFF and scgKK. These mycelia were 50 m apart, so it is unlikely that there was any mycelial connection. Similarly factor A1 was shared by both scgA and scgL at site LA in Pichilingue. These genets were found on different liana vines and there was no apparent connection, although the fact that only one of the parental B factors from scgL was identified, leaves the possibility that these two mycelia were also sib-related.

Matings between SSIs from Pichilingue and Jauneche (Fig. 6*d*) revealed that mating type factor A2 was shared by both scgA and scgII. Pairings were also conducted between tester SSIs from coastal and Amazonian Ecuador (Fig. 6*e*). Although these cross-Andean pairings were incomplete, there was no direct indication that any mating type factors were common to both populations. It was therefore estimated that 23 A and 25 B mating type factors were present from a theoretical maximum of 28 A and 28 B factors (i.e. 5 A factors and 3 B factors were common to more than one SCG).

A number of non-sib matings consistently displayed atypical mating reactions, such as the production of secondary mycelium on one side of the plate only ('unilateral mating') and/or delayed secondary mycelium formation (up to 9 wk before clamp connections were observed). For instance, the progeny derived from basidiocarp LX1 all exhibited delayed or unilateral mating, while matings involving the progeny of LC3 (scgQ) progressed normally (Griffith, 1989; data not shown). These two sets of SSIs contained identical mating type factors and were probably derived from the same mycelial genet (see above), so the only significant difference between them was that the progeny of LX1 had been in culture for over 18 months when the matings were conducted. It may be significant that the following repeated subculture of primary mycelia of *Stereum hirsutum*, reduced access migration associated with

enhanced somatic rejection has been observed (Rayner *et al.*, 1984; Coates & Rayner, 1985).

Some SSIs, which had not undergone such prolonged periods in culture, also exhibited similar behaviour (e.g. SSI LJ17.3 from scgKK, Fig. 6*d*). The frequency of unilateral or delayed mating reactions was higher in cross-Andean pairings than that observed for matings between less geographically distant SSIs (Fig. 6*e*). However, the fact that confirmatory pairings involving different SSIs with the same mating genotype were fully compatible suggests that this phenomenon was unrelated to the mating type factors. Studies with other basidiomycete species (Boidin, 1986; Ainsworth, 1987; Brezinsky *et al.*, 1987; Chase & Ullrich, 1990) have indicated that genetic factors responsible for partial or total intersterility between allopatric populations are unlinked to mating type loci.

DISCUSSION

These results confirm previous observations that, in addition to its well-documented occurrence in Ecuador on members of the genera *Theobroma* and *Herrania*, *C. perniciosa* is also found in association with lianas and plant debris in the forests of both coastal and Amazonian Ecuador. Furthermore it was observed that many of these basidiocarps arose directly from the bark of living lianas. More detailed examination of the substrata of L-biotype basidiocarps at Pichilingue and Jauneche suggested that only one liana species, *Arrabidaea verrucosa*, was involved. Much of the debris on which basidiocarps were found also appeared to belong to this species. Recent re-examination of the remains of liana stems on which basidiocarps of *C. perniciosa* were recorded by Evans (1977) and Hedger *et al.* (1987) has found that these were also *A. verrucosa* (C. Dodson, personal communication).

Despite the evidence suggesting the host specificity of the L-biotype, basidiocarps were also found on other (non-liana) debris. However, some connection via PSPs from the substratum to either live or dead lianas was usually present, although it was not possible to confirm, by isolation into pure culture, that the PSPs were formed by *C. perniciosa*. Nonetheless, whilst other tropical agarics, including several *Marasmius* species, produce PSPs (Hedger, Lewis & Gitay, 1993), the yellow-purple pigmentation has only been found in association with *C. perniciosa*. The location of the PSPs indicated the route by which the debris was probably colonized, although subsequent disturbance events may have disrupted some of these connections.

At two sites (LF and LG), both in an area of cleared forest at Pichilingue, L-biotype basidiocarps were not found near lianas of *A. verrucosa*. It is quite likely, given its widespread distribution elsewhere in the *Bosque Viejo*, that this species was present at or near these two sites prior to the clearing of the forest. The occurrence of basidiocarps of the L-biotype at these sites suggests that, once established, the mycelium of the L-biotype can persist in the absence of its putative host and can colonise quite substantial portions of forest debris. Evans (1978) noted the abundance of L-biotype basidiocarps in recently cleared areas of forest. Such disturbance may promote increased basidiocarp production and possibly the mycelial spread of the fungus.

