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Genetic Stability and Diversity of *Pneumocystis carinii* Infecting Rat Colonies

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There is increasing molecular and antigenic evidence that *Pneumocystis carinii* organisms isolated from humans, ferrets, and rats are different species. In contrast, little is known about the extent of genetic diversity among *P. carinii* strains found within a single mammalian species. In the present study, electrophoretic karyotypes were obtained from *P. carinii* prepared from 10 chronically immunosuppressed rat colonies to investigate diversity at the chromosomal level. Most organism preparations produced patterns with 13 to 15 bands, but as many as 24 bands were observed in a few preparations. All bands separated between 700 and 300 kbp. Four distinct karyotype forms emerged from among the 13- to 15-band karyotypes of the 10 colonies sampled. Form 1 was shared by five rat strains from two vendors; form 2 was shared by two rat strains from the same vendor; and forms 3 and 4 were unique to their vendor colonies. Within a given rat colony, most rats harbored the same *P. carinii* karyotype. A survey of selected rat colonies showed that the karyotype within a vendor colony could remain stable over a period of 2 to 3 years. Hybridization of the blotted karyotypes with a repetitive DNA element isolated from rat-derived *P. carinii* and with single-copy gene probes showed that every chromosome in the karyotypes contained some repetitive DNA, and there was a general size concordance among the chromosomes carrying the unique gene loci. Differences in gene sequences, electrophoretic karyotypes, and hybridization profiles suggested that the immunosuppressed rats were infected by genetically distinct *P. carinii* strains. A provisional system of nomenclature for *P. carinii* that will permit differentiation of *P. carinii* organisms from the same mammalian host is discussed. These data show that all rats were not infected by a single type of *P. carinii*, that pulsed-field gradient electrophoresis can detect sufficient genetic diversity among the organism preparations to allow for characterization of the organisms, and that the genome of the organism within the rat host is relatively stable over time.

Pneumocystis carinii organisms are eukaryotic protists found in the lungs of a wide variety of mammals. These organisms are able to cause a lethal pneumonia in hosts with compromised immune status. The natural history of *P. carinii* is poorly understood in large part because of a lack of a continuous in vitro culture system for its propagation. Although it was once considered a protozoan parasite, recent comparisons of rRNA and DNA sequences from several genes have shown that *P. carinii* is more closely related to fungi than to protozoans (2, 3, 9, 10, 42, 52, 56, 61).

The life cycle of *P. carinii* has not been completely elucidated. At least one phase takes place in the mammalian lung, in which a multitude of morphological forms can be observed (1, 6, 35, 36, 55). Serological studies have shown that >85% of the human populations in North America and Europe are reactive to *P. carinii* organisms and antigens and that seropositivity occurs by the age of 4 years (38, 40, 41). These findings suggest that the organism may have a ubiquitous distribution in nature, although an environmental cycle has not been identified.

Methods that can distinguish *P. carinii* preparations will make it possible to better understand the infective process, transmission of infection, and relapse and latency associated with recurrent infection. Serological assays and molecular biological techniques such as restriction fragment length polymorphisms have been used to characterize other microbial isolates for epidemiological purposes. Use of serological techniques to distinguish *P. carinii* preparations is complicated by issues of sensitivity and specificity. Increasing knowledge of the genomic organization of *P. carinii* provides the rationale for application of molecular methods.

Any technique used to differentiate organism isolates is subject to certain requirements. The technique must have the power to distinguish the microbe of interest from other species or groups of organisms. The basis of the variation must be relatively stable to provide reproducible results. There must be sufficient diversity to allow recognition of related members of a microbial species or group. Diversity at the genomic level can arise from gross or subtle chromosomal rearrangements, gene duplications, or deletions, or from any number of processes used during nuclear replication.

We have chosen to explore the technique of pulsed-field gradient electrophoresis (PFGE) as a means to characterize *P. carinii* preparations. PFGE is a technique that resolves large DNA molecules. It was originally used to separate the chromosomes of the yeast *Saccharomyces cerevisiae* (47), but modifications of the system have been used to produce distinct yet reproducible chromosome patterns (electrophoretic karyotypes) of protistan pathogens (14, 23, 24, 26, 29, 32–34, 39, 45, 49). *P. carinii* preparations from immunosuppressed rats have been analyzed by several PFGE techniques, including orthogonal-field-alternation gel electro-

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phoresis (12, 60), contour-clamped homogeneous electric field electrophoresis (CHEF) (19, 59, 60), pulsed-field gel electrophoresis according to the method of Lundgren et al. (31), transverse alternating field electrophoresis (31), and field inversion gel electrophoresis (FIGE) (8, 18, 19, 63). The reported number of bands among the rat electrophoretic karyotypes ranged from a low of 8 (59) to a maximum of 20 to 22 bands (8, 18, 19, 60). In our own studies, we identified three electrophoretic patterns of *P. carinii* obtained from four immunosuppressed rat colonies populated with two strains of rats, Sprague-Dawley and Lewis (19). Two of the three karyotypes contained 15 separate bands, while the third displayed a 22-band pattern.

Collectively, these studies strongly suggest that distinct genetic types of *P. carinii* were present in rats obtained from commercial rodent vendors. In the present study, we extended the survey of rat-derived *P. carinii* to include the sampling of several rat colonies with the following goals: (i) to determine the extent of genetic variation among *P. carinii* preparations obtained from a single mammalian species, the rat, by electrophoretic karyotyping; (ii) to investigate the basis of variation among the electrophoretic karyotypes by hybridization with chromosome-specific probes; and (iii) to evaluate the stability of the electrophoretic karyotypes over time.

MATERIALS AND METHODS

Rat colonies. A total of 15 to 27 rats of each of 10 viral antibody-positive rat colonies were received in filtered containers and immediately placed under barrier housing conditions in our facilities. Rats raised in conventional colonies, those with cages open to the room environment, are exposed to rodent viruses (e.g., Sendai virus and rat corona virus) and produce antibodies to viral antigens that can be detected by serological assays. Exposure to these viruses has been associated with the presence of latent *P. carinii* infection (57). All rats were male and received at weights ranging from 125 to 150 g. Upon receipt, three to five rats were immediately sacrificed, and the lungs were removed and homogenized for evaluation of the presence of *P. carinii* organisms by tinctorial staining methods (7). No organisms were detected by these staining methods, but about 90% of the sera tested showed reactivity to rat-derived *P. carinii* antigens by immunoblotting (data not shown). The following rat strains were received: (i) Sprague-Dawley from Hilltop Laboratories (Scottdale, Pa.) colony 23 (HSD), (ii) Long Evans from Charles River Breeding Laboratories (COBS; Wilmington, Mass.) area 083 (CRLE), (iii) Lewis from Charles River Breeding Laboratories area 083 (CRL), (iv) Brown Norway from Charles River Breeding Laboratories area 083 (CRBN), (v) Sprague-Dawley from Zivic Miller (Zelienople, Pa.) room 011 (ZMSD), (vi) Holtzman from Sasco, Inc. (St. Louis, Mo.), room 2 (SH), (vii) Holtzman from Harlan Industries (Indianapolis, Ind.) colony 219 (HH), (viii) Fischer 344 from Hilltop Laboratories colony 24 (HF), (ix) Wistar from Hilltop Laboratories colony 11 (HW), and (x) Sprague-Dawley from Sasco, Inc. (Omaha, Nebr.), O'Fallon colony (SSD).

Housing. Three rats were placed in each polycarbonate shoebox cage fitted with a microisolator top with 3-μm exclusion (Lab Products, Inc., Federalsburg, Md.). The cages were placed on horizontal flow hoods (Germ Free Industries, Miami, Fla.). All bedding (Anderson Bed O'Cobs Combo; Industrial Products, Maumee, Ohio) and water were autoclaved prior to entry into the animal room; the food (rodent chow 20; PicoLab, St. Louis, Mo.) was irradiated at the vendor. The water was supplemented with cephradine (Velosef; E. R. Squibb & Sons, Inc., Princeton, N.J.) solution by injection into the water bottles for a final concentration of 0.200 mg/ml. All manipulations of the animals (e.g., cage change-out and injections) were performed in a horizontal flow changing station by personnel wearing sterile gowns, gloves, masks, shoe covers, and hair caps. This apparel was changed after treatment of a given rat colony. Cages were changed on a weekly basis, and water bottles (500-ml total volume) were changed twice a week.

Provocation of *P. carinii* pneumonia. After 7 days of acclimation, the rats were started on an immunosuppressive regimen consisting of weekly injections of methylprednisolone acetate (DepoMedrol; The Upjohn Co., Kalamazoo, Mich.) at 4 mg/week and sacrificed beginning at 6 weeks and continuing to 15 weeks of immunosuppression (4).

Preparation of *P. carinii* organisms from rats. In our previous studies, we have observed fulminant *P. carinii* infection to develop after about 8 weeks of immunosuppressive therapy in the latently infected rat model of pneumocystosis. Since different strains of rats were included in the present study, some of which we had not previously used, examination of the lungs began at 6 weeks and extended throughout a 15-week period. Rats were sacrificed by CO_2 narcosis, and the lungs were removed and processed for pulsed-field gel electrophoresis according to our earlier studies, with some modifications (19). Each set of lungs was prepared individually. After homogenization in a laboratory blender (Stomacher 80; Tekmar Inc., Cincinnati, Ohio), the preparations were filtered through gauze to remove large particulate host material and treated with a lysis solution (0.85% aqueous ammonium chloride) followed by at least two passes through 10-μm-pore-size filters (Mitex; Millipore Corp., Bedford, Mass.) to further reduce the host cell contaminants. The preparations were then sampled for the number of organisms by enumeration of *P. carinii* nuclei with the Diff-Quik stain (Fisher Scientific, Cincinnati, Ohio) (5). The Diff-Quik stain produces results similar to those of Wright-Giemsa stain. The nuclei appear purple, and the cytoplasms of cells are blue. All life cycle stages of *P. carinii* are enumerated by quantification of the nuclei. Thus, a fully mature cyst would yield up to eight nuclei, while trophic forms are counted individually. Surveillance cultures for microbial contaminants were conducted as previously described (19). No evidence of other eukaryotic microbes in the preparations was detected by culture or staining techniques. Some preparations contained bacterial contamination, which was shown previously to have no influence on the karyotypes (19).

Preparation of organisms for PFGE. After treatment with DNase I (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) at 10 to 20 μg/ml in a solution of 150 mM NaCl–10 mM $MgCl_2$–10 mM Tris at pH 7.2 for 30 min at 37°C to digest extracellular DNA, the preparations were washed successively with 0.250 and 0.125 M EDTA. Organisms were embedded in agarose by reconstituting the pellets in 1 volume of 0.125 M EDTA to 2 volumes of a solution of 1.2% low-melting-point agarose (Boehringer Mannheim Biochemicals) in 0.125 M EDTA (approximately 0.8% final agarose concentration). The total volume of the individual preparations was determined by the number of organisms present in the rat lung; homogenates from rats with fewer than 10^8 organism nuclei were prepared in volumes of less than 1.0 ml; 10^8 to 10^9 organism nuclei were prepared in 1.0 to 2.0 ml; and preparations with $>10^9$ nuclei were prepared in 4.0 ml or more. The final parasite density ranged from 5×10^7 to $5 \times$

10^8/ml. Volumes of less than 1.0 ml were pipetted into Plexiglas custom molds holding 240 μl each for FIGE analysis or into the CHEF 10-well sample plug mold (Bio-Rad, Melville, N.Y.) for CHEF analysis. Larger volumes were placed in disposable spectrophotometer cuvettes (Fisher Scientific). Gel-embedded organisms were treated with 0.25 mg of proteinase K (Boehringer Mannheim Biochemicals) per ml in a solution of 1% *N*-lauroylsarcosine (Sigma Chemical Co., St. Louis, Mo.)–0.45 M EDTA–0.01 M Tris and digested at 55°C for 18 h (small plugs) or 24 to 48 h (larger volumes). Digested agarose samples were stored in 0.5 M EDTA (pH 9.0) at 4°C. Samples stored under these conditions remained intact and produced karyotypes without signs of degradation for at least 3 years.

FIGE and CHEF conditions. Gels for FIGE contained 1% FMC SeaKem genetic technology-grade agarose (Hoeffer Scientific Instruments, San Francisco, Calif.) prepared in 0.5× TBE (45 mM Tris hydrochloride, 45 mM boric acid, 1.25 mM EDTA) for a total volume of 200 ml. Final gel dimensions were 20 by 25 cm. Velcro tape was fastened to the bottom of the casting tray, and the molten agarose was poured over it to anchor the gel to the tray. FIGE was performed with a PC 750 Pulse Controller (Hoeffer Scientific Instruments) and horizontal gel electrophoresis systems (Bethesda Research Laboratories). The buffer system was 0.5× TBE supplemented with 0.1 M glycine (Boehringer Mannheim Biochemicals). FIGE gels were run in a 4°C cold room; buffer temperature was maintained at 6 to 8°C by circulation through an ice bath. The gel was run for 48 h at 5.3 V/cm with a 50-s forward and 25-s reverse pulsing, after which time the buffer was changed and the gel was run for an additional 96 h at 4 V/cm with the 50-s forward and 25-s reverse pulse times (19, 63).

CHEF gels were made in the same manner as for FIGE except that 300 ml was used with a casting stand of 14 by 21 cm, without Velcro tape (Bio-Rad, Richmond, Calif.). CHEF gels were run with a Bio-Rad CHEF DRII apparatus under conditions slightly different from those in our previous studies (19). Gels were run in 0.5× TBE at 135 V, 14 to 16°C, with a 50-s initial pulse that was gradually increased to 100 s over the 144-h run time.

Photodocumentation and estimation of chromosome size. Gels were stained with ethidium bromide and photographed with Kodak professional T Max 400 film (loaded in film holders of 4 by 5 in. [ca. 10 by 13 cm]; Fidelity Manufacturing Co., Burbank, Calif.). Lambda multimers, 48.5-kbp increments (Hoeffer Instruments and Bio-Rad, Melville, N.Y.) were used to produce standard curves from which the *P. carinii* band sizes were estimated. *S. cerevisiae* chromosomes prepared as previously described (44) were also included on some gels as additional size markers. The schematic of the karyotypes (see Fig. 4) was generated by the Harvard Graphics 3.1 program.

DNA transfer conditions. For hybridization with radioactive probes, DNA was transferred from the FIGE and CHEF gels by capillary flow onto Hybond N+ (Amersham, Amersham, United Kingdom) membranes with 0.4 M NaOH following treatment of the gels with 0.25 M HCl and 0.5 M NaOH (19). For digoxigenin probes, DNAs were depurinated and transferred by capillary action to positively charged membranes (Boehringer Mannheim Biochemicals) with 10× SSC buffer (1.5 M NaCl, 150 mM sodium citrate, pH 7.0), following vendor specifications.

Hybridization probes. The DNA probes to *P. carinii* genes were isolated from a genomic library constructed from rat-derived *Pneumocystis* DNA partially cut with *Sau*3A and ligated in Lambda Fix (Stratagene Inc., La Jolla, Calif.) (51). The characterization of the probes is described in detail in the references cited. The following probes were used: transcription factor IID (53); alpha-tubulin (64); thymidylate synthase (11); a 12-kbp fragment of the rat *P. carinii* genome that includes the 18S rRNA gene, the intergenic transcribed sequence, the 5.8S rRNA gene, and the 26s rRNA gene (51); cation-transporting ATPase (37); and two anonymous clones, T1 and T2 (62). Hybridization conditions for radioactive probes were 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 0.015 M sodium citrate), 0.5% sodium dodecyl sulfate (SDS), 100 μg of sheared herring sperm DNA per ml, and 5× Denhardt's solution for 16 to 18 h at 65°C (46). Washing was done in 2× SSC with 0.1% SDS at 65°C twice for 30 min each time and then in 0.2× SSC at 65°C twice for 15 min each time. The blots were exposed to Fuji RX film with intensifying screens, placed at −70°C for 18 to 24 h, and then developed. The hybridization conditions for digoxigenin-labeled probes followed the vendor's specifications and included a prehybridization in 5× SSC (1× solution is 0.15 M NaCl plus 15 mM sodium citrate, pH 7.0)–1.0% (wt/vol) blocking reagent (Boehringer Mannheim Biochemicals)–0.1% *N*-lauroylsarcosine–0.02% SDS for 2 h at 65°C, followed by hybridization in the same solution overnight at 65°C. The membranes were then washed twice for 5 min each time in 2× SSC with 0.1% SDS at 65°C and twice for 15 min each time in 0.5× SSC with 0.1% SDS at the same temperature. The blots were equilibrated in 100 mM Tris-HCl–150 mM NaCl, pH 7.5, and then incubated in 2% blocking reagent (Boehringer Mannheim Biochemicals) for 30 to 60 min. Antidigoxigenin labeled with alkaline phosphatase was added to the blots in the 2% blocking reagent and incubated for 30 min. Hybridization signals were detected by the addition of Lumi-Phos 530 directly to the blot sandwiched between two sheets of acetate. The blots were then exposed to Fuji RX film for 10 to 120 min.