A. verrucosa is widely distributed in South America (Dodson & Gentry, 1978) but absent in the main part of the Amazon Basin, where it is replaced by the closely related species *A. japurensis* D. C. Bur. & K. Schum. (Gentry, 1979). *A. verrucosa* is common in Amazonian Ecuador and has been found as far east as Iquitos in Peru (A. Gentry, personal communication) suggesting that it could have been the host species at San Carlos. The L-biotype has been found near Manaus in Amazonian Brazil (Bastos *et al.*, 1981). *A. japurensis* and other members of the genus *Arrabidaea* could provide alternative hosts in these more easterly regions.

Information from somatic incompatibility pairings between isolates obtained from the field has been used in a number of earlier studies to map the local distribution of fungal genets (Thompson & Rayner, 1982; Holmer & Stenlid, 1991; Kay & Vilgalys, 1992; Smith, Bruhn & Anderson, 1992). Genetic control of somatic compatibility in certain ascomycetes, including *Aspergillus nidulans* and *Neurospora crassa*, is known to be complex and to involve several loci (Croft & Jinks, 1977; Croft & Dales, 1984), and the same situation probably applies in many basidiomycetes (e.g. Rayner *et al.*, 1984).

When 22 sib-composed secondary mycelia derived from sib-matings of the L-biotype isolate LZ1 (scgU) were paired in all combinations (I. M. Rodgers and G. W. Griffith, data not shown), over 96% (213/221) of the pairings were incompatible and produced distinct barrage interactions. This figure is comparable to that obtained by Kay & Vilgalys (1992) for *Pleurotus ostreatus* and suggests that the genetic control of somatic incompatibility in the L-biotype is also complex. Two mycelia obtained from the field are therefore only likely to be compatible if they belong to the same genet and are therefore genetically identical. The fact that isolates belonging to the same SCG were also found to be morphologically identical in agar culture, to contain identical mating type factors and to have identical isoenzyme profiles (data not shown; Griffith, 1989) provides further confirmation. Somatic compatibility phenotypes, in the case of the L-biotype and also of other outcrossing basidiomycetes, therefore represent a highly discriminating system for identifying mycelial genets.

In addition to providing confirmation that members of the same SCG contained identical comple-

ments of mating type factors, the non-sib matings also provided some information about the level of inbreeding within L-biotype populations and the dynamics of mycelial establishment. They also demonstrated that no significant barriers to fertility were present within the three Ecuadorian populations of the L-biotype were present.

A total of eight mating type factors (5A and 3B) were found to be common to more than one genet. It is likely that in most cases the common factors indicate close relatedness between the genets involved rather than random segregation of mating factors, as discussed earlier. Nevertheless, the formula of Lewontin & Prout (1956), as applied by Ullrich (1973) when investigating *Sistotrema brinkmannii* mating type factors, provides an estimate of 56A and 88B for the number of mating type factors present within the L-biotype population. As previously noted by Ullrich (1973) and Williams & Todd (1985), the inclusion of data from basidiocarps found on the same substratum is likely to lead to underestimation of the total number of factors, due to non-random distribution of mating factors. Therefore the actual number of mating factors in the population is likely to be larger than that calculated above.

The formula of Stamberg & Koltin (1973) gives an estimate of 97·1 % for the outbreeding potential of the L-biotype (assuming that 56A and 88B factors are present within the population). Despite the fact that the total number of factors within the population represents an underestimation, the outbreeding potential at each site is probably significantly lower than this, since it is unlikely that all the factors are present within each subpopulation. The occurrence of both binucleate (potentially self-fertile) basidiospores and of intrafactor recombination (leading to the generation of non-parental mating type factors) observed in these L-biotype populations (Griffith & Hedger, 1994) are also predicted to lead to increased levels of inbreeding. A more accurate estimate of either outbreeding potential or the actual level of outcrossing would therefore require further information about the level of gene flow both within and between populations.

Basidiospores of the C-biotype are known to be sensitive to both sunlight and low humidity and an upper estimate of 150 km for spore dispersal has been quoted (Evans & Solorozano, 1982). Assuming that L-biotype basidiospores are equally short-lived, it is highly unlikely that any gene flow via basidiospores could have occurred between coastal and Amazonian populations of the fungus since the formation of the Andes. The observation that matings between coastal and Amazonian populations were frequently delayed or unilateral and therefore not always fully intercompatible suggests that some divergence may have occurred. Similarly atypical mating reactions have been observed between allopatric populations of *Stereum*, *Phanerochaete* and *Coniophora* (Ainsworth, 1987; Ainsworth & Rayner, 1989; Ainsworth *et al.*, 1990). The recent but considerable deforestation that has occurred in coastal Ecuador is likely to have greatly reduced the distribution of *A. verrucosa* and by implication that of the L-biotype. The sites at Pichilingue and Jauneche are 50 km apart, so it is reasonable to speculate that the destruction of intervening forest areas has also resulted in a reduction in the level of gene flow between the two populations.