Two additional probes were isolated from a cDNA expression library made from rat-derived *P. carinii* poly(A)$^+$ RNA (48): Bluescript clone pSK790 (obtained from A. G. Smulian, University of Cincinnati College of Medicine), which contain a 695-nucleotide insert which encodes the 3′ portion of an antigen in the 45- to 55-kDa size range, and Bluescript clone 1410 (obtained from M. J. Linke, University of Cincinnati College of Medicine), with a 1.8-kbp insert that contains part of a gene encoding one of the major surface glycoproteins of rat-derived *P. carinii* (28). Clone 1410 was found to be homologous to a repetitive DNA element, Rp3-1 (50a), previously characterized from the rat-derived genomic library described above (51).

The DNA probes were prepared by random-primed labeling with [α-^{32}P]dCTP or digoxigenin-dUTP according to previous protocols (8, 19, 46, 51) or vendor specifications (Genius System; Boehringer Mannheim Biochemicals).

RESULTS

General characteristics of *P. carinii* karyotypes. *P. carinii* organisms were isolated from individual rats in 10 colonies after 6 to 15 weeks of immunosuppression (Table 1). Representative ethidium bromide-stained chromosome-sized DNAs of the *P. carinii* preparations from these colonies are shown after separation by CHEF in Fig. 1 and 2. All the bands separated between approximately 700 and 300 kbp, as we and others have previously reported (19, 31). Bands migrating above 850 kbp hybridized with total rat cell DNA made radioactive and used as a probe but not with a rat-*P.*

TABLE 1. Rat colonies from which *P. carinii* was isolated

Rat no.	Rat strain	Vendor source	Colony abbreviation	Prototype form
1025–1039	Sprague-Dawley	Zivic-Miller (Zelienople, Pa.), room 011	ZMSD	3
1040–1054	Holtzman	Sasco, Inc. (Omaha, Nebr.), colony OM1	SH	1
1055–1069	Holtzman	Harlan Industries (Indianapolis, Ind.), colony 219	HH	4
1070–1090	Fischer 344	Hilltop Laboratories (Scottdale, Pa.), colony 024	HF	2
1091–1105	Sprague-Dawley	Hilltop Laboratories, colony 023	HSD	2
1106–1120	Wistar	Hilltop Laboratories, colony 011	HW	2
1121–1132	Lewis	Charles River Laboratories (Wilmington, Mass.), area 083	CRL	1
1133–1147	Long Evans	Charles River, area 083	CRLE	1
1148–1162	Brown Norway	Charles River, area 083	CRBN	1
12/90–6/92	Sprague-Dawley	Sasco, Inc., O'Fallon colony	SSD	1

carinii repetitive element (Rp3-1 or 1410), suggesting that this DNA was from the rat host (data not shown). Three general sizes of *P. carinii* bands were separated. The largest group of bands migrated between 700 and 575 kbp; each preparation had four or five bands within this range. The number of intermediate-size bands (550 to 400 kbp) varied between six and seven among the preparations, while the smallest-size bands (350 to 300 kbp) numbered between two and four. In certain karyotypes, some bands stained more brightly than others. For example, the ZMSD pattern (Fig. 1 and 2) contained bands at 600 and 460 kbp that were at least twofold brighter than the rest of the bands in the karyotype. This nonstoichiometric staining was likely due to comigration of similarly sized DNAs. In fact, partial resolution of the 460-kbp band into two separate bands in another preparation from the same colony by FIGE can be observed in Fig. 5A, ZMSD 1032.

The total number of chromosome size bands contained in the genomes of the *P. carinii* harbored within the rat colonies was 14 to 16, disregarding the faint bands (discussed below) and considering the more brightly staining bands as two comigrating chromosomes. This predicts the total genome size of rat-derived *P. carinii* to be between 7 and 8 megabase pairs (Mbp), which is in agreement with earlier predictions based on PFGE analyses (19, 31).

Colonies may be infected with one karyotype of *P. carinii* or a mixture. Bands that stained much more lightly than the 13 to 15 predominant bands could be observed in a few karyotypes. In Fig. 1, faint bands are apparent in the CRL preparation at about 530 kbp and in the CRLE preparation at 650 kbp and between 350 kbp and 400 kbp (marked by white squares). These bands appeared among the *P. carinii* preparations with variable frequency, depending on the colony sampled. In previous work, polymerase chain reaction, DNA sequencing, and Southern hybridization experiments determined that these additional bands were from the coinfection of a single rat with two types of *P. carinii*, called prototype and variant (8). The total number of bands in a karyotype from a coinfected rat varied from 20 to 24 bands, while the prototype patterns produced 13 to 15 bands. Examples of two coinfected rat preparations from the CRLE and CRBN colonies and the HF-V 1087 variant preparation are shown in Fig. 3. Note that the CRLE 1140a preparations show the populations of variant and prototype to have almost equal ethidium bromide-stained band intensities, indicating that these populations were present in similar numbers. In contrast, the CRBN rat 1160 contained mostly prototype *P. carinii*, as evidenced by the 13 bright bands contributed by the prototype and several very light bands contributed by the variant population (marked by black arrows). The brightly staining bands in the preparation from HF rat 1087 were produced from a population of variant *P. carinii* in greater number than the population of prototype organisms, which produced the more lightly staining bands.

Comparison of rats 1087 and 1160 shows the karyotypic difference between a prototype and variant. The five larger DNA bands of the variant organisms were significantly smaller than their five counterparts in the prototype organisms. Conversely, the smallest three to four bands of the variant were larger than those of the prototype organisms.

The frequency of coinfections within the rat colonies surveyed is discussed below.

Similar and distinct karyotypes among the rat colonies. Sixty-seven karyotypes were visualized. Most (85%) of the karyotypes produced patterns with 13 to 15 bands that were identified as prototype *P. carinii* on the basis of homology with the repetitive element Rp3-1 (described below) and hybridization of representative karyotypes with a prototype-specific oligonucleotide probe (8).

Four prototype band patterns were observed. Fig. 4 shows the karyotype profiles in schematic form. All of the profiles in Fig. 4 are consensus karyotypes resulting from averaging of the individual bands from the karyotypes produced by the different gel runs. To evaluate the reproducibility of the band pattern differences, representative preparations from each rat colony were run at least three times on FIGE or CHEF gels and side by side with representative preparations from the other rat colonies.

Organisms infecting CRL, CRLE, CRBN, SSD, and SH rats displayed similar karyotypes composed of 14 to 15 bands that varied little in size or number (Fig. 1 and 2, CRL, CRLE, CRBN, and SH; see Fig. 6, CRL and SSD). The differences were slight, usually involving the absence of one or two bands and band size disparities of less than 15 kbp. All of these karyotypes contained five large DNA bands of approximately 686, 648, 626, 612, and 600 kbp; six or seven bands of 534, 500, 480, 452, 430, and 412 kbp; and three small bands of 340, 330, and 318 kbp. Because of the similarities in karyotype patterns produced by these organism preparations and the localization of unique gene probes to the same-size chromosomes (discussed below), we have designated this common karyotype form 1; the consensus form 1 karyotype is shown in Fig. 4. The form 1 karyotype was not restricted to a certain rat strain or vendor colony, as shown in Table 2. Form 1 was found in the Lewis, Brown Norway, and Long Evans rat strains from Charles River and in the Sprague-Dawley and Holtzman rat strains from Sasco.

Rats from HF and HW colonies harbored form 2 *P. carinii* (Fig. 1 and 2, for example). The form 2 patterns contained a distinctive arrangement of the five large DNA bands: one band at 680 kbp and two sets of band pairs at approximately 658 and 600 kbp. Six to seven bands were apparent between

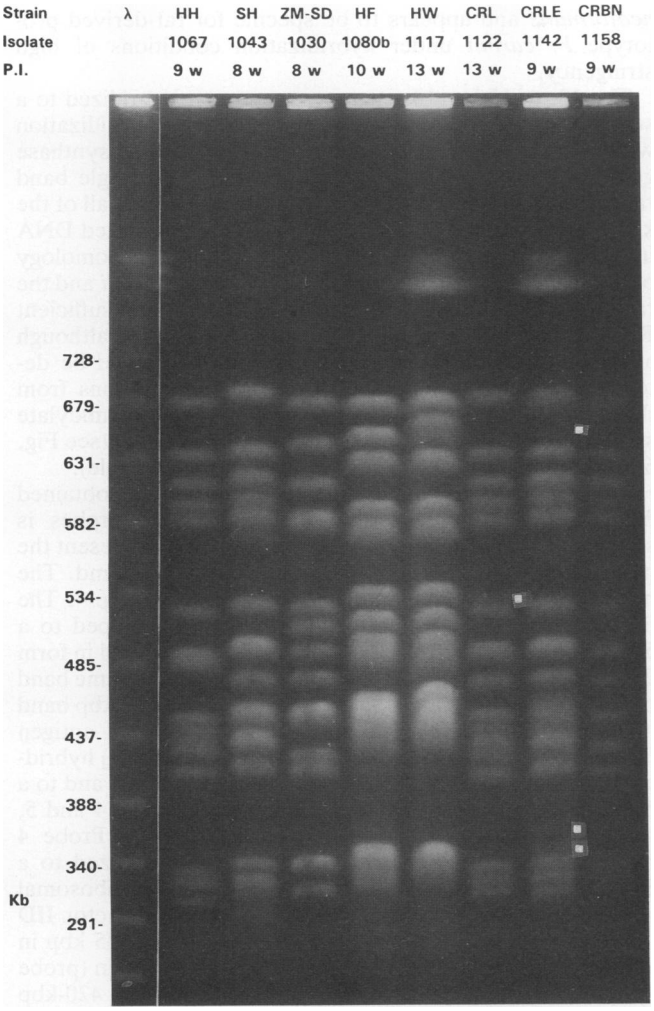

FIG. 1. Electrophoretic karyotypes of representative rat *P. carinii* preparations. *P. carinii* was prepared from rats in several different colonies that were immunosuppressed for 6 to 13 weeks. Electrophoretic karyotypes stained with ethidium bromide are shown. The times at which the organisms were obtained from the rats are expressed as weeks (w) postimmunosuppression (P.I.). The CHEF gels were run according to the conditions described in Materials and Methods. Lambda ladder (48.5-kbp increments) served as size markers. White squares indicate bands that stained faintly with ethidium bromide.

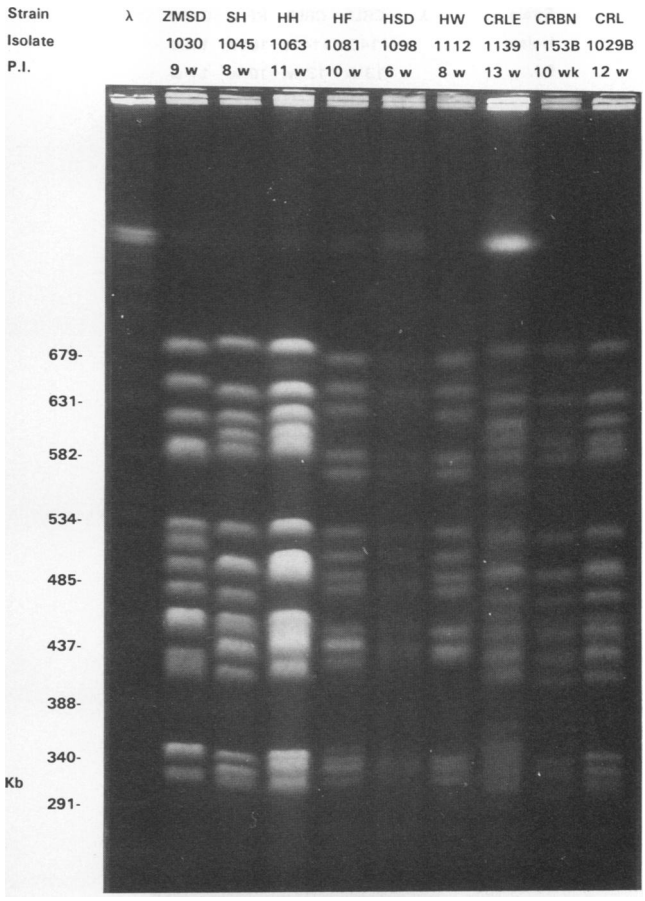

FIG. 2. Electrophoretic karyotypes of representative rat *P. carinii* preparations. Electrophoretic karyotypes (stained with ethidium bromide) of additional preparations from the colonies in Fig. 1 and the single preparation from the HSD colony. Conditions of the CHEF run and size markers are the same as those for Fig. 1.

550 and 420 kbp. In some cases, smears of DNA were observed in this region because of overloading of the lanes with DNA, as seen in Fig. 1. The three smallest bands were approximately 345, 328, and 318 kbp in size. Unlike the form 1 karyotype, the form 2 karyotype was found only in the Hilltop colonies, although it was not restricted to a particular rat strain, as it was harbored by Fischer, Wistar, and Sprague-Dawley rats (Table 2).

Organisms from the ZMSD and HH colonies each produced a karyotype unlike those from any of the other colonies (Fig. 1 and 2, for example). The ZMSD karyotype displayed 13 or 14 bands and was characterized by four large DNA bands of >600 kbp; seven to eight bands between 550 and 410 kbp, with the 460-kbp band resolved in preparation 1032 only (Fig. 5A); and two bands at 340 and 320 kbp. This karyotype was designated form 3 and was found only in the ZMSD colony (Table 2).

The HH karyotype contained five large DNA bands that were similar in size to those of the form 1 karyotypes, but this band pattern differed from form 1 in the sizes of the intermediate-size bands. Doublets at 500 and 450 kbp were in contrast to single bands of that size in the form 1 karyotype. This karyotype was designated form 4 and was found only in HH rats (Table 2).

Hybridization with unique and repetitive probes reflects electrophoretic karyotyping. To explore further the karyotypic differences among *P. carinii* preparations, the blotted karyotypes were hybridized to a series of DNA probes. The first probe to be used was a repetitive element, which has previously been shown to hybridize to every chromosome-sized band in the prototype rat *P. carinii* preparations separated by PFGE (19). The repetitive element has recently been reported to encode a major surface glycoprotein of rat *P. carinii* (28). Chromosomes from rat-derived variant *P. carinii* preparations showed little hybridization to the repetitive probe under conditions of high stringency, although under reduced stringency some homology was apparent (data not shown). Chromosomes from human-derived organisms also showed no homology to this probe under conditions of high stringency (50). It was our intent to use the

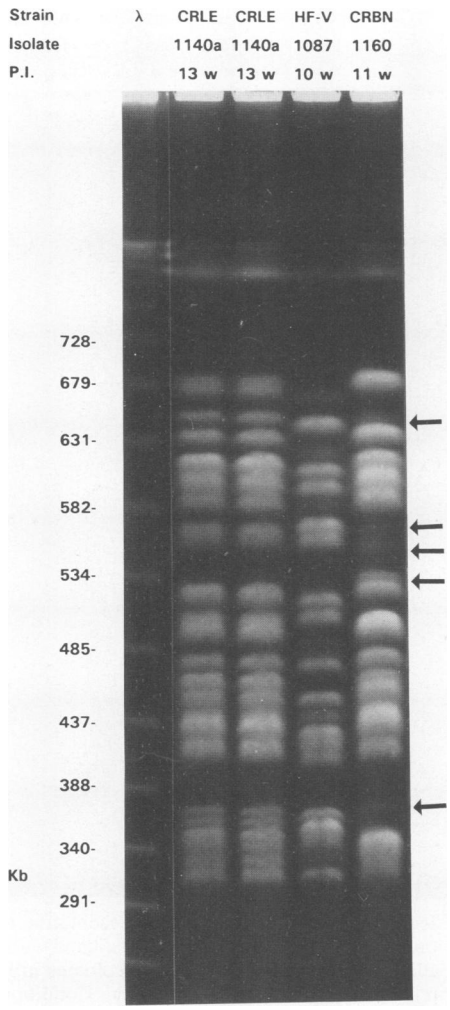

FIG. 3. Electrophoretic karyotypes of mixed populations and variant *P. carinii*. Electrophoretic karyotypes (stained by ethidium bromide) of examples of mixed populations of organisms prepared from a single rat were found in CRLE colonies (rat 1140a) and CRBN rat 1160. These rats were immunosuppressed for 13 and 11 weeks, respectively. A population composed almost entirely of variant *P. carinii* was found in a single preparation from an HF rat, HF-V 1087, immunosuppressed for 10 weeks. CHEF conditions were the same as for Fig. 1 and 2. The leftmost lane contains lambda size markers. Arrows indicate bands that stained faintly with ethidium bromide.

repetitive DNA probe to evaluate the preparations for the presence of prototype and variant populations, as well as to visualize the individual chromosomes in the prototype populations by autoradiography.

Figure 5B shows the results of hybridization with repetitive probe 1410 to representative karyotypes from the rat colonies produced by FIGE. Every band of karyotype forms 1 to 4 had homology with repetitive clone 1410. This hybridization pattern is shown schematically in Fig. 4, as probe R. The intensity of the hybridization signal was not always reflective of the ethidium bromide staining intensity, indicating that different amounts of the element were interspersed among the chromosomes or that there were homology differences. This repetitive element did not hybridize with chromosomal DNA of either *S. cerevisiae* or *Cryptococcus neoformans* and appears to be specific for rat-derived prototype *P. carinii* under hybridization conditions of high stringency.