The distribution of somatically incompatible mycelia in the L-biotype is similar to that observed in several outcrossing basidiomycete species, where the extent of each genet is limited by a combination of factors including resource availability, mycelial growth rate and time elapsed since colony establishment (e.g. Anderson *et al.*, 1979; Thompson & Rayner, 1982; Rayner, Boddy & Dowson, 1987). All L-biotype SCGs were restricted to a very small area and, unlike the C-biotype, the distribution of all somatically compatible mycelia could reasonably be accounted for by vegetative growth. Compatible mycelia occupying different substrata were often found to be connected via PSPs. L-biotype genets were restricted to an area of only a few square metres, so it is unlikely that the L-biotype is capable of any other form of long or medium range dispersal, such as asexual spores or rhizomorphs. Although Delgado & Cook (1976*b*) have reported the occurrence of arthroconidia in plate cultures of some C-biotype isolates, no such structures were observed during field studies or in pure cultures of the L-biotype.

The outbreeding strategy of the L-biotype clearly has implications for mycelial establishment, since mating is normally a prerequisite for fruiting. No primary mycelia were found in nature, although this may be due to the limited success of bark isolation. In *Coriolus versicolor* primary mycelia are rapidly dikaryotized in nature, by mating with spores, secondary mycelia or other primary mycelia (Williams, Todd & Rayner, 1981). However, the process of secondary mycelium formation in the L-biotype is slow in culture and homokaryon–heterokaryon matings have not been observed (Griffith & Hedger, 1994).

Correlation between the results of somatic compatibility pairings and the field distribution of basidiocarps from which mycelial isolates were obtained demonstrated that some of the L-biotype genets occupied lengths of liana stem several metres long. The fact that the L-biotype is capable of biotrophic growth in potato callus (Griffith, 1989) raises the possibility that establishment of new mycelia from basidiospores may involve latent colonization of living liana tissues. The presence, in senescent tree branches, of extensive mycelia of several other host-specific basidiomycetes has been

interpreted as evidence of latent invasion (Boddy & Rayner, 1983; Chapela & Boddy, 1988; Boddy & Griffith, 1989).

The L-biotype appears to exhibit a novel mechanism of vertical vegetative spread by means of falling debris. The mycelium in such debris provides an opportunity for the colonization of non-liana debris via PSPs, representing a much more potent inoculum than basidiospores. This strategy may compensate for the poor competitive/combative ability of the L-biotype compared with other fungi which colonize similar substrate (Bravo & Hedger, 1988). The mycelia in some dead but attached liana sidebranches were compatible with adjacent mycelia on the living vines, although it was not clear whether these sidebranches became colonized before or after death. Since loss of apical dominance leading to the production of swollen, branched brooms is involved in the biotrophic phase of infection by the C-biotype (Thorold, 1975), it is possible that the production of sidebranches on the woody parts of vines is stimulated by the presence of the fungus.

Tropical forests are characterized by large numbers of epiphytic and climbing plants (Longman & Jenik, 1976), and debris falling from the canopy is often trapped in the understorey (Hedger, 1985). In all the forest areas visited during this study, masses of aerial debris was seen, the total amount usually being greater in the wetter habitats where larger numbers of lianas and epiphytic plants were also present. Hedger (1985) found that the mycoflora of this aerial debris is dominated by taxonomically distinct communities of small litter-trapping agarics. Newly arrived debris from the canopy is colonized by means of rhizomorphic structures (e.g. *Marasmius* spp.) or by PSPs, as has been observed for *C. perniciosa* and other members of this genus (Hedger *et al.*, 1993). In this way the debris is prevented from descending to ground level and can be exploited by these fungi. Bravo & Hedger (1988) have shown that these fungi are capable of withstanding the large fluctuations in atmospheric humidity, caused by uneven rainfall distribution and high daytime temperatures, and can thus be considered to exhibit a stress-tolerant ecological strategy (Cooke & Rayner, 1984).

The C-biotype of *C. perniciosa* differs from the other litter-trapping agarics in that it is known to have a biotrophic establishment phase. However, the weak pathogenicity of the L-biotype on cocoa prompted Evans (1977) to speculate that it produced a latent infection of lianas, which then awaited, but did not induce or accelerate, the death of its host, prior to saprotrophic exploitation. There are many instances of non-pathogenic, often asymptomatic biotrophic infections of higher plants (Petrini, 1986; Carroll, 1988) and the fungi involved in these associations are often closely related to important plant pathogens (e.g. Hepperly, Kirkpatrick & Sinclair, 1980; Kulik, 1984; Ogle, Irwin & Cameron, 1986). The evidence presented above provides further weight to Evans's speculation, although further investigation is required before the role of latent invasion in the mycelial establishment of the L-biotype, or indeed of other litter-trapping agarics can be clearly demonstrated.

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