The 13- to 15-band karyotypes were next hybridized to a series of single-copy probes. An example of a hybridization with a single-copy gene probe, the thymidylate synthase gene, is shown in Fig. 5C. Hybridization to a single band ranging in size from 340 to 330 kbp was observed in all of the karyotypes. Hybridization to the megabase pair-sized DNA in a few of the samples was likely due to the homology between the thymidylate synthase genes of *P. carinii* and the rat host. The CRL and CRLE lanes did not contain sufficient DNA to produce a signal under these conditions, although after increased exposure times a faint signal could be detected. In other experiments, organism preparations from these same colonies were probed with the same thymidylate synthase probe and produced a hybridization signal (see Fig. 6, CRL preparations 6/89 through 10/92, for example).

A diagrammatic representation of the results obtained from hybridization with the single-copy gene probes is shown in Fig. 4. The numbers beside the bands represent the hybridization of a gene or gene fragment to the band. The numbered probes are identified in the legend to Fig. 4. The probe to the ATPase gene (Fig. 4, probe 1) mapped to a 500-kbp band in forms 1, 3, and 4 and a 520-kbp band in form 2. The ribosomal locus (probe 2) was found on the same band of 500 kbp in form 1 and 4 karyotypes and on a 516-kbp band in forms 2 and 3. The probe to a 45- to 55-kDa antigen isolated from a rat *P. carinii* cDNA library (probe 3) hybridized to a band of about 630 kbp in forms 1, 3, and 4 and to a 640-kbp band in form 2. Two anonymous probes, 4 and 5, were hybridized only to karyotypes of form 1. Probe 4 hybridized to 650-kbp band, and probe 5 hybridized to a 500-kbp band which also carried the ATPase and ribosomal gene loci. A probe to the gene for transcription factor IID (probe 6) hybridized to a band of approximately 535 kbp in all karyotype forms, while the probe to alpha-tubulin (probe 7) hybridized to a 430-kbp band in forms 1 and 4, a 420-kbp band in form 2, and a 460-kbp band in form 3. Generally, the single-copy gene probes were found to hybridize with single bands of similar size among the four forms of the karyotypes, indicating that gross genome rearrangements were not characteristic of the rat *P. carinii* populations surveyed and that the variations in band size were due to minor changes in chromosome length. In a few cases, two bands hybridized to a single-gene probe. These karyotypes were later found to contain both prototype and variant *P. carinii*.

Frequency of rat colonies infected with a single karyotype of *P. carinii* versus a mixture. Inspection of the karyotypes made from the 10 infected rat colonies surveyed revealed that an entire colony could be infected with a single 13- to 15-band karyotype or that a colony could be infected with both a 13- to 15-band karyotype and a 20- to 24-band karyotype. As shown in Table 3, the rats in the ZMSD, HH, and SH colonies were each infected with a single *P. carinii* prototype form. That these karyotypes were prototype *P. carinii* was verified by hybridization with a cloned repetitive DNA element, Rp3-1 or 1410 (described above); hybridization with a prototype-specific oligonucleotide probe; and sequencing of a portion of the 18S ribosomal subunit gene of selected karyotypes from each of the colonies (reference 8 and data not shown). Since prototype forms 1, 3, and 4 were all found as the only detectable *P. carinii* organism in these colonies, it does not appear that single infections are restricted to a particular *P. carinii* prototype karyotype form.

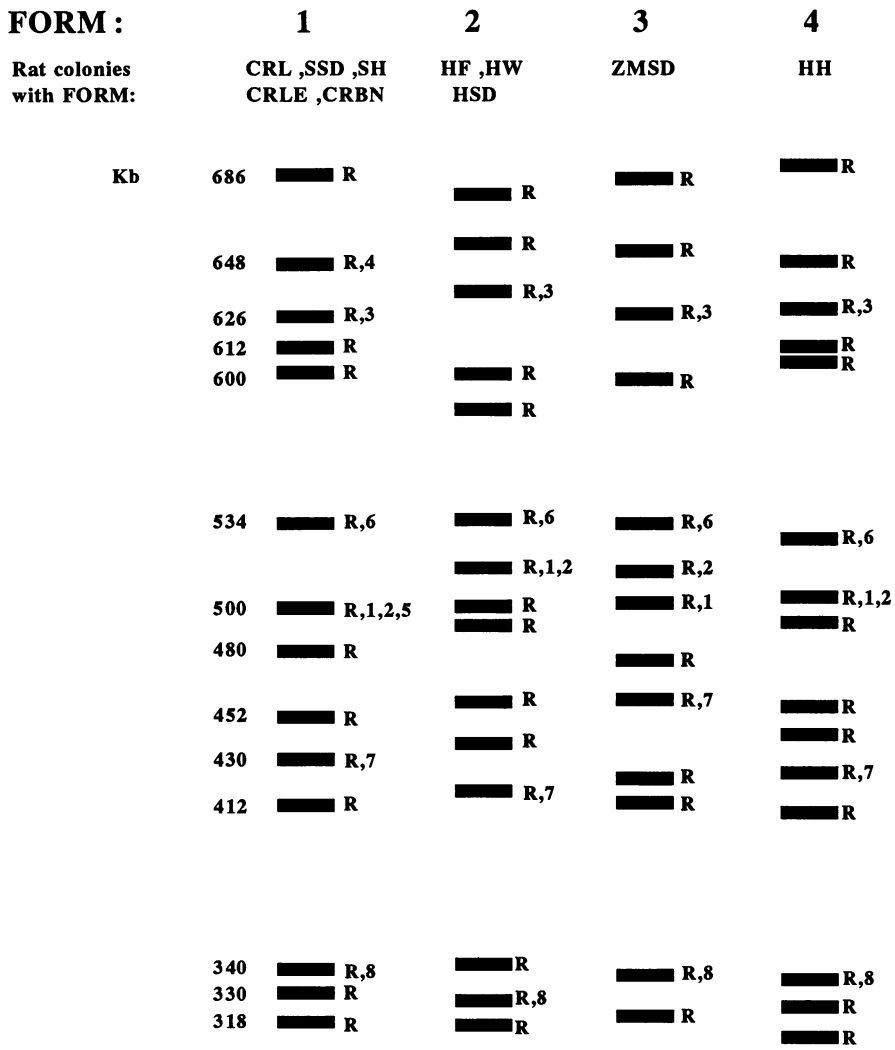

FIG. 4. Schematic representation of rat *P. carinii* karyotype forms. Consensus karyotypes were constructed by averaging the band sizes from at least three CHEF gels of the *P. carinii* prepared from the various rat colonies. Assignment of form type is discussed in the text. Repetitive and unique gene probes were mapped to the bands in the different karyotypes by Southern hybridization as described in Materials and Methods. The following designations correspond to the probes used in the schematic: R, repeated DNA element (51); 1, ATPase (37); 2, rRNA locus (51); 3, 45- to 55-kDa antigen (48); 4, anonymous probe T1; 5, anonymous probe T2; 6, transcription factor IID (53); 7, alpha-tubulin (64); 8, thymidylate synthase (11).

Ten examples of mixed infections with prototype and variant *P. carinii* were detected in rats from the CRL, CRBN, and CRLE colonies and in the HW and HF rats. The frequency of mixed infection varied from a low of 1 in 16 preparations from the HF colony (6%) to a high of about 42% in the CRLE colony. It is important to note that the Long Evans, Lewis, and Brown Norway strains of rats were housed in the same area (083) at the vendor facilities during the time of this survey. All of these rat strains showed the presence of mixed infections, and in the absence of the variant *P. carinii*, all of the rats were infected with *P. carinii* prototype form 1. It is not difficult to envision the capacity for transmission of the infections in such an area where the cages are of the standard type, open to the air (as opposed to barrier cages), and are linked by a common entry and work station. The Brown Norway rats have resided in area 083 since January 1978, while the Lewis and Long Evans strains were moved into this area in April 1991 from area 081 (11a).

Mixed infections occurred with a reduced frequency in the HW and HF colonies, where a single rat in each colony had a mixed infection. Most of the rats in these colonies were infected with prototype *P. carinii* form 2. This form was also

TABLE 2. Distribution of prototype forms among rat colonies

Prototype form	No. of rats that produced form	Rat strains in which form was detected	Vendor
1	33	Lewis, Brown Norway, Long Evans	Charles River
		Sprague-Dawley, Holtzman	Sasco
2	22	Wistar, Fischer, Sprague-Dawley	Hilltop
3	5	Sprague-Dawley	Zivic-Miller
4	7	Holtzman	Harlan

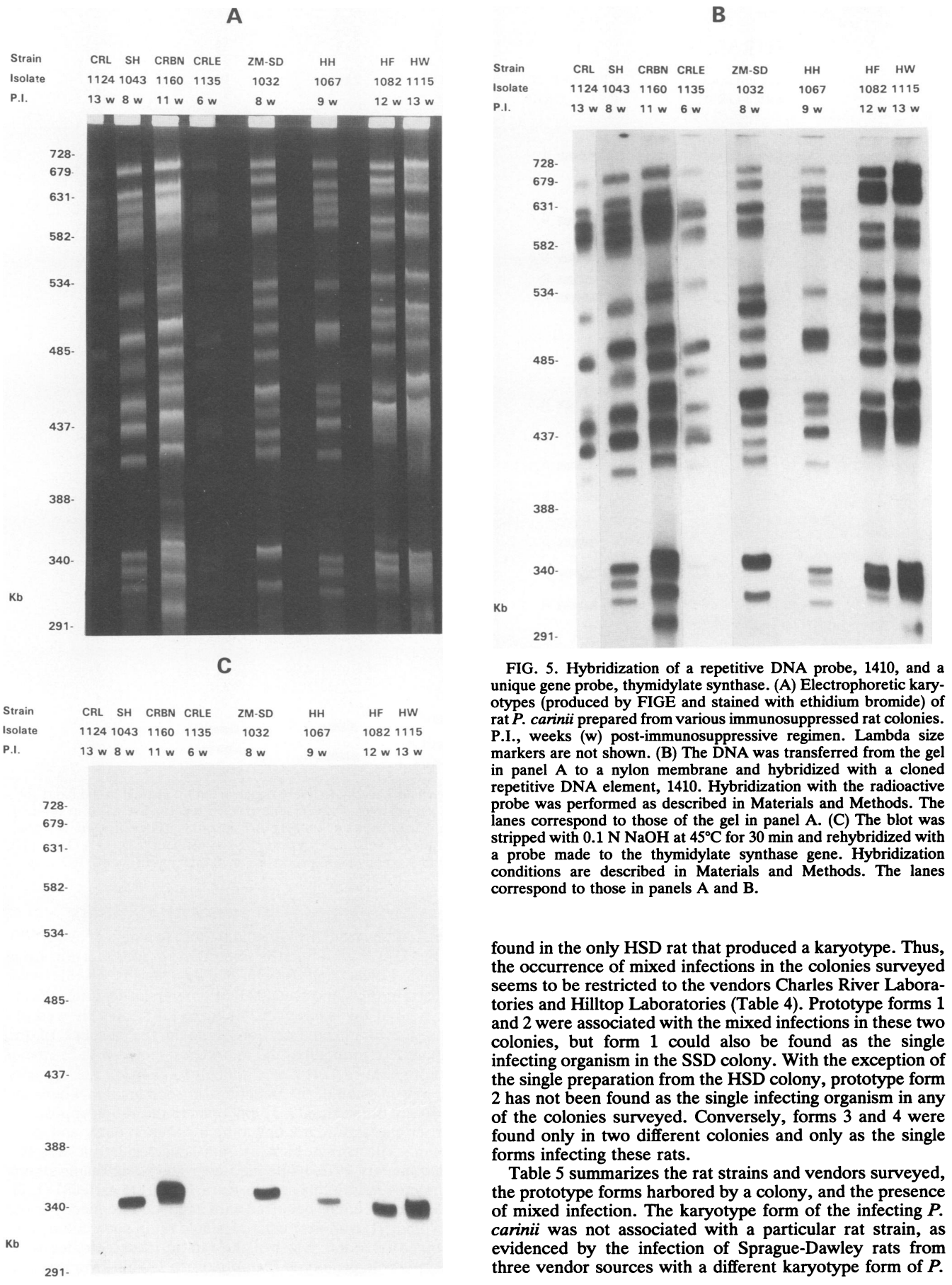

FIG. 5. Hybridization of a repetitive DNA probe, 1410, and a unique gene probe, thymidylate synthase. (A) Electrophoretic karyotypes (produced by FIGE and stained with ethidium bromide) of rat *P. carinii* prepared from various immunosuppressed rat colonies. P.I., weeks (w) post-immunosuppressive regimen. Lambda size markers are not shown. (B) The DNA was transferred from the gel in panel A to a nylon membrane and hybridized with a cloned repetitive DNA element, 1410. Hybridization with the radioactive probe was performed as described in Materials and Methods. The lanes correspond to those of the gel in panel A. (C) The blot was stripped with 0.1 N NaOH at 45°C for 30 min and rehybridized with a probe made to the thymidylate synthase gene. Hybridization conditions are described in Materials and Methods. The lanes correspond to those in panels A and B.

found in the only HSD rat that produced a karyotype. Thus, the occurrence of mixed infections in the colonies surveyed seems to be restricted to the vendors Charles River Laboratories and Hilltop Laboratories (Table 4). Prototype forms 1 and 2 were associated with the mixed infections in these two colonies, but form 1 could also be found as the single infecting organism in the SSD colony. With the exception of the single preparation from the HSD colony, prototype form 2 has not been found as the single infecting organism in any of the colonies surveyed. Conversely, forms 3 and 4 were found only in two different colonies and only as the single forms infecting these rats.

Table 5 summarizes the rat strains and vendors surveyed, the prototype forms harbored by a colony, and the presence of mixed infection. The karyotype form of the infecting *P. carinii* was not associated with a particular rat strain, as evidenced by the infection of Sprague-Dawley rats from three vendor sources with a different karyotype form of *P.*

TABLE 3. Distribution of prototype and mixed infections among the rat colonies surveyed

Rat colony	Prototype form	No. of rats that produced:	
		Only prototype	Mixed infections
ZMSD	3	5	0
HH	4	7	0
SH	1	9	0
CRL	1	6	3
CRBN	1	6	2
CRLE	1	4	3
HW	2	6	1
HF	2	15	1
HSD	2	1	0

TABLE 5. Association of karyotype forms with rat strains or vendors

Rat strain	Vendor(s) surveyed	Prototype form(s) present	Mixed infection
Sprague-Dawley	Zivic Miller, Hilltop,[a] Sasco	1, 2, 3	No
Holtzman	Sasco, Harlan	1, 4	No
Fischer	Hilltop	2	Yes
Wistar	Hilltop	2	Yes
Lewis	Charles River	1	Yes
Long Evans	Charles River	1	Yes
Brown Norway	Charles River	1	Yes

[a] A single preparation was analyzed.

carinii. Holtzman rats from two vendors each harbored a different karyotype form (form 1 or 4), supporting the observation that a specific rat strain is not a requirement for infection by a given *P. carinii* form. Also apparent from Table 5 was the absence of mixed infections in the Sprague-Dawley and Holtzman strains of rats. All of the other rat strains surveyed supported the mixed infection. As noted above, only *P. carinii* prototype forms 1 and 2 were associated with the presence of mixed infection. However, these apparent differences could be due to the absence of the variant *P. carinii* from the Sasco and Harlan facilities.

The karyotype harbored by a rat colony can remain stable over time. To evaluate the stability of the karyotype of *P. carinii* infecting a rat colony, two vendor colonies of rats were selected for sampling over a 2- to 3-year period. During this period, male Lewis rats and male Sprague-Dawley rats were purchased every 3 to 4 months from the commercial suppliers (Charles River and Sasco), brought into our housing facilities in filtered containers, and subsequently immunosuppressed while being housed under barrier conditions. The experiment began with the Lewis colony in January 1989 and the Sprague-Dawley colony in February 1991. The colonies were sampled several times at random, and representative karyotypes from various time points are shown in Fig. 6. Very little change occurred in the karyotype of the *P. carinii* harbored by the rats in either of the two colonies. One band deletion was observed among the smallest group of bands (350 to 300 kbp) in preparation SSD 2/91. Hybridization with a probe to the thymidylate synthase gene showed that the band carrying this gene was not lost but was decreased in size by about 7 kbp (Fig. 6B). Minor size variations in all of the bands in the second lane, SSD 12/90, are evident. We have observed that the migration of bands is affected by the amount of DNA loaded; overloading causes a slight retardation of migration, as has been reported by other investigators (14, 54). In the CRL 5/91 and CRL 10/92 karyotypes, nonstoichiometric staining bands were observed at about 460 kbp and at 360 and 380 kbp (CRL 5/91) (marked by white squares). The presence of these bands may indicate that a second type of *P. carinii* had taken up residence in this colony of rats. The housing arrangement at the vendor facilities, Charles River, could be responsible for this, since the Lewis rats are housed in the same area as the CRLE and CRBN colonies. These last two colonies have been shown to harbor coinfections with two *P. carinii* types, as discussed above and reported recently (8).

TABLE 4. Presence of mixed infections among rats from different vendors

Vendor	Prototype form	Mixed infection
Charles River	1	Yes
Hilltop	2	Yes
Zivic-Miller	3	No
Harlan	4	No
Sasco	1	No

DISCUSSION

It has been known for some time that rats in different colonies can harbor forms of *P. carinii* that differ in karyotype, but the extent and basis of such variation have not been well studied. It is important to better characterize karyotypic variation and stability in rat-derived *P. carinii* for several reasons. Karyotypes can be used to define strains of *P. carinii* that are found in various rat colonies. This is important information for those working with the rat model. Knowledge of the forms of *P. carinii* contained in colonies from commercial breeders will permit a correlation of the various forms of *P. carinii* to be made with a particular research application, such as responsiveness to drugs, ability to adapt to in vitro cultivation, and phenotypic differences recognized by antigenic analyses. In addition, identification of karyotypic forms is important in establishing archives of frozen stocks that represent as many of the forms of the organism as possible. Such archival stocks will allow preservation of natural variants that could be lost because of changes in breeding practices. It is now the trend of animal vendors to phase out the viral antibody-positive stocks of rats, in which the diverse *P. carinii* forms are harbored, and replace them with barrier-maintained, viral antibody-negative rats that do not harbor the organism. One of the rat colonies that we had originally sampled by PFGE (19) has been phased out by the vendor, and to date we have not detected this karyotype form among the colonies that have been sampled subsequently. Preservation of diverse *P. carinii* stocks seems prudent, since it is not known whether karyotypically distinguishable *P. carinii* stocks are functionally different. Natural variants might exhibit different properties with respect to virulence or structure of macromolecules studied as potential drug targets. In addition, access to *P. carinii* stocks with distinct karyotype patterns will facilitate research on the biology of *P. carinii* infection and its life cycle. The mechanism of transmission and the role of sexual reproduction are two areas that can be more thoroughly explored by understanding and using karyotypic variation.

The results of this study indicate that rat-derived *P. carinii* occurs in at least four karyotypic forms, each of which is associated with a different commercial breeder colony. The

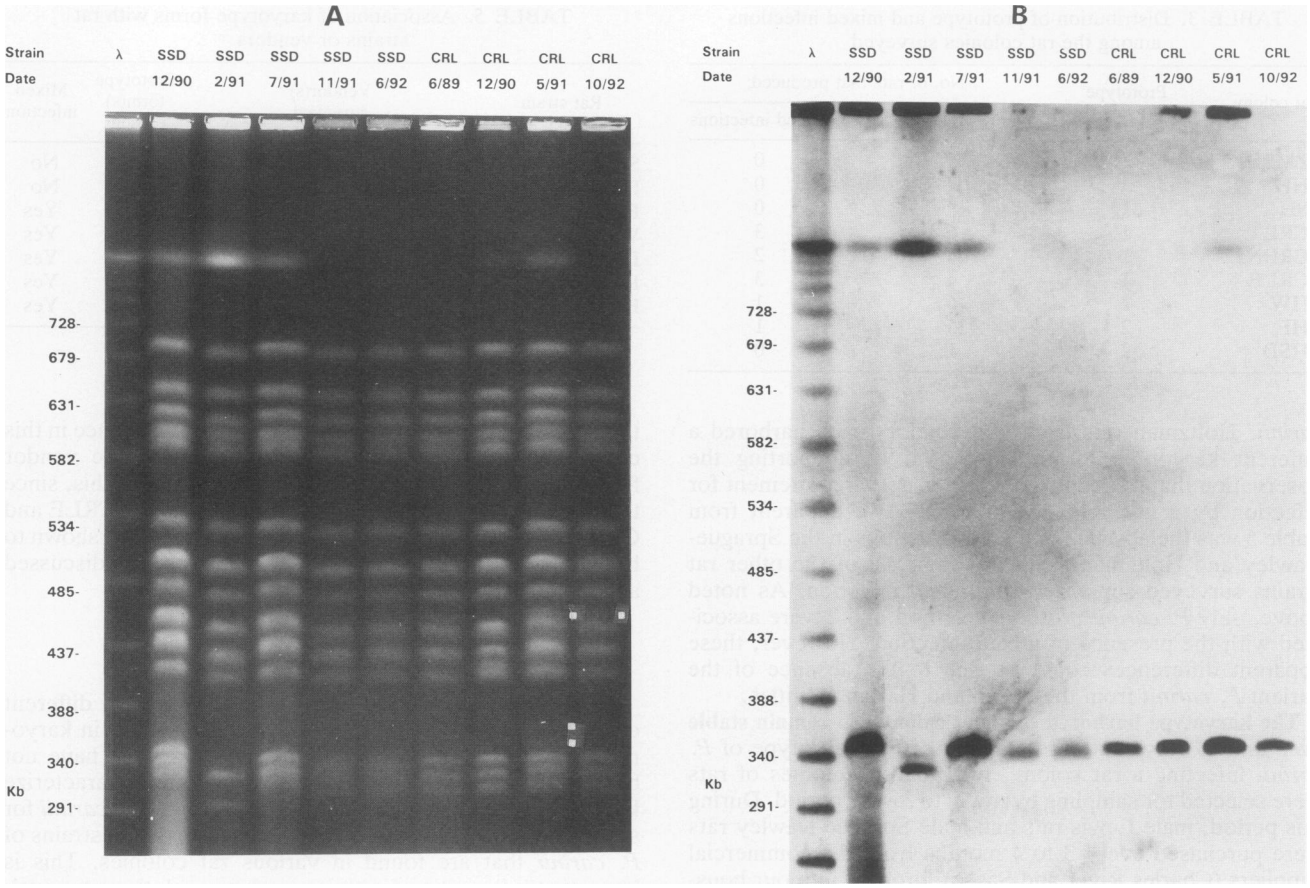

FIG. 6. Stability of *P. carinii* karyotypes infecting two colonies over time. Rats were received into our facilities from two vendor sources (Sasco and Charles River), immunosuppressed, and randomly sampled by electrophoretic karyotypic analysis over a 2- or 3-year period, respectively. (A) Ethidium bromide-stained karyotypes; (B) hybridization of the Southern blotted DNAs to a thymidylate synthase probe. CHEF and hybridization conditions were as described in Materials and Methods. Lambda size markers are in the leftmost lane. White squares indicate bands that stained faintly with ethidium bromide.

karyotypes were similar enough to support the conclusion that the genome of rat-derived *P. carinii* consists of 14 to 16 chromosomes that range in size from 300 to 700 kbp and that the size of the genome is 7 to 8 Mbp. There was little evidence of chromosomal length polymorphisms, suggesting that the genome is haploid or that if it is diploid, the sizes of the homologs were almost identical.

Rats from each breeder colony carried a particular form of prototype *P. carinii*. This phenomenon seems to be due to differences in exposure of the rats in each colony rather than to differences in susceptibility, because forms 1 and 2 were each found in more than one strain of rat. The host range of prototype forms 3 and 4 could not be assessed because the colonies containing these forms each consisted of a single rat strain. Consistent with this finding were the results from a recent study that showed immunosuppressed Sprague-Dawley, Fischer, and Wistar rat strains that were housed in a single room were infected with the same *P. carinii* karyotype (18). A second observation supporting the idea that *P. carinii* forms are associated with breeding facilities and not with particular rat strains was that Sprague-Dawley rats harbored three forms and Holtzman rats harbored two forms of prototype *P. carinii*. Although the susceptibility of other rat strains to different forms of *P. carinii* could not be determined because these strains were not in colonies containing more than one prototype form, it is noteworthy that Wistar, Fischer, Lewis, Brown Norway, and Long Evans rats all sustained coinfections with prototype and variant *P. carinii*. The variant variety of *P. carinii* was found only in the Charles River and Hilltop facilities. Again, the apparent absence of the variant in rats from the other breeding facilities was probably due to lack of exposure to this organism, but we cannot rule out the possibility that Sprague-Dawley rats and Holtzman rats are resistant to it. The HSD rat did not contain detectable amounts of variant, but only one rat was analyzed, and coinfection was rare in the HW and HF rats. Coinfections were seen with prototype forms 1 and 2. The capacity of the variant to coinfect rats carrying prototype forms 3 and 4 could not be determined from the animals available, but this question can be addressed by transmission or inoculation experiments.

Infections caused by organisms displaying a 20- to 24-band karyotype have been reported by at least one other group (18). In that other study, rats harboring two distinct 13-band karyotypes produced by FIGE and designated types I and II were placed in the same housing area. After 7 to 8 weeks of immunosuppression, the 20- to 24-band karyotypes were detected in some of the rats. The authors concluded that these infections arose by a mixing of pattern types I and II. Although hybridization data were not included to support

this assumption, it is conceivable that these rats harbored a coinfection with two genetically distinct *P. carinii* strains. Coinfection with two strains of *P. carinii* would then be considered a widespread event and not confined to the vendors of the United States, since this particular report evaluated rats in South Korea (18).

The stability and uniformity of *P. carinii* within a particular breeder colony have implications regarding the source of the variation seen among *P. carinii* strains in different breeder colonies. The diverse prototype forms of rat-derived *P. carinii* do not seem to be the result of extreme genomic instability, since a given karyotype was essentially the same for 2 to 3 years and the differences in karyotype band patterns among prototype forms could be ascribed to slight shifts in the sizes of chromosomes rather than to radical rearrangements such as translocations. The forms and varieties of *P. carinii* residing in different rat colonies might be representatives of organisms that preexisted in the rats that founded the colony. These forms may in turn represent a sample of what is present in the wild, either colonizing or parasitizing rats, or in some as yet undiscovered free-living form. Given the existence of numerous karyotypic forms of *P. carinii*, the karyotype obtained from *P. carinii* derived from each rat in a given colony is strikingly simple and consistent. This suggests that new infections are acquired exclusively from the *P. carinii* strains that are in the colony and either that rats are not exposed to other *P. carinii* strains or that these organisms do not often establish an infection of a magnitude similar to that caused by *P. carinii* indigenous to the colony.

Discussion of the organism preparations would be simplified by a system of nomenclature. Two separate suggestions have been offered for the differentiation of *P. carinii* organisms isolated from various mammalian sources. Frenkel's suggestion of *P. carinii* for rat-derived organisms and "*Pneumocystis jiroveci*" for organisms from humans offers only a distinction between organisms from those two mammalian hosts (13). Hughes and Gigliotti suggested retaining the binomial *P. carinii*, with the host of origin following as a descriptor (20). Neither of these suggestions directly addresses the need to differentiate *P. carinii* preparations obtained from a single mammalian host. Furthermore, we have recently described the coinfection of immunosuppressed rats with two genetically distinct *P. carinii* strains (8). These two genetic variants were called prototype and variant on the basis of differences in electrophoretic karyotype pattern, sequence within a region of the small ribosomal subunit, presence of the 390-bp intron in the small-rRNA gene, and homology with repetitive DNA (Rp3-1 or 1410). The present study focused on the karyotypes of the *P. carinii* prototypes present in the rat colonies from commercial breeders. These prototype *P. carinii* preparations could be differentiated by electrophoretic karyotype and hybridization profile. Following the suggestions of previous investigators, as well as the botanical code which is used to name fungi (21, 22), we propose a provisional system of nomenclature for *P. carinii* that will permit differentiation of *P. carinii* preparations isolated from the same mammalian host. Under this system, *Pneumocystis* preparations from rats would be designated by the binomial *P. carinii*, followed by a subspecies designation, *rattus*, to identify the host of origin. Use of the host of origin to differentiate organisms is permitted by the botanical code, under the provision for recognition of special forms (formae speciales) that have a parasitic existence. There are no standardized rules governing the choice of subspecies versus species as a hierarchical designation given to physiologic variants. Rather, the definitions are dependent upon the organisms being evaluated. At this time, we prefer the use of subspecies to differentiate the preparations of *P. carinii* obtained from the various mammalian hosts until a more complete dossier of information is available to support the distinction of species, since such a designation would require change of the binomial. A designation of varietas would further subdivide the rat preparations on the basis of the aforementioned molecular biological criteria into prototype or variant, and electrophoretic karyotype variation would provide the basis by which to differentiate formae, which would be distinguished by an arabic numeral. There are no rules governing the definition of names below the subspecies level in the botanical code. Thus, we have suggested these names as a practical approach to distinguish the various *P. carinii* strains found in rats. Application of this system of nomenclature to the preparations in this study would yield the following: *P. carinii* subsp. *rattus* var. prototype form 1, to include the CRL, CRLE, CRBN, SH, and SSD organisms; *P. carinii* subsp. *rattus* var. prototype form 2, to include the organisms prepared from HF and HW rats; *P. carinii* subsp. *rattus* var. prototype form 3, to include ZMSD preparations; *P. carinii* subsp. *rattus* var. prototype form 4, to include organisms isolated from HH rats; and *P. carinii* subsp. *rattus* var. variant form 1, for the single representative of this genetic variant of *P. carinii* prepared from an HF rat, 1087. *P. carinii* subsp. *rattus* var. prototype form 1 has been deposited in the American Type Culture Collection and will be available for propagation by inoculation into viral antibody-negative rats.

Whether the electrophoretic karyotypes reported by other *P. carinii* investigators fall under any of the forms reported here remains to be evaluated. Direct comparison of the karyotypes is made difficult by the different PFGE techniques used. In our experience, the CHEF technique combined with the extended gel format is able to provide very good resolution of the chromosome-sized DNA of *P. carinii* in a reproducible manner. We have found these conditions to produce the same results between the two CHEF systems in our own laboratories and expect a high degree of reproducibility among the commercial units available. Thus, by exchange of *P. carinii* preparations or by the simple use of appropriate size markers, comparison of karyotypes from different laboratories under the CHEF conditions reported here should be feasible.

Although we have focused on the differences among *P. carinii* preparations from a single mammalian host in this study, molecular and antigenic evidence suggests that *P. carinii* organisms from different mammalian hosts are genetically distinct entities. The electrophoretic karyotype from ferret-derived *P. carinii* appears to differ dramatically from those of organisms from rats (59). In our laboratory, ferret *P. carinii* obtained from Francis Gigliotti produced karyotypes of about 15 bands that separated between 500 to 950 kbp by CHEF, a profile quite distinct from rat, human, or mouse karyotypes (data not shown). Some ferret *P. carinii* antigens appear to have epitopes that diverge from those of *P. carinii* from other mammalian hosts, indicating phenotypic differences (15, 16). The electrophoretic karyotype from human *P. carinii* separates within the 300- to 700-kbp range observed for rat-derived organisms, although the sizes of the bands produce a pattern different from the rat *P. carinii* karyotypes and the hybridization profiles and gene sequence variation also suggest distinct differences (50, 63). Likewise, antigenic differences between human- and rodent-derived *P. carinii*

have been long appreciated (17, 27, 58). Thus, this same system of nomenclature could be applied to organisms from different hosts. For example, organisms from a human source would be called by the trinomial *P. carinii* subsp. *humanus*, as we have previously suggested (50). Distinction at the species level would require the change of the binomial. Such changes are appropriately discussed at sessions during congresses convened for the express purpose of nomenclature.

To be useful as an epidemiologic tool, electrophoretic karyotypes of a microbe should provide sufficient variability for characterization of preparations yet should not exhibit extensive variation so that every preparation, even within a strain, shows differences. Ideally, the karyotype should remain stable within a given strain. Unlike the genomes of some other protists, such as *Leishmania* spp. (39), *Candida albicans* (45), or *Kluyveromyces marxianus* var. marxianus (29), the rat-derived *Pneumocystis* genome does not appear to undergo extensive chromosomal rearrangements in the confines of this mammalian host. Differences in karyotype have been shown to occur in vitro for some protists like *Plasmodium falciparum* (24, 54) and among isolates of the same genus and species of yeast (29). In contrast to these organisms, the present study showed little variation in the electrophoretic karyotype of a population of organisms harbored by a rat colony. Thus, karyotypes of *P. carinii* preparations provide sufficient diversity and stability that warrant their use as markers in future experiments.

The similarity of *Pneumocystis* karyotypes isolated from several rat colonies seems to suggest that the *P. carinii* subsp. *rattus* genome maintains its chromosome sizes within this mammalian host. Such size regulation has been associated with the ability of an organism to undergo meiosis (25), although size differences among homologs does not preclude this process (43). An ultrastructural study showing synaptonemal complexes in a developmental form of *P. carinii* subsp. *rattus* supports the contention that this organism is able to undergo meiosis (35).

It is also apparent from the data presented here that all members of a rat colony may be infected with a *P. carinii* population that produces the same karyotype or that some members may be infected with at least two types of the organism. Presently, the biological implications of a mixed infection versus a homogeneous one are not understood. However, the potential for relapse of infection, the susceptibility of drugs, and the question of virulence should be examined in light of these data.

